

**Identification of regulatory factors in the signal
transduction pathway in herbivore-induced
maize**

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Summary

In order to survive in a changing environment, plants are able to communicate with other organisms. One aspect of chemical communication is the production of complex mixtures of volatile organic compounds (VOC) after the plants are attacked by an herbivore enemy. Those volatiles can be used as cues by parasitic wasps to locate their hosts. Therefore, this tritrophic interaction has been termed “indirect defense”.

The ecological aspects of the indirect defense and the biosynthesis of the terpene volatiles are well studied in maize (*Zea mays*). Two terpene synthases, TPS10 and TPS23, are responsible for the biosynthesis of the major sesquiterpene volatiles produced after attack by the larvae of the lepidopteran herbivore *Spodoptera littoralis*. TPS10 converts the precursor farnesyl diphosphate (FPP) into the sesquiterpenes (*E*)- β -farnesene and (*E*)- α -bergamotene. The enzyme TPS23 synthesizes (*E*)- β -caryophyllene from the same substrate. The signal transduction pathways responsible for the regulation of volatile induced defenses remain mostly unclear. However, damage by different types of herbivores results in specific volatile signals, which suggests a complex regulatory network.

This work focused on the identification of transcription factors involved in the signaling cascade between herbivore damage and volatile emission. Microarray hybridization identified several transcription factors affected by herbivory, mechanical damage, and mechanical damage plus caterpillar regurgitate. Also, the effects of herbivore-damaged roots upon leaves and vice versa (systemic effects) were assayed. The majority of transcription factors reacted in a similar fashion to several types of induction which included caterpillar feeding and mechanical damage. Nevertheless, maize plants responded to mechanical damage by emitting only ~50 % of sesquiterpenes found in herbivore-induced plants, indicating a separate recognition of caterpillar regurgitate.

The analysis of plant hormones after herbivore damage confirmed their roles in specific signal transduction pathways. While an increase of jasmonic acid (JA) concentration was observed after all types of mechanical damage to the plant, salicylic acid (SA) concentrations were only increased when plants were treated with caterpillar regurgitate. Therefore, SA might have a role in maize herbivore signal transduction pathways that is different from its role in signal transduction after pathogen attack.

The expression patterns and product formation of the terpene synthases TPS10 and TPS23 were investigated in response to herbivory. Transgenic Arabidopsis plants with promoter::reporter gene constructs of both terpene synthases displayed an herbivore-regulated activity similar to the expression patterns in maize. This heterologous expression system was utilized to further characterize the promoters of both genes. The promoter of *tps10* was only induced when plants were treated with both mechanical damage and JA. The promoter of *tps23* was induced by either treatment alone. A promoter deletion analysis revealed an essential area with a WUN-motif in the promoter of *tps10*. An area with the same motif in the promoter of *tps23* was not crucial for gene expression. Overall, this study indicated that maize is able to respond to a variety of cues of herbivory and contribute to the formation of herbivore-specific volatile signals via several signal transduction pathways. The exact components involved in each of the signal transduction cascades and their functions still need to be determined.

Zusammenfassung

Um in einer sich veränderten Umwelt zu überleben, nutzen Pflanzen unterschiedliche Arten der Kommunikation. Die Produktion von komplexen Gemischen volatiler organischer Verbindungen ist ein Aspekt der chemischen Kommunikation. Diese flüchtigen Substanzen werden von Pflanzen nach Fraßbefall abgegeben und können als Lockstoffe für parasitäre Schädlinge der Fraßfeinde genutzt werden. Diese tritrophische Interaktion kann der indirekten Verteidigung der Pflanze dienen.

Die ökologischen Gesichtspunkte der indirekten Verteidigung und die Biosynthese der flüchtigen Terpene sind in Mais (*Zea mays*) gut untersucht. Zwei Terpensynthasen synthetisieren die Mehrheit der Sesquiterpene, die durch den Befall mit der Larve eines Eulenfalters (*Spodoptera littoralis*) gebildet werden. Die Terpensynthase TPS10 katalysiert die Umsetzung des Substrates Farnesyldiphosphat (FPP) zu den Sesquiterpenen (*E*)- β -Farnesen und (*E*)- α -Bergamoten. (*E*)- β -Caryophyllen wird aus dem gleichen Ausgangsprodukt von der Terpensynthase TPS23 gebildet. Die Signalkaskade, die nach der Verletzung der Pflanze zur Bildung der flüchtigen Terpene führt, ist weitgehend unbekannt. Die Abhängigkeit der Zusammensetzung der volatilen Signale vom Typ des angreifenden Herbivoren deutet auf ein komplexes regulatorisches Netzwerk hin.

Der Fokus dieser Arbeit lag in der Identifizierung von Transkriptionsfaktoren, die an diesen Signalwegen beteiligt sein könnten. Durch die Hybridisierung von Microarrays wurden mehrere Transkriptionsfaktoren gefunden, deren Genexpression durch Raupenfraß, mechanische Verwundung und Elizitoren differenziell reguliert wurde. Der systemische Effekt eines Herbivorenbefalls der Wurzeln auf die Genexpression der Blätter und umgekehrt wurde ebenfalls untersucht. Die Mehrzahl der Transkriptionsfaktoren zeigte eine ähnliche Genexpression nach unterschiedlichen Induktionsmethoden. Dennoch wurden aber nur etwa 50 % der Sesquiterpene von der Maispflanze abgegeben, nachdem diese nur mechanisch verwundet wurde. Das deutet auf separate Signalwege für die mechanische Verwundung und für die Reaktion auf Elizitoren von Insekten hin. Eine Analyse der Pflanzenhormone bestätigte deren Rolle bei der Signaltransduktion nach Herbivorie. Während Jasmonsäure auch in mechanisch verwundeten Pflanzen gefunden wurde, war Salicylsäure nur nach Zugabe von Elizitoren aus dem Regurgitat von *S. littoralis* messbar. Daher scheint sich die

Funktion der Salicylsäure in Mais nach Herbivorenbefall von der Funktion bei der Pathogenabwehr, die in anderen Pflanzen charakterisiert wurde, zu unterscheiden.

Die Genexpression und die Synthese der volatilen Produkte von TPS10 und TPS23 nach Insektenfraß wurden ebenfalls untersucht. Transgene Arabidopsispflanzen mit Promotor::Reportergenkonstrukten beider Terpensynthesen zeigten dieselbe herbivorie-regulierte Aktivität wie in Mais. Dieses heterologe Expressionssystem wurde zur weiteren Charakterisierung der Promotoren genutzt. Während der Promotor von *tps10* sowohl die mechanische Verwundung als auch Jasmonsäure für die Aktivierung benötigte, konnte der Promotor von *tps23* jeweils einzeln durch mechanische Verwundung und Jasmonsäure aktiviert werden. Promoter-Deletionsstudien von *tps10* deuteten auf einen essentiellen Promotorbereich hin, der ein WUN-Motiv enthält. Das WUN-Motiv ist ebenfalls im Promotor von *tps23* vorhanden, allerdings scheint dieser Bereich hier keine exklusive Rolle bei der Genexpression zu spielen. Insgesamt zeigen die Ergebnisse, dass die Verletzung der Maispflanze durch Herbivoren verschiedene fraßbedingte Signale erzeugt, die durch mehrere Signalkaskaden zur Bildung von herbivor-spezifischen Volatilen führt. Die Funktion der identifizierten Komponenten in den jeweiligen herbivor-spezifischen Signalkaskaden benötigt eine weitere Aufklärung.

Abbreviations

aa	amino acid
ABA	abscic acid
ABRE	abscic acid responsive element
<i>a.dest.</i>	distilled water
Amp ^r	ampicillin resistance
Amu	atomic mass unit
AP2/EREBP	apetala2/ethylene responsive element binding protein
APS	ammonium persulfate
ARF	auxin responsive factor
BCIP	5-bromo-4-chloro-3-indolyl phosphate
bHLH	basic helix loop helix
BLAST	basic local alignment search tool
BSA	bovine serum albumin
CAF1	CCR4-associated factor 1
CAPS	N-cyclohexyl-3-aminopropansulfonacid
CCR4	carbon catabolite repressor 4
cDNA	copy DNA
Cm ^r	chloramphenicol resistance
CoA	coenzyme A
cpm	counts per minute
cps	counts per second
CTR1	constitutive triple response 1
DAPI	4',6-diamidino-2-phenylindol
DMNT	(3, <i>E</i>)-4,8-dimethyl-1,3,7-nonatrien
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DMAPP	dimethylallyl pyrophosphate
DRE	drought responsive element
dsDNA	double stranded DNA

DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
EIN	ethylene insensitive
EMSA	electrophoretic mobility shift assay
ERE	ethylene responsive element
ERF	ethylene response factor
FPLC	fast protein liquid chromatography
FPP	farnesyl diphosphate
GC-MS	gas chromatography-mass spectrometry
Gent ^r	gentamycin resistance
GGPP	geranylgeranyl diphosphate
GLV	green leaf volatiles
GO	Gene Ontology
GPP	geranyl diphosphate
GUS	β -glucuronidase
IPP	isopentenyl diphosphate
IPTG	isopropyl-1-thio- β -D-galactoside
JA	jasmonic acid
JAI3	jasmonate insensitive 3
JA-Ile	jasmonic acid isoleucin
JAR	JA amino acid conjugate synthase
JAZ	jasmonate-ZIM-domain proteins
JERE	JA- and elicitor responsive element
k_{cat}	turn over number
kDa	kilodalton
Km ^r	kanamycin resistance
LB	Luria-Bertani
LHY	late elongated hypocotyl
LTR	long terminal repeat
MeJA	methyl jasmonate
MEP	2C-methyl-D-erythritol-4-phosphate

MES	(2-N-morpholino)ethanesulphonic acid
MeSA	methyl salicylate
MPK	mitogen-activated protein kinase
mRNA	messenger RNA
MTTF	membrane-tethered transcription factor
MU	4-methylumbelliferone
MUG	4-methylumbelliferyl-beta-D-glucuronide
NAC	no apical meristem (NAM), ATAF1,2 and cup-shaped cotyledon (CUC2)
NBT	nitro-blue tetrazolium
NLS	nuclear localization signal
NPR1	nonexpressor of pathogenesis-related genes 1
Nt	nucleotide
NTA	nitrilotriacetic acid
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethyleneglycol
phi	phosphate induced
RACE	rapid amplification of cDNA ends
RNA	ribonucleic acid
rRNA	ribosomal RNA
SA	salicylic acid
SAR	systemic acquired resistance
SARE	salicylic acid responsive element
SCF ^{COI1}	skip-cullin-F-box
SDS	sodium dodecyl sulfate
Sm/Sp ^r	streptomycin/spectinomycin resistance
SPME	solid phase micro extraction
TAE	tris-acetate-EDTA

TF	transcription factor
TEMED	N,N,N',N'-tetramethylethylenediamine
TIC	total ion chromatogram
TMV	<i>tobacco mosaic virus</i>
TPS	terpene synthase
Tris	tris(hydroxymethyl)aminomethane
UV	ultra-violet
VOC	volatile organic compound

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1. Introduction

1.1 The role of volatiles in plant defense

Organisms have evolved a way of communication with other organisms in order to survive in their environment. The chemical nature of plant communication has recently developed into a subject for intense research.

Plants are able to produce a complex mixture of volatile organic compounds (VOC) which are means to exchange information with its environment. These VOCs can be grouped into several chemical families: phenylpropanoid/benzenoids, fatty acid derivatives, amino acid derivatives, sulfur-containing compounds, and terpenes (Dudareva *et al.* 2006). Most volatile terpenes belong to mono- (C_{10}) and sesqui- (C_{15}) terpenes and can be released from flowers and vegetative organs. Since the production of volatiles is cost-intensive for plants (Wright *et al.* 1979), these compounds were suspected to play an important role. Volatile compounds that are emitted from flowers can attract pollinators and seed dispersers to provide a reproductive advantage (Raguso and Pichersky 1995; Pichersky and Gershenzon 2002; Reinhard *et al.* 2004). Also, vegetative organs can emit volatiles. Sharkey and coworkers showed that isoprene, a simple C_5 - terpene volatile, is released from the foliage of many tree species. Isoprene facilitates the photosynthetic apparatus to recover from brief episodes of temperatures over 40°C (Sharkey *et al.* 2001). The function of volatiles emitted from herbivore-attacked plants, has been well studied. Sometimes, these compounds are released when feeding ruptures pre-existing internal or external secretory structures in which the volatiles are synthesized and stored (Franceschi *et al.* 2005). In this case, the volatiles can directly repel or otherwise kill the invader (microbes, animals) due to their toxic nature (De Moraes *et al.* 2001; Kessler and Baldwin 2001; Vancanneyt *et al.* 2001). In other cases, airborne volatiles are produced from herbivore-damaged plants several hours after plant damage (Paré and Tumlinson 1997). Here, the volatiles serve as a chemical signal that attracts the enemies of the herbivore (Dicke 1994; Degenhardt *et al.* 2003; Howe and Jander 2008), thereby reducing the number of attacking herbivores (Kessler and Baldwin 2001). Belowground, the volatiles released from roots can act as antimicrobial and antiherbivore substances. They can also recruit enemies of root-feeding herbivores (Rasmann *et al.* 2005). Interestingly, plant volatiles do not only have the potential to affect animals but also other

plants in the direct proximity (Pichersky and Gershenzon 2002). It was shown that tobacco (*Nicotiana attenuata*) plants growing next to wounded sagebrush (*Artemisia tridentata*) suffered reduced levels of herbivore damage and exhibited increased levels of transcriptional responses (Kessler *et al.* 2006). In a different experiment, undamaged bean plants (*Phaseolus vulgaris*) exposed to volatiles from herbivore-infested neighbors showed an elevated expression of several genes involved in defense metabolism (Arimura *et al.* 2000). This so called “priming” was also found in poplar (Frost *et al.* 2008) and maize (Engelberth *et al.* 2004) when plants were exposed to green leaf volatiles, methyl jasmonate (MeJA), or volatile terpenes. An overview of the functional aspects of plant volatiles is depicted in Fig.1.1.

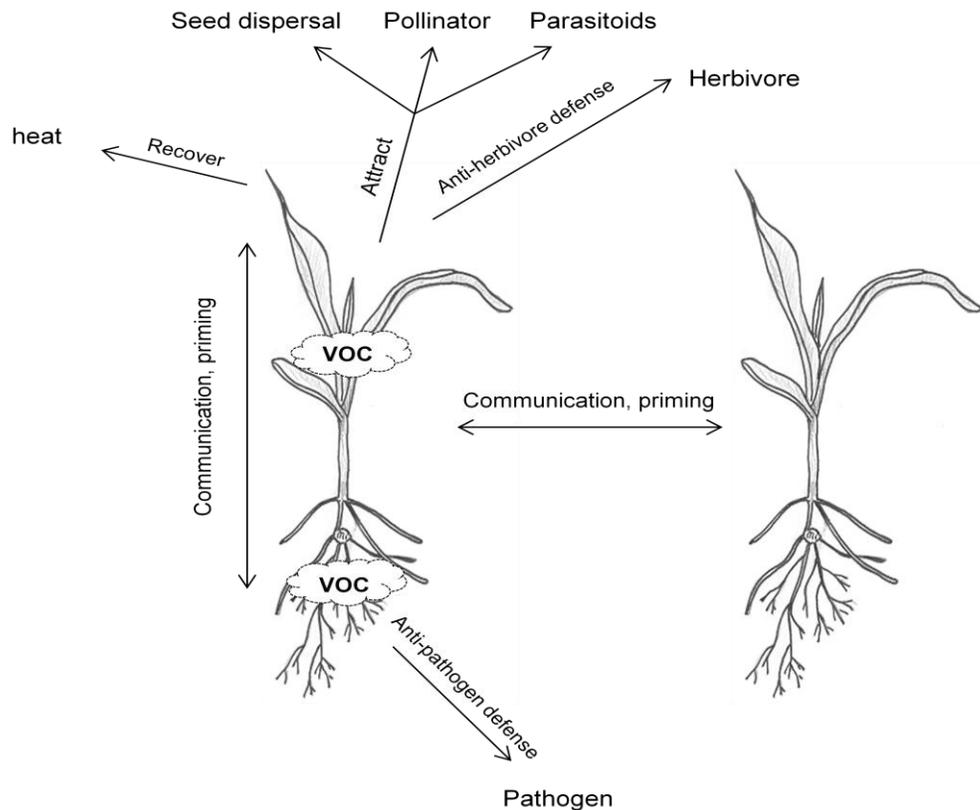


Fig.1.1: Volatile-mediated plant interactions with its environment. VOCs emitted from the plant can attract pollinators, seed disseminators, and parasitoids. Defense against herbivore attackers takes place above- and belowground. Communication between different organs of the plant and in between plants (priming, allelopathy) can be mediated by VOCs. The volatile terpene isoprene has a function in photoprotection and thermotolerance.

Systemic signaling has been described in several plant species (Howe and Jander 2008). Within hours, inducible defenses are found in undamaged parts of herbivore-attacked plant, providing a better resistance to future insect attacks (Karban and Baldwin 1997; Bostock 2005). These defenses include increased leaf concentration of terpenoids in cotton (*Gossypium herbaceum*) and *Zea mays* (Bezemer *et al.* 2003; Bezemer *et al.* 2004; Rasmann *et al.* 2005), certain glucosinolates in *Brassica spp.* (Van Dam *et al.* 2004; Van Dam and Raaijmakers 2006), and phytoectosteroids in *Spinacia oleracea* (Schmelz *et al.* 1998) upon root attack. Also, increased defenses in roots have been observed after leaf herbivory (Baldwin *et al.* 1994; Van Dam *et al.* 2001). These interactions indicate a possible effect of root herbivory upon defenses in the leaves by changing the performance of leaf herbivores (Wäckers and Bezemer 2003; Van Dam *et al.* 2005).

1.2 The indirect defense in *Zea mays*

Maize is one of the major crop plants, a nutrition base for many cultures and used as a source for bioethanol (Wheals *et al.* 1999). Therefore, pests damaging maize fields can cause major economical losses. Consequently, many researchers investigated the natural defense mechanisms in *Zea mays*. It was found that maize is able to defend itself by releasing a complex mixture of volatiles after herbivore damage through the larvae of the Egyptian cotton leaf worm (*Spodoptera littoralis*, (Turlings *et al.* 1990)). These volatiles can be used as cues by the parasitic wasp *Cotesia marginiventris* to locate its host for oviposition (Turlings *et al.* 1990; Turlings *et al.* 2005). Upon parasitization, the lepidopteran larvae feed less and die eventually due to the growing parasitic larvae within. This can result in a significant reduction in the damage to the plant (Hoballah *et al.* 2002; Hoballah *et al.* 2004). This tritrophic interaction, termed “indirect defense”, was recognized in the 1980s and studied since (Fig 1.2). Similar interactions were also found belowground. Parasitic nematodes locate their host through the volatiles released by herbivore-damaged maize roots (Rasmann *et al.* 2005). Maize volatiles consist mainly of terpenoids and products of the lipoxygenase pathway as well as some minor aromatic compounds (Turlings *et al.* 1990; Degen *et al.* 2004; Köllner *et al.* 2004).

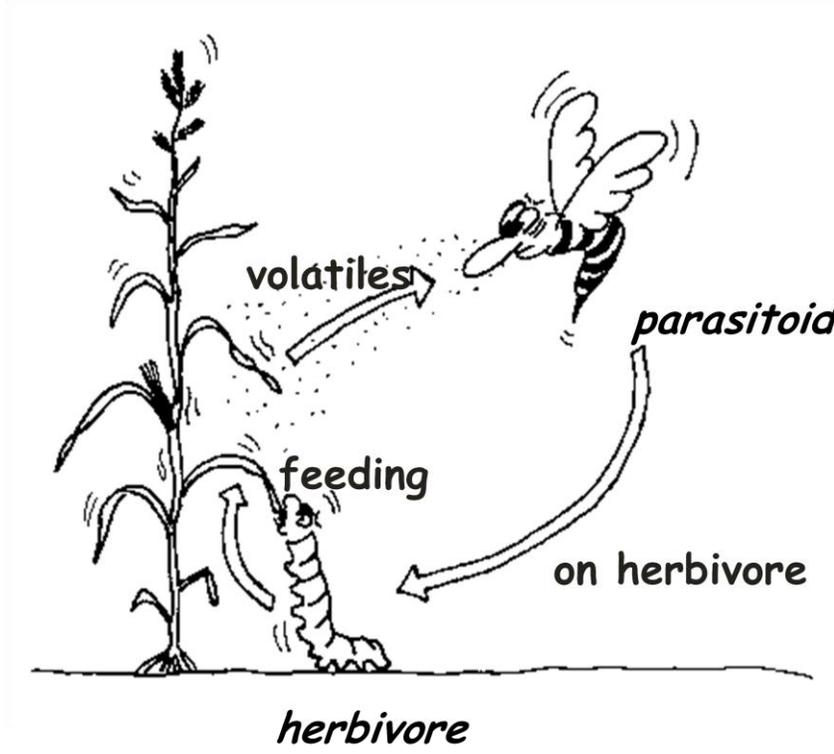


Fig 1.2: Schematic overview of the tritrophic interaction in maize. When maize is fed upon by the larvae of *S. littoralis*, it releases a complex mixture of volatiles which act as cues for the parasitic wasp *C. marginiventris* to locate its host (picture by Tobias Köllner).

Overall, there are more than 100 terpene synthase genes identified today (Tholl 2006; Degenhardt *et al.* 2009). All of them appear to have evolved from an ancestral diterpene synthase (Trapp and Croteau 2001; Martin *et al.* 2004). Furthermore, hemi-, mono-, sesqui-, and diterpene synthases from plants are more related to each other than to tri-, and tetraterpene synthases (Wu *et al.* 2005). All terpenoids originate from the precursor isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP, (Dudareva *et al.* 2006). Synthesis of these compounds occurs via the MEP pathway in plastids or the mevalonate pathway in the cytosol and mitochondria (Gershenzon and Croteau 1993; Rodríguez-Concepción and Boronat 2002). The enzymes responsible for the production of the maize terpenes, especially (*E*)- β -caryophyllene, (*E*)- α -bergamotene, and (*E*)- β -farnesene, have been identified and characterized. The terpene synthase TPS23 uses the substrate farnesyl diphosphate (FPP) to produce (*E*)- β -caryophyllene as major product, and δ -elemene and α -humulene as minor products (Fig.1.3, (Köllner *et al.* 2008)).

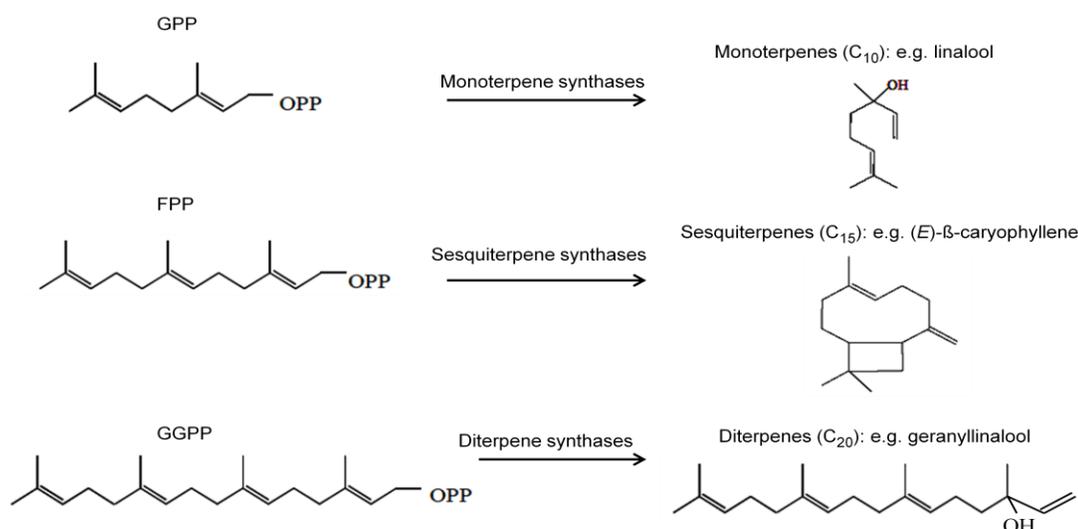


Fig.1.3: The production of terpenes is mediated by different kinds of terpene synthases. Monoterpene synthases use the substrate GPP to produce monoterpenes. The substrate FPP is the starting point for the synthesis of sesquiterpenes and is converted by sesquiterpene synthases. Diterpenes are produced by diterpene synthases using the precursor GGPP.

The terpene synthase TPS23 is induced after damage by the larvae of *S. littoralis* in the leaves and attack by larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*) at the roots. The terpene synthase TPS10 which converts FPP into (*E*)-α-bergamotene and (*E*)-β-farnesene (Schnee *et al.* 2006) is only induced after aboveground herbivory. In herbivore-induced roots, the terpenes specific for TPS10 were not found. This observation suggested differences in the signaling pathways that regulate the activity of both terpene synthases.

The composition of emitted volatiles differs between organs, developmental stages, and maize lines (Köllner *et al.* 2004). While the maize variety Delprim produces not only the sesquiterpenes (*E*)-α-bergamotene and (*E*)-β-farnesene, but also (*E*)-β-caryophyllene, the maize cultivar B73 does not produce (*E*)-β-caryophyllene. This behavior is not only present in the leaves but also in infested roots (Rasmann *et al.* 2005). Roots of the maize line Delprim produce (*E*)-β-caryophyllene after root attack by the larvae *D. virgifera*, but B73 does not. Since (*E*)-β-caryophyllene can act belowground as a signal for pathogenic nematodes which feed upon the *D. virgifera* larvae, the production of (*E*)-β-caryophyllene might be an important defense mechanism (Degenhardt *et al.* 2009).

1.3 Plant signal transduction in response to herbivory

1.3.1 The function of plant hormones in signal transduction after herbivore attack

Plant hormones such as jasmonic acid (JA), salicylic acid (SA), ethylene, and abscisic acid (ABA) not only regulate plant growth and development, but the responses to abiotic and biotic stresses with considerable signaling cross talk (Walley et al. 2007). ABA, a sesquiterpenoid with one asymmetric carbon at C-1 (Rock and Zeevaart 1991), is known for the control of germination, seedling growth and development (Fig.1.4). It plays a key role in the adaptive responses to abiotic stresses such as drought (Davies and Zhang 1991), high salinity, and cold. It also mediates adaptive responses like stomata closure (Tardieu and Davies 1992). The functional aspects of ABA, calcium, and the signaling cascade leading to the closing of the guard cells have been studied intensively. There are two signaling cascades leading to stomata closure, one via Ca^{2+} and one via ABA which are both interconnected. Both cascades lead to the activation of anion channels and thereby reduce the osmotic potential of the cell (Marten *et al.* 2007). ABA might also play a role in herbivore defense (Anderson *et al.* 2004). ABA deficiency in tomato (*Solanum lycopersicum*) and *Arabidopsis thaliana* leads to an enhanced performance of larvae of the beat armyworm (*Spodoptera exigua*) and the Egyptian cotton leaf worm (*Spodoptera littoralis*, (Thaler and Bostock 2004; Bodenhausen and Reymond 2007). However, upon root attack ABA and ABA-induced signaling do not play an exclusive role in leaf defense. It is rather the ABA-independent hydraulic changes upon herbivory that induces plant defenses. Overall, the increase of ABA concentration after belowground attack might be a plant response to reduce the negative effects of water loss (Erb *et al.* 2011).

Ethylene, the only volatile phytohormone, has many biological functions (Fig.1.4). It triggers fruit ripening, influences senescence and the abscission of plant organs, plays a role in seed germination, root formation, pollination, cell elongation, sex determination, and flowering (Abeles *et al.* 1992). It also regulates responses to biotic and abiotic stresses. The importance of ethylene to biotic stress on aboveground parts of the plants after attack by arthropod herbivores has also been demonstrated (Kendall and Bjostad 1990; von Dahl and Baldwin 2007). Because of its volatility, ethylene is highly transportable by either direct diffusion

through the vascular tissue or diffusion from the rhizosphere to the phyllosphere (Jackson and Campbell 1975). Ethylene might also be involved in volatile defense signaling within and between plants (Ruther and Kleier 2005).

The plant hormone most strongly associated with defense against pathogen attack is salicylic acid (SA, Fig.1.4). This hormone modulates plant responses to pathogen infection and regulates responses to abiotic stresses through signaling crosstalk with other plant hormones (Achard *et al.* 2006; Horváth *et al.* 2007; Spoel and Dong 2008; Vlot *et al.* 2009). Additionally, SA plays a role in plant adaptive responses to osmotic stress (Singh and Usha 2003), chilling, drought (Senaratna *et al.* 2000), heat stress (Clarke *et al.* 2004), and salt stress (Khodary 2004). Through the interactions with gibberellins, ABA, JA, and ethylene, SA exerts a role in a variety of plant development processes (Yasuda *et al.* 2008; Alonso-Ramírez *et al.* 2009; Leon-Reyes *et al.* 2009). The importance of SA for the induction of systemic acquired resistance (SAR) has been well studied (Ross 1961; Kuc 1982). However, SA-responsive genes were also found to be up-regulated after herbivore attack (Zarate *et al.* 2007). The methylated form of salicylic acid, methyl salicylate (MeSA) is released after pathogen infection and might be a potential signal for the induction of defense-related genes in neighboring plants (Shulaev *et al.* 1997).

Jasmonic acid is synthesized via the octadecanoid pathway (Vick and Zimmerman 1984; Feussner and Wasternack 2002) from α -linolenic acid or hexadecatrienoic acid (Ishiguro *et al.* 2001; Hyun *et al.* 2008). This hormone is a key regulator for stress-induced genes in all plants (Reymond and Farmer 1998; Schaller 2001). It is implicated in systemic plant responses against herbivory aboveground (Farmer and Ryan 1992; Howe *et al.* 1996) and likely also belowground (Schmelz *et al.* 1999; Puthoff and Smigocki 2007). Jasmonic acid is also induced in the tissue surrounding the site of wounding (Felix and Boller 1995; O'Donnell *et al.* 1996) and is actively transported or diffuses from its site of synthesis (Farmer *et al.* 1992). Its role in long-distance wound signaling (Stratmann 2003; Wasternack *et al.* 2006) is supported by the fact that exogenous JA moves easily through the phloem (Zhang and Baldwin 1997). Experiments in tomato showed that jasmonate signaling is required for a functional recognition of a long distance wound signal while jasmonate biosynthesis is required for the generation of such a long distance wound signal (Li *et al.* 2002). Thus, Li and coworkers demonstrated that jasmonate is an essential component of the

transmissible wound signal and may act as intercellular signal or trigger for the production of such signal (Li *et al.* 2002). An overview of structures of the major hormones found in plants is shown in Fig.1.4.

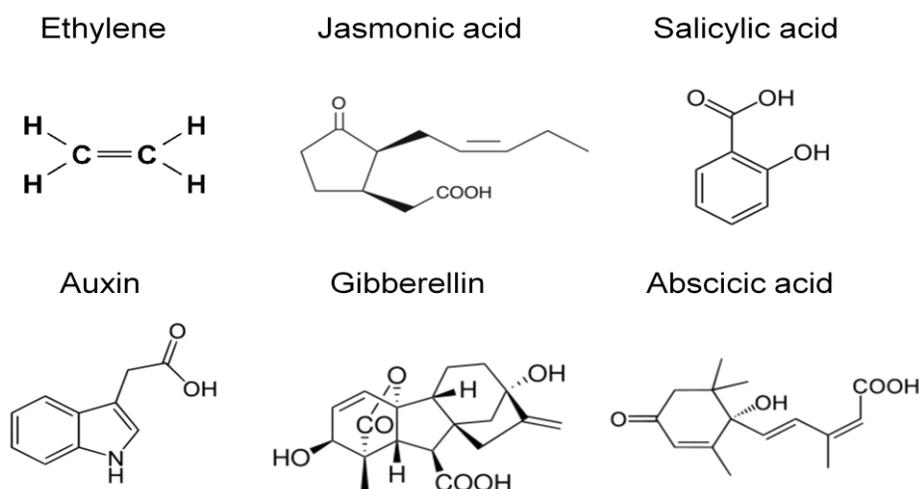


Fig.1.4: Overview of major plant hormones: ethylene, jasmonic acid, salicylic acid, auxin, gibberellins, and abscisic acid with the regarding chemical structure, respectively.

The production of plant hormones varies greatly depending on the nature of the attacking pathogen or insect (Mur *et al.* 2006). In addition to JA, SA, ET, and ABA, further plant hormones seem to have a function in the immune response of plants. Unfortunately, the role of brassinosteroids (Nakashita *et al.* 2003), gibberellins (Navarro *et al.* 2008) and auxin (Navarro *et al.* 2006; Wang *et al.* 2007) in plant defense is less studied. Since hormone-dependent pathways are differentially effective against specific types of attackers (Thomma *et al.* 2001; Glazebrook 2005; Thatcher *et al.* 2005), there has to be a cross talk between the signaling pathways that induces plant defense (Reymond and Farmer 1998; Koornneef and Pieterse 2008; Spoel and Dong 2008). This cross talk can be either antagonistic or synergistic in order to fine tune the response to the invading attacker (Reymond and Farmer 1998; Pieterse *et al.* 2001; Kunkel and Brooks 2002; Bostock 2005). JA and ethylene are both required for the activation of wound responses and induce each other's synthesis (Ross 1961), while SA inhibits the synthesis and the signaling pathway of JA and ET (Dong 1998).

1.3.2 Components of the signaling transduction pathways of plant defenses

The signal transduction pathway from herbivore attack to the production of volatile terpenes is mostly unknown. It is evident that a defense response depends on the developmental stage of a plant as well as on the strategy of the invading attacker (De Vos *et al.* 2005). Also, the quantity, composition and timing of a phytohormonal blend produced by the plant varies among different attackers (De Vos *et al.* 2005). For the primary defense response to a pathogen, plants must be able to recognize common features of microbial pathogens, such as flagellin, chitin, glycoproteins and lipopolysaccharides, so-called PAMPs (Göhre and Robatzek 2008; Nürnberger and Kemmerling 2009). The positive outcome of an immune reaction to pathogen invasion results in a hypersensitive response (De Wit 1995; De Wit 1997) and leads to a SAR which is dependent on a SA signaling (Durner *et al.* 1997). This SAR is induced throughout the plant and is relevant for an enhanced and long-lasting resistance to secondary challenges by the same or unrelated pathogen (Wobbe and Klessig 1996). While the receptor type for SA is unknown (Santner and Estelle 2009), one key regulator of SA-mediated signaling has been characterized. NPR1 (nonexpressor of pathogenesis-related genes 1) is an ankyrin repeat-containing protein (Cao *et al.* 1997) that interacts with TGA transcription factors to activate pathogen-related gene expression (Fan and Dong 2002; Després *et al.* 2003). Its nuclear localization has been implicated in the activation of SA-responsive genes (Kinkema *et al.* 2000; Dong 2004). For the antagonistic effect of SA on the JA-signaling pathway, NPR1 has to be localized in the cytosol (Spoel *et al.* 2003; Yuan *et al.* 2007). The picture becomes even more complicated since the cytosolic function of NPR1 can be bypassed by ethylene (Leon-Reyes *et al.* 2009).

The jasmonate signaling pathway has been investigated intensively and some of the key elements are known. Jasmonic acid is induced after wounding or herbivore feeding (Farmer and Ryan 1992). After JA formation, the JAR1 (JA amino acid conjugate synthase) protein binds isoleucin to jasmonic acid (Staswick and Tiryaki 2004), which represents the biologically active signal. In the presence of jasmonic-isoleucin (JA-Ile), the SCF^{COII} (skip-cullin-F-box) complex promotes the ubiquitination of JAI3 (jasmonate insensitive3) and JAZ (jasmonate-ZIM-domain proteins) which leads to their degradation by the 26S proteasome (Chini *et al.* 2007; Thines *et al.* 2007). Without the JAZ3 protein, the transcription factor MYC2 is de-repressed and can activate the transcription of jasmonate-responding genes including JAZ3 (Lorenzo *et al.* 2004). Therefore, jasmonic acid signaling contains a negative self-regulation. Jasmonate-inducible genes include proteinase inhibitors such as insect antifeedants (Ryan 1990), antifungal proteins like Thi (thionins), and PDF1.2 (defensin) (Penninckx *et al.* 1998; Vignutelli *et al.* 1998) that is often used as molecular marker for herbivory. Interestingly, the signaling pathways of ethylene, gibberellin, and auxin are also mediated via SCF complexes (Devoto and Turner 2005). An overview of the signaling pathway of JA and the crosstalk with SA and ET is depicted in Fig.1.5.

1.4 Regulatory elements of wound-inducible promoters

Promoters contain sequence elements that regulate gene activity in response to environmental cues. Those regulatory elements are bound by transcription factors which control the activation of gene expression. Several *cis*-acting elements have been identified in connection with the regulation of defense genes. A methyl jasmonate-responsive element was identified in the promoter of lipoxygenase in barley with the sequence TGACT (Rouster *et al.* 1997). Table 1.1 gives some examples of further *cis*-elements involved in plant defense.

Tab.1.1: Examples of identified regulatory elements induced by hormones or wounding.

Name	Sequence	Response to	Reference
ABRE	ACGTG	ABA	(Hattori <i>et al.</i> 1995)
as-1	TGACG	SA/pathogen	(Strompen <i>et al.</i> 1998)
ATMYC2	CAYRTG	ABA	(Abe <i>et al.</i> 2003)
ERE	ATTTCAAA	Ethylene	(Itzhaki <i>et al.</i> 1994)
G-box	CACGTG	JA	(Chini <i>et al.</i> 2007)
G-box	AGCCGCC	Ethylene	(Ohme-Takagi and Shinshi 1995)
GT-1 motif	GAAAAA	Pathogen/salt	(Park <i>et al.</i> 2004)
JERE	GCC core	JA/MeJA	(Menke <i>et al.</i> 1999)
MYBR	TGGTTAG	ABA	(Abe <i>et al.</i> 2003)
MYCR	CACATG	ABA	(Abe <i>et al.</i> 2003)
S-box	AGCCACC	Fungal elicitor	(Kirsch <i>et al.</i> 2000)
W-box	TGACY	Wound	(Nishiuchi <i>et al.</i> 2004)
WUN-box	TCATTACGAA	Wound	(Matton <i>et al.</i> 1993; Pastuglia <i>et al.</i> 1997)
SARE	TTCGATC	SA	(Hayashi <i>et al.</i> 2003)

In addition, Hayashi and colleagues presented several inducible elements found in the promoters of *ngr1* and *ngr3*, two enzymes encoding S-like ribonucleases. Here, a tobacco mosaic virus (TMV)-responsible element, the GT1 binding site, is described. The same promoter also contains many elicitor-responsive elements like ERE, TC-rich repeats, an ELI-box, and an EIR-element (Hayashi *et al.* 2003).

Two additionally jasmonate-responsive elements, JASE1 and JASE2, have been reported. Both elements have been found in the promoter of the *opr1* gene in Arabidopsis (He and Gan

2001). The LTR (long terminal repeat) promoter of the tobacco retrotransposon element *TtoI* contains a L/AC-I or H-box-like motif, which is responsive to MeJA (Takeda *et al.* 1999). Overall, a number of jasmonate-responsive elements exist. Well-studied promoter elements are the G-box and motifs with a GCC-core. Promoters containing a G-box (e.g. *PDF1* (Lorenzo *et al.* 2003)) respond to jasmonates while they are negatively influenced by ethylene. On the other hand, promoters with a GCC-motif (e.g. vegetative storage protein (Benedetti *et al.* 1995)) are activated by JA with a synergistic effect of ET (Memelink 2009). In several studies, inducible promoters have been used to generate transgenic plants that are more resistant to various diseases. Moreno and colleagues used the promoter of a fungal-induced class II chitinase fused to the antifungal protein of *Aspergillus giganteus* to create rice plants with resistance to the blast fungus *Magnaporthe grisea* (Moreno *et al.* 2005).

1.5 Transcription factors involved in the regulation of plant defense

An important part of plant stress responses is the regulation of specific stress genes that primarily occurs at the level of transcription (Rushton and Somssich 1998). Transcription factors regulate early steps of gene expression. They are defined as proteins that contain a specific DNA-binding domain (Mitsuda and Ohme-Takagi 2009). A genome-wide analysis identified over 2000 putative transcription factors in the Arabidopsis genome (Davuluri *et al.* 2003; Mitsuda and Ohme-Takagi 2009) while the fruit fly (*Drosophila melanogaster*) only contained 600 transcription factors. This suggests that transcriptional regulation plays a more important role in plants than in animals (Riechmann *et al.* 2000). Even between different organs of the same plant, there are differences in the activation of transcription factors. Roots of Arabidopsis express more stress-related transcription factor genes than leaves (Chen *et al.* 2002).

Different families of transcription factors have been identified in Arabidopsis: AP2/EREBP, bZIP/HD-ZIP, MYB, and zinc finger domain proteins. The expression of these factors is often induced or repressed under stress conditions and therefore has most likely a role in plant stress responses (Shinozaki and Yamaguchi-Shinozaki 2000). Transcription factors can activate or repress gene expression depending on the structure of their activation domain (Mitsuda and Ohme-Takagi 2009). Members of the AP2/EREBP (apetala2/ethylene

responsive element binding protein) family are induced after abiotic stress, bacterial infection, infection with different pathogens including bacteria, fungi, oomycetes, and viruses (Chen *et al.* 2002). BZIP/HD-ZIP transcription factors are induced after abiotic stress like cold, salt, osmoticum, and JA, and abiotic stress plus bacterial infection (Chen *et al.* 2002). These basic leucine zipper proteins play a role in phytomorphogenic development and hormone signaling (Jakoby *et al.* 2002) and show similar features to other bZIP proteins of plants (Cheong *et al.* 1998). The bZIP transcription factors recognize C-boxes and C/T or C/A-boxes over G-boxes in the promoters of their target genes (Song *et al.* 2008).

The expression of MYB transcription factors can be up-regulated after abiotic stress, abiotic stress plus bacterial infection, bacterial infection, as well as infection with different pathogens (e.g. fungi, oomycetes, viruses) (Chen *et al.* 2002). Also, MYB transcription factors were implicated as key regulators in aliphatic glucosinolate biosynthesis in *Arabidopsis* (Hirai *et al.* 2007). Since the MYB domain consists of up to 3 imperfect repeats forming a helix-turn-helix structure (Frampton *et al.* 1991), it is thought that MYB proteins interact with basic helix loop helix proteins (bHLH) to form complexes through the conserved amino acid sequence [DE]L_x₂[RK]_x₃L_x₆L_x₃R (Zimmermann *et al.* 2004). In maize, two R2R3-MYB proteins interact with bHLH transcription factors that are required for the production of the purple anthocyanin pigments (Petroni *et al.* 2000). bHLH proteins form a ubiquitous family of regulators in eukaryotes. Functional analyses predicted different roles in plant cell, tissue development, and plant metabolism (Heim *et al.* 2003).

Transcription factors that have a strictly conserved amino acid sequence and bind to the W-box motif belong to the group of WRKY transcription factors (Eulgem *et al.* 1999). They contribute to the control of some of the stress responses and are up-regulated after pathogen infection or wounding (Eulgem *et al.* 2000). Several studies indicate a role of WRKY factors from *Arabidopsis thaliana* during senescence (Eulgem *et al.* 2000). Interestingly, all WRKY factors contain an intron within the region encoding the C-terminal region, and share a zinc-finger-like motif (Eulgem *et al.* 2000). In tobacco plants, WRKY transcription factors are involved in the early events of the hypersensitive response against tobacco mosaic virus (Yoda *et al.* 2002). Members of the WRKY group III transcription factors in *Arabidopsis* are induced after salicylic acid treatment (Kalde *et al.* 2003) and have a functional role in the cross talk between SA and JA pathway (Li *et al.* 2004). In this regard, it was published that

NPR1 is controlled by WRKY factors (Yu *et al.* 2001) and that those transcription factors are downstream components of the MAPK pathway (Petersen *et al.* 2000; Miao *et al.* 2007). WRKY transcription factors are the substrates for MAP kinases, which can regulate activation of SA-dependent resistance as well as block the induction of PDF1.2 by JA (Petersen *et al.* 2000). A further example of WRKY factors is OsWRKY from rice that is regulated by multiple factors to achieve disease resistance (Cai *et al.* 2008).

Another group of transcription factors are zinc-finger transcription proteins which are implicated after abiotic stress, bacterial infection, and infection with pathogens (Chen *et al.* 2002). An important example for this group is the CaZFP, an Cys₂/His₂-type zinc-finger transcription factor suggested in early defense of pepper plants against pathogens and abiotic stress (Kim *et al.* 2004). It binds to two *cis*-acting elements, AGT core motifs, separated by 13 bp (Takatsuji and Matsumoto 1996).

Transcription factors with an implicated role in the JA-ET crosstalk are AtMYC2, a helix-loop-helix-leucine zipper, and the ERF1 ethylene responsive factor (Felton and Korth 2000; Lorenzo *et al.* 2004). ERF1 binds to GCC-box in the promoter of target genes (Fujimoto *et al.* 2000) and is induced after infection with pathogens (Solano *et al.* 1998). An essential transcription factor in the signaling pathway of JA and ET that can activate PDF1.2 gene expression is ORA59 which belongs to the AP2/ERF domain transcription factors (Pré *et al.* 2008).

SA biosynthesis is influenced by the transcription factor NtWIF (Waller *et al.* 2006) that is activated upon phosphorylation by wound-induced protein kinase (WIPK) in tobacco (Maffei *et al.* 2007). Also, the CaRAV1 transcription factor from pepper (*Capsicum annum* L.) is involved in the SA-dependent signaling pathway (Sohn *et al.* 2006) and contains two or more distinct types of DNA-binding domains (Kagaya *et al.* 1999). This transcription factor belongs also to the family of AP2/EREBP. In pepper, the important transcription factor CaATL1 was found. CaATL1 contains an AT-hook motif and increases in response to SA and/or ethylene treatment. Therefore, it has been implicated in the response against pathogen attack (Kim *et al.* 2007). GBF3 (G-box factor 3) belongs to the family of bZIP transcription factors and functions downstream of SA and NPR1 in the SA dependent signaling pathway (Chen *et al.* 2002). Additionally, NPR1 is known to respond to ABA (Lu

et al. 1996). Therefore, it was thought that the signaling pathway activated by senescence may overlap with stress signaling pathway (Chen *et al.* 2002).

The activity of transcriptions factors is not only regulated at the level of transcription but by proteolytic activation. Some transcription factors were found to be anchored in the ER or golgi membranes in a dormant state and are activated upon proteolysis (Chen *et al.* 2008). This group of MTTFs (membrane-tethered transcription factors) contains some bZIP and NAC transcription factor, but only one was found in response to ABA (Chen *et al.* 2008).

1.6 Objectives of this work

The crop plant *Zea mays* is known to respond to herbivore attack with the emission of terpene volatiles. The composition of the volatile blend is specific for the type of herbivore and the site of attack, suggesting a complex signal transduction network that is involved in the formation of the volatile signal. Since terpene biosynthesis and its key enzymes, the terpene synthases, have already been investigated in maize, we chose this plant as a model system to investigate the signal transduction pathway for herbivore-induced genes and elucidate their temporal and spatial patterns. The project starts with a transcriptome analysis to identify genes that are altered in their expression patterns after herbivore attack. The analysis focuses on transcription factors which may play an important role in the regulation of plant defense genes. An investigation of the spatial and temporal expression pattern in response to several herbivore-related stimuli provides insight into the major cues and nature of the plant regulatory networks for volatile terpene biosynthesis.

A second approach to elucidate the regulation of volatile terpene biosynthesis focuses on the regulation of the terpene synthases *tps10* and *tps23*. A promoter deletion analysis will be performed to identify important regulatory *cis*-elements for both genes. Heterologous expression of promoter::reporter gene constructs in *Arabidopsis thaliana* provides a method to overcome the limitations of maize transformation.

2. Materials and methods

2.1 Cultivation and treatment of *Zea mays* L.

Seeds of the four maize lines B73, Delprim, W22, and UFMu-02709 were grown in climate-controlled chambers (York Int., York, USA) under the following conditions: 16 h photoperiod, $1 \text{ mmol m}^{-2} \text{ s}^{-1}$ of photosynthetic active radiation, a temperature cycle of 22°C/18°C (day/night), and 65 % relative humidity (Tab.2.1). Clay substrate potting soil (Klasmann, Gross-Hesepe, Germany) with Osmocote fertilizer (Scotts, Nordhorn, Germany) in seed flats (400x600x60 mm) or pots (160 mm in diameter) were used to cultivate plants.

Tab.2.1: Description of maize lines used in this work

Name	Description	Origin
B73	Inbred line [Reid Yellow Dent]	KWS (Einbeck, Germany)
Delprim	Hybrid line ([DSP1387C[Dent] x DSP1743A[Flint])	DSP AG (Delley, Switzerland)
W22	NSL 30053 - NC7, inbred line	US National Plant Germplasm System (Beltsville, USA)
UFMu-02709	Mu transposon insertion line with W22 as background	US National Plant Germplasm System (Urbana, USA)

Twelve to fourteen day-old plants with 3 to 4 expanded leaves were used in all experiments. For herbivore treatment, three third instar larvae (*Spodoptera littoralis*) were enclosed in the lower part of the middle leaf of each plant in a clipped cage. The larvae were able to feed at least 12 h or according to the time course experiment: 0.5 h, 1 h, 2 h, and 4 h, respectively.

For mechanical damage, the upper side of 2 young leaves was scraped with a razor blade on each side of the midvein in an area of about 5x10 mm. For elicitor treatment, 10 µl of *S. littoralis* regurgitate (1:2 diluted with sterile water and boiled for 5 min at 95°C) or jasmonic acid (in 0.05 % DMSO) as an aqueous solution was applied to the wounded area. Additionally, plants were damaged with the mechanical caterpillar Mecworm (MPI for Chemical Ecology, Jena, Germany) for the same time points and induced again with 10 µl

regurgitate (Fig.2.1). The metal punch ($\text{Ø}=0.5\text{ mm}$) of this unit was programmed to damage 30 mm^2 of the leaf material (Mithöfer *et al.* 2005).

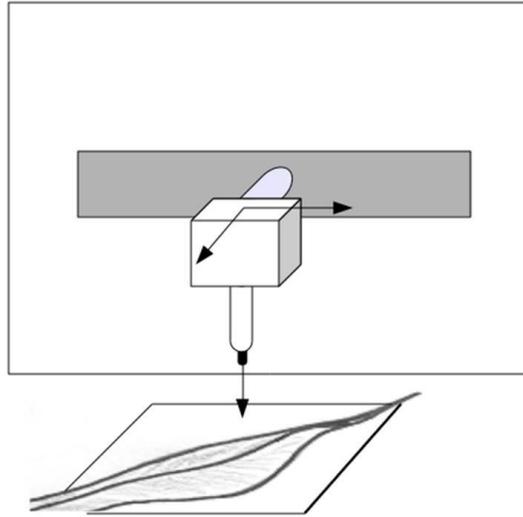


Fig.2.1: Schematic design of the Mecworm. The punching unit can be moved in two dimensions and results in a damaged area according to the programmed instructions.

2.2 Cultivation of *Spodoptera littoralis* (Boisd.) [Lepidoptera: Noctuidae]

Eggs of the Egyptian cotton worm (*Spodoptera littoralis*) were obtained from Syngenta (Basel, Switzerland) and raised on an artificial wheat germ diet (Heliothis mix, Stonefly Industries, Bryan, USA) for about 10-15 days at 22°C under an illumination of $750\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$. Third instar larvae were used for herbivore induction experiments.

2.3 Cultivation, treatment and transformation of *Arabidopsis thaliana* L.

Arabidopsis thaliana variety Columbia was used for all experiments. Transformed *Arabidopsis* plants were used in the T2 generation and were grown like wild type plants under standard conditions, including a 3 day cold period to obtain a simultaneous germination of the seeds. Plants were sown in a $100\times 100\times 110\text{ mm}$ pots in clay substrate potting soil (Klasmann, Gross-Hesepe, Germany) with Osmocote fertilizer (Scotts, Nordhorn,

Germany) in a climate controlled room at $150 \mu\text{mol (m}^2)^{-1} \text{ s}^{-1}$ of photosynthetic active radiation at 22°C and 65 % relative humidity at long-day conditions (16 h light). After starting to develop basal leaves, plants were separated into 60x60x80 mm pots.

Five week-old plants were induced by scratching the upper leaf with a razor blade and application of 10 μl 250 μM jasmonic acid (in 0.05 % DMSO) or by adding 2 ml 250 μM jasmonic acid to the plant soil over 3 days.

Arabidopsis was transformed using the floral dip method (Clough and Bent 1998; Zhang *et al.* 2006). The transformed *Agrobacterium tumefaciens* (2.4.4) were inoculated in a LB medium with spectinomycin, gentamycin and rifampicin for 48 h at 25°C . 25 ml of the pre-cultures cells were shaken in 500 ml LB medium containing the three antibiotics for 24 h at 27°C ($\text{OD}_{600}=0.9$). After centrifugation at 5500 g for 20 min at room temperature, the pellet was redissolved in 400 ml infiltration medium and OD_{600} was adjusted to around 9.0 by adding infiltration medium.

The inflorescence of *A. thaliana* variety Columbia was transformed by dipping the whole upper parts of the plants for 10 s into the infiltration suspension. Plants were then wrapped in blue plastic hoods and kept overnight without direct sunlight. The transformed seeds were harvested and dried for several days. Positive transformed seeds were selected using a 1:3000 dilution of Basta® herbicide (Bayer Cropscience, Monheim am Rhein, Germany). The herbicide was applied every second day after the plants had reached a size of about 5 mm.

Infiltration medium: 5 % [w/v] sucrose
 0.005 % [v/v] Silwet L-77

2.4 Microbiological methods

2.4.1 Bacterial strains and cultivation

Escherichia coli and *Agrobacterium tumefaciens* were cultured in LB medium in a shaker or grown on LB-agar plates in an incubator at 37°C, unless noted otherwise (Tab.2.2). Stock cultures were obtained by adding 300 µl 100 % sterile glycerol to 1 ml liquid culture and frozen at -80°C.

Tab.2.2: Bacterial strains and their application

Strains	Genotype	Application
<i>E. coli</i> BI21 (DE3) (Novagen, Madison, USA)	F ⁻ <i>ompT gal dcm lon hsdS_B</i> (r _B ⁻ m _B ⁻) (DE3)	protein overexpression in pHIS8-3 (T7 promoter)
<i>E. coli</i> TOP10 (Invitrogen, Carlsbad, USA)	F ⁻ <i>mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 deoR recA1 araD139 Δ (ara-leu)7697 galU galK rpsL (Str^R) endA1 nupG</i>	general cloning of PCR products for sequencing and subcloning
<i>A. tumefaciens</i> GV3101 (van Larebeke et al., 1974)	with nopaline vir helper plasmid pMP90	introduction of <i>tps</i> promoter constructs into <i>Arabidopsis thaliana</i>

LB culture: 25 g LB medium (AppliChem, Darmstadt, Germany) in 1 l *a.dest.*

LB agar: 32 g LB-medium (AppliChem, Darmstadt, Germany) in 1 l *a.dest.*

2.4.2 Antibiotics

Tab.2.3: Concentrations of applied antibiotics

Antibiotic	Stock solution (mg ml ⁻¹ in <i>a.dest</i>)	End concentration (µg ml ⁻¹ media)
Kanamycin	50	50
Ampicillin	100	100
Gentamycin	50	50
Rifampicin	30 (in methanol)	30
Spectinomycin	100	100

2.4.3 Transformation of *Escherichia coli*

50 µl of chemically competent *E. coli* cells were thawed on ice and mixed with 3 µl ligation solution. After 30 min incubation on ice, the transformation mixture was heat-shocked for 45 s at 42°C and transferred to ice again for 1 min. Subsequently, 150 ml SOC medium was added and the cultures were shaken for 1 h at 37°C. Half of the transformed culture was spread on a prewarmed agar plate with the corresponding antibiotic and incubated at 37°C over night (B6120 Kendro, Heraeus). Colonies were picked and checked for successful transformation with vector or insert specific PCR primers.

SOC-medium: 2 % Trypton [g/v] (Sigma-Aldrich, St. Louis, USA)
 0.5 % yeast extract [g/v] (Sigma-Aldrich, Munich, Germany)
 0.5 % glucose [g/v]
 10 mM NaCl
 2.55 mM KCl
 21.6 mM MgCl₂
 20 mM Mg₂SO₄

LB-agar: 32 g LB-medium (AppliChem, Darmstadt, Germany) in 1 l *a.dest*.

2.4.4 Transformation of *Agrobacterium tumefaciens*

The freeze-thaw method (Hofgen and Willmitzer 1988) was used to transform *Agrobacterium tumefaciens* with the destination vector containing the reporter gene GUS and a promoter fragment of *tps10* and *tps23*. 200 µl chemo-competent cells were thawed on ice and incubated with 0.5-1 µg plasmid DNA for 5 min on ice, in liquid nitrogen, and at 37°C, respectively. One ml LB medium was added, the reaction incubated for 30 min on ice, and then transferred to 28°C and shaken for 3 h. After centrifugation for 1 min at 7000 g, the cells were resuspended in 100 µl LB medium and 30 or 70 µl were plated on an agar plate containing spectinomycin, gentamycin and rifampicin. The agar plates were incubated at 28°C for 3 days.

LB-agar: 32 g LB-Agar (AppliChem, Darmstadt, Germany) in 1 l *a.dest.*

2.5 Nucleic acid techniques

2.5.1 Isolation of total RNA from maize

RNA for cDNA synthesis and QRT-PCR was extracted from leaves and roots with the RNeasy Plant Mini kit from Qiagen (Hilden, Germany) according to the manufacturer's protocol. Tissue was disrupted in liquid nitrogen, lysed in the presence of a denaturing guanidine isothiocyanate containing buffer and homogenized by centrifugation through a shredder column. Ethanol was added and samples were applied to silica-gel membrane columns which selectively bind the RNA. After several washing steps with ethanol-containing buffers, total RNA was eluted in 40 µl nuclease free water. Quantification of the total RNA was measured by UV spectrophotometers Ultrospec 2100pro (Amersham Biosciences, Uppsala, Sweden) and Infinite 200 Nano Quant (Tecan Group Ltd, Männedorf, Switzerland) and on an agarose gel.

RNA extraction for microarray experiments was also performed using the RNeasy Plant Mini kit from Qiagen with the following protocol. 50-80 mg frozen plant material was mixed with 450 µl RLT buffer, vortexed vigorously for 5 min and incubated at 21°C for 15 min in an Eppendorf Thermomixer (Eppendorf, Hamburg, Germany) at 1400 rpm. The extract was

transferred to a QiaShredder Column and centrifuged for 2 min at 16000 g. The supernatant was mixed with 225 μ l 100 % ethanol, transferred to the RNeasy mini Column and centrifuged for 25 s at 8000 g. Flow through was discarded, 350 μ l RW1 buffer was added to the column and again centrifuged for 15 s at 8000 g. DNA digestion was performed on column by adding 90 μ l DNase buffer (Promega, Madison, USA) and 10 μ l DNase (Promega, Madison, USA). After the incubation of 15 min at room temperature, 350 μ l RW1 buffer was added and centrifuged for 15 s at 11000 g. The flow-through was discarded and 500 μ l RPE buffer was added. After the centrifugation (15 s at 11000 g) the flow through was discarded and the washing step was repeated. The column was dried for 2 min at 11000 g and RNA was eluted with 40 μ l 70°C preheated nuclease free water and centrifuged at 9000 g for 1 min.

2.5.2 Isolation of genomic DNA from plant material

Plant material was frozen in liquid nitrogen and ground into a fine powder. Genomic DNA was isolated with the DNeasy Plant kit from Qiagen (Hilden, Germany) according to the manufacturer's instruction.

2.5.3 Isolation of plasmid DNA from bacteria

For the isolation of plasmid DNA from bacteria, the Nucleo Spin Plasmid kit from Macherey&Nagel (Düren, Germany) was used following manufacturer's instruction.

2.5.4 DNA Digestion

DNA was digested using Promega DNase (Promega, USA) according to the manufacturer's protocol unless noted otherwise. 2.75 μ g RNA was incubated for 30 min at 37°C with the appropriate buffer and DNase. The reaction was stopped by adding 1 μ l stop solution and incubation for 10 min at 65°C.

2.5.5 Oligonucleotides

Oligonucleotide primers used in this work were purchased from MWG-Biotech (Ebersbach, Germany) and are listed in Tab.2.4.

Tab.2.4: Oligonucleotide Primers used in this work

Name	Sequence	Application
HG 3	GGTCAGGATAGAGAAGGGAGAGC	Housekeeping gene: RNA polymerase II large subunit fwd
HG 4	GAAGAAGCCAGTAGTTTACAAGCC	Housekeeping gene: RNA polymerase II large subunit rev
HG 5	AGGCGTCCGTGACACCATC	Housekeeping gene: putative APT1A fwd
HG 6	CTGGCAACTTCTTCGGCTTCC	Housekeeping gene: putative APT1A rev
S53	CCGGGTGAGATCACCAAGATA	TPS2 QRT-PCR
S54	GGGAAGTGAATCAGCAGCCA	TPS2 QRT-PCR
S57	GGCAGCACTCCAAGGATCTTCT	TPS3 QRT-PCR
S58	TGGGAAGTGAATCAGCAGCCG	TPS3 QRT-PCR
AM120	AGGGAACTTCGTGGTGGATGATAC	TPS10 QRT-PCR fwd
AM121	TGGCGTCTGGTGAAGGTAATGG	TPS10 QRT-PCR rev
BH51	TCTGGATGATGGGAGTCTTCTTTG	TPS23 QRT-PCR fwd
BH52	GCGTTGCCCTTCTCTGTGG	TPS23 QRT-PCR rev
SF9	CCGACTTAGGCTGCTCATCA	ZmOMT1 QRT-PCR fwd
SF10	GCTGCATCTCCCTTGTGTGTC	ZmOMT1 QRT-PCR rev
SK10	GATGTTGTGCATAACAGTATTAGGAGC	ZmOMT3 QRT-PCR fwd
SK11	ATGCCAAGCGAGATAGTGAGAAAC	ZmOMT3 QRT-PCR rev
SM1	CCGACTTAGGCTGCTCATCG	ZmOMT4 QRT-PCR fwd
SM2	GCCTACCCCTTGTGGTTTCTT	ZmOMT4 QRT-PCR rev
TF3	CCACAGGACAGGACACACAGAG	TF1 QRT-PCR fwd
TF4	CTGGATTGCTGGCGATGAACC	TF1 QRT-PCR rev
TF5	TGAGCGTGGAGTGGTGGAC	TF2 QRT-PCR fwd
TF7	GGGAGGGTGGTGGATGACG	TF2 QRT-PCR rev
TF13	AGATGAACAGGAAGCCAGGAGAC	TF4 QRT-PCR fwd
TF14	AAGCCACCGCCGAAGGAG	TF4 QRT-PCR rev
TF17	ACACCCGCCAGATTTGAAGTAAG	TF5 QRT-PCR fwd
TF18	CAGTGGAGTGGATACAGTGATGAG	TF5 QRT-PCR rev
TF23	CTACCGCCGCCGTTGTTC	TF7 QRT-PCR fwd
TF24	TGCCTTCTGCTTGCTTGC	TF7 QRT-PCR rev
TF25	ATGGAGTTGACCTGCTGAATTAC	TF8 QRT-PCR fwd
TF26	CGGCGTGTACTGCTCTGG	TF8 QRT-PCR rev
TF31	CACTACCCGCCCTCCTC	TF9 QRT-PCR fwd
TF32	TGCCGTCGCCCAGAATCG	TF9 QRT-PCR rev
TF40	TTCACAGAGGAGGAAGAAGAGC	TF12 QRT-PCR fwd
TF41	TGGACGGCGAGGAGGATG	TF12 QRT-PCR rev
TF42	ACAGCGATACAAGATTAGCATACG	TF13 QRT-PCR fwd
TF43	CAACTACACAAGCAGCGATGG	TF13 QRT-PCR rev
TF46	TGCGGTTGGTGGCTCATAAG	TF14 QRT-PCR fwd
TF47	AAGGTTCTCAGGCTTCAAGTCC	TF14 QRT-PCR rev
TF50	GAAGGGCAAGAAGAAGAAGATC	TF15 QRT-PCR fwd
TF51	GGTGAAGCAGGCGGATGG	TF15 QRT-PCR rev
TF53	ACTGCTGTCTACTTGGC	TF16 QRT-PCR fwd
TF54	AGTCCTCTCGGTCTTGGC	TF16 QRT-PCR rev
TF55	GGAAGCCTTAGTGGAAGCAGTAG	TF17 QRT-PCR fwd
TF56	CGTAGTTGATGGAAGCAAGTTGTG	TF17 QRT-PCR rev
TF65	GCAGTCTGTCCATTTGATAGGC	TF20 QRT-PCR fwd
TF66	ACTCAGGCTCATAGGCTACTTCC	TF20 QRT-PCR rev
TF70	CTCTGGATGATGGATGGCGAAGG	TF21 QRT-PCR fwd
TF71	ACAAGGGAAGCACGGCACTG	TF21 QRT-PCR rev
TF72	ACTCCACTCCCAATCACACTC	TF22 QRT-PCR fwd
TF73	GCCGTCACCTGCTCCTC	TF22 QRT-PCR rev
TF78	TGGACGGAGATGAATGTGGAGAC	TF23 QRT-PCR fwd
TF79	CCCAGGACGACAAGAGGAACC	TF23 QRT-PCR rev
TF94	GCCTCGTCTCGTCTCGTCTTC	TF28 QRT-PCR fwd
TF95	GGTCAGCCTTCGTCTCTTCC	TF28 QRT-PCR rev
TF96	GCAGAGCAGAAGCCAGCAG	TF29 QRT-PCR fwd
TF97	GGTGGAGATGGTGAGGAGGAAC	TF29 QRT-PCR rev
TF100	GACGCTTCTCACGCCCTCTG	TF30 QRT-PCR rev
TF101	CCACACCACCTCGCTGAACC	TF30 QRT-PCR fwd
TF104	GGCAGCGGTGGAGGAGAC	TF31 QRT-PCR fwd

TF105	TCACAAGTTCAGAAGAGCAGAAGC	TF31 QRT-PCR rev
TF108	CATCTGCTCCAACTCTCCTTCTC	TF32 QRT-PCR fwd
TF109	GCTGCCCGTCTCCTGAACC	TF32 QRT-PCR rev
TF118	CACGCCCGCGGAGGAGTTG	TF34 QRT-PCR rev
TF119	GCGCAAGTACGGGCAGAAGCAGG	TF34 QRT-PCR fwd
TF122	CGGTGGGCTCAAGACAAAACA	TF35 QRT-PCR rev
TF123	TTCCAAAGCAAACAGGCAGAT	TF35 QRT-PCR fwd
TF141	TCACTGGGGCAAGTGGGTGG	TF40 QRT-PCR fwd
TF142	TTGGCATCGCCTTGCTCTTGG	TF40 QRT-PCR rev
TF147	GATGTTGATCACCATGCCGTCC	TF42 QRT-PCR fwd
TF148	CAACTCCGCCTCGCAGTGCC	TF42 QRT-PCR rev
TF161	TCAAGCTGCCGACCCTCC	TF46 QRT-PCR fwd
TF162	CGCCTCCGTCATAGTGCTTCC	TF46 QRT-PCR rev
TF169	ATGACGCCCGGGAACCTCCGT	TF2 RACE rev
TF170	ACCGCCACCACCACCTCC	TF2 RACE fwd
TF171	GCATACTCCGATCCACGCAGG	TF35 RACE fwd
TF172	AAGCATGGGAGTTGGGCATC	TF35 RACE rev
TF173	GCTTGAGCTGATGACCACGATG	TF8 RACE fwd
TF174	GTACCCGGTGGTGGTGGTCTGT	TF8 RACE rev
TF175	GGAAGACCCGCACACACACGC	TF30 RACE fwd
TF176	CCCATGCCACGCCTCCGAC	TF30 RACE rev
TF177	CAGGTTAAGGGGAGCGGAGAAC	TF34 RACE fwd
TF178	GGCGCTGGACAGCGGCTTGG	TF34 RACE rev
TF179	ACGGTGAGCAGCACTCTCGCC	TF23 RACE fwd
TF180	CCGCGTGGTTTAGCTCCAGTGTG	TF23 RACE rev
TF181	CAGCTGAGCTGATAGTGACCC	TF29 RACE fwd
TF182	GGAACCAAAAAGAAAAGAAAAC	TF29 RACE rev
TG1	GTGACGCATGGGTCTGAA	TF1 cloning fwd
TG2	TCAATGACAGCGCAGAACC	TF1 cloning fwd
TG3	CTAAAAAATTCCTCGTTCA	TF1 cloning rev
TG4	TGAGCTCACTCAGAAGGG	TF1 cloning rev
TG5	AGAAGAATTCAGTTTGGGAG	TF2 cloning rev
TG6	TATTCTTGATCATCTCAGCA	TF2 cloning rev
TG7	TCGCGTTACATGTCGCCGT	TF8 cloning rev
TG8	ATGAGTTTTAGGACTCGTC	TF20 cloning fwd
TG9	AAGTAGCATCACATTCCAA	TF20 cloning rev
TG10	ATGCAGCTGGGGAAGACCGC	TF22 cloning fwd
TG11	CCTCGCCTCAGCTCAGAACT	TF22 cloning rev
TG12	GACCTCCCGCCTGGCTGCTCC	TF34 cloning fwd
TG13	AGCAGCGACATGGTAGGAG	TF34 cloning fwd
TG14	ATGTCGCCCTCCTCCTACTTCT	TF34 cloning fwd
TG15	AGTAGCTGACAGTGGTCTGT	TF34 cloning fwd
TG16	AGATTGGGTGCGTGCGTCA	TF34 cloning rev
TG17	TCGCCAGCTCTAGCAGAGGA	TF34 cloning rev
TG18	AAATGCAGATGGACTCCTA	TF23 cloning fwd
TG19	ATGCCGGCCTCGTTCAG	TF23 cloning fwd
TG20	GAAGTGGATCAGTCATTGTCA	TF23 cloning rev
TG21	TTGTTTGGTTCGACGGGCAT	TF35 cloning fwd
TG22	CCTGCCTAGTTCCTGACC	TF30 cloning rev
TG23	CGATCATGCGCTAGCTAGTTCAC	TF8 cloning rev
TG24	ATGGGACAGCCGGTGACGAG	TF1 cloning fwd
TG25	TCAGAAGGGGCCCAACCCC	TF1 cloning rev
TG26	ATGGGTCTGCCGATGAGGAG	TF1 cloning fwd
TG27	TCAGAAGTGTCCCAACCCG	TF1 cloning rev
TG28	ATGCCTTCAGTCGAGGTCG	TF2 cloning fwd
TG29	TCAGTTTGGGAGCTCGAGG	TF2 cloning rev
TG30	ATGGAGTTTGCCTGCTG	TF8 cloning fwd
TG31	TTACATGTCGCCGTGGGG	TF8 cloning rev
TG32	ATGTTCCAAGGAATGATGT	TF20 cloning fwd
TG33	TCACATTCCAAAGGGGGCA	TF20 cloning rev
TG34	ATGGGTCTGCCGATGAGGA	TF22 cloning fwd
TG35	TCAGAAGTGTCCCAACCCC	TF22 cloning rev
TG36	ATGCAGATGGACTCCTAC	TF23 cloning fwd
TG37	GGAGGATCGCGTGTCAAAAC	TF23 cloning fwd
TG38	TCAGTCATTGTGACAAAAG	TF23 cloning rev
TG39	ATGGCGCCCCGGGTGGCGGA	TF30 cloning fwd

TG40	CTAGTTCTCTGCCGGCGG	TF30 cloning rev
TG41	ATGAGCTGCGCGGGGCGAG	TF32 cloning fwd
TG42	ATGGTCGACGACCCGGCC	TF32 cloning fwd
TG43	CTAGGCGACCGGCGGCCAGT	TF32 cloning rev
TG44	ATGGCGTCTCGACGGGGA	TF34 cloning fwd
TG45	TCACCGGTAGAGGGGCGCC	TF34 cloning rev
TG46	ATGGACTTCCACCACAGCA	TF35 cloning fwd
TG47	TCAAGGGGTGGTGATGTC	TF35 cloning rev
TG48	ATGGGTCATGATGAAGCTGTAG	TF21 cloning fwd
TG49	GGCACAAGGGAAGCACGGCAC	TF21 cloning rev
TH1	TCCGAATTCATGACAGCGCAGAACCC	TF1 expression fwd
TH2	GCGGCCGCTAAAAAATTCCTCGTTCACC	TF1 expression rev
TH3	AGCTTGC GGCCGCTAAAAAATTCCTCGTTCACC	TF1 expression rev
TH4	TCCGAAATTCATGCAGCTGGGGAAGACCGCCGTC	TF22 expression fwd
TH5	TCCGAAATTCATGCAGCTGGGGAAGACCGCCGTC	TF22 expression fwd
TH6	GCGGCCGCTAGATCAGAACTGTCCCAACCC	TF22 expression rev
TH7	AGCTTGC GGCCGCTAGATCAGAACTGTCCCAACCC	TF22 expression rev
TH8	TCCGAATTCATGTTCCAAGGAATGATGT	TF20 expression fwd
TH9	GCGGCCGCAAGTAGCATCACATTCCAAAGGG	TF20 expression rev
TH10	AGCTTGC GGCCGCAAGTAGCATCACATTCCAAAGGG	TF20 expression rev
TH11	TCCGAATTCATGAGTTTTAGGACTCGTCATGTTCC	TF20 expression fwd
TH12	TCCGAATTCATGAGCTGCGCGGGGCGAG	TF32 expression fwd
TH13	AGCTTGC GGCCGCTAGGCGACCGGCGGCCAGT	TF32 expression rev
TH14	TCCGAATTCATGGAGTTTGACCTGCTG	TF8 expression fwd
TH15	AGCTTGC GGCCGCTTACATGTCGCCGTGGGG	TF8 expression rev
TP1	CATCAGCTGCCATGAAAGTATA	TPS23 promoter studies 1651-1800 rev
TP2	GCTGGTACCTATATTAAGAAC	TPS23 promoter studies 1651-1800fwd
TP3	CAAGCACCTGGATCCTATGGT	TPS23 promoter studies 1511-1693 rev
TP4	TGTGTTGCACATGCCCTGTGC	TPS23 promoter studies 1511-1693 fwd
TP5	GATCTGCCGACAGGCATGTG	TPS23 promoter studies 1378-1537 rev
TP6	AACATTTGAGGTGTATGGTTG	TPS23 promoter studies 1378-1537 fwd
TP7	GCATTTTCATGCATAACAAC	TPS23 promoter studies 1260-1413 rev
TP8	ACGAGTACCGGGTGGCGGTC	TPS23 promoter studies 1260-1413 fwd
TP9	CATCGATCCTTGCATTAGACC	TPS23 promoter studies 1136-1296 rev
TP10	CTATACATGTGATCTTTAGAGGT	TPS23 promoter studies 1136-1296 fwd
TP11	CTCTAAAAATGTGAACAATATGTAC	TPS23 promoter studies 1026-1180 rev
TP12	CAAAGGTTGTATGCACAAAAGGC	TPS23 promoter studies 1026-1180 fwd
TP13	GAGTATCAAATCTGATTAATTTTC	TPS10A promoter studies 8-161 fwd
TP14	CAAATGGTCTGATCGGTTAATG	TPS10A promoter studies 8-161 rev
TP15	AACATACACCGCGTTAGCATC	TPS10A promoter studies 115-291 fwd
TP16	TGTGTTGATCGTTTTGTCTTG	TPS10A promoter studies 115-291 rev
TP17	CAAGACAAAACGATCAACACACC	TPS10A promoter studies 271-421 fwd
TP18	ATGCAATGAACAACCATTGCGG	TPS10A promoter studies 271-421 rev
TP19	CATATCCCGAATGGTTGTTTC	TPS10A promoter studies 395-566 fwd
TP20	GCTGAGACAAAATGGAGTTGC	TPS10A promoter studies 395-566 rev
TP21	GTCTCAGCTAGGATAAAATACTGG	TPS10A promoter studies 559-718 fwd
TP22	GTACTTCTCTCAACTATTACTTC	TPS10A promoter studies 559-718 rev
TP23	GAAGTAATAGTTGAGAGGAAGTACCA	TPS10A promoter studies 712-855 fwd
TP24	CTTCTGATTGTTTCAGTGTGTTG	TPS10A promoter studies 712-855 rev
TP25	GAAACAATCAGAAGCTGTAGACG	TPS10A promoter studies 842-1005 fwd
TP26	CTTGGGTGGAAGCGGTGGCAT	TPS10A promoter studies 842-1005 rev
TP27	ACCACAACCTTAATTAATATACACC	TPS10B promoter studies 1-174 fwd
TP28	AGAATCTCTTAGGAATCTACTTC	TPS10B promoter studies 1-174 rev
TP29	AACCATATATATACGGAGCGG	TPS10B promoter studies 458- fwd
TP30	ATTACCAGACTTGTGAGCTTGTGG	TPS10A promoter studies 622 fwd
TPG1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAAAATTAAT ACTGGGAAAAGG	TPS23 promoter Gateway cloning 1.8 kb
TPG2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTGCAGATGG AAGAAACACAAC	TPS23 promoter Gateway cloning 600 bp
TPG3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTTAAGCTTG TAGAATGGCAG	TPS23 promoter Gateway cloning 1.2 kb
TPG4	GGGGACCACTTTGTACAAAGAAAGCTGGGTCGAAAGTATAG TATACTAGCTA	TPS23 promoter Gateway cloning rev
TPG5	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTTGCCTA GTGTCAAAAAAACACTC	TPS10 promoter Gateway cloning 1.5 kb
TPG6	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTAGTGTC	TPS10 promoter Gateway cloning 1.5 kb-b

	AAAAAAACACTCGGC	
TPG7	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAAAGAAGCT CTTTGGCGAGTG	TPS10 promoter Gateway cloning 1.5 kb-c
TPG8	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAGTATCAA AATCTGATTAATTTTC	TPS10 promoter Gateway cloning 800 bp
TPG9	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCATTGCATA ATGTGACTCC	TPS10 promoter Gateway cloning 500 bp
TPG10	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGTAATA GTTGAGAGGAAGTACCA	TPS10 promoter Gateway cloning 200 bp
TPG11	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTGCAGCAGC CCCTGCACATC	TPS10 promoter Gateway cloning rev
TPG12	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTGCACGCAG AAGTAATAGTTGAGAG	TPS10 promoter Gateway cloning 200 bp new
TPG13	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGGATGATGT AACATTTGAGGTG	TPS23 promoter Gateway cloning 400 bp
TPG14	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAATATAATG CAAATTGAGTAC	TPS23 promoter Gateway cloning 200 bp

2.5.6 Amplification of DNA

DNA was amplified by “polymerase chain reaction” (PCR) in a thermocycler Primus 96 (MWG Biotech, Ebersberg, Germany) or Peclab (Peclab, Germany). Standard PCR was performed using Go Taq-DNA polymerase (Promega, Madison, USA). Amplification reactions of inserts for cloning experiments were carried out with Advantage2 Polymerase Mix (BD Biosciences Clontech, Palo Alto, USA) or with *Pfu*Turbo DNA Polymerase (Stratagene, La Jolla, USA). A 50 μ l standard PCR mix contained 1 μ l primer 1, 1 μ l primer 2 (10 pmol μ l⁻¹ each), 1 μ l dNTP (10 mM), 5 μ l reaction buffer, 0.5 μ l polymerase (0.1-2.5 U) and 5-200 ng DNA template. Cycling conditions were as follows:

	94°C for 3 min	
Denaturation	94°C for 30 s	} 25-35 cycles
Annealing	50-68°C for 30 s	
Extension	72°C for 30 s - 3 min	
	72°C for 5 min	

Denaturation conditions remained constant in all PCRs, however annealing temperatures and extension times were adapted depending on the melting temperatures of primers (annealing temperature = melting temperature of primers minus 2-5 °C) and the expected fragment sizes (extension time = 1 min per 1 kb).

2.5.7 RACE-PCR

A 5'- and 3'-RACE cDNA library were constructed from mRNA using the SMARTer RACE cDNA amplification kit (BD Biosciences Clontech, Palo Alto, USA) according to the instructions provided by the manufacturer. The procedure forms first-strand cDNA with specific adaptor sequences either at the 5'- or at the 3'-end of the cDNA that allows for RACE- (rapid amplification of cDNA-ends) PCR. Following RACE PCR, nested PCR was conducted to obtain specific single bands using inner primers.

2.5.8 cDNA synthesis

cDNA for applications in QRT-PCR reactions was synthesized with the reverse transcriptase Superscript III (Invitrogen, Carlsbad, USA). 0.75 µg of DNA digested RNA (2.5.4) was mixed with 4 µl dNTPs, 0.5 µl oligo(dT)primers and 0.5 µl random primers, and filled to a volume of 13 µl with *a. dest.* The reaction was incubated for 5 min at 65°C and shortly cooled down on ice. Four µl reaction buffer, 1 µl DTT, as well as, 1 µl RNase and 1 µl SuperscriptIII was added to the sample and incubated at room temperature for 5 min and afterwards transferred to 50°C for 1 h. Reverse transcription was terminated by an 70°C incubation period of 15 min and the cDNA was stored in aliquots at -20°C.

2.5.9 Quantitative RT-PCR

Quantitative real time PCR was performed to analyze transcript accumulation of specific genes in different samples. In order to observe the accumulation of our DNA fragments, 10 µl Brilliant Sybr Green QPCR Master Mix (Stratagene, La Jolla, USA) was added to 1 µl cDNA and 0.5 µl of each primer (0.25 mM). Since Sybr Green is a chemical compound that fluoresces when unspecifically bound into the minor groove of double-stranded DNA, it can be measured by its fluorescence after excitation at 497 nm. The signal intensity depends on the initial concentration of target present in the PCR reaction and is therefore a tool to calculate the present mRNA amount of one specific gene.

QRT-PCR reactions were carried out in an Mx3000P (Stratagene, La Jolla, USA) or the CFX96 (Bio-Rad, Munich, Germany) with the following reaction scheme:

	95°C for 10 min	
Denaturation	95°C for 30 s	} 40 cycles
Annealing	56-68°C for 30 s	
Extension	72°C for 1 min	
Melting Curve:	95°C for 1 min	
	56°C to 95°C for 30 s each	

Gene expression levels were quantified using the standard curve method. The standard curve for each gene was generated using pooled cDNA in equal amounts from all samples. Two reference genes, a putative adenine phosphoribosyltransferase 1 (TA172777_4577) and a maize RNA polymerase II largest subunit (AF519538), were used to quantify the cDNA in each sample. The expression level of both genes was similar in control and herbivore-induced plants, with a cycle threshold value difference from 1 to 2. Using the automated threshold determination given by the Mx3000P software, the starting amount of cDNA was calculated for each sample. Relative expression levels were calculated as the expression level of the respective gene divided by the geometric mean of the expression levels of the two reference genes.

2.5.10 Restriction of DNA, plasmids and PCR products

Restriction of DNA with restriction endonucleases was applied to produce compatible inserts and vectors for ligation and to screen vectors for successful insertion of PCR products. Enzymes and the appropriate buffers (10x) were purchased from NEB (Schwalbach, Germany) or Fermentas (St. Leon-Rot, Germany). Reactions were carried out with 2-20 U enzyme and 0.5-3 µg DNA with the suitable amount of buffer in reaction volumes of 20 to 60 µl and incubated for 1-2 h at 37°C.

2.5.11 Isolation of DNA fragments from agarose gels and PCR reactions

The DNA fragments of interest were cut under UV illumination (260 nm) and extracted with the NucleoSpin Extract kit from Macherey&Nagel (Düren, Germany). The pieces of agarose were dissolved in a buffer containing chaotropic salts which promotes the binding of DNA to the silica membrane of a column. After washing the column with ethanol-containing buffers,

purified DNA was eluted with 35 μ l 5 mM Tris-HCl (pH8.5). The same kit was used to purify PCR products from reaction solutions.

2.5.12 Ligation of DNA fragments

DNA fragments with compatible or blunt ends were ligated using 1 U T4-DNA-Ligase from Fermentas (St. Leon-Rot, Germany) with the provided 10x ligase buffer. Vector and inserts were mixed in an estimated molecule ratio of 1:5 and incubated at 37°C for 1 h or at 16°C over night.

2.5.13 Cloning and transformation of DNA fragments

DNA fragments were cloned with the TOPO TA Cloning® kit from Invitrogen (Carlsbad, USA) or the CloneJET™ PCR Cloning kit from Fermentas (St. Leon-Rot, Germany) according to the manufacturer's instructions. For expression analysis, DNA fragments and corresponding vectors (Tab.2.5) were digested with the specific restriction enzyme to produce compatible overhangs (2.5.10), and ligated as described above.

Tab.2.5: Vectors and their application in this work

Vector	Characteristics	Application
pHIS8-3 (Joseph P.Noel, The Salk Institute, La Jolla, USA)	Bacterial expression vector with N-terminal His(8)-tag, Km ^r , T7-promoter	Overexpression of His-tagged proteins in <i>E. coli</i>
pJet1.2/blunt (Fermentas, St. Leon-Rot, Germany)	Bacterial cloning vector, 2974 bp, Amp ^r , P _{lac} -promoter, T7 RNA-promoter	Cloning of PCR products
pCR®4-TOPO® (Invitrogen, Carlsbad, USA)	Bacterial cloning vector, 3900 bp, Amp ^r , Km ^r , P _{lac} -promoter, T7 RNA-promoter	Cloning of PCR products
pDONR™ vector 207 (Invitrogen, Carlsbad, USA)	Transformation vector, 5585 bp, Gent ^r , Cm ^r , pUC origin	Donor vector for Gateway cloning system
pBGWFS7.0 (Invitrogen, Carlsbad, USA)	Transformation vector, 12451 bp, GUS gene, GFP gene, Sm/Sp ^r , CoIE1, pVS1	Destination vector for Gateway cloning system (Karimi <i>et al.</i> 2002)

2.5.14 Sequencing and sequence analysis

Sequencing of plasmids and DNA fragments was performed by either Eurofins MWG (Ebersberg, Germany) or GATC (Konstanz, Germany). Samples were diluted to the concentrations and volumes specified by these companies and send via mail.

DNA and protein sequence data were evaluated with the software DNASTar (Madison, USA) and the web-based software Multalin at <http://multalin.toulouse.inra.fr/multalin/multalin.html>. Data bank searches were performed using BLAST (basic local alignment search tool) at <http://www.ncbi.nlm.nih.gov/>, maize sequence database at <http://www.maizesequence.org/index.html> and TIGR at <http://www.tigr.org/> as well as the plantGDB database at <http://www.plantgdb.org/>. Analysis of regulatory elements was performed using PLACE (<http://www.dna.affrc.go.jp/PLACE/>) and plant CARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

2.5.15 Gateway Cloning System

The Gateway® Technology is a universal cloning method based on the site-specific recombination properties of bacteriophage lambda (Invitrogen, <http://www.invitrogen.com>, (Landy 1989)). This method was used to clone the promoter fragments of *tps10* and *tps23* fused to the GUS gene into the destination vector in order to transform the resulting vector into *Agrobacterium tumefaciens*.

The promoter fragments were cut into sizes starting from 1.5 kb for *tps10* and 1.8 kb for *tps23* using PCR (Tab.2.6) and cloned according to the manufacturer's instructions via BP reaction into the donor vector pDONR™ vector 207. After selection on a kanamycin containing LB plate and colony PCR, the resulting entry clone was cloned using the LR reaction in the destination vector pBGWFS7.0. This destination vector contained the reporter gene β -glucuronidase. The resulting expression clone was used for transformation into *A. tumefaciens*.

The promoter constructs were cloned and transformed with support by Kurt Stampniok and Ulschan Scheler (AG Degenhardt).

Tab.2.6: Overview of the promoter sizes of *tps10* and *tps23* used for the Gateway cloning system.

Name of the construct	Size in [nt]	Primers used
tps10 1.5 kb::GUS	1297	TPG5, 6, 7; TPG11
tps10 800 bp::GUS	965	TPG8; TPG11
tps10 500 bp::GUS	559	TPG9; TPG11
tps10 200 bp::GUS	293	TPG10, 12 ; TPG11
tps23 1.8 kb::GUS	1779	TPG1; TPG4
tps23 1.2 kb::GUS	1186	TPG2; TPG4
tps23 600 bp::GUS	602	TPG3; TPG4
tps23 400 bp::GUS	397	TPG13, TPG4
tps23 200 bp::GUS	172	TPG14, TPG4

2.5.16 Microarray hybridization

The Maize Long Oligonucleotide 46k Array (version 1) provided by the University of Arizona was used for global expression profiling.

Material from plants treated according to the time course experiment (2.1) was ground in liquid nitrogen into a fine powder and total RNA was extracted as described above. A DNA digestion using Promega DNase (Promega, Madison, USA) was performed on-column according to the manufacturer's instructions.

Utilizing the Amino Allyl MessageAmpTMII aRNA Amplification Kit (Ambion, Carlsbad, USA), amplified RNA was produced and labeled with Cy3/Cy5 Mono-Reactive Dye Pack (GE Healthcare, Freiburg, Germany) according to the instruction of the manufacturer's. The labeled RNA probes were purified (RNeasy MinElute Cleanup, Qiagen, Hilden, Germany), mixed and hybridized with the Long Oligonucleotide Microarray with the protocols provided by the University of Arizona (<http://ag.arizona.edu/microarray/methods.html>). Reverse labeling experiments were performed to eliminate dye-specific bias. For each treatment, the RNA was labeled with Cy3 and Cy5 in the reverse experiment. Considering the reverse labeling experiments, a total of 4 biological replicates and 2 technical replicates were included. After hybridization, the microarray slides were washed and scanned immediately with the DNA microarray scanner (system: G2565AA/BA from Agilent, Santa Clara, USA) and images were processed by Feature Extraction software (Agilent, Santa Clara, USA).

The R-based open source software Bioconductor (<http://www.bioconductor.org>) was used to analyze the resulting data files. Local background subtraction and Lowess normalization was applied for each microarray slide (Yang 2002). The p-values and \log_2 -ration between arrays were calculated using the linear model and empirical Bayes methods from the limma package of Bioconductor. Genes with a ratio cut-off of two and a p-value <0.05 were used for further analysis.

2.6 Electrophoresis and transfer techniques

2.6.1 Electrophoresis

DNA fragments were separated by electrophoresis in 1.5x TAE agarose gels (1.5 % agarose [g/v]; Bio-Rad Laboratories, Hercules, USA, 1 $\mu\text{g ml}^{-1}$ ethidium bromide). DNA probes were mixed with 1/4 volume loading buffer and separated at a constant voltage of 100 V in an *i*-Mupid mini electrophoresis unit (Helixx, Ontario, Canada) containing 0.5x TAE buffer. Nucleic acid was visualized under UV-light due to the intercalation of ethidium bromide into the DNA. To characterize the fragment sizes, an additional 3 μl of a 1 kb or 100 bp marker was applied to the gel (Invitrogen, Carlsbad, USA).

Loading buffer: 50 % glycerin [v/v]
 0.05 % bromphenol blue [g/v]
 100 mM EDTA

0.5x TAE-Puffer: 40 mM Tris-acetate pH8.0
 20 mM sodium acetate
 1 ml EDTA

2.6.2 SDS-PAGE

Proteins were separated under denaturing conditions using the polyacrylamide electrophoresis with sodium dodecyl sulfate (SDS) on 12 % polyacrylamide gels. The production of the gels was carried out after Laemmli (1970).

Protein probes were mixed with 2x buffer and denatured for 10 min at 90°C and concentrated in the stacking gel at a constant voltage of 100 V. In the separation gel, protein samples were separated at 200 V. Following electrophoresis, proteins were either blotted on a nitrocellulose membrane (Western blot 2.6.3) or directly stained for 30 min with Coomassie Brilliant Blue solution. Destaining of the gel was obtained by incubation with destaining solution for several hours. For the estimation of the protein sizes fragments were compared to the “Pre-Stained” protein marker (Invitrogen, Carlsbad, USA).

2x buffer:	100 mM Tris-HCl pH6.0
	4 % SDS [g/v]
	10 % 2-mercaptoethanol [v/v]
	20 % glycerol [v/v]
	0.1 % bromphenol blue [g/v]
Stacking gel 4 %:	0.125 M Tris-HCl pH6.8
	4 % acrylamide/bisacrylamide (29:1) Roth (Karlsruhe)
	0.1 % SDS [g/v]
	0.075 % APS [g/v]
	0.05 % TEMED [v/v]
Separation gel 12 %:	0.375 M Tris-HCl pH8.8
	12 % acrylamide/bisacrylamide (29:1)
	0.1 % SDS [g/v]
	0.075 % APS [g/v]
	0.05 % TEMED [v/v]
10x SDS-buffer:	200 mM Tris
	1.9 M glycine
	10 % SDS [g/v]

Coomassie Brilliant

Blue solution: 45 % methanol [v/v]
 7 % acetic acid [v/v]
 0.1% Coomassie Brilliant Blue R250® [g/v] (Sigma-Aldrich, St. Louise, USA)

Destaining solution: 5 % methanol [v/v]
 7 % acetic acid [v/v]

2.6.3 Western-Blot

2.6.3.1 Transfer of proteins

Proteins were transferred from the polyacrylamide gels onto nitrocellulose membranes (Millipore Corporation, Bedford, USA) using a tank blotting system („Mini Trans-Blot Cell“, Bio-Rad Laboratories, Hercules, USA). Therefore, membranes were washed with ethanol and equilibrated for 15 min in blotting buffer with filter paper and pads. The setup is shown in Fig.2.2. The transfer was carried out by constant voltage of 100 V for 3 h.

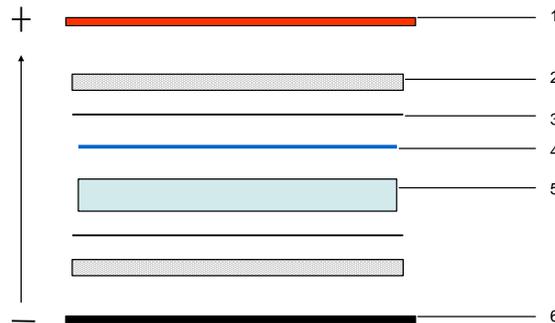


Fig.2.2: Western-Blot setup. 1: cathode; 2: pads; 3: filter paper; 4: nitrocellulose membrane; 5: SDS-Gel; 6: anode.

Blot-buffer: 25 mM Tris
 192 mM glycine
 20 % methanol [v/v]

2.6.3.2 Protein detection

To block unspecific binding of the antibody, the nitrocellulose membrane was incubated with the protein-side facing up for 30 min at room temperature in blocking solution.

A specific monoclonal anti-polyhistidine antibody from mouse (Sigma-Aldrich, St. Louis, USA) was used as primary antibody in a dilution 1:6000 in blocking solution. The membrane was incubated over night with constant shaking and then rinsed 3 times 5 min each in TBST-buffer. The secondary antibody anti-mouse IgG from goat which is conjugated to alkaline phosphatase (Sigma-Aldrich, St. Louis, USA) was added to the membrane with a working dilution of 1:30000. After an incubation of 1 h, the membrane was washed 3 times for 5 min in TBST-buffer. The colored precipitate was obtained by adding NBT/BCIP solution („BCIP/NBT Liquid Substrate System“, Sigma-Aldrich, St. Louis, USA). This reaction, where the alkaline phosphatase converts the NBT/BCIP solution into a blue pigment, was carried out in darkness and was stopped by adding H₂O.

Blocking solution: 2 % BSA [g/v] in TBST-Puffer

TBST-Puffer: 10 mM Tris-HCl pH8.0
150 mM NaCl
0.05 % Tween 20 [v/v]

2.6.4 Band shift assays (EMSA)

EMSA was performed using the Gel Shift Assay System from Promega (Madison, USA) according to the manufacturer's instruction. Ten mCi ml⁻¹ [γ ³²-P] ATP was attached to 1.75 pmol μ l⁻¹ of the truncated promoter fragments during incubation with the T4 Polynucleotide Kinase 10x buffer and T4 polynucleotide kinase for 10 min at 37°C. The specific concentration of the oligonucleotide-fragments was calculated after this formula:

$$c(\text{pmol}) = \frac{c\left(\frac{\text{ng}}{\text{ml}}\right) * 10^6}{MW}$$

$$MW \text{ of } dsDNA = (\text{length in nt} * 667.4) + 157.9$$

c(pmol)	= concentration of the oligofragment in pmol
c(ng/ml)	= concentration of the oligofragment in ng/ml
MW	= molecular weight of the oligofragment

The reaction was stopped by adding 1 μ l of 0.5 M EDTA. To remove unincorporated label from the oligonucleotide, 89 μ l of TE buffer was added to the sample and purified using a G-25 spin column (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions. One μ l of the purified oligofragments was measured using a scintillation counter. A binding reaction was set up using 250 ng of the purified transcription factor (2.7.1) and 2 μ l Gel Shift Binding 5x buffer. After 10 min incubation at room temperature, 2 μ l of the labeled promoter fragment were added and incubated again for 20 min at room temperature. One μ l gel loading buffer was added to the negative control only because this buffer might inhibit the *in vitro* binding ability between DNA and proteins. Before loading 10 μ l of each probe, the 3.8 % 0.5x TBE nondenaturing polyacrylamide gel was pre-run for 10 min at 250 V. The gel was run at 200 V for 20 min, removed from the cassette, placed on a filter paper, sealed in a plastic film and exposed to a phosphor sensitive screen for 90 min. Pictures were developed using a phosphor imager. Unlabeled promoter fragments were used as specific competitor and SP1 consensus oligo, provided from the kit, as unspecific competitor.

TE buffer:	10 mM Tris-HCl pH8.0 1 mM EDTA
10x TBE buffer:	0.9 M Tris base 0.9 M boric acid 0.02 M disodium EDTA·2H ₂ O pH8.3
3.8 % gel:	1x TBE buffer 3.8 % acrylamide/bisacrylamide (19:1) 3 % glycerin 0.005 % TEMED [g/v] 0.075 % APS [v/v]

2.7 Protein techniques

2.7.1 Heterologous expression of transcription factors in *E. coli* and purification of recombinant protein

The open reading frames (ORFs) of the identified transcription factors were cloned into the bacterial expression vector pHIS8-3 with an N-terminal His-tag (Tab.2.5) and expressed in *E. coli* BL21 (DE3). 400 ml LB-medium were inoculated with a 3 ml overnight culture of *E. coli* containing the expression constructs and incubated at 37°C with constant shaking to an OD₆₀₀ of 0.7. The vector-mediated expression was induced by adding IPTG to a final concentration of 2 mM to the medium. Cultures were shaken for 3 h at 37°C and harvested by centrifugation (20 min at 5000 g, 4°C). The pellet was resuspended in 10 ml chilled lysis buffer and disrupted by a 5 x 30 s treatment with a sonicator (Branson sonifier 250, Schwäbisch Gmünd). 15 mg lysozyme (Appli Chem, Darmstadt) was added and incubated for 1 h at 4°C. The *E. coli* cell fragments were removed by centrifugation at 14000 g for 20 min, 4°C, 1 ml Ni-NTA-Agarose (50 %, Qiagen, Hilden) was added to the supernatant and shaken for 2 h at 4°C. The supernatant was added to an equilibrated column (Poly-Prep® Chromatography column, BioRad (Munich) and washed twice with 3 ml washing buffer. His-tagged proteins bound to the Ni-NTA-matrix were eluted with 3 ml elution buffer. One ml fractions were collected. The protein-containing elution fractions were combined after the protein quantification (2.7.2) and used as probes in the band shift assays (2.6.4).

LB-culture: 25 g LB -medium (AppliChem, Darmstadt) in 1 l *a.dest.*
Lysis buffer in PBS: 500 mM NaCl
2.7 mM KCl
1.7 mM KH₂PO₄
10.1 mM Na₂HPO₄
20 mM imidazol
1 % Tween 20
10 % glycerol
10 mM β-mercaptoethanol

Washing buffer

in PBS: 500 mM NaCl
2.7 mM KCl
1.7 mM KH₂PO₄
10.1 mM Na₂HPO₄
20 mM imidazol
10 % glycerol
10 mM β-mercaptoethanol

Elution buffer in PBS: 500 mM NaCl
2.7 mM KCl
1.7 mM KH₂PO₄
10.1 mM Na₂HPO₄
250 mM imidazol
10 % glycerol
10 mM β-mercaptoethanol

PBS-buffer: 137 mM NaCl
2.7 mM KCl
7 mM Na₂HPO₄ · 2 H₂O
1.5 mM KH₂PO₄
pH7.4 with HCl

2.7.2 Protein quantification

Purified protein was quantified according to Bradford (1976). Protein samples were diluted 1:20 and added with 200 µl 1.8x Roti®-Quant (Roth, Karlsruhe) to 250 µl and incubated for 5 min at room temperature. OD was measured at 590 nm (NanoQuant, Tecan, Crailsheim) and compared with the standard curve prepared with 0, 20, 30, 40, 50, 60, 80 and 100 µg ml⁻¹ bovine serum albumin (Sigma-Aldrich, St. Louis, USA), respectively.

2.8 Chromatographic analysis

2.8.1 Volatile collection

For the analysis of volatile terpenes, plant material was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. 200 mg of the ground material was transferred to a glass vial with a septum in the lid. A 100 µm PDMS SPME fiber was inserted through the

septum and incubated for 30 min at 40°C. The volatile spectrum was analyzed by gas chromatography-mass spectrometry.

2.8.2 Gas chromatography – mass spectrometry (GC-MS)

GC-MS was performed on a Shimatsu GC-MS2010Plus gas chromatograph (Duisburg, Germany) with H₂ as carrier gas at 1 ml min⁻¹. The injection temperature was set to 220°C and samples were added directly using a SPME fiber without split. The temperature on the column was raised from 60°C (constant for 3 min) to 200°C with a rate of 7°C per minute and then finally raised to 300°C with a rate of 100°C per minute. The mass spectrometer was run with the following settings: interface temperature: 250°C, ion source temperature: 200°C, ionization potential: 70 V, and a scan range of 46-350 amu. An EC-5 column (length: 30.0 m, diameter: 0.25 mm and film thickness: 0.25 µm (Grace, Deerfield, USA)) was used as chromatographic column.

The data were analyzed with Shimatsu Labsolution software and the mass spectrum libraries “Wiley8” (Hewlett-Packard) and “Shim2205” (Shimatsu).

2.8.3 Identification of plant hormones

Approximately 200 mg of leaf material was used for hormone extraction and mixed with 1 ml ethyl acetate spiked with 40 ng ml⁻¹ labeled phytohormones, D₆-abscisic acid (ABA), D₄-salicylic acid (SA), jasmonic acid-¹³C₆ isoleucin (JA-Ile) and 200 ng ml⁻¹ D₂-jasmonic acid (JA), respectively. The mixture was vortexed for 10 min and then centrifuged at 4°C for 20 min at 16000 g. Extraction was repeated with 500 µl ethyl acetate without internal standard and supernatants were combined and dried in the speedvac at 30°C. The pellet was dissolved in 500 µl 70 % methanol and centrifuged at 16000 g to remove precipitates. Ten µl supernatant was used for the analysis on the Varian 1200L Triple-Quadrupol-MS (Palo Alto, USA). Hormones were separated on a ProntoSil C18-ace-EPS column (50x2 mm, 5 µm, 120 Å) with 0.05 % formic acid and methanol as solvent solutions with a flow rate of 0.4 ml/min in a gradient mode. The compounds were detected in the ESI negative mode. Molecular ions (M-H) with *m/z* 137, 209, 263 and 322 for SA, JA, ABA and JA-Ile and 141, 213, 269 and 328 for the respective internal standards were fragmented. Daughter ions 93, 59, 153 and 130 for the compounds and 97, 59, 159 and 136 for the internal standards were recorded for

quantification. Collision energy was 15 V for SA, 12 V for JA, 9 V for ABA and 19 V for JA-Ile. Needle, shield and detector were set at 4500 V, 600 V and 1800 V, respectively.

2.9 Histochemical methods

2.9.1 GUS staining

GUS staining was used to analyze the activity of promoter fragments of *tps10* and *tps23*. The β -glucuronidase gene was fused to the promoter fragments using the Gateway cloning system (2.5.15) into an expression vector. The promoter containing plasmid was stable transformed into *Arabidopsis thaliana* using *Agrobacterium* (2.4.4).

The GUS enzyme converts its substrate, X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-gluconic acid, cyclohexylammonium salt), into glucuronic acid and the colorless 5-bromo-4-chloro-indoxyl intermediate which spontaneously undergoes an oxidative dimerization to form the insoluble and highly colored indigo dye (5,5'-dibromo-4,4'-dichloro-indigo).

Arabidopsis material was harvested after different induction treatments (2.3) and transferred to glass tubes containing sufficient GUS-staining solution to cover the whole leaf. Vacuum was applied to the vials to ensure complete exposure of the plant cells to the X-Gluc substrate. Leaves were incubated in this solution at 37°C over night.

To remove the chlorophyll from the plant material, the leaves were transferred to 80 % ethanol for several hours at 37°C until all chlorophyll was extracted. Leaves were then washed and scanned.

GUS-staining solution: 50 mM sodium dihydrogen phosphate (pH7.0)
 10 mM EDTA
 0.1 % Triton [v/v]
 0.1 % N-lauryl-sarcosine [g/v]
 0.7 $\mu\text{g ml}^{-1}$ β -mercaptoethanol
 1 mg ml^{-1} X-Gluc (added freshly)

2.9.2 Quantitative GUS assay

The β -glucuronidase activity was measured quantitatively using 4-methylumbelliferyl-beta-D-glucuronide (MUG) as its substrate (Kay *et al.* 2007). MUG is converted by the β -glucuronidase to 4-methylumbelliferone (4-MU) which has an excitation wavelength at 365 nm and an emission wavelength at 345 nm and can therefore be used in fluorescence measurements.

20 mg of frozen plant material was ground in liquid nitrogen, placed in 300 μ l GUS-extraction buffer, and redissolved. After centrifugation for 5 min at 20000 g at 4°C, 200 μ l of the supernatant was transferred to a 96-well plate and centrifuged again for 10 min at 1610 g and 4°C. 100 μ l of the supernatant were kept for a Bradford assay (2.7.2). Another 20 μ l were transferred to a new 96-well plate and 180 μ l 5 mM MUG (dissolved in fresh GUS-extraction buffer) was added and incubated in the dark for 90 min at 37°C. 20 μ l of this reaction was transferred to a black 96-well PCR plate (Greiner, Frickenhausen, Germany) and stopped by adding 180 μ l 0.2 M sodium carbonate. The fluorometric endpoint measurement was conducted using the infinite 200 Pro (Tecan, Crailsheim, Germany).

For each measurement, a standard curve with 4-MU with the concentrations of: 0.1 μ M; 1 μ M; 10 μ M and 100 μ M was prepared. GUS activity was calculated in pmol MU μ g protein⁻¹ min⁻¹ according to the formula below (Boch *et al.* 2009).

$$A_{GUS} \left[\frac{\text{pmol MU}}{\mu\text{g protein} * \text{min}} \right] = \frac{F * dF * 2000 \text{ pmol MU}}{90 \text{ min} * \text{protein} [\mu\text{g}] * F_{2000 \text{ pmol MU}}}$$

A_{GUS}	GUS activity
F	fluorescence of sample
dF	dilution factor of sample
$F_{2000 \text{ pmol MU}}$	fluorescence of standard
GUS extraction buffer:	50 mM sodium dihydrogene phosphate (pH7.0) 10 mM EDTA 0.1 % SDS [g/v] 0.1 % Triton [v/v] 10 mM MUG (added freshly)

2.10 Statistical analyses

To calculate the statistical relevance of the given data, the appropriate statistical methods were used dependent on the experimental setup. In most cases, a two way ANOVA was performed and Post-Hoc tests were conducted with the Holm-Sidak method to calculate pairwise comparisons. Normal distribution of the data and equality of variances was verified. In case of no normality, data were transformed if possible or analyzed by an ANOVA based on ranks. Significant differences of the data are indicated for $p < 0.05$. The respective method for each experiment is provided in the text.

3. Results

3.1 Identification of genes regulated by herbivore treatment

Maize plants produce a complex mixture of terpene volatiles upon herbivory (Turlings *et al.* 1990; De Moraes *et al.* 1998), but the regulatory mechanisms and signaling cascade leading to the production of volatile terpenes is mainly unknown. In this work, the identification of essential signaling compounds involved in defense responses in *Zea mays* was one main focus.

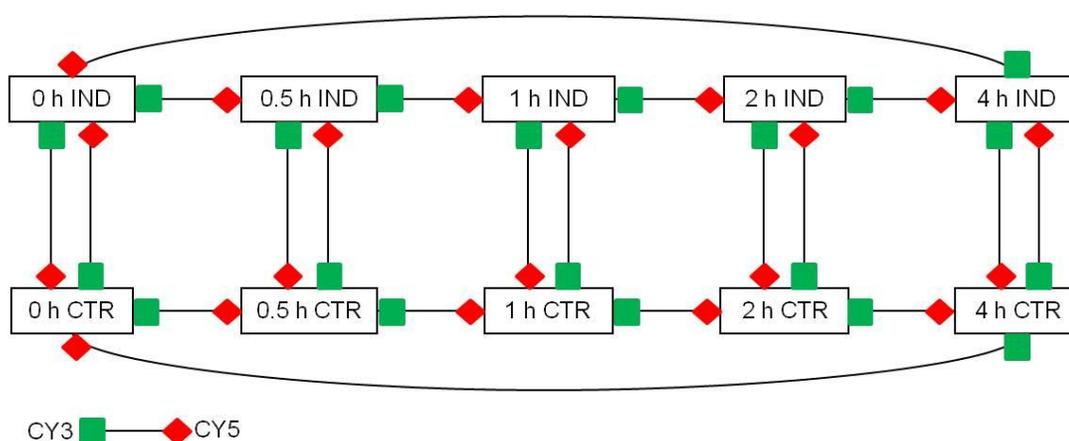


Fig.3.1: Experimental setup for the microarray hybridization experiment used in this work. Maize plants were left unharmed (CTR) or treated for 30 min with larvae of *S. littoralis* (IND). Leaves were harvested after 0.5 h, 1 h, 2 h, and 4 h. Additionally, a 0 h time point was included. Four biological replicates were included for each measurement. RNA was obtained from all plants and for all time points and labeled with the fluorescent dyes CY3 and CY5 according to the setup. Each line represents one microarray hybridization experiment using two RNA probes. Thereby, one probe was labeled with CY3 and one with CY5. Dye swaps were performed to guarantee minimal effects of different dye incorporations.

To detect genes regulated by herbivore feeding, we conducted a microarray hybridization experiment with a 46k oligo microarray from the University of Arizona. This microarray was printed by the Maize Oligonucleotide Project Team (<http://www.maizearray.org/>) on a glass slide and contains 46128 70mer oligos based on cDNA and EST sequence information from *Zea mays* var. B73. Therefore, the chip represents a large fraction of the transcriptome of the maize line B73. The experimental design chosen, described as “loop-design”, included 4

biological replicates and dye swaps for each sample (Fig.3.1). Dye swaps minimize the effect of different incorporations of fluorescent molecules into RNA, whereas the loop design provides a higher statistical power than a simple reference design (Churchill 2002).

To induce *Zea mays* var. Delprim, two second instar larvae of *S. littoralis* were placed on the lower part of maize leaves for 30 min. Both the herbivore-damaged and the undamaged plants were harvested after 0 h, 0.5 h, 1 h, 2 h, and 4 h to determine the transcriptional changes. The undamaged plants were harvested at each time point to account for circadian changes and other factors that might affect plant gene expression throughout the 4 h experiment. A reliable test for the successful herbivore induction of the plant material is the expression of the terpene synthases TPS10 and TPS23, since those enzymes produce the main volatiles released after herbivore attack in maize (Köllner *et al.* 2004). To check for efficient herbivore induction in damaged plants, terpene production was determined by GC-MS in induced leaf material at 4 h after induction (Fig.3.2).

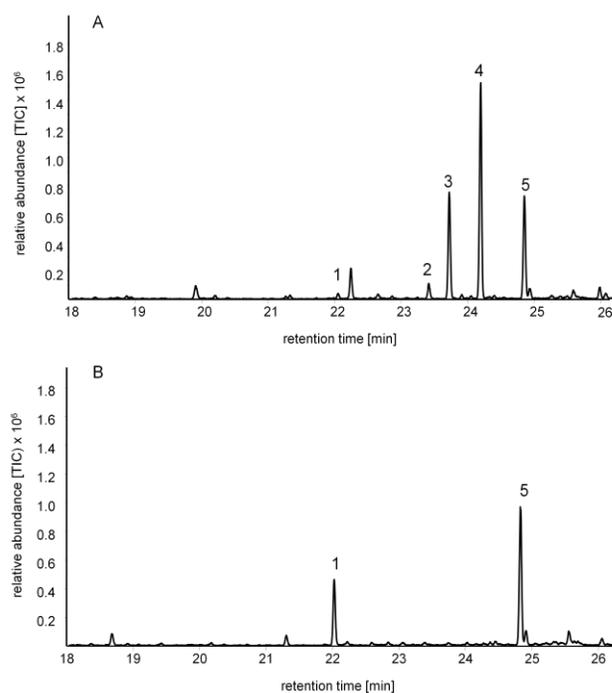


Fig.3.2: Gas chromatographic analysis of herbivore-damaged and undamaged maize leaves. (A) Maize leaves were induced by herbivore feeding for 30 min and material was harvested after 4 h or (B) undamaged material harvested after 4 h. Plant material was ground into a fine powder and 200 mg were incubated for 30 min with a SPME fiber. Peaks were identified as 1: unknown, 2: (*E*)- β -caryophyllene, 3: (*E*)- α -bergamotene, 4: (*E*)- β -farnesene and 5: β -Ionone, respectively.

The sesquiterpenes (*E*)- β -caryophyllene, (*E*)- α -bergamotene and (*E*)- β -farnesene were emitted after the herbivore induction period while no sesquiterpenes were visible in the control plants. This result confirmed the induction of the maize plants was appropriate for microarray analysis.

To evaluate the up- and down-regulated genes, the raw data from the microarray were analyzed using the statistical program R as described in section 2.5.16. Only genes with a relative induction higher than 2-fold were chosen for further analysis. Induced oligo fragments were run through different databases to find a putative gene function. A list of all genes differentially regulated after herbivory can be found in the appendix (7.6). To generate a simple overview of the molecular function and cell processes the affected genes were involved in, the program Blast2GO (Conesa *et al.* 2005) was used.

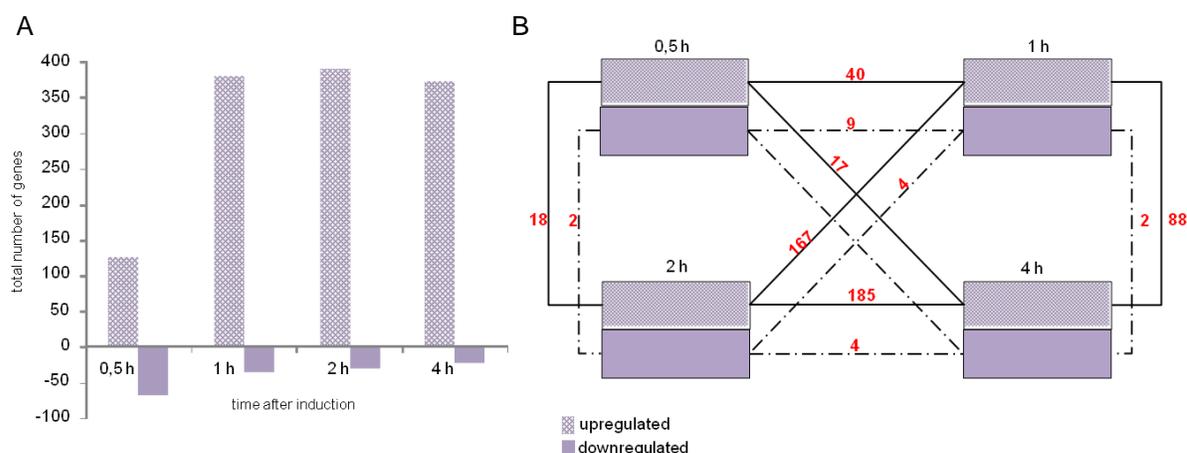


Fig.3.3: Overview of all differential regulated genes by herbivory. Plants were treated with *S. littoralis* for 30 min and harvested at 0.5 h, 1 h, 2h, and 4 h. A: The total number of genes that showed a differential fold of ≥ 2 are displayed. B: The number of genes that shared a same regulation pattern is displayed.

An overview of all positive- and negative-regulated genes found after herbivore treatment is shown in Fig.3.3. Half an hour after the caterpillar treatment, 125 transcripts displayed increased expression within the leaves while 68 genes showed a reduced expression. After one hour, the number of up-regulated genes increased to 381 and did not drop dramatically at 2 h and 4 h. Overall, the majority of differentially regulated genes showed an increased expression level. Only 12% of the genes displayed a decreased expression. For better

visualization, identified genes were displayed regarding their biological process (Fig.3.4). The functional annotation of the sequences was given by Gene Ontology.

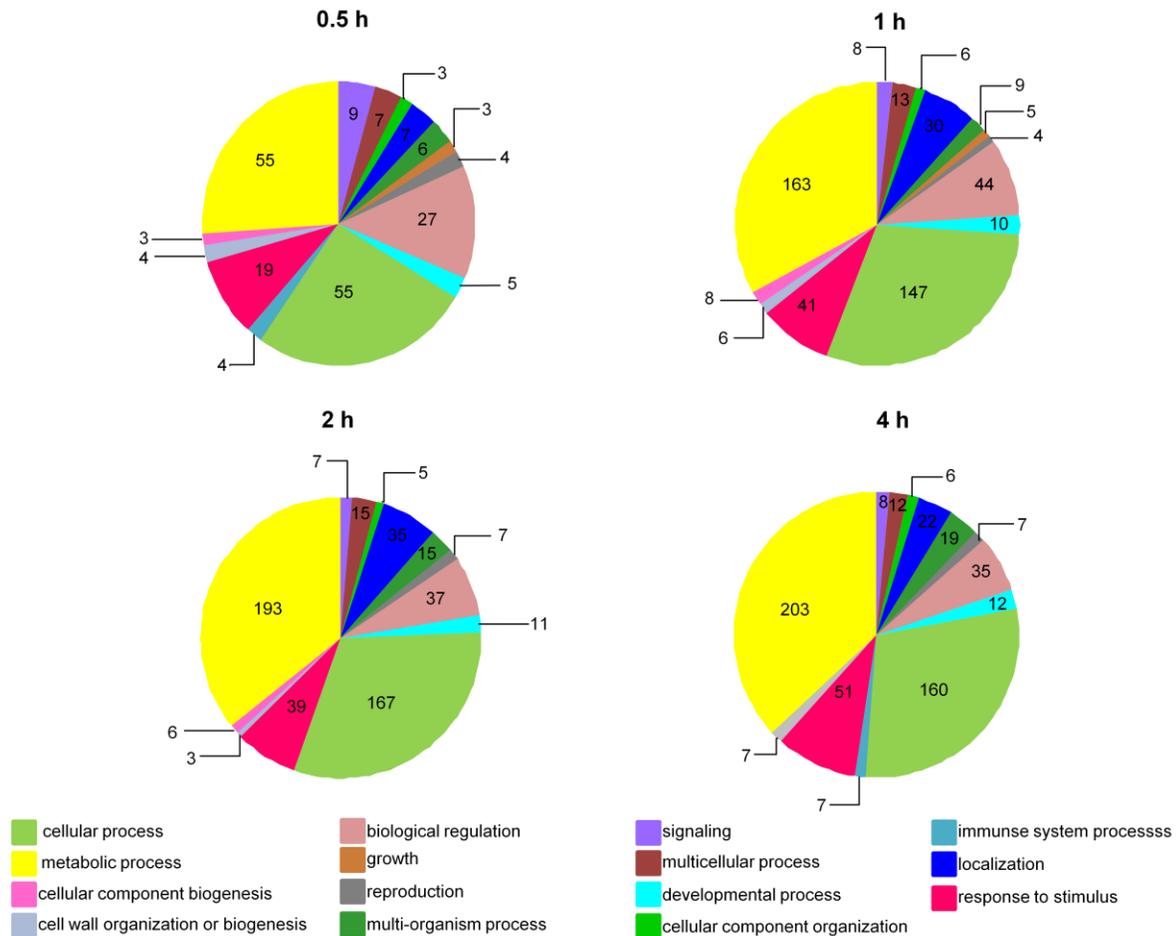


Fig.3.4: Classification of herbivore-regulated genes in regard to the biological processes of the plant.

Genes regulated by herbivory and identified with the Long Oligo Microarray, were mapped and associated with their biological role within the plant using Blast2Go (sequence filter = 2; level = 2). Plants were treated with *S. littoralis* for 30 min and harvested at 0.5 h, 1 h, 2 h, and 4 h. The numbers indicate the number of associated sequences.

At the four time points, the majority of the identified genes belonged to the group of “metabolic and cellular processes”. The genes belonging to the group “response to stimulus” increased over the 4 h period from 19 to 51 sequences, while genes with a function in plant growth were completely missing after 1 h.

Next to the biological function, the identified transcripts were compared regarding their molecular function. Within this analysis, the sequences could be grouped into: binding activity, enzyme regulator activity, transporter activity, transcription regulator activity, etc. Fig.3.5 shows an overview of the genes which were differentially regulated within the microarray hybridization experiment. The graphs were obtained from Blast2Go with a sequence filter setting of 2, level 2. At the four time points, genes belonging to the group of “catalytic activity” and “binding” took up the major portion. It is noteworthy that genes can belong to more than one functional group, depending on the Gene Ontology numbers provided by Blast2Go. The figure shows that genes having a binding function were doubled from 0.5 h to one hour after herbivore attack. Genes mapped to “transcription regulator activity” showed the highest amount after 0.5 h after the treatment and were then limited to 3 genes. This suggests that the plant was able to respond very quickly to the herbivore damage by activating a specific set of transcription factors.

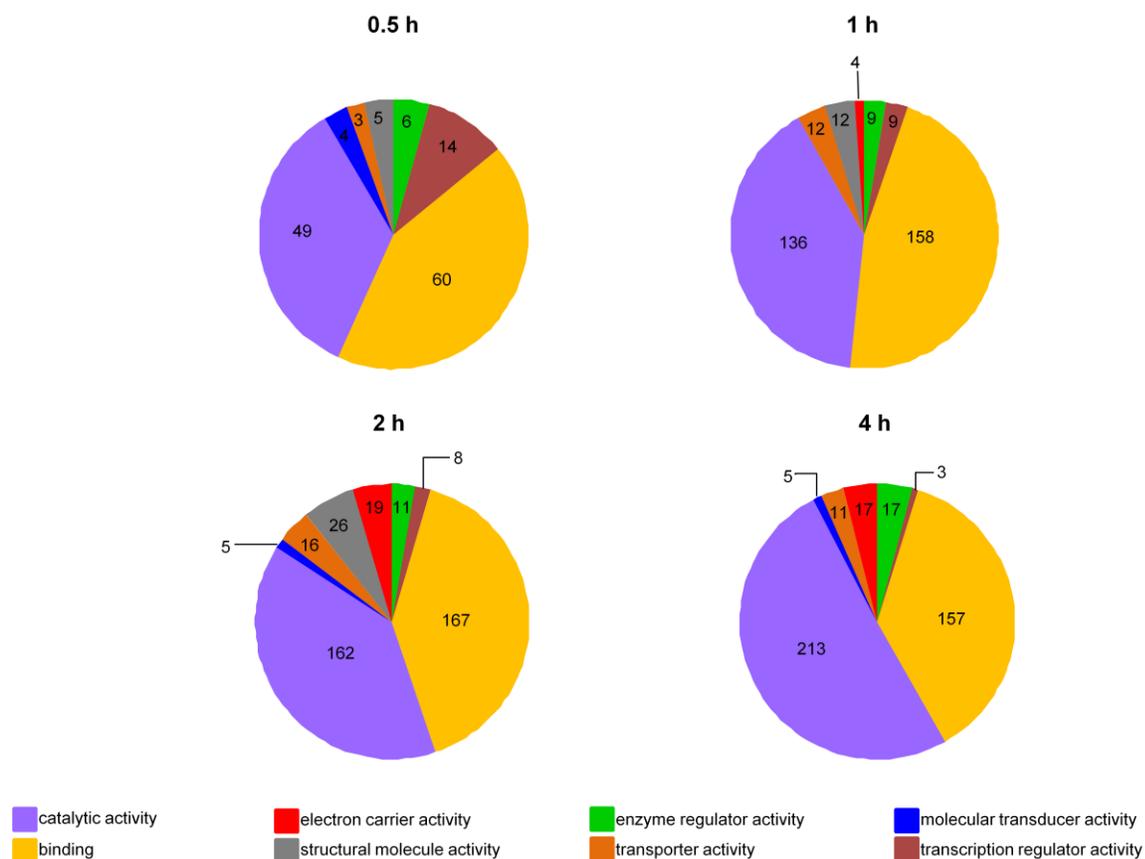


Fig.3.5: Classification of herbivore-regulated genes in regard to the molecular function of the plant. Plant material was harvested at 0.5 h, 1 h, 2 h, and 4 h after a 30 min treatment with *S. littoralis*. Transcripts with concentration differences (>2 fold) were detected after herbivory and assembled into molecular functional groups. Figures were obtained from Blast2GO (Sequence filter = 2, level = 2). The numbers indicate the number of associated sequences.

To analyze the functional distribution of the herbivore-induced genes more specifically, several biological processes were investigated for positive and negative-regulated genes. The functional classifications chosen were transport, transcription, signaling, cell wall metabolism, secondary metabolism, photosynthesis, stress response, amino acid metabolism, carbon metabolism, nucleotide metabolism, plant development, and hormone metabolism (Fig.3.6). While the total numbers of herbivore-regulated genes increased strongly between the 0.5 h and 1 h time points, the highest number of negatively regulated genes were present at 0.5 h. Nine transcripts with a putative function in stress response were negatively regulated and ten transcripts were up-regulated. Among the genes with putative functions in gene

transcription, seven transcripts were down-regulated and 13 were up-regulated. One hour after herbivore induction, the total number of genes induced by herbivory increased dramatically, while only few genes were still reduced. 39 genes functioning as regulatory factors showed an increased transcript accumulation, also 17 genes of hormone metabolism were differentially expressed. Genes of this group were not involved 0.5 h after induction. There are only few changes between 1 h and 2 h after herbivore induction, mainly the number of transcripts involved in the amino acid metabolism rose. This could be explained with the need of amino acids for the translation of proteins necessary for defense. The main difference between 2 and 4 h after the herbivore treatment was the suppression of genes having a role in transcriptional activity and the negative-regulation of transcripts playing a role in the photosynthesis of the plant. While a plant is attacked by an herbivore, resources may rather be put into defense than into photosynthesis.

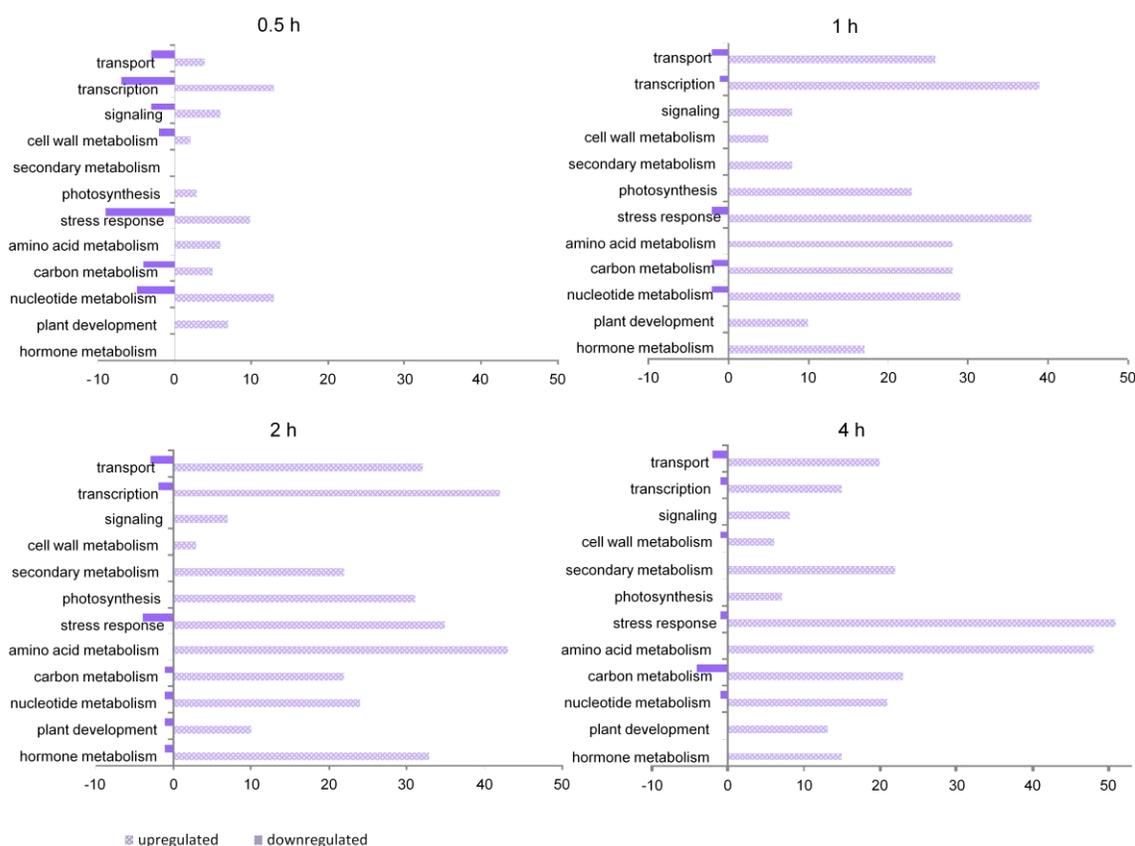


Fig.3.6: Functional classification of genes with herbivore-regulated transcript levels. Plants were treated with *S. littoralis* for 30 min and harvested at 0.5 h, 1 h, 2 h, and 4 h. Total numbers of genes with enhanced and repressed transcript accumulation were distributed into functional groups.

Transcription factors affected by herbivore treatment were the main focus of this study, assuming that those play a critical role in the induction of plant defense. Transcription factors are small proteins which regulate the gene expression through binding of the promoter of specific target genes, either by itself or in co-operation with other regulatory proteins. The Long Oligonucleotide chip was based on the ESTs of the maize inbred line B73 for which the genome sequence is available in genomic databases like TIGR, PlantGDB, NCBI and Maizesequence. Oligofragments of putative transcription factors on the microarray chip were aligned to the genomic sequence in the databases to obtain the full length sequences. Unfortunately, this method was not successful for all putative transcription factors. Thus, RACE PCR had to be performed where the information from those databases could not solve the genomic sequence of the transcription factors of interest. Table 3.1 gives an overview of annotated transcription factors identified by the Long Oligonucleotide Microarray, their accession number and their putative genomic annotation.

Tab.3.1: Putative transcription factors (TF) and their annotation.

Synonyms	Accession number	Annotation
TF1	MZ00026661 / TC280937	putative NAC-domain protein
TF2	MZ00031423 / TC314449	CAF1 like protein
TF3	MZ00044429 / TC326357	MYB-like DNA binding
TF4	MZ00006968 / TC297179	Zn-finger protein
TF5	MZ00014652 / TC284178	CHY Zn-finger protein
TF6	MZ00017631 / TC298582	putative transcription factor
TF7	MZ00018761/ AZM5_85107	MYB-like DNA binding protein
TF8	MZ00017826/ AZM5_89531	bHLH-like protein
TF12	MZ00056923/ AZM5_18508	MYB-like protein
TF15	MZ00031032/ AZM5_10006	putative PRLI-interacting factor
TF18	MZ00029591/ AZM5_14769	DNA binding protein
TF19	MZ00024627/AZM5_32315	ETTIN-like auxin response factor
TF20	MZ00024626/ AZM5_31563	ETTIN-like auxin response factor
TF21	MZ00049092 /AZM5_95253	G-Box binding protein
TF22	MZ00035947/ AZM5_27326	NAC-domain protein
TF23	MZ00005265/ AZM5_98744	helix-loop-helix DNA-binding protein

TF24	MZ00003819/ AZM5_142343	ethylene responsive transcriptional coactivator
TF25	MZ00035924/ AZM5_43253_1	response regulator Cip1
TF26	MZ00042739/ AZM5_152884, AZM5_108687	WRKY transcription factor
TF27	MZ00017310/ AZM5_31855, AZM5_24906	Zn-finger protein
TF28	MZ00048444/ AZM5_15878	MYB factor protein
TF29	MZ00041804/ AZM5_5103	ZN-finger protein
TF30	MZ00026596/ AZM5_13093	ethylene responsive element binding factor3
TF31	MZ00025601/ AZM5_10813	putative leucine zipper protein
TF32	MZ00025832/ AZM5_7677	bHLH transcription factor
TF33	MZ00025058/ AZM5_85092	transcription factor like protein
TF34	MZ00020619/ AZM5_6856	transcription factor WRKY12
TF35	MZ00023008/ AZM5_416	putative I-box binding factor
TF36	MZ00028957/ AZM5_14321	calmodulin-binding protein-like
TF37	MZ00041802/ AZM5_5103	Zn-finger protein
TF38	MZ00018541/ AZM5_87053	Putative EREBP-like protein
TF39	MZ00032877/ AZM5_50726	putative phi-1
TF40	MZ00015673/ AZM5_90421	DRE binding factor 1
TF41	MZ00001133/ AZM5_93171	putative LHY-protein
TF42	MZ00056523/ AZM5_37193	putative phi-1
TF43	MZ00029551/ AZM5_16115	Zn-finger protein 1
TF44	MZ00004193/ AZM5_107126	putative bZIP protein HY5
TF45	MZ00014137/ AZM5_98313	transcription factor-like
TF46	MZ00056566/ AZM5_16172	Zn-finger transcription factor ZF1
TF47	MZ00035095/ AZM5_18076	putative nucleoid DNA-binding protein cnd41
TF48	MZ00037615/TC329977	putative NLI interacting factor
TF49	MZ00016101/TC295701	putative RAB7A protein
TF50	MZ00000664/TC308252	RING finger-like protein
TF51	MZ00036019/TC280937	putative NAC-domain protein

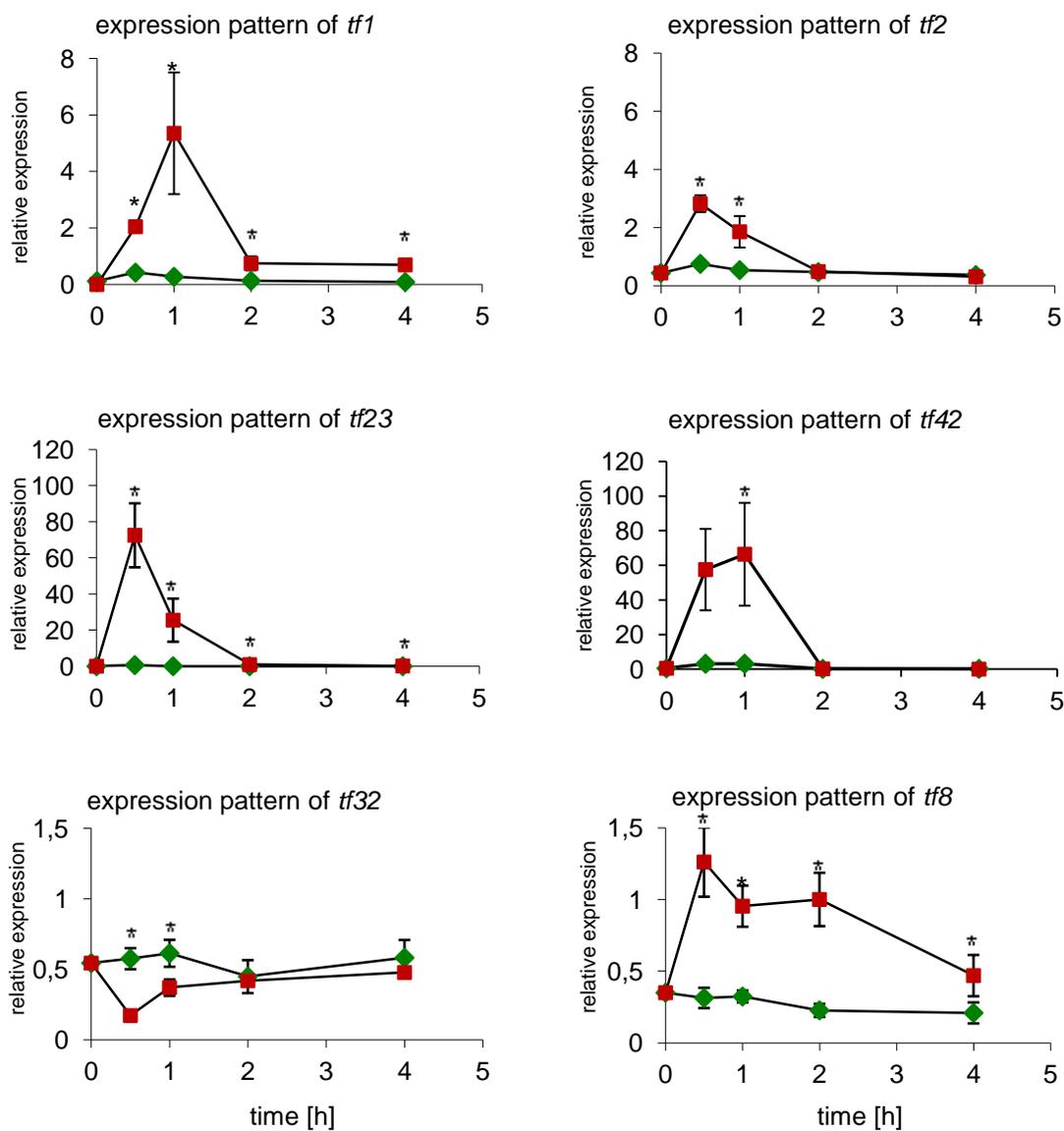
3.2 The induction of transcription factors depends on environmental cues

3.2.1 The transcript patterns of transcription factors respond to herbivore feeding

To confirm the expression patterns of the identified transcription factors and investigate their spatial distribution, primers for the chosen transcription factors were designed from the oligonucleotide fragments given by the microarray and the genome databases. Results from the microarray experiment were verified with QRT-PCR using plant material that was also harvested at 0 h, 0.5 h, 1 h, 2 h, and 4 h after herbivore damage. Verification of this data was important due to the low threshold of 2 used for statistical analysis. This low threshold was necessary to identify a large fraction of the transcription factors involved in plant defenses. Compared to enzymes, transcription factors are usually present in a low amount to regulate gene expression and therefore do not rise that strongly. On the other hand, the chance of false positives rises. Only for 18 of the selected transcription factors, the microarray results could be affirmed. To test for successful induction of the plant material, five additional herbivore-regulated genes, the terpene synthases *tps10* and *tps23*, and the O-methyl transferases: *omt1*, *omt3* and *omt4* were analyzed with QRT-PCR.

Transcription factors of several classes were affected by herbivore feeding in the leaf: MYB-like binding proteins, WRKY binding proteins, I-Box binding proteins, G-Box binding proteins, zinc-finger protein, bHLH binding proteins and bZIP binding proteins. Most of the herbivore-induced transcription factors exhibited a transient increase in transcript levels. The kinetics of this increase differed between the factors and was used to classify them into early and late responding factors. The transcript accumulation profiles of six transcription factors and two terpene synthases are shown in Fig.3.7. Transcription factors *tf1*, *tf2*, *tf8* and *tf23* showed a significant expression maximum already after half an hour of induction, indicating a fast response after herbivory. *Tf42* also had a highly induced expression at 30 min after induction but reached its maximum at 1 h after the onset of treatment. In contrast, *tf32* demonstrated a negative regulation pattern. In this case, the expression level between control plants and induced plants showed a significant down-regulation of gene expression for the time points half an hour and 1 h after treatment. Overall, transcription factors were induced from half an hour to two hours after the treatment, while the expression of the terpene

synthases started at around 2 h after herbivore feeding and then slowly increased. This correlated with the volatile measurement which was detected at 4 h after herbivore treatment (Fig.3.2). The results for all further investigated transcription factors are in the appendix (7.1).



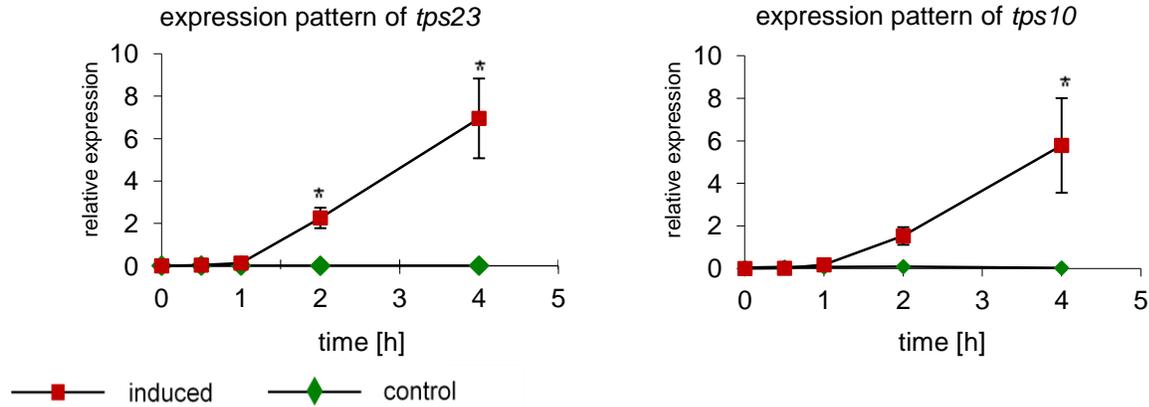


Fig.3.7: Relative expression pattern of selected transcription factors and terpene synthases. Plants were treated with *S. littoralis* caged for 30 min on a single leaf. Material was harvested after: 0 h, 0.5 h, 1 h, 2 h, and 4 h. Means and standard error are shown (n=4). The relative expression levels were calculated as described in 2.4.9. Two-Way ANOVA was used to calculate statistical differences with time as factor one and treatment as second variable. * indicate significant differences between treatments within time points ($p < 0.05$).

3.2.2 Transcript accumulation is affected by the duration of caterpillar feeding

After testing the induction kinetics of transcription factors in response to an herbivore attack of 0.5 h, we investigated the effect of different durations of herbivore feeding. In the following experiments, the caterpillar larvae were caged on a single leaf and were allowed to feed until the plant material was harvested. To obtain a good feeding performance, caterpillar larvae were not able to feed the night before. Leaf material was harvested after 0 h, 0.5 h, 1 h, 2 h, and 4 h and ground in liquid nitrogen. cDNA was obtained and used to test the expression level of several transcription factors by QRT-PCR. The relative expression was calculated against the expression of the housekeeping genes.

The transcript levels of the transcription factors *tf1* and *tf35* in response to different lengths of feeding time are shown in Fig.3.8. The expression kinetics of further factors can be found in the appendix (7.2). While the expression level for *tf1* increased to its maximum at 1 h after a feeding time of 30 min, the expression pattern with the continuous feeding showed significant differences. Here, transcript accumulated within 0.5 h, but then dropped and

increased again for the remaining time points. The same pattern could be observed for the transcription factor *tf35*.

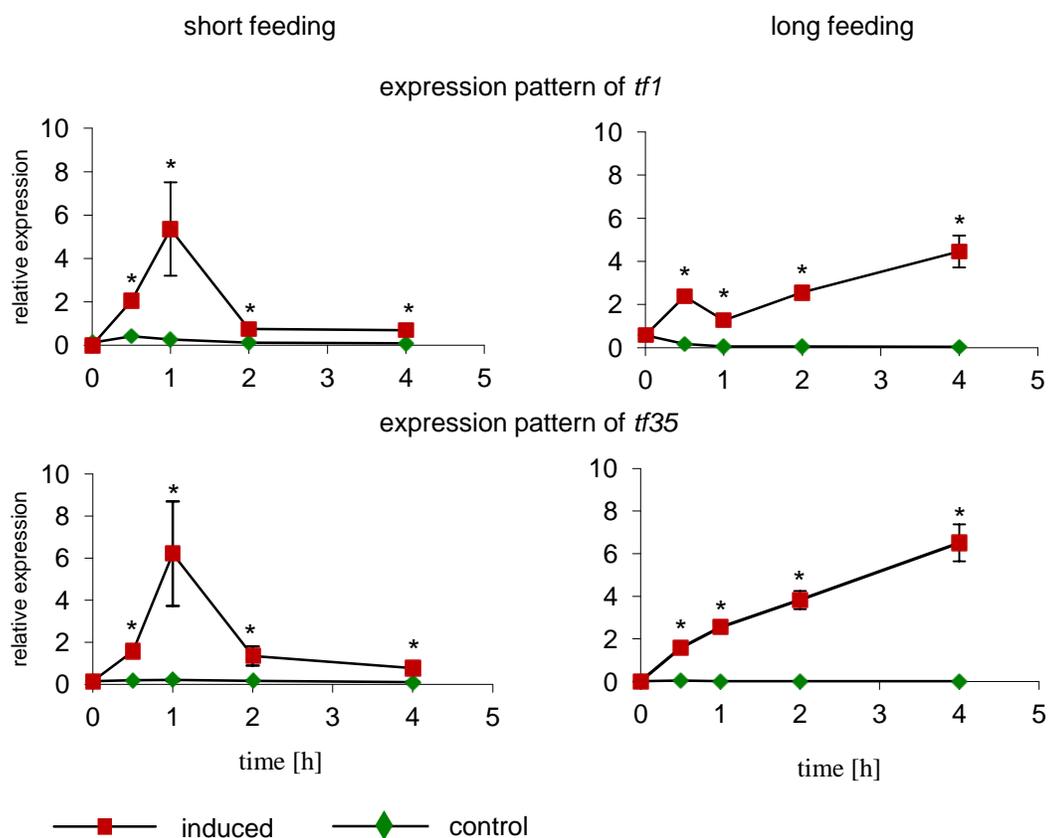


Fig.3.8: Relative expression pattern of *tf1* and *tf35* after short and long term feeding. Delprim maize leaves were treated either for half an hour with the caterpillar larvae *S. littoralis* (short feeding) or continuous feeding until plant material was harvested (long feeding). The expression patterns are shown over a 4 h interval. Induced expression pattern is marked with a red square, control points with a green diamond. Expression was calculated against the housekeeping genes. Standard error (n=4 for short term feeding, n=3 for long term feeding) is shown for each measurement. Two-Way ANOVA was used to test significant differences with $p < 0.05$ defined as significant for the treatment within the time points (*).

3.2.3 The transcript patterns of transcription factors are similar in Delprim and B73 despite differences in volatile production

While most European maize lines have the ability to produce (*E*)- β -caryophyllene after root damage, most American maize lines have lost the ability to do so (Köllner *et al.* 2008).

In our study, we used the maize variety Delprim as an example for an European, (*E*)- β -caryophyllene-producing maize line and B73 as an American, non-producing maize line. Both maize lines are able to produce the sesquiterpenes (*E*)- β -farnesene and (*E*)- α -bergamotene which are formed by the sesquiterpene synthase TPS10 (Bohlmann *et al.* 1998; Schnee *et al.* 2006). (*E*)- β -caryophyllene is produced by the terpene synthase TPS23 (Köllner *et al.*, 2008). Although the maize line B73 contains a functional *tps23* structural gene, the gene is not transcribed in B73. This suggests that some components in the signaling cascade for the expression of *tps23* are missing in B73.

To study the molecular base of *tps23* regulation, the transcript accumulation of nine of the herbivore-induced transcription factors was tested in both maize lines using QRT-PCR. The plants were induced using 3rd instar larvae. The results are shown in Fig.3.9 with the stacked columns of the control and the induced expression levels for each plant cultivar.

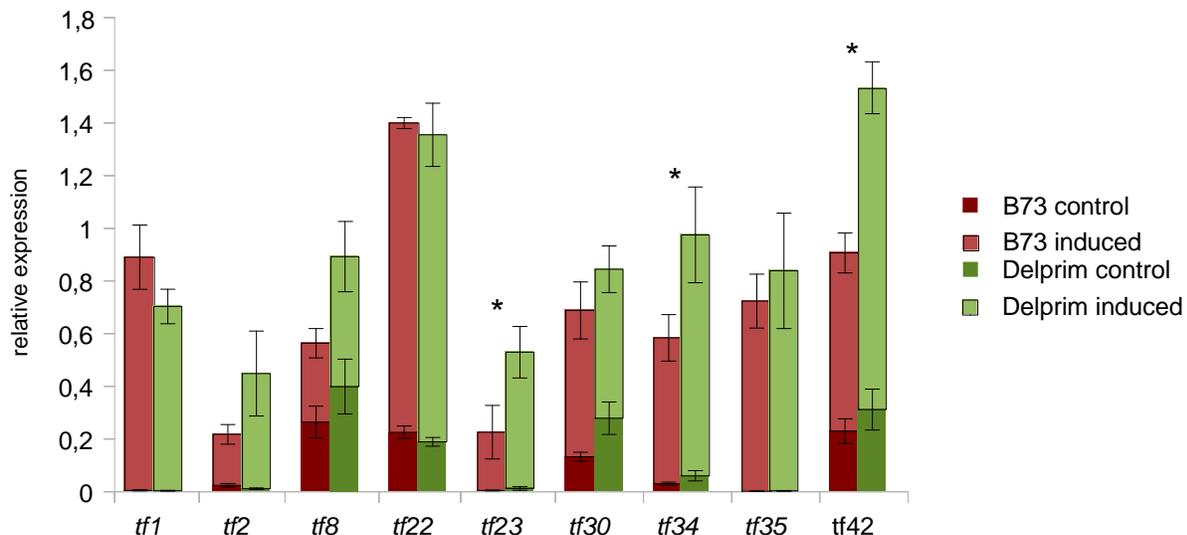


Fig.3.9: Relative expression levels of transcription factors after herbivore damage in the maize varieties B73 and Delprim. Four biological replicates were used for each control and induced sample. Plants were treated in the afternoon with the caterpillar larvae and harvested the next morning. The relative expression level was calculated against the two housekeeping genes as described in 2.5.9. Means and standard error bars are shown. Two-Way ANOVA was used to calculate statistical significance between B73 and Delprim. P-values <0.05 are highlighted with (*).

The transcript levels of the transcription factors were always induced after herbivory. Only transcription factors *tf23*, *tf34*, and *tf42* showed a significant difference between the two maize cultivars, but were still expressed in the B73 cultivar after herbivory. Therefore, none of the factors seems to be directly associated with the loss of the (*E*)- β -caryophyllene signal in B73.

3.2.4 The application of jasmonic acid causes transcriptional changes

JA is one of the major plant hormones formed in response to many abiotic and biotic stresses (Turner *et al.* 2002). To test whether the herbivore-regulated transcription factors are located upstream or downstream of JA in the signal transduction cascade, we measured the effect of JA on those transcription factors. Plants were induced with jasmonic acid by cutting young maize plants and transferring them into glass vials containing either water or a 250 μ mol jasmonic acid solution. Since cutting of maize plants also induces low levels of JA formation (Engelberth *et al.* 2004), a second control group with undamaged plants was added. In these experiments, transcript levels were only studied after 0.5 h and 1 h induction. This is the time window in which JA is known to influence the signaling cascade (Mandaokar *et al.* 2006) that leads to the production of volatile terpenes.

Plant material was harvested and QRT analysis was performed. In addition to the transcription factors, the expression profile of *tps23* was measured to determine the effect on the TPS target gene as well. The JA-induced expression profile of four transcription factors (*tf1*, *tf2*, *tf23*, and *tf30*) and *tps23* are shown in Fig.3.10. The relative expression was calculated against the housekeeping gene HG2 (Phillips *et al.* 2009). After jasmonic acid treatment, cDNA accumulation of the four transcription factors as well as *tps23* was found. Surprisingly, the transcript levels were similarly induced in mechanically treated plants where only water was added to the wounding site. This indicates that the cutting the maize plant already results in activation of JA-mediated plant defenses. The presence of jasmonic acid in the aqueous solution did not seem to have a strong impact on the expression kinetics of these transcription factors.

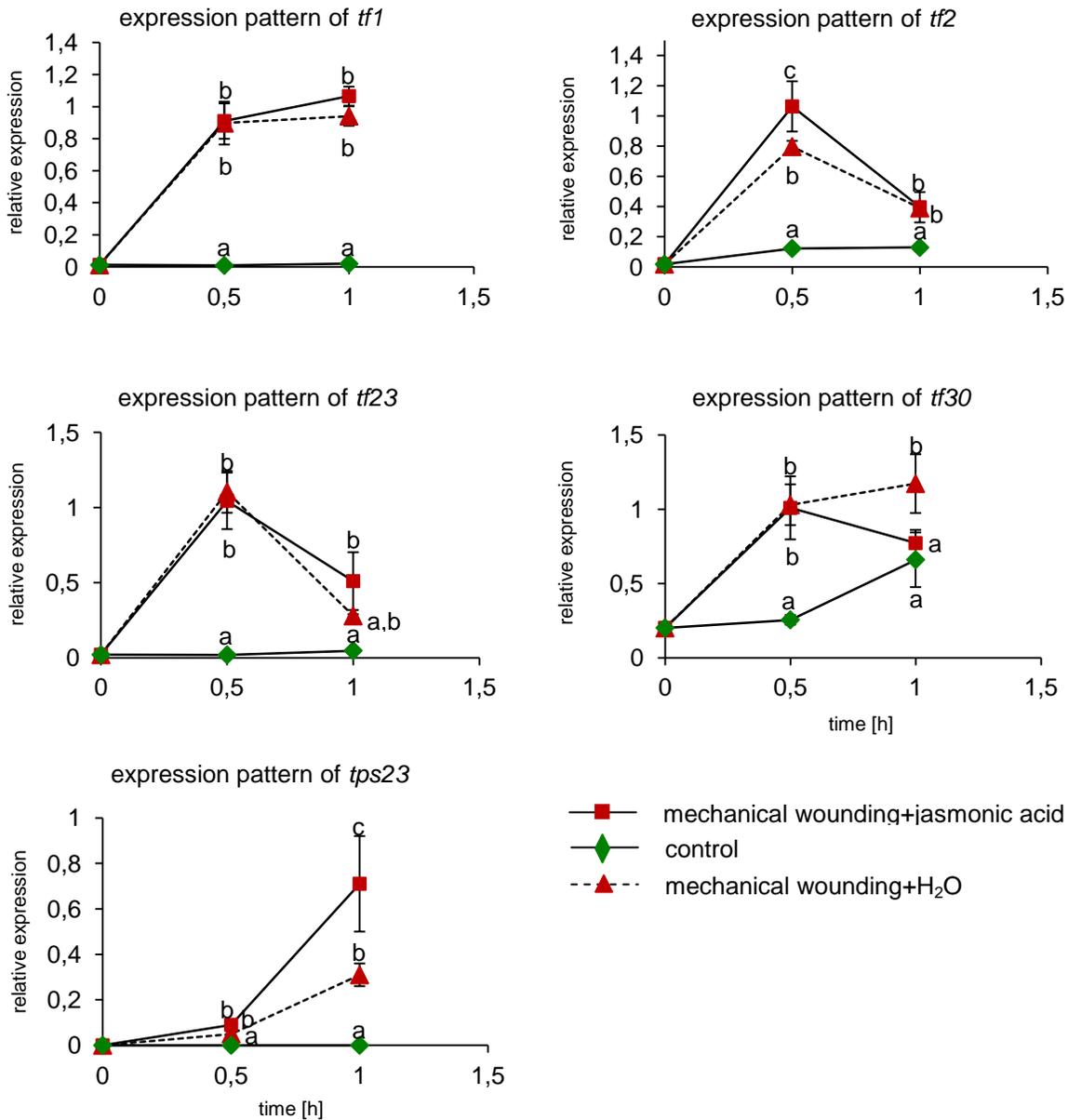


Fig.3.10: Expression profile of transcription factors *tf1*, *tf2*, *tf23*, *tf30*, and *tps23* after induction with jasmonic acid. Plants were cut and put into water or water containing jasmonic acid, to analyze early transcriptional changes of transcriptions factors during the first hour by QRT-PCR. The relative expression was calculated as the expression of the housekeeping gene HG2. Means and standard errors are shown (n=3). Two-Way ANOVA was performed with time as the first variable and treatment as second factor. Different letters indicate significant differences between treatments within time points (p<0.05).

In all cases, the transcript levels rose significantly after half an hour of induction. No increase was detected in the undamaged control plants. While *tf1* transcript levels did not change between 0.5 h and 1 h, transcript levels of *tf2* and *tf23* decreased somewhat after 1 h. For *tf23*, the treatment with jasmonic acid displayed a tendency to retain a higher expression level when compared to the mechanically wounded plants. In the case of *tf30* however, the cutting of the leaf alone seemed to have a higher impact on the expression level than JA treatment. After one hour, the relative expression of *tf30* decreased only when plants were treated with jasmonic acid. The behavior of all transcription factors was very specific upon mechanical wounding and only few transcription factors responded to the additional JA treatment (see appendix 7.3). The transcript levels of terpene synthase *tps23* responded much more slowly to the induction treatment than the transcription factors. Still, a significant amount of transcript was already visible after half an hour of induction and increased after one hour of induction. Only the expression level of *tps23* increased significantly in response to JA. Still, a conclusion cannot be drawn whether the identified transcription factors responded to internal or externally applied JA. To elucidate the specific function of transcription factors, further experiments have to be conducted.

3.3 Plant responses to mechanical damage – The Mecworm

The induction experiments with jasmonic acid (3.2.4) indicated the importance of leaf mechanical damage in the regulation of gene expression in maize. In lima bean, mechanical damage alone was shown to induce volatile production (Mithöfer *et al.* 2005). Since there is no comparable study in maize, an experiment was set up to differentiate between the effects of mechanical treatment, mechanical treatment plus application of caterpillar regurgitate, and completely unharmed plants. The time course was similar to that of the microarray hybridization experiment to obtain comparable results.

Mechanical treatment was performed with the Mecworm, an instrument which imitates caterpillar feeding by damaging the plant surface with a metallic pin. In addition to the mechanical damage, plants were also treated with 10 μ l caterpillar regurgitate per leaf to imitate both induction mechanisms of herbivore feeding. Plant material was analyzed for

transcript accumulation of the transcription factors, the production of volatiles and the induction of several plant hormones.

3.3.1 The influence of mechanical damage on the transcript accumulation of transcription factors

The expression patterns of the transcription factors were tested after the Mecworm treatment and compared to those of undamaged controls. Relative expression was calculated as the expression of the housekeeping genes HG1 and HG2 (Tab.2.4) to exclude changes in the expression patterns due to diurnal rhythms.

Most of the transcription factors showed similar expression levels after mechanical damage with and without regurgitate (Fig.3.11). The transcript levels rose after the induction stimulus within the first hour and then decreased to the control level. The expression patterns of *tf1* and *tf2* demonstrated a very short response to the induction. Also *tf34*, *tf42* and *tf46* showed a similar expression pattern (figures in the appendix 7.4). Overall, the mechanical wounding seemed to induce the expression of the transcription factors by itself.

Tf23 and *tf30* were the only factors that displayed a difference between mechanical damage and the treatment with caterpillar regurgitate. The transcript level of *tf23* was highly induced after application of oral secretion, while the transcript accumulation of *tf30* showed a higher induction after mechanical wounding alone. In both cases, expression levels of the two induction types converged after 2 h and showed an expression pattern similar to most of the other transcription factors. A factor in the oral secretion of caterpillars must be responsible for the differential expression of those transcription factors.

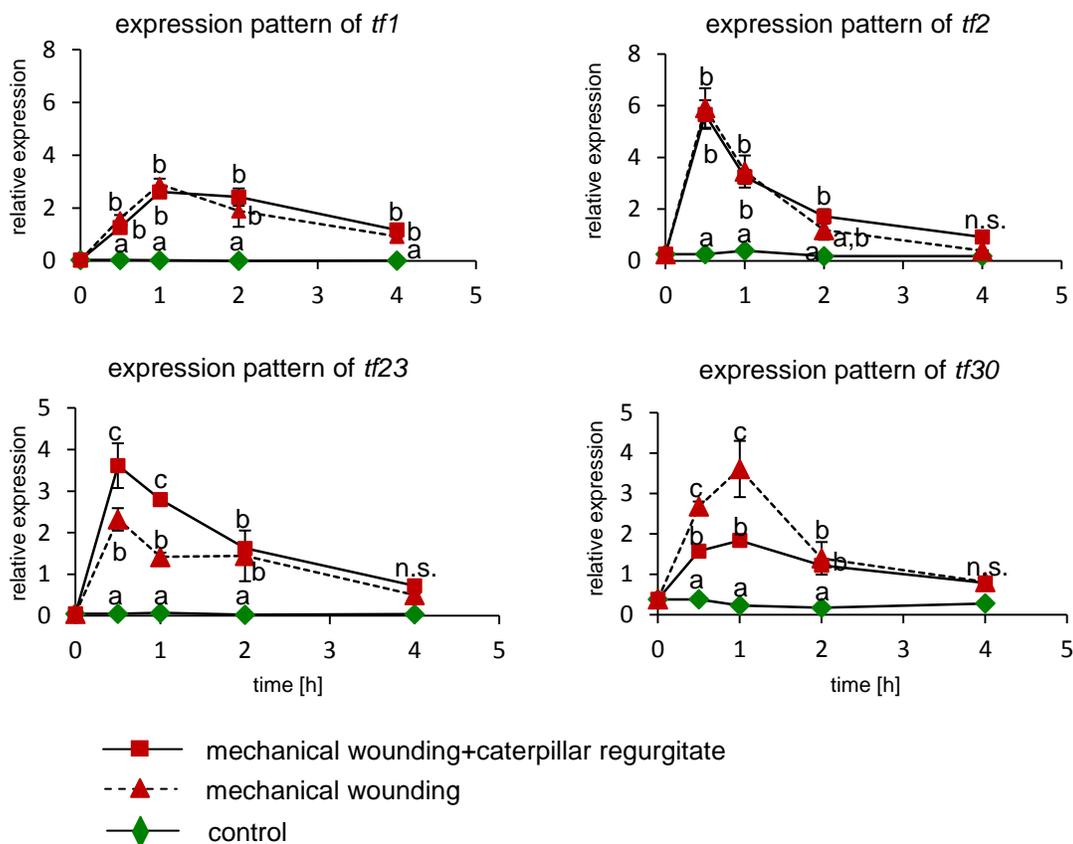


Fig.3.11: QRT-PCR analysis of transcription factors *tf1*, *tf2*, *tf23*, and *tf30* after mechanical induction with and without caterpillar regurgitate. Plants were treated with mechanical damage and mechanical damage plus the application of caterpillar regurgitate and harvested after 0 h, 0.5 h, 1 h, 2 h, and 4 h. Means and standard errors are shown (n=4). The relative expression was calculated as described in 2.5.9. Two-Way ANOVA was performed with time as the first variable and different induction types as second factor. Different letters indicate significant differences between treatments within time points (p<0.05).

3.3.2 The type of damage affects the amount of terpene production

Next, it was studied whether the constant mechanical damage of the caterpillar, simulated by the Mecworm, is sufficient not only for transcription factor induction but also for volatile terpene emission. To confirm the induction of plant material, the transcript level of *tps23* was measured. The expression profile showed a significant increase of cDNA for the time points 1 and 2 hours after the induction and accumulated consistently up to 4 hours after induction (Fig.3.12). Interestingly, there is no difference in the amount of *tps23* cDNA measured after

mechanical damage and mechanical damage + caterpillar regurgitate, matching the transcription profile of most transcription factors measured. Still, this result was in contrast to the transcript expression profile where plants had been treated with jasmonic acid (3.2.4). The differences could result from the different types of induction. While JA-treated plants were cut before the treatment, the Mecworm-experiment simulated a more natural herbivore attack.

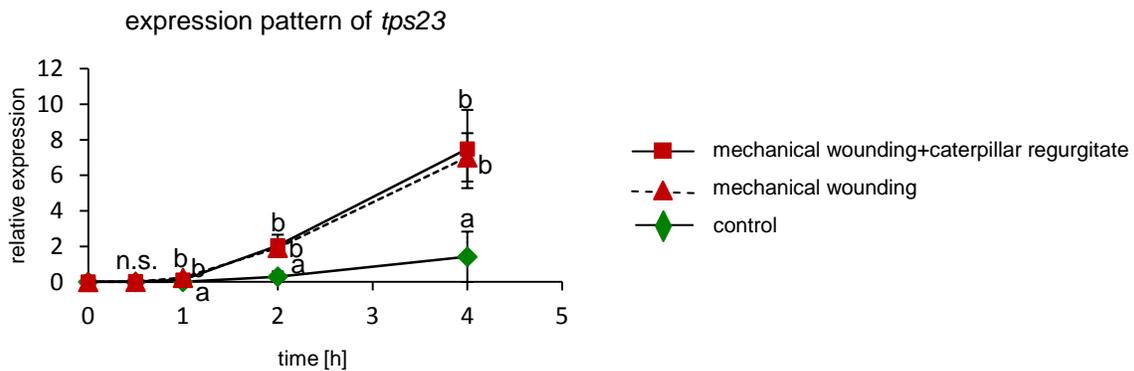


Fig.3.12: Expression pattern of *tps23* after mechanical damage, mechanical damage plus caterpillar regurgitate and in control plants. Leaves were treated with the Mecworm and plant material was harvested after the given time points. Means and standard errors are shown (n=4). Two-way ANOVA was performed with time as the first variable and treatment types as second factor. Different letters indicate significant differences between treatments within time points ($p < 0.05$).

The same plant material was also analyzed for terpene volatiles by a GC-MS (Fig.3.13). The plant material was incubated with a SPME fiber which has the advantage of a high sensitivity compared to hexane extracts.

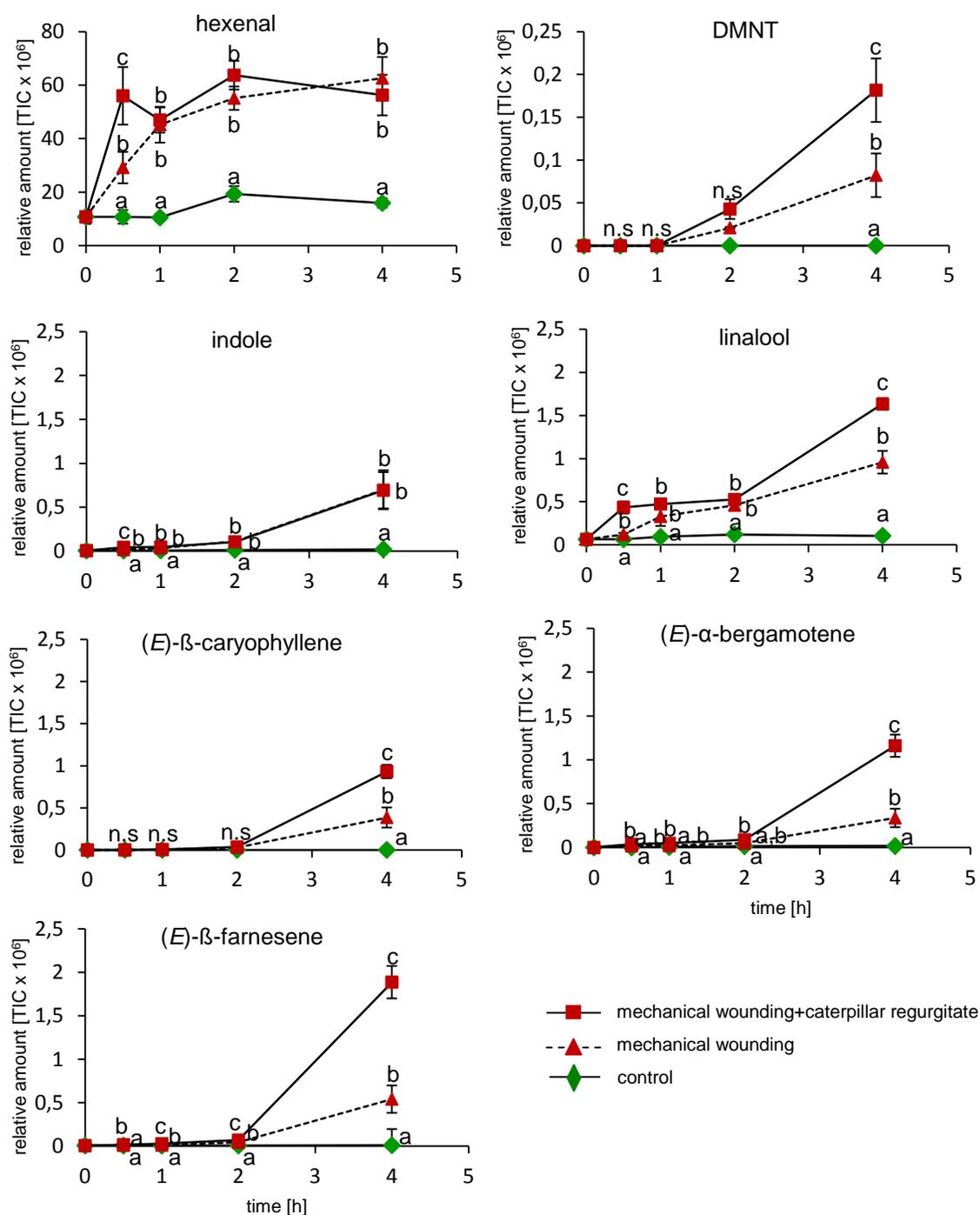


Fig.3.13: Relative amount of volatile emission after treatment of seedlings with Mecworm and regurgitate. Leaves of maize plants var. Delprim were treated with mechanical damage (Mecworm), mechanical damage+caterpillar regurgitate and control, respectively, and analyzed after 0 h , 0.5 h , 1 h , 2 h, and 4 h. Plant material was ground and 200 mg were measured for volatile production using SPME. Means (n=4) and standard error is shown. Two-Way ANOVA was performed with time as the first variable and treatment types as second factor. Different letters indicate significant differences between treatments within time points (p<0.05).

Looking at the volatile sesquiterpene (*E*)- β -caryophyllene, the induction profile showed a picture different from the *tps23* transcript levels. While the transcript levels were detectable after 0.5 h, the corresponding volatile of *tps23*, (*E*)- β -caryophyllene, was observed after 2 h. The amount of (*E*)- β -caryophyllene increased between 2 h and 4 h after induction. At 4 h, plants treated with mechanical damage emitted only ~50 % (*E*)- β -caryophyllene compared to plants treated with caterpillar regurgitate.

The sesquiterpenes (*E*)- α -bergamotene and (*E*)- β -farnesene illustrated a similar behavior. In both cases, the terpene production was induced at 4 h but more volatiles were emitted after application of regurgitate than mechanical damage alone. The same pattern was observed for the homoterpene DMNT. The monoterpene linalool was also emitted in a significant higher amount in plants treated with caterpillar regurgitate, but could already be measured after half an hour of induction. The leaf aldehyde hexenal showed the highest production, of all VOCs measured, but with only small differences between the two treatments. This profile matched that of the heterocyclic compound indole which was released after 1 h of induction independently from the induction type. This indicated that mechanical wounding alone was sufficient to induce the production of these volatile compounds and was not elevated by the application of caterpillar regurgitate. This is in contrast to the other volatiles where extra treatment with regurgitate “boosted” the production of those volatiles.

3.3.3 The induction of plant hormones is dependent on the type of induction

Plant hormones play an important role in the regulation of plant growth, development, and in the response to biotic stresses (Davies 2010). In order to investigate the effect of herbivory on the induction of the plant hormones ABA, JA, JA-Ile, and SA, the hormone concentrations were measured after mechanical damage and application of caterpillar regurgitate. Previous unpublished experiments by Tobias Köllner showed that JA, JA-Ile and ABA were induced around and above the wounding site when caterpillars were able to feed on maize plants (Tobias Köllner, paper in progress). SA, on the other hand, showed increased levels towards the base of the injured leaf and was repressed in the tip of the leaf.

In the following experiments, the Mecworm was used as a method to mimic herbivore damage. The wounded area was restricted to the base of the leaves and the whole leaf was collected for analysis. The hormone analysis of the leaf is shown in Fig.3.14.

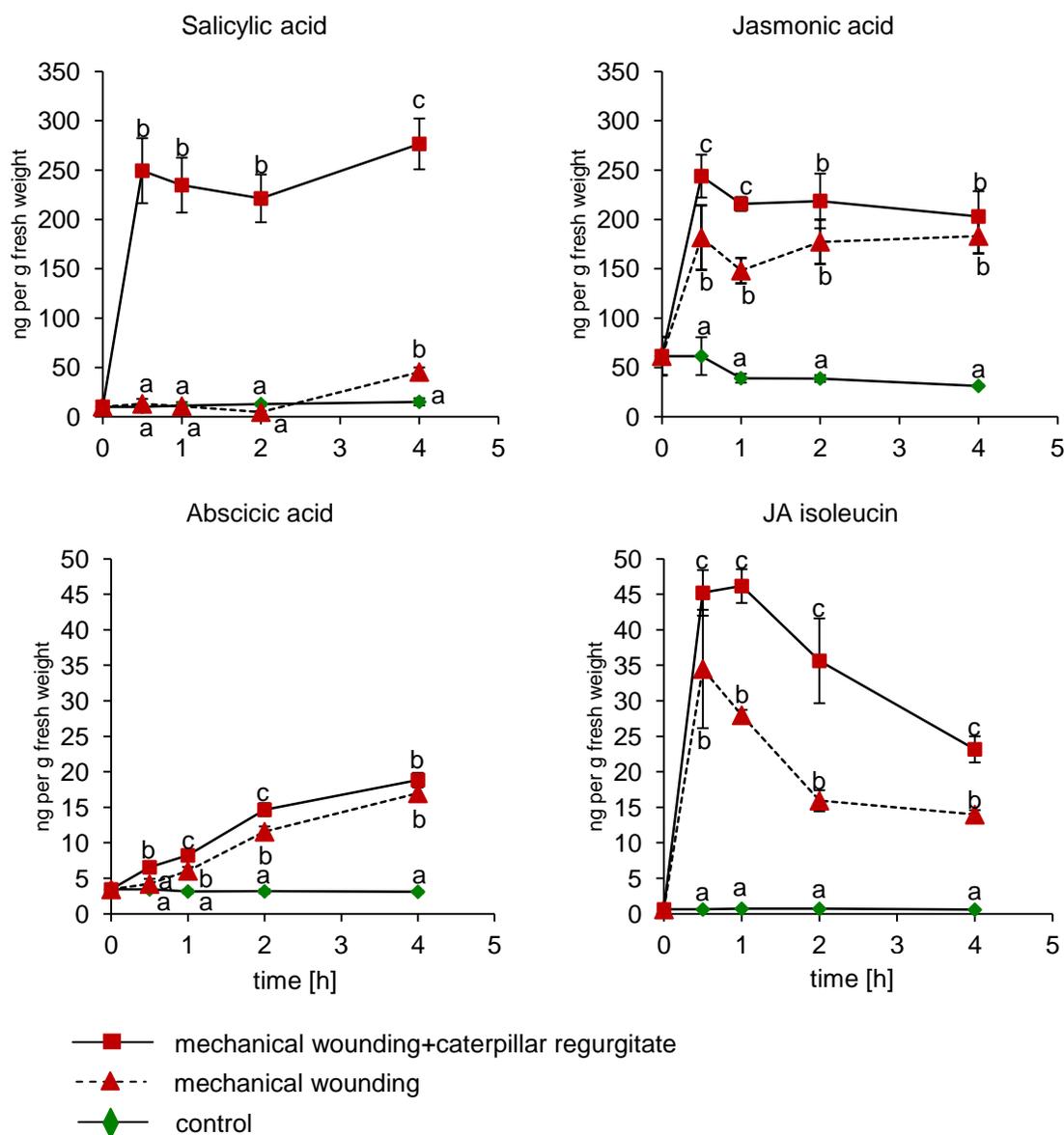


Fig.3.14: Phytohormone concentration in damaged maize leaves. The concentration of phytohormones were measured in ng per g fresh weight after mechanical damage, damage and caterpillar regurgitate and the controls. Leaf material was harvested at 0 h, 0.5 h, 1 h, 2 h, and 4 h after the treatment. Means and standard errors are shown (n=4). Two-way ANOVA was performed with time as the first variable and treatment as second factor. Different letters significant differences between treatments within time points ($p < 0.05$).

The levels of JA as well as JA-Ile increased significantly after the treatment. While the amount of JA rose to 250 ng per g fresh weight, only up to 50 ng per g fresh weight of the JA-Ile conjugate was found in leaves. Both plant hormones showed a similar pattern for the two treatments. The addition of regurgitate to the damage seemed to enhance the production of JA and JA-Ile. The increase of JA and JA-Ile after mechanical damage suggested that the damaged leaf was sufficient to induce the hormone levels.

Upon wounding, plants contained up to 20 ng per g fresh weight of ABA. The concentration of ABA is not further increased after application of regurgitate. In contrast to the hormones JA and JA-Ile, it is worth to notice that the concentration of ABA rose constitutively after wounding while the level of JA and JA-Ile already reached their maximum level after 0.5 h and then started to decline.

For JA, JA-Ile and ABA, the pattern of hormone induction was similar with the addition of regurgitate, yet the differences in the concentrations for each time point was mostly significant ($p < 0.05$). In the case of SA, the hormone level after mechanical wounding was close to the hormone level of the control plants with only a slight increase at the 4 h time point. In contrast, the hormone concentration increased significantly to 300 ng per g fresh weight when the plants were wounded and regurgitate was applied. Like jasmonic acid, SA concentrations stayed elevated upon treatment with caterpillar regurgitate. SA is a signaling molecule commonly associated with pathogen attack and is often down-regulated upon herbivory in *Arabidopsis thaliana* (Lee et al. 2010). In maize, SA also seemed to play a role in the defense against herbivores and it is selectively induced by caterpillar regurgitate.

3.4 Promoter analysis of the terpene synthases *tps10* and *tps23*

3.4.1 Promoters of *tps10* and *tps23* are dissimilar among maize lines

The promoters of *tps10* and *tps23* of several maize lines were sequenced and analyzed for putative regulatory sequences. Promoter sequences were obtained by PCR or with the help of the Institute of Genomic Diversity from Cornell University. The maize lines selected for sequence analysis differed in (*E*)- β -caryophyllene production. The lines Delprim, NC358, Du101, and F476 possess an active TPS23 enzyme and produced herbivore-induced (*E*)- β -

caryophyllene. In the lines B73, CML247, OH7B, and MS71, on the other hand, transcript of *tps23* could not be detected (Degen *et al.* 2004; Köllner *et al.* 2008). Sequence analysis of ~800 bp revealed only minor nucleotide exchanges among the *tps23* promoter of all lines (Fig.3.15).

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-766                                     -697
Dul01  CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTGGTGT AGCAGCTTTG TTTGGTCTGA TTTGGCTTTG
NC358  CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTGGTGT AGCAGCTTTG TTTGGTCTGA TTTGGCTTTG
F476   CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTAGTGT TGGCGCTTTG TTTGGTCTGA TTTGGCTTTG
OH7B   CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTAGTGT TGGCGCTTTG TTTGGTCTGA TTTGGCTTTG
Delprim CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTAGTGT TGGCGCTTTG TTTGGTCTGA TTTGGCTTTG
B73    CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTAGTGT TGGCGCTTTG TTTGGTCTGA TTTGGCTTTG
MS71   CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTAGTGT TGGCGCTTTG TTTGGTCTGA TTTGGCTTTG
CML247 CAAAGGTTGT ATGCACAAA AGGCTGCTTC TTTCTAGTGT TGGCGCTTTG TTTGGTCTGA TTTGGCTTTG

-696                                     -627
Dul01  CCGTAATGAT GTGGTCTTTA ATCATAACCA TATATCGTCA ATTATACACG TGATCTTTAG AGATACATAT
NC358  CCGTAATGAT GTGGTCTTTA ATCATAACCA TATATCGTCA ATTATACACG TGATCTTTAG AGATACATAT
F476   CCGTAATGAT GTGGTCTTTA ATCATAACCA TATACCGTCA ATTATACATG TGATCTTTAG AGGTACATAT
OH7B   CCGTAATGAT GTGGTCTTTA ATCATAACCA TATACCGTCA ATTATACATG TGATCTTTAG AGGTACATAT
Delprim CCGTAATGAT GTGGTCTTTA ATCATAACCA TATACCGTCA ATTATACATG TGATCTTTAG AGGTACATAT
B73    CCGTAATGAT GTGGTCTTTA ATCATAACCA TATACCGTCA ATTATACATG TGATCTTTAG AGGTACATAT
MS71   CCGTAATGAT GTGGTCTTTA ATCATAACCA TATACCGTCA ATTATACATG TGATCTTTAG AGGTACATAT
CML247 CCGTAATGAT GTGGTCTTTA ATCATAACCA TATACCGTCA ATTATACATG TGATCTTTAG AGGTACATAT

-626                                     -557
Dul01  TGGTTTCAGAT TTTGGGGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
NC358  TGGTTTCAGAT TTTGGGGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
F476   TGTTTCAGAT TTTAGAGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
OH7B   TGTTTCAGAT TTTAGAGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
Delprim TGTTTCAGAT TTTAGAGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
B73    TGTTTCAGAT TTTAGAGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
MS71   TGTTTCAGAT TTTAGAGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT
CML247 TGTTTCAGAT TTTAGAGACT CCTGCAGAA G AAGAAAACAT AACAGCAAAT CTTAGTCGTG TATCAGACTT

-556                                     -487
Dul01  TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
NC358  TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
F476   TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
OH7B   TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
Delprim TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
B73    TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
MS71   TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA
CML247 TGGAGGTTAT GGCCATGGAG TTCTTCACGA GTCACGGGTG CCGGTCTAAT GCAAGGATCG ATGCGGTTTA

-486                                     -417
Dul01  GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
NC358  GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
F476   GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
OH7B   GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
Delprim GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
B73    GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
MS71   GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG
CML247 GTGTTTATGA TCTTTTATTC CATATCATGT TTTAAGTTGA AGTTTGTTG ATCCATGAAT TCTTGGGATG

-416                                     -347
Dul01  ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
NC358  ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
F476   ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
OH7B   ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
Delprim ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
B73    ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
MS71   ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA
CML247 ATGTAAGATT TGAGGTGTAT GGTGTATTC ATGAAAATGC AAAAGTTCGG AACGATATTT CCATTTCAAA

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DNA was not successful (data provided by Jeff Glaubitz and Edward Buckler). Unfortunately, data from that database did not cover promoter fragments long enough for useful comparisons (Fig.3.16).

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-1491
B73 GGCAAAGAGC TCCTTTAC TA AAT TTTTAT TTTAC CGAGG GTTTTTTT T GCTTAGCAC TCGGCAAGGA
OH7B GGCAAAGAGC TCCTTTAC TA AAT TTTTAT TTTAC CGAGG GTTTTTTT -- GCTTAGCAC TCGGCAAGGA
MS71                                     CCG CCCCCAAGAA
Delprim GGCAAAGAGC TCCTTTAC TA AAT TTTTAT TTTAC GAGG GTTTTTTT -- GCTTAGCAC TCGGCAAGGA
NC358
CML247

-1421
B73 GTTTATTTC CGAGTGTCCG AAAAAACAC TCGGCAAAAC ATTGGCACT CGGCAAGAG CCGAATCCG
OH7B GTTTATTTC CGAGTGTCCG AAAAAACAC TCGGCAAAAC ATTGGCACT CGGCAAGAG CCGAATCCG
MS71 GTTT--TTAC CGAGTGTCCG AAAAAACAC TCGGCAAAAC AATTCGT--- --GTGAAGTT TTCAAATTTA
Delprim GTTTATTTC CGAGTGTTCG AAAAAACAC TCGGCAAAAC GC----- ---CAAATC CGGTAATGCG
NC358
CML247

-1351
B73 GTAATGTTTA GTATTAATAT TAACATTTAG GAATCTTCGT ATTGAATATT TTAGCTCCC CAAGATCTCA
OH7B GTAATGTTTA GTATTAATAT TAACATTTAG GAATCTTCGT ATTGAATATT TTAGCTCCC --AAGATCTCA
MS71 GTAATGTTTA GTATTAATAT TAACATTTAG GAATCTTCGT ATTGAATATT TTAGCTCCC CAAGATCTCA
Delprim GTAATGTTTA GTATTAATAT TAACATTTAG GAATCTTCGT ATTGAATATT TTAGCTCCC CAAGATCTCA
NC358
CML247

-1281
B73 AATGAAACT ATTTTAACTA TAGAGTTTTA GATCGATCTT ATCAAAGCAC TACATTTTTT GACATAAGTA
OH7B AATGAAACT ATTTTAACTA TAGAGTTTTA GATCGATCTT ATCAAAGCAC TACATTTTTT GACATAAGTA
MS71 AATGAAACT ATTTTAACTA TAGAGTTTTA GATCGATCTT ATCAAAGCAC TACATTTTTT GACATAAGTA
Delprim AATGAAACT ATTTTAACTA TAGAGTTTTA GATCGATCTT ATCAAAGCAC TACATTTTTT GACATAAGTA
NC358                                     TTT GACATAAGTA
CML247                                     TCTT ATCAAAGCAC TACATTTTTT GACATAAGTA

-1211
B73 TGTCTCATCC AAGATAATTT TAAATTTTGA GTATCAAAAT CTGTTAATT TCATAATTAA TATTTAGGAC
OH7B TGTCTCATCC AAGATAATTT TAAATTTTGA GTATCAAAAT CTGTTAATT TCATAATTAA TATTTAGGAC
MS71 TGTCTCATCC AAGATAATTT TAAATTTTGA ATATCAAAAT CTGTTAATT TCATAATTAA TATTTAGGAC
Delprim TGTCTCATCC AAGATAATTT TAAATTTTGA GTATCAAAAT CTGTTAATT TCATAATTAA TATTTAGGAC
NC358 TGTCTCATCC AAGATAATTT TAAATTTTGA GTATCAAAAT CTGTTAATT TCATAATTAA TATTTAGGAC
ML247 TGTCTCATCC AAGATAATTT TAAATTTTGA GTATCAAAAT CTGTTAATT TCATAATTAA TATTTAGGAC

-1141
B73 TAAATGATTT CAGATGAAA GCTTACCAAT TATAATGCGA TAAATCACGT TGAATACCAC AACCTTAATA
OH7B TAAATGATTT CAGATGAAA GCTTACCAAT TATAATGCGA TAAATCACGT TGAATACCAC AACCTTAATA
MS71 TAAATGATTT CAGATGAAA GCTTACCAAT TATAATGCGA TAAATCACGT TGAATACCAC AACCTTAATA
Delprim TAAATGATTT CAGATGAAA GCTTACCAAT TATAATGCGA TAAATCACGT TGAATACCAC AACCTTAATA
NC358 TAAATGATTT CAGATGAAA GCTTACCAAT TATAATGCGA TAAATCACGT TGAATACCAC AACCTTAATA
CML247 TAAATGATTT CAGATGAAA GCTTACCAAT TATAATGCGA TAAATCACGT TGAATACCAC AACCTTAATA

-1071
B73 TACACCGCGT TAGCATCTGG GCATTAACCG ATCAGACCAT TTGAAGGTGG TGGCCAGGCT GCCTCTAAAA
OH7B TACACCGCGT TAGCATCTGG GCATTAACCG ATCAGACCAT TTGAAGGTGG TGGCCAGGCT GCCTCTAAAA
MS71 TACACCGCGT TAGCATCTGG GCATTAACCG ATCAGACCAT TTGAAGGTGG TGGCCAGGCT GCCTCTAAAA
Delprim TACACCGCGT TAGCATCTGG GCATTAACCG ATCAGACCAT TTGAAGGTGG TGGCCAGGCT GCCTCTAAAA
NC358 TACACCGCGT TAGCATCTGG GCATTAACCG ATCAGACCAT TTGAAGGTGG TGGCCAGGCT GCCTCTAAAA
CML247 TACACCGCGT TAGCATCTGG GCATTAACCG ATCAGACCAT TTGAAGGTGG TGGCCAGGCT GCCTCTAAAA

-1001
B73 ATGAGTTTTG TAATAGTAGA TTAGTAGCGT AAGGAACCCT AAACCATATA TATATAFATA TATATATATA
OH7B ATGAGTTTTG TAATAGTAGA TTAGTAGCGT AAGGAACCCT AAACCATATA TATATAFATA TA-----
MS71 ATGAGTTTTG TAATAGTAGA TTAGTAGCGT AAGGAACCCT AAACCATATA TATATAFATA TATATATATA
Delprim ATGAGTTTTG TAATAGTAGA TTAGTAGCGT AAGG----- ---CGTTTG TATATAFATA -----
NC358 ATGAGTTTTG TAATAGTAGA TTAGTAGCGT AAGGAACCCT AAACCATATA TATATAFATA TATATATATA
CML247 ATGAGTTTTG TAATAGTAGA TTAGTAGCGT AAGGAACCCT AAACCATATA TATATAFATA -----

-931
-862

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B73	TATATATATA	TATATATATA	TATATATATA	TATAGTGAAC	AAATTAGTAT	GGAATAGAAA	AAAAATCAAG
OH7B	-----	-----	-----	---GTGAAC	AAATTAGTAT	GGAATAGAAA	AAAA-TCAAG
MS71	TATATATATA	TATATATATA	TATATATATA	TATAGTGAAC	AAATTAGTAT	GGAATAGAAA	AAAAATCAAG
Delprim	-----	-----	-----	---GTGAAC	AAATTAGTAT	GGAATAGAAA	AAAAATCAAG
NC358	TATATATATA	TATATATATA	TATATATATA	TATAGTGAAC	AAATTAGTAT	GGAATAGAAA	AAAAATCAAG
CML247	-----	-----	-----	---GTGAAC	AAATTAGTAT	GGAATAGAAA	AAAAATCAAG
-861							
B73	ACAAAACGAT	CAATATAGAT	CACGTTGAAT	ACCACAACCT	TAATTAATAT	ACACCGCGTT	AGCATCTGGG
OH7B	ACAAAACGAT	CAATATAGAT	CACGTTGAAT	ACCACAACCT	TAATTAATAT	ACACCTGT	AGCATCTGGG
MS71	ACAAAACGAT	CAATATAGAT	CACGTTGAAT	ACCACAACCT	TAATTAATAT	ACACCGCGTT	AGCATCTGGG
Delprim	ACAAAACGAT	CA-----	-----	-----	-----	-----	-----
NC358	ACAAAACGAT	CA-TATAGAT	CACGTTGAAT	ACCACAACCT	TAATTAATAT	ACACCTGT	AGCATCTGGG
CML247	ACAAAACGAT	CAATATAGAT	CACGTTGAAT	ACCACAACCT	TAATTAATAT	ACACCTGT	AGCATCTGGG
-791							
B73	CATTAACCGA	TCAGACCATT	TGAGGAAGGT	GGCCAGGCTG	CCTCTAAAAA	TGAGTTTGT	AATAGTAGAT
OH7B	CATTAACCGA	TCAGACCATT	TGAGGACGGT	AGCC-----	--TCTAAAAA	TGAGTTTGT	AATAGTAGAT
MS71	CATTAACCGA	TCAGACCATT	TGAGGAAGGT	GGCCAGGCTG	CCTCTAAAAA	TGAGTTTGT	AATAGTAGAT
Delprim	-----	-CATACC	-----	-----	-----	-----	-----
NC358	CATTAACCGA	TCAGACCATT	TGAGGACGGT	AGCC-----	--TCTAAAAA	TGAGTTTGT	AATAGTAGAT
CML247	CATTAACCGA	TCAGACCATT	TGAGGACGGT	AGCC-----	--TCTAAAAA	TGAGTTTGT	AATAGTAGAT
-721							
B73	TAGTAGCGTA	AGGCGTTTGT	GTAATTAAT	TAGTTANA--	-----TA	TGAAGTAGAT	TCCTAAGAGA
OH7B	TAGTAGCGTA	AGGCGTTTGT	GTAATTAAT	TAGTTANA--	-----TA	TGAAGTAGAT	TCCTAAGAGA
MS71	TAGTAGCGTA	AGGCGTTTGT	GTAATTAAT	TAGTTANAAT	TAGTTAAA	TGAAGTAGAT	TCCTAAGAGA
Delprim	-----	-----	GTAATTAAT	TAGTTANA	-----TA	TGAAGTAGAT	TCCTAAGAGA
NC358	TAGTAGCGTA	AGGCGTTTGT	GTAATTAAT	TAGTTANA--	-----TA	TGAAGTAGAT	TCCTAAGAGA
CML247	TAGTAGCGTA	AGGCGTTTGT	GTAATTAAT	TAGTTANA--	-----TA	TGAAGTAGAT	TCCTAAGAGA
-651							
B73	TTCTAGGGCA	CAACTGT--C	TCTCTGTAGC	AAAAACTAGT	TTAATCAGTA	GGCATGATGT	CATATCCCA
OH7B	TTCTAGGGCA	CAACTGT--C	TCTCTGTAGC	AAAAACTAGT	TTAATCAGTA	GGCATGATGT	CATATCCCA
MS71	TTCTAGGGCA	CAACTGT--C	TCTCTGTAGC	AAAAACTAGT	TTAATCAGTA	GGCATGATGT	CATATCCCA
Delprim	TTCTAGGGCA	CAACTGT--C	TCTCTGTAGC	AAAAACTAGT	TTAATCAGTA	GGCATGATGT	CATATCCCA
NC358	TTCTAGGGCA	CAACTGT--C	TCTCTGTAGC	AAAAACTAGT	TTAATCAGTA	GGCATGATGT	CATATCCCA
CML247	TTCTAGGGCA	CAACTGT--C	TCTCTGTAGC	AAAAACTAGT	TTAATCAGTA	GGCATGATGT	CATATCCCA
-581							
B73	A-TGGTTGTT	CATTGCATAA	-TGTGACTCC	TATAACCAAC	TAGCTTGAGC	CTAAAGAAGC	TCACACACA-
OH7B	AATGGTTGTT	CATTGCATAA	ATGTGACTCC	TATAACCAAC	TAGCTTGAGC	CTAAAGAAGC	TCACACACA-
MS71	AATGGTTGTT	CATTGCATAA	-TGTGACTCC	TATAACCAAC	TAGCTTGAGC	CTAAAGAAGC	TCACACACA-
Delprim	A-TGGTTGTT	CATTGCATAA	-TGTGACTCC	TATAACCAAC	TAGCTTGAGC	CTAAAGAAGC	TCACACACA-
NC358	AATGGTTGTT	CATTGCATAA	-TGTGACTCC	TATAACCAAC	TAGCTTGAGC	CTAAAGAAGC	TCACACACA-
CML247	AATGGTTGTT	CATTGCATAA	ATGTGACTCC	TATAACCAAC	TAGCTTGAGC	CTAAAGA-GC	TCACACNCA-
-511							
B73	-GCAATCTAG	CTCAATTAGT	TGTACACATG	TGAGCATAACA	TCACGTGCAA	TACAATGGCA	GTTCTGATTT
OH7B	-GCAATCTAG	CTCAATTAGT	TGTACACATG	TGAGCATAACA	TCACGTGCAA	TACAATGGCA	GTTCTGATTT
MS71	-GCAATCTAG	CTCAATTAGT	TGTACACATG	TGAGCATAACA	TCACGTGCAA	TACAATGGCA	GTTCTGATTT
Delprim	AGCAATCTAG	CTCAATTAGT	TGTACACATG	TGAGCATAACA	TCACGTGCAA	TACAATGGCA	GTTCTGATTT
NC358	-GCAATCTAG	CTCAATTAGT	TGTACACATG	TGAGCATAACA	TCACGTGCAA	TACAATGGCA	GTTCTGATTT
CML247	-NCAATCTAG	CTCAATTAGT	TGTACACATG	TGAGCATAACA	TCACGTGCAA	TACAATGGCA	GTTCTGATTT
-441							
B73	AATTTGCAAC	TCCATTTTGT	CTCACAGGTA	GGATAAATAC	TGGTGCCAGC	CCATGCATCC	TTATTGTTCT
OH7B	AATTTGCAAC	TCCATTTTGT	CTCA--GATA	GGATAAATAC	TGGTGCCAGC	CCATGCATCC	TTATTGTTCT
MS71	AATTTGCAAC	TCCATTTTGT	CTCA--GATA	GGATAAATAC	TGGTGCCAGC	CCATGCATCC	TTATTGTTCT
Delprim	AATTTGCAAC	TCCATTTTGT	CTCA--GATA	GGATAAATAC	TGGTGCCAGC	CCATGCATCC	TTATTGTTCT
NC358	AATTTGCAAC	TCCATTTTGT	CTCACAGGTA	GGATAAATAC	TGGTGCCAGC	CCATGCATCC	TTATTGTTCT
CML247	AATTTGCAAC	TCCATTTTGT	CTCACAGGTA	GGATAAATAC	TGGTGCCAGC	CCATGCATCC	TTATTGTTCT
-371							
B73	TCAGTGCGAA	ACCAACCATA	TATATACGGA	GCGGAGCTTG	TGGCGGCAAT	TCTCCCGTCT	TGGACGGCCC
OH7B	TCAGTGCGAA	ACCAACCATA	TATATACGGA	GCGGAGCTTG	TGGCGGCAAT	TCTCCCGTCT	TGGACGGCCC
MS71	TCAGTGCGAA	A---ACCAT	---TAC---	---	---	---	---
Delprim	TCAGTGCGAA	A---CCAT	ACCAGACTTG	T---GAGCTTG	TGGCGGCAAT	TCTCCCGTCT	TGGACGGCCC
NC358	TCAGTGCGAA	ACCAACCATA	TATATACGGA	GCGGAGCTTG	TGGCGGCAAT	TCTCCCGTCT	TGGACGGCCC
CML247	TCAGTGCGAA	ACCAACCATA	TATATACGGA	GCGGAGCTTG	TGGCGGCAAT	TCTCCCGTCT	TGGACGGCCC
-302							

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-301                                     -232
B73 AACGTTTTGC ACGCAGACGT AATAGTTGAG AGGAAGTACC AGACTTGTGT ACATCAGCCG TCTCTATCTT
OH7B AACGTTTTGC ACGCAGAAGT AATAGTTGAG AGGAAGTACC AGACTTGTGT ACATCAGCCG TCTCTATCTT
MS71 ----- ---CAGACGT AATAGTTGAG AGGAAGTACC AGACTTGTGT ACATCAGCCG TCTCTATCTT
Delprim AACGTTTTGC ACGCAGACGT AATAGTTGAG AGGAAGTACC AGACTTGTGT ACATCAGCCG TCTCTATCTT
NC358 AACGTTTTGC ACGCAGACGT AATAGTTGAG AGGAAGTACC AGACTTGTGT ACATCAGCCG TCTCTATCTT
CML247 AACGTTTTGC ACGCAGAAGT AATAGTTGAG AGGAAGTACC AGACTTGTGT ACATCAGCCG TCTCTATCTT

-231                                     -162
B73 CTGCATGCAT CATGCATCAT TATTTGACT GCTGGTCTCT GCTGCTTCAT TGTGCACAGC GCCCTATAAG
OH7B CTGCATGCAT CATGCATCAT TATTTGACT GCTGGTCTCT GCTGCTTCAT TGTGCACAGC GCCCTATAAG
MS71 CTGCATGCAT CATGCATCAT TATTTGACT GCTGGTCTCT GCTGCTTCAT TGTGCACAGC GCCCTATAAG
Delprim CTGCATGCAT CATGCATCAT TATTTGACT GCTGGTCTCT GCTGCTTCAT TGTGCACAGC GCCCTATAAG
NC358 CTGCATGCAT CATGCATCAT TATTTGACT GCTGGTCTCT GCTGCTTCAT TGTGCACAGC GCCCTATAAG
CML247 CTGCATGCAT CATGCATCAT TATTTGACT GCTGGTCTCT GCTGCTTCAT TGTGCACAGC GCCCTATAAG

-161                                     -92
B73 TACCTGGCGC AATGCAACAC ACTGAAACAA TCAGAAGCTG TAGACGTACT ACCAAAGATC GATCTCAAAA
OH7B TACCTGGCGC AATGCAACAC ACTGAAACAA TCAGAAGCTG TAGACGTACT ACCAAAGATC GATCTCAAAA
MS71 TACCTGGCGC AATGCAACAC ACTGAAACAA TCAGAAGCTG TAGACGTACT ACCAAAGATC GATCTCAAAA
Delprim TACCTGGCGC AATGCAACAC ACTGAAACAA TCAGAAGCTG TAGACGTACT ACCAAAGATC GATCTCAAAA
NC358 TACCTGGCGC AATGCAACAC ACTGAAACAA TCAGAAGCTG TAGACGTACT ACCAAAGATC GATCTCAAAA
CML247 TACCTGGCGC AATGCAACAC ACTGAAACAA TCAGAAGCTG TAGACGTACT ACCAAAGATC GATCTCAAAA

-91                                     -22
B73 CAAAAGGGTA ATA----CCA TTACCAGCTA GAAGATGTTC AGG--GCTG CTGCAATTTG CTCCGTCAAC
OH7B CAAAAGGGTA ATA----CCA TTACCAGCTA GAAGATGTTC CTG CTGCAATTTG CTCCGTCAAC
MS71 CAAAAGGGTA ATA----CCA TTACCAGCTA GAAGATGTTC CTGACGACGC CTGCAATTTG CTCCGTCAAC
Delprim CAAAAGGGTA ATATATACCA TTACCAGCTA GAAGATGTTC CTGACGACGC CTGCAATTTG CTCCGTCAAC
NC358 CAAAAGGGTA ATA----CCA TTACCAGCTA GAAGATGTTC CTGACGACGC CTGCAATTTG CTCCGTCAAC
CML247 CAAAAGGGTA GAG----CG T--CSTG

-21                                     -1
B73 ATGTGCAGG GGCTGCTGCA AATG
OH7B ATGTGCAGG GGCTGCTGCA AATG
MS71 ATGTGCAGG GGCTGCTGCA AATG
Delprim ATGTGCAGG GGCTGCTGCA AATG
NC358 ATGTGCAGG GGCTGCTGCA AATG
CML247 ATGTGCAGG GGCTGCTGCA AATG

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Fig.3.16: Sequence comparison of the promoter fragment of *tps10* in different maize lines. Start codon is underlined. Differences in the sequences are marked in red.

Interestingly, far more nucleotide changes could be observed in the *tps10* promoter than in the promoter of *tps23*. While line MS71 and CML247 are not able to produce TPS10-specific sesquiterpenes, all other lines have an active TPS10 enzyme. Looking at the raw promoter sequence, an essential difference between TPS10-producing and non-producing cannot be predicted. In a next step, both promoter fragments from line Delprim were analyzed for regulatory sequences. Database analysis was conducted using either PLACE (plant *cis*-acting regulatory DNA elements) or PlantCARE (*cis*-acting regulatory element). 530 potential boxes were revealed in the promoter of *tps23* and 379 in the promoter of *tps10*. An overview of all identified regulatory elements is attached in the appendix (7.8, 7.9). Several core promoter elements were identified in both promoters as well as stress- and defense-related

cis-acting elements. Truncation experiments were conducted to isolate essential regulatory elements involved in the herbivore-induced responses (3.4.3).

3.4.2 Analysis of the promoters of *tps10* and *tps23* for binding activity to TF1, TF8, TF20 and TF22

To investigate whether the transcription factors TF1, TF8, TF20, and TF22 bind to the promoters of *tps10* and *tps23*, 1 kb sequence upstream of the start codon of both genes from the maize line Delprim was cut into ~200 bp fragments and used as targets for band shift assays. This technique serves to identify *cis*-acting promoter elements involved in the regulation of *tps10* or *tps23* after herbivore attack. Herbivore-regulated transcription factors with a transcript accumulation between 1 and 2 hours (Tab.3.1) were selected for analysis. The full length sequences for TF1, TF8, TF20, and TF22 were obtained by PCR or RACE and can be found in the appendix (7.5). The sequences were cloned into a pHis vector containing the codons for 8 histidine residues at the N-terminal end of the inserted sequence. The constructs were transformed into the *E. coli* strain BL21 which allowed the IPTG-induced overexpression of the transcription factor genes. The expressed and partially purified transcription factors were analyzed using SDS-PAGE and Western Blot with His-tag specific antibodies.

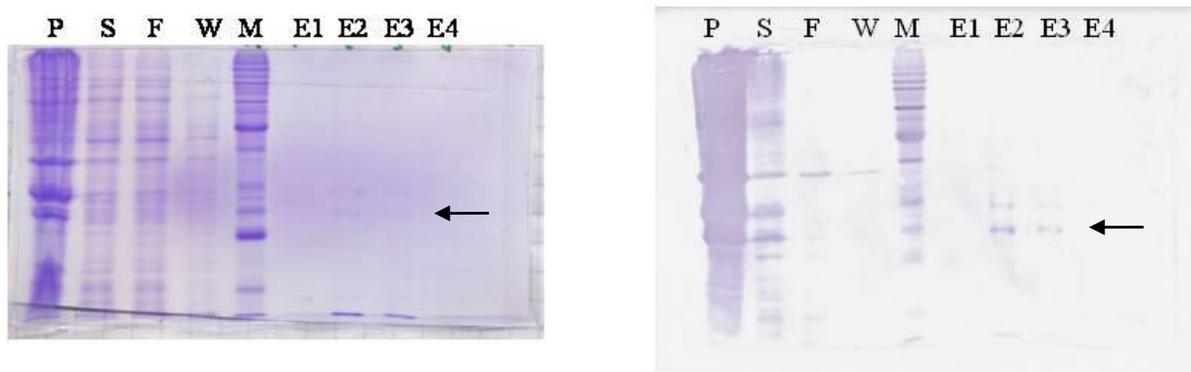


Fig.3.17: SDS-Page and Western blot of transcription factor TF1. Protein was overexpressed in BL21 cells and purified using a Ni-NTA-column. 4 elution fractions were collected for analysis. The left picture displays the SDS-gel, the right picture shows the western blot. M = Bench Mark from Invitrogen, P = pellet, S = supernatant, F = flow through, W = washing step, E1-E4 = elution fraction 1-4.

In Fig.3.17, the Western blot and SDS-PAGE of transcription factor TF1 are shown. A distinct band in elution fraction 3 and 4 with the approximate size of the protein (28 kDa with 239 aa) was observed. TF22 consisted of 162 amino acids and was calculated to a protein size of 17.4 kDa including the HIS-tag. TF20 has a molecular weight of 28.7 kDa with HIS-tag (266 aa), and TF8 runs at 13.9 kDa including the HIS-tag and 129 aa (data not shown). After expression and purification of the protein, 250 ng of the purified transcription factors were added to isotope-labeled promoter fragments. Binding results were evaluated on a non-denaturing polyacrylamide gel and visualized with a phosphor imager. Theoretically, DNA fragments bound to a specific protein would travel at a lower speed through the acrylamide gel while unbound DNA would move faster through the matrix. The transcription factor TF1 was analyzed for the binding ability to the promoter fragments of *tps23* (Fig.3.18). The promoter fragments were designed with overlapping ends to each other, so that the chance of cutting an important region site of recognition would be limited.

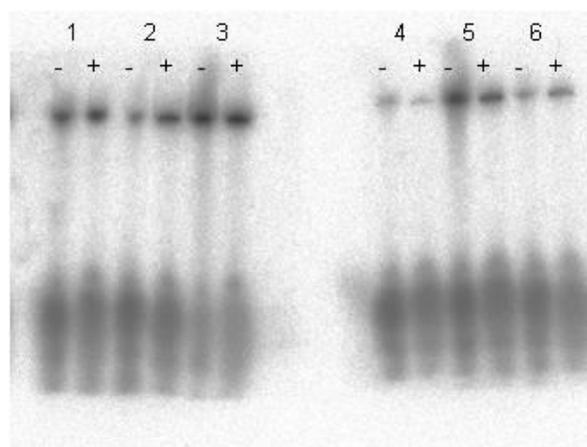


Fig.3.18: Band shift assay of transcription factor TF1 with the promoter fragments of *tps23*. Binding reaction was analyzed on a nondenaturing acrylamide gel and a phosphor imager. For each promoter fragment, a positive and a negative (no labeled fragment was added) probe was run. Fragments represent the complete *tps23* promoter from nucleotide 1:-762- (-607), 2: -675- (-491), 3: -528- (-374), 4: -410- (-250), 5: -277- (-115), 6: -137- (+13).

The unbound promoter fragments appeared at the bottom of the gel as a smeared band. A shifted band could be observed in each lane. Because this particular band appeared in each

lane, it was probably not a specific shift of bound promoter-protein complex. It is possible that the band consisted of probe artefacts of the rather long oligonucleotides.

The results for the promoter fragments of *tps10* with the transcription factor TF1 showed a similar picture (Fig.3.19). Each promoter of both alleles of *tps10* was cut into several fragments. Promoter A was divided into 8 fragments while only 2 fragments represent promoter B due to the high similarity between the two alleles. The unbound, labeled fragments could be seen at the base of the gel, while the previously observed unspecific band ran at the top of the gel. All tested transcription factors showed similar results (data not shown), so that a specific binding between the transcription factors and promoters could not be shown.

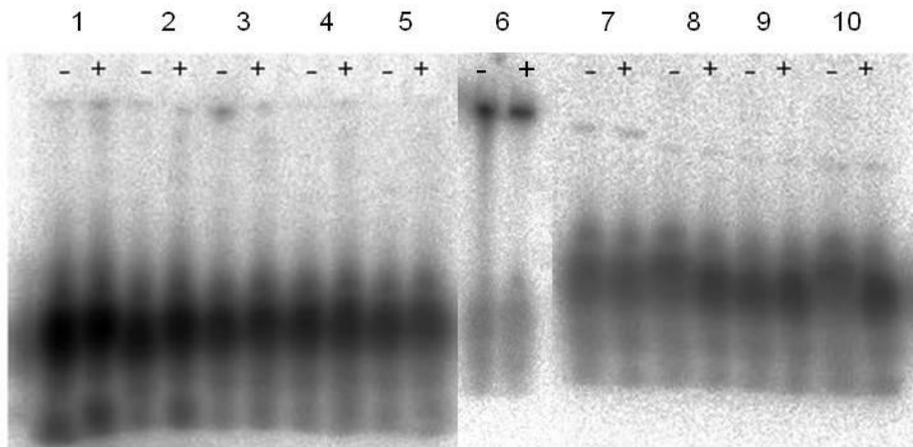


Fig.3.19: Band shift assay of promoter fragments of *tps10* with the transcription factor TF1. Labeled fragments were added to 250 ng of purified protein. Binding reaction was analyzed on a ^{32}P sensitive screen and a phosphor imager. For each fragment, a positive (with protein) and a negative (without protein) sample was run. Overlapping fragments run from nucleotide 1: -976- (-822), 2:-869- (-692), 3: -713- (-592), 4: -589- (-417), 5: -362- (-127), 6:-424- (-264), 7: -289- (-127), 8: -142- (+22) of promoter A and 9: -817- (-643), 10: -360- (-127) of promoter B.

3.4.3 Analysis of *tps10* and *tps23* promoter constructs in Arabidopsis

To gather information as to how the promoters of *tps10* and *tps23* are regulated and to narrow down important regulatory elements, promoter deletion analysis was performed. In this set of experiments, Arabidopsis was chosen as a heterologous system because it is an

easy transformable plant with a shorter reproduction time compared to maize. Several plasmid constructs were produced containing different promoter fragments fused to the reporter gene β -glucuronidase (GUS). This reporter gene enabled us to observe whether the promoter fragments are active after different types of treatment in the Arabidopsis-system. The resulting plasmids were cloned using the Gateway cloning system into the destination vector pBGWFS7.0. These constructs were then transferred into *Arabidopsis thaliana* using *A. tumefaciens* as a vector system. Transformed plants were cultivated to the T2 generation. For induction experiments, the leaves of the plants were induced by scratching with a razor blade with or without addition of jasmonic acid to the wounded area. This type of induction was used to mimic mechanical damage as well as local herbivore damage. Promoter activity was tested using GUS-staining and GUS-assay. As a negative control, wild-type plants (var. Columbia) were used and induced analogous to the transformed plants.

The longest promoter fragment of both terpene synthases *tps10* and *tps23* was inactive in undamaged plants, but displayed activity when plants were treated with mechanical damage and jasmonic acid. This indicated that the herbivore-induced signal transduction pathway between the evolutionary distinct plants maize and Arabidopsis are somewhat conserved. Consequently, we investigated the promoters of *tps10* and *tps23* using promoter::reporter gene constructs in the Arabidopsis-system.

In the first set of experiments, the transformed plants were treated with mechanical damage alone. Deletion analysis revealed an active, damage-regulated *tps23* promoter in the longer promoter fragments 1.8 kb, 1.2 kb and 600 bp (Fig.3.20). Here, GUS-staining was distributed throughout the whole leaf. Unwounded leaves of damaged plants (systemic leaves) and control leaves showed a weak staining in the midvein of the leaf. The promoter constructs *tps23* 400 bp::GUS and *tps23* 200 bp::GUS showed no visible promoter activity in the undamaged and control leaves. Systemic leaves and the controls displayed a slight GUS-staining along the midvein only with the 1.8 kb, 1.2 kb, and 600 bp promoter constructs. This could be a response to the wounding that resulted from the cutting of the leaf. Plant material incubated with GUS-staining solution was not frozen in liquid nitrogen and therefore was still able to respond to environmental cues while it was stained.

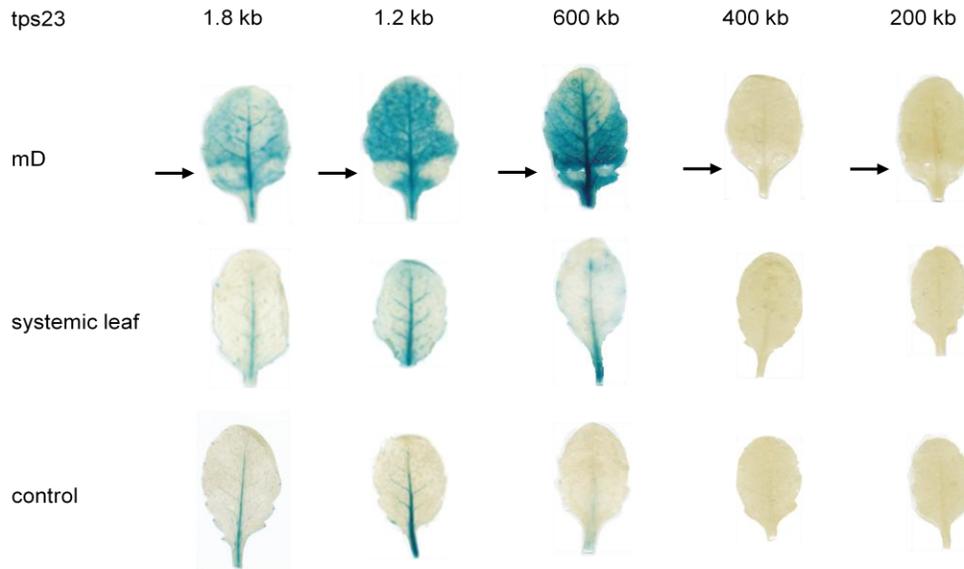


Fig.3.20: Gus-staining of *tps23* promoter constructs after mechanical damage. Arabidopsis plants containing a *tps23* promoter fragment fused to the β -glucuronidase were induced by scratching the leaf with a razor blade (mD). A leaf of an induced plant was taken as reference (systemic) as well as a transformed plant without treatment as control. Arrows indicate the wounding site.

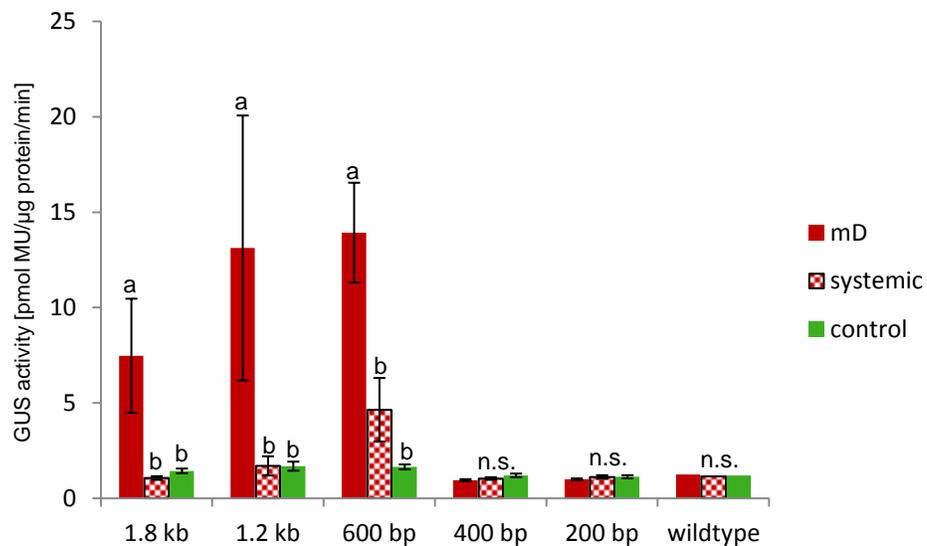


Fig.3.21: Promoter activity of the *tps23* constructs 1.8 kb, 1.2 kb, 600 bp, 400 bp, 200 bp, and Columbia wild-type, respectively, after mechanical treatment. Arabidopsis plants (n=4) were treated with mechanical damage (mD) by scratching the leaves with a razor blade. Undamaged leaves were taken as well as not treated plants as control. Means and standard error are shown. Statistical analysis was performed using t-test for the treatment mD and control and a paired t-test for mD and undamaged treatment. Undamaged and control were tested using a t-test. Different letters indicate significant activity ($p < 0.05$) between the treatments.

There was a significant difference in the activity of the promoter constructs after mechanical damage (Fig.3.21). The three longest promoter fragments, 1.8 kb, 1.2 kb, and 600 bp, were able to induce the reporter gene β -glucuronidase, while the shorter fragments of the *tps23* promoter and control plants didn't show reporter gene activity. In systemic leaves and control plants, the promoters were not active.

After testing the effect of mechanical damage, the role of jasmonic acid on the promoter activity was investigated. Plants were damaged according to the previous experiment but jasmonic acid was added. The *tps23* promoter was active in the constructs with 1.8 kb, 1.2 kb and 600 bp in the wounded leaves of transformed Arabidopsis plants (Fig.3.22). The shorter constructs with only 400 bp or 200 bp of the *tps23* promoter displayed no GUS staining and were therefore considered to be too short to induce expression. In treated plants, the longer fragments showed promoter activity along the veins of the leaves as well as in the surrounding cells. The activity decreased towards the top of the leaf. Interestingly, the promoter was active not only around and above the wounding site but below as well. Here, the promoter activity of *tps23* might correspond to the distribution of the jasmonic acid in the leaf. Jasmonic acid is transported via the phloem (Li *et al.* 2002) and could therefore act as an activator on the *tps23* promoter.

The promoter activity of *tps23* was confirmed with the GUS-assay. A significant difference in activity was found between treated and control plants in the constructs *tps23* 1.8 kb::GUS, 1.2 kb::GUS, and 600 bp::GUS (Fig.3.23). Systemic leaves of plants treated with damage and JA showed no significant induction of promoter activity, similar to those treated with mechanical damage only.

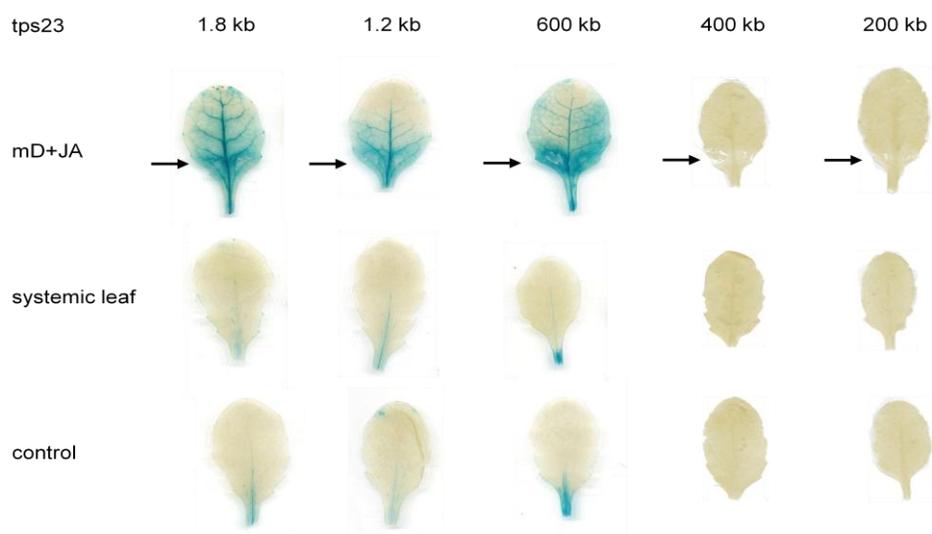


Fig.3.22: Gus-staining of *tps23* promoter constructs after mechanical damage and application of jasmonic acid. Arabidopsis plants containing a *tps23* promoter fragment fused to the β -glucuronidase were induced by scratching the leaf with a razor blade and adding 250 μ M jasmonic acid (mD+JA). A leaf of an induced plant was taken as reference (systemic) as well as a transformed plant without treatment as control. Arrows indicate the wounding site.

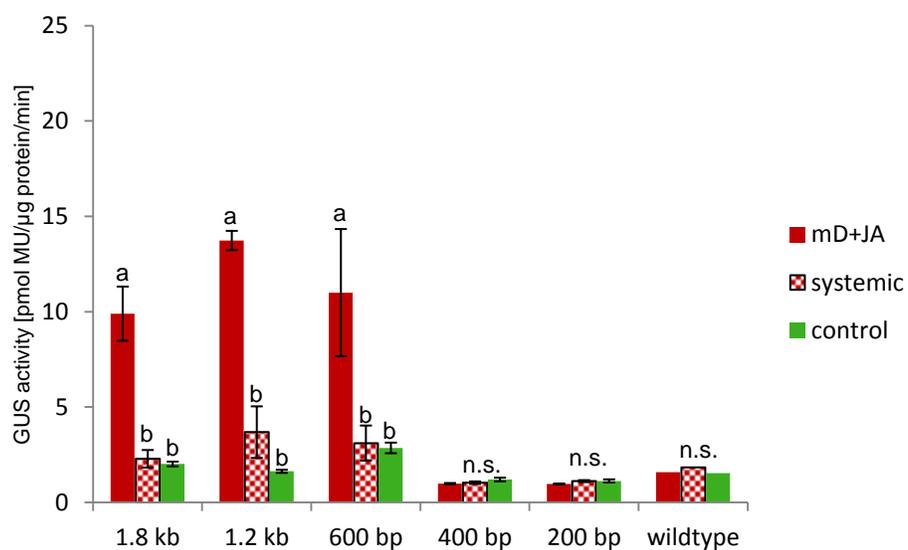


Fig.3.23: Quantitative GUS-activity in leaves of Arabidopsis transformed with *tps23* promoter constructs and Columbia wild-type. Arabidopsis plants were induced by mechanical damage plus the application of jasmonic acid (mD+JA). Undamaged leaves were taken from damaged plants to reflect systemical damage. Standard error and means are shown (n=4). Statistical analysis was performed using t-test for the treatment mD+JA and control and a paired t-test for mD+JA and undamaged treatment. Undamaged and control were tested using a t-test. Different letters indicate significant activity ($p < 0.05$) between the treatments.

To analyze the effect of JA to the promoter activity, plants were induced without mechanical damage. Because the induction methods of plants without harming the leaves were not successful, plants were watered with a jasmonic acid-containing solution. After 3 days of watering with a JA-solution, the leaves were harvested and treated as described above. Here, the promoter activity (Fig.3.24) was limited to the veins of the leaf, corresponding to the transport of jasmonic acid. Unlike the damaged leaves, treatment with jasmonic acid induced the 1.8 kb and 1.2 kb promoter fragments. The 600 bp promoter constructs displayed some minor staining along the mid vein while the 400 bp and 200 bp promoter fragments were completely inactive.

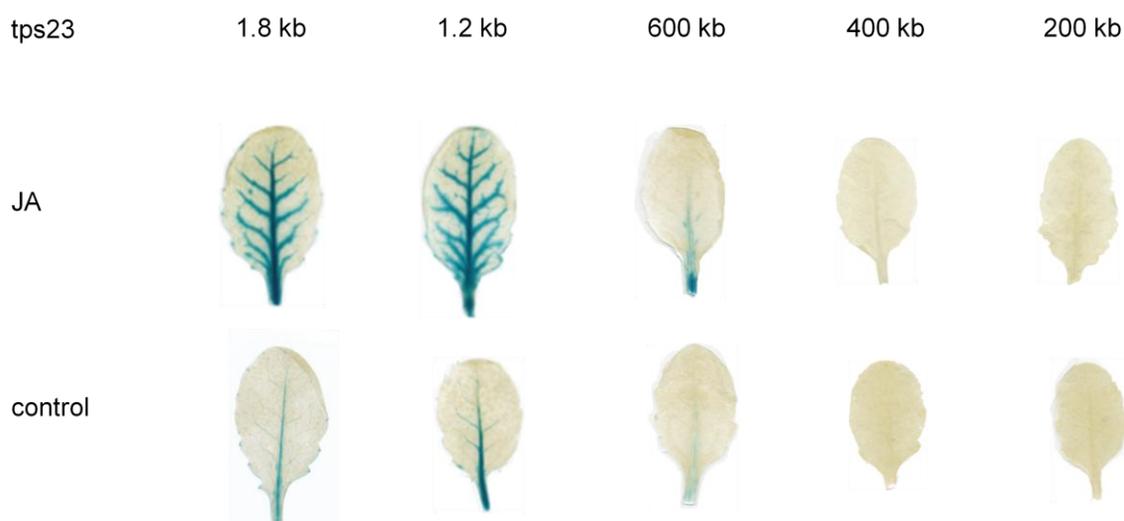


Fig.3.24: GUS-staining of transformed Arabidopsis leaves treated with jasmonic acid over 3 days (JA). Transformed Arabidopsis plants containing the *tps23* promoter constructs were induced by watering with a jasmonic acid containing solution over 3 days.

Additionally, the activity of the *tps23* promoter was tested using a quantitative GUS-assay (Fig.3.25). The behavior of the promoter activity was similar in both analysis methods.

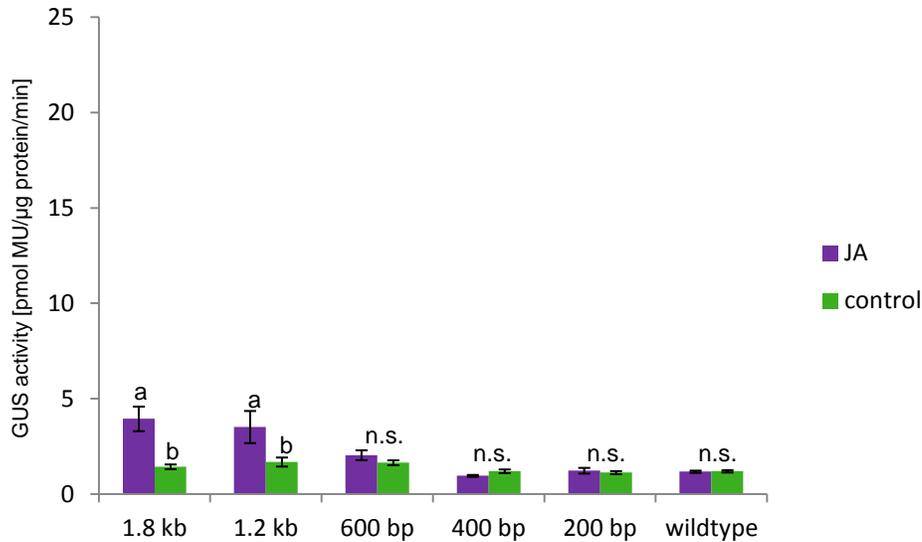


Fig.3.25: Quantitative GUS-assay in Arabidopsis plants transformed with different *tps23* promoter::GUS constructs after induction with jasmonic acid. Plants (n=4) were watered with jasmonic acid for 3 days. Means and standard errors are shown. To calculate the statistical relevance, One-Way ANOVA was performed for the different treatment within the constructs. Undamaged leaves were left out in this analysis because material is not independently from the mechanical damage treatment. Different letters indicate significant activity ($p < 0.05$) between the treatments.

In a next step, the promoter constructs of *tps10*::GUS were tested. Analog to the experiments with *tps23*, promoter fragment lengths of 1.5 kb, 800 bp, 500 bp and 200 bp were fused to the β -glucuronidase gene and treated with mechanical damage with or without addition of jasmonic acid. In the first round of experiments, transformed Arabidopsis plants were mechanically damaged with a razor blade. The longest *tps10* promoter fragment of 1.5 kb::GUS showed minor GUS staining around the wounded area (Fig.3.26). Promoter activity was not found in systemic leaves and only slightly present at the cutting site in control plants. The more reliable, quantitative GUS assay revealed no promoter activity in the 1.5 kb construct, indicating that the longest promoter construct cannot be induced after mechanical damage (Fig.3.27). Therefore, smaller fragments were not included in this experiment.

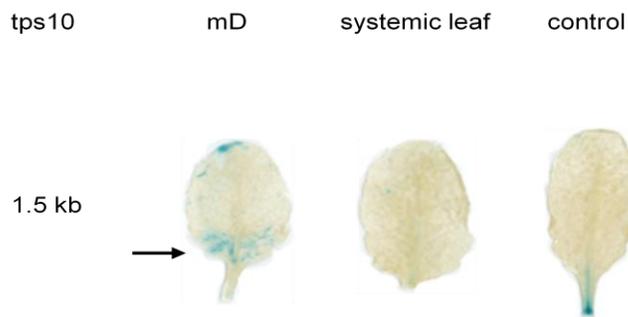


Fig.3.26: GUS-staining of Arabidopsis plants transformed with the construct *tps10* 1.5 kb::GUS. Plants were induced by scratching the leaf with a razor blade (mD). Systemic leaves of induced plants and uninduced plants were taken as controls. Arrows indicate the wounding site.

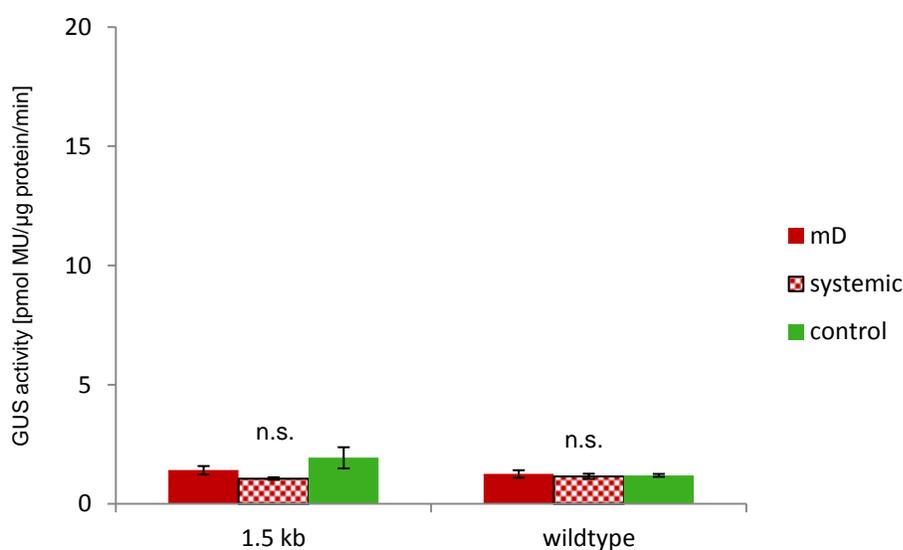


Fig.3.27: Promoter activity *tps10* 1.5kb::GUS after mechanical damage. Wounding of the leaves resulted from scratching the leaves with a razor blade (mD). Systemical induced leaves from the mechanical wounded plants and untreated plants were taken as controls (control). Means (n=4) and standard errors are shown. One-Way ANOVA was performed for statistical analysis.

Different results were obtained when transformed Arabidopsis plants were treated with mechanical damage and jasmonic acid. In these experiments, all *tps10* promoter constructs were included. However, the 1.5 kb promoter construct was the only one exhibiting a promoter activity above and around the wounding site in the cells next to the harmed area (Fig.3.28). GUS staining was slightly distributed along the veins, without spreading to the top of the leaf. The 800 bp and 500 bp fragments showed no promoter activity, while the 200 bp fragment of the *tps10* promoter displayed a very weak promoter activity next to the wounding site. The systemic leaves as well as the control plants showed no promoter activity.

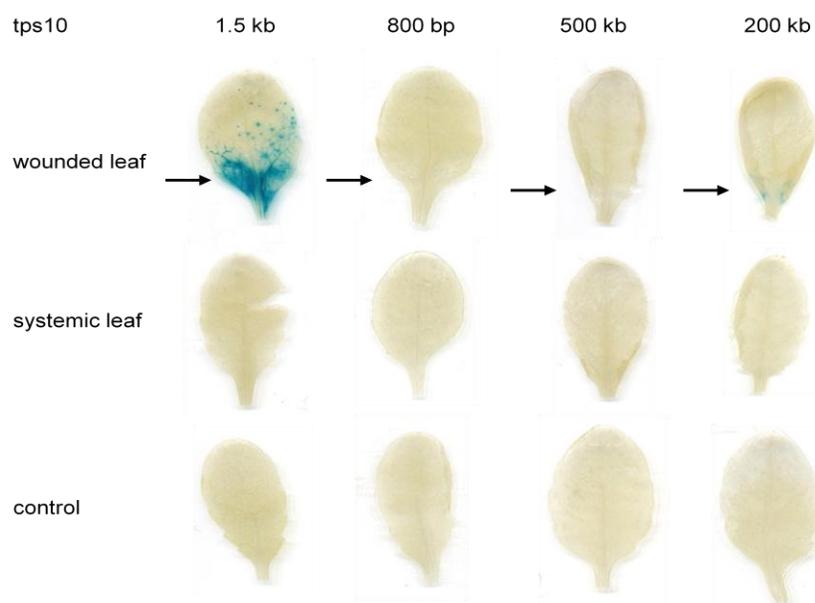


Fig.3.28: Gus-staining of *tps10* promoter constructs after mechanical damage and application of jasmonic acid. Arabidopsis plants containing a *tps23* promoter fragment fused to the β -glucuronidase were induced by scratching the leaf with a razor blade and adding 250 μ M jasmonic acid (mD+JA). A leaf of an induced plant was taken as reference (systemic) as well as a transformed plant without treatment as control. Arrows indicate the wounding site.

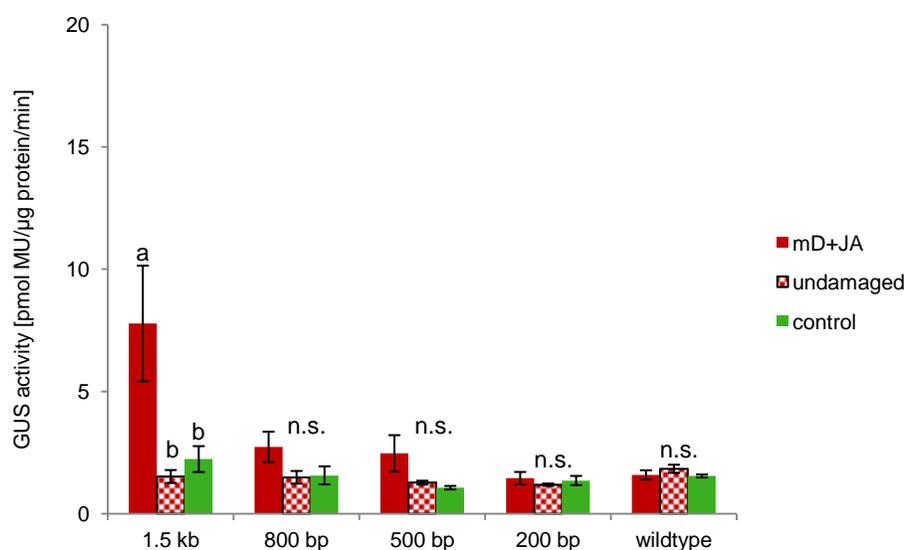


Fig.3.29: Quantitative analysis of GUS-activity in the different *tps10*::GUS constructs. Arabidopsis plants were induced with mechanical damage by scratching the leaf and adding jasmonic acid. Systemic leaves were taken from induced plants. The control plants were left unharmed. Means ($n=4$) and standard errors are shown with the statistical differences indicated by different letters between the treatments for each construct ($p<0.05$). Analysis was performed using a regular t-test for the damaged plants versus the control plants. The significance between damaged and undamaged leaves was calculated using a paired t-test.

Analog to the GUS-staining, the quantitative analysis (Fig.3.29) showed a significant promoter activity with the induced *tps10* 1.5 kb::GUS construct after treating the plants with mechanical damage plus application of JA. Shorter promoter fragments were not active. Systemic leaves and control plants didn't show a significant promoter activity between the treatments.

Arabidopsis plants transformed with the construct *tps10* 1.5 kb::GUS were induced with jasmonic acid by watering over 3 days analog to the former experiment. Similar to the results of the GUS-staining method (data not shown), this promoter construct showed no significant induction of reporter gene activity after the treatment with jasmonic acid (Fig.3.30). These results indicate that the *tps10* promoter cannot be induced by mechanical damage or jasmonic acid alone, but needs the combination of both stimuli.

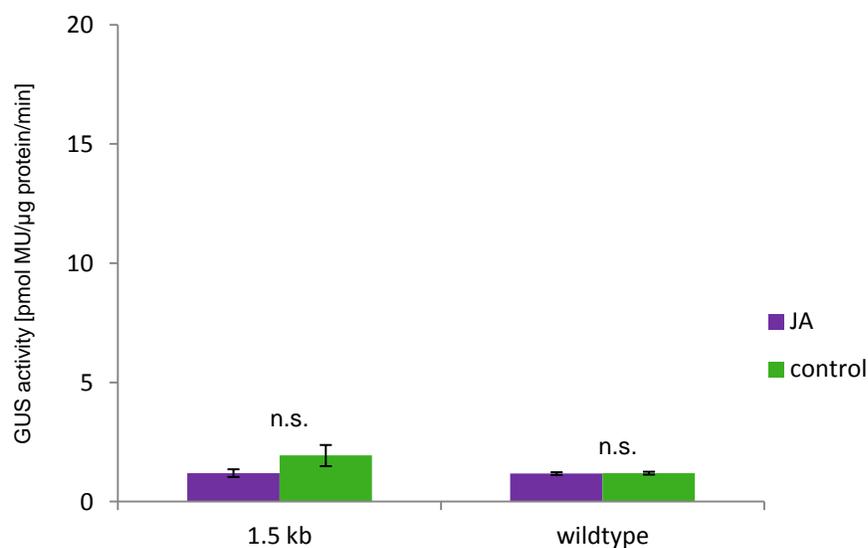


Fig.3.30: GUS-analysis of *tps10* 1.5 kb::GUS and wild-type plants after jasmonic acid treatment. Plants were watered with jasmonic acid over 3 days. Control plants were left untreated. Means and standard error are shown (n=4). One-Way ANOVA was performed for statistical analysis.

3.5. Identification of transcription factors induced by local, systemical and mechanical wounding in roots and leaves

In order to respond to an herbivore threat, plants react to herbivore damage in their non-damaged parts (Erb *et al.* 2009). Therefore, we wanted to investigate the cross talk between plant organs after herbivory and mechanical damage. Using the Long Oligo Microarray, the interaction between roots and shoots were analyzed. In particular, the influence of *D. virgifera* infested roots upon uninfested leaves (systemic induction) and the influence of *S. littoralis* infested leaves upon uninfested roots was tested. To investigate whether the transcriptional changes caused by *D. virgifera* attack are a general wound response or a specific reaction of the plant, roots were also damaged mechanically. Plant material was harvested 18 h after the treatment. Infested or mechanically damaged roots and herbivore-induced leaves as well as the systemically induced material were provided by Matthias Erb and analyzed with the Long Oligo Microarray for differences in gene expression. Since the main focus of this work were herbivore-regulated transcription factors, only the differentially expressed transcription factors will be presented here. The analysis comprised all proteins annotated as transcription factors although some might not be functional. The total number of differentially regulated transcription factors is displayed in Fig.3.31.

After treatment with the *D. virgifera* larvae, 29 putative transcription factors showed a higher expression in roots while only 3 genes were suppressed. Systemic induction by *S. littoralis* larvae resulted in an induced expression of 10 transcription factors while 9 showed a decreased expression. After roots were treated with mechanical damage, 20 transcription factors were up-regulated while 4 transcription factors were down-regulated. Overall, the roots of *Zea mays* react to local herbivory by altering the expression of more transcription factors than upon systemic induced leaves. Leaves on the other hand, showed far less differentially expressed transcription factors. Eight putative transcription factors were repressed in the leaves when roots were infested with *D. virgifera* while only one factor was induced. Even after wounding by *S. littoralis* larvae for 18 h, only 6 transcription factors in the damaged leaf showed a transcript accumulation while one gene was negatively regulated. After mechanical damage in roots, only 2 induced and 1 suppressed transcription factors were found in leaves.

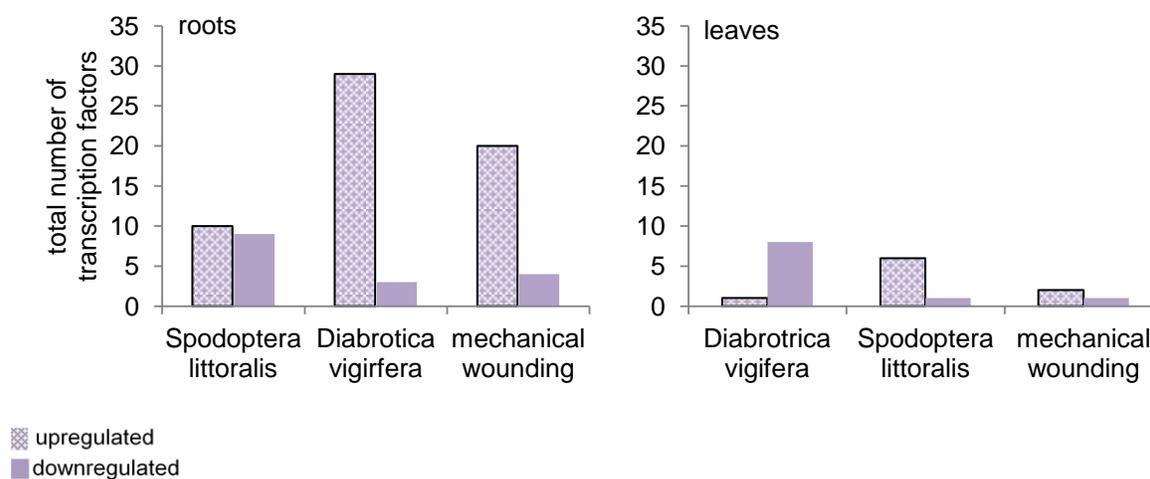


Fig.3.31: Overview of transcriptional changes of transcription factors in herbivore-infested plants. *S. littoralis* feeding upon the leaves resembles the systemical wounding to the roots, while *D. virgifera* feeding upon the roots displays the local wounding on the roots and vice versa. Mechanical wounding was performed by stabbing the roots several times with a scalpel.

A closer analysis of differentially regulated transcription factors in roots revealed some similarities between the treatments (Fig.3.32). A total number of 32 transcription factors showed changes in gene expression. Nine of them were also affected after mechanical damage to the roots while 2 transcription factors showed different transcript levels after *S. littoralis* attack. Only 2 transcription factors displayed a difference in gene expression after all three treatments. Eleven and 13 transcription factors were regulated in response to mechanical wounding or feeding of *S. littoralis*, respectively. Their corresponding MZ-numbers are listed in Tab.3.2. Annotation of these accession numbers can be found in the appendix (7.7).

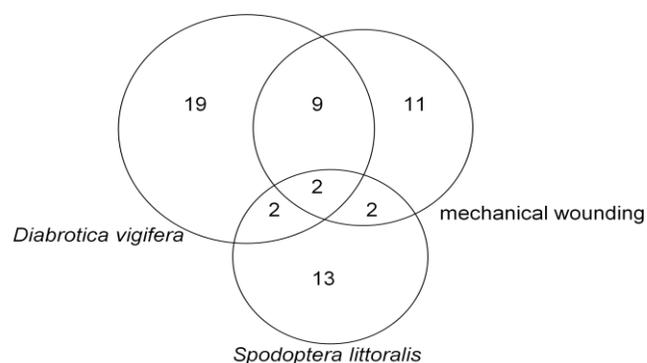


Fig.3.32: Venn-diagram of transcription factors with herbivore-regulated expression in root material after local damage by *D. virgifera*, damage in the leaves by *S. littoralis* larvae, and mechanical damage. Numbers in the circles represent the numbers of equal MZ-numbers between the treatments. Diagram was constructed using <http://bioinforx.com>.

Tab.3.2: List of shared MZ-numbers from the roots after different treatments.

<i>S. littoralis-D. virgifera</i>	<i>S. littoralis-D. virgifera</i> -mechanical damage	<i>S. littoralis-mechanical</i> damage	<i>D. virgifera-mechanical</i> damage
MZ00004381	MZ00004381	MZ00004381	MZ00004381
MZ00017495			MZ00020499
MZ00026127	MZ00026127	MZ00026127	MZ00026127
MZ00036067		MZ00028351	MZ00026538
		MZ00054846	MZ00026661
			MZ00028044
			MZ00035959
			MZ00036019
			MZ00041768
			MZ00042353
			MZ00046918

Two transcription factors, MZ00004381 and MZ00026127 were expressed after local, systemical and mechanical wounding. MZ0004381 is a putative bZIP transcription factor and MZ00026127 is only annotated as development regulation gene. Interestingly, there were no overlapping transcription factors within the leaf material, while there were several shared transcription factors between root and leaf material. The graphical outline can be viewed in

Fig.3.33 and annotation is presented in Tab.3.3. After local damage in leaves and local damage in roots, five transcription factors showed the same transcriptional changes. Local herbivory in shoots and systemic herbivory in roots had only one transcription factor in common. Also one transcription factor with the same transcriptional change was found in leaves and roots after mechanical root-damage.

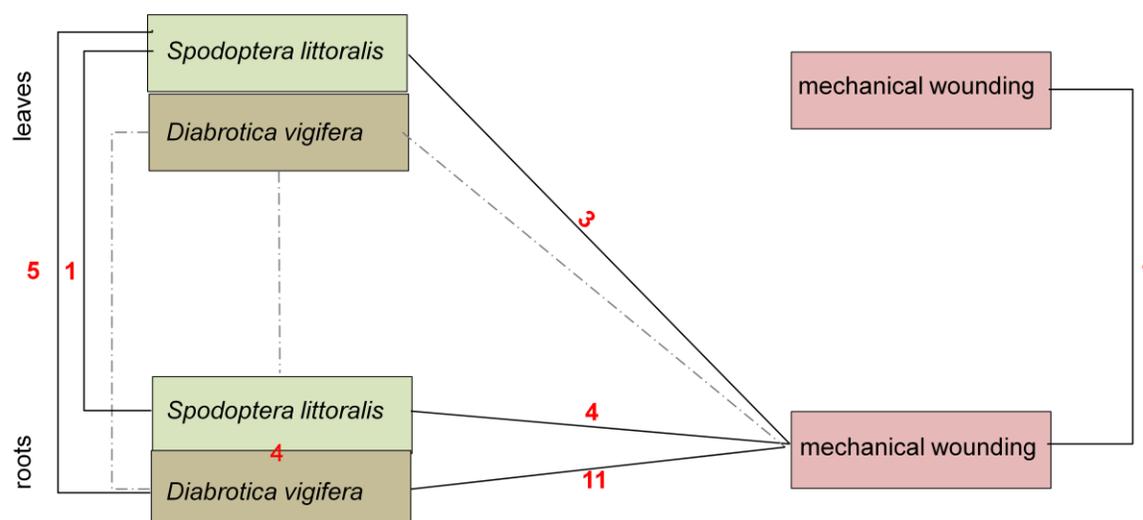


Fig.3.33: Similarities in gene expression patterns of transcription factors between leaves and roots after different types of induction. Leaves were investigated after attack of *S. littoralis*, infestation of roots by *D. virgifera* or mechanical damage. Root material was harvested after local damage by *D. virgifera*, mechanical damage and after *S. littoralis* herbivory upon the leaves (systemical damage). Bold lines indicate equal MZ-number between two treatments with the total number in red. Dashed lines indicate no similarity.

Tab.3.3: List of shared transcription factors between leaves and roots after different induction treatment.

<i>S. littoralis</i> : leaf-root	<i>S. littoralis</i> : leaf- <i>D. virgifera</i> : root	leaf-mechanical damage: root	<i>S. littoralis</i> : leaf-mechanical wounding:root
	MZ00012674	MZ00028419	
MZ00017495	MZ00017495		
	MZ00026538		MZ00026538
	MZ00036019		MZ00036019
	MZ00041768		MZ00041768

While MZ00017495 was induced in both roots and leaves after different induction stimuli, the ZIM-motif family protein MZ00026538 was also found in roots and leaves after mechanical damage in roots, as well as in earlier time course experiments. The putative zinc finger protein MZ00041768 was highly induced after local wounding and mechanical damage of the roots and was also found after 2 h after herbivore induction by *S. littoralis* in the leaves. The data of this microarray experiment were only evaluated using the program R. Validation of these results by QRT-PCR is necessary to verify the complex regulatory patterns of these transcription factors.

An overview of all other differentially regulated genes of this experiment can be found in Erb *et al.* (Erb *et al.* 2009).

4. Discussion

4.1 Microarray analysis as a tool to find genes in the plant responses against herbivore damage

Plants and insects have been coexisting for over 350 million years and therefore evolved a diversity of complex interactions (Gatehouse 2002). When plants recognize herbivore-associated molecular patterns (HAMPs; (Mithöfer and Boland 2008)), several reactions take place in plant cells, including changes in the ion flux, protein phosphorylation, formation of ROS and oxylipins, and initiation of various defense reactions (Kessler and Baldwin 2002; Maffei *et al.* 2007). The defense reactions include the generation of the phytohormones JA, SA, and ET (Pieterse and van Loon 1999; Glazebrook 2001; Dicke and Van Poecke 2002), as well as the synthesis of secondary compounds (Baldwin and Callahan 1993; De Moraes *et al.* 1998), among them volatile terpenes (Turlings *et al.* 1990; Kessler and Baldwin 2001).

Microarray hybridization has been used to study the transcriptome in several plant species. The transcript profiles responding to insect feeding, mechanical wounding, JA, H₂O₂, and herbivore-induced volatiles were investigated using microarray technology in lima beans, *Arabidopsis thaliana*, and *Nicotiana attenuata*, (Arimura *et al.* 2000; Reymond *et al.* 2000; Halitschke *et al.* 2003; Reymond *et al.* 2004). In Rice, more than half of the genes identified in insect-induced indirect defense belonged to the category “involved in secondary metabolism”. The genes included terpene synthases, P450 enzymes, methyltransferases, and BAHD acyltransferases (Yuan *et al.* 2008). Transcription factors were the second largest group of genes influenced by herbivory. Temporal changes in expression patterns were only conducted in tobacco and *Arabidopsis* (Durrant *et al.* 2000; Reymond *et al.* 2000).

One aim of this work was the identification of early transcriptional changes in maize after herbivore induction. With a large fraction of the transcriptome of the maize line B73, the microarray chip from the University of Arizona enabled us to investigate gene expression after herbivory. The results of the microarray experiment were verified by QRT-PCR which confirmed the microarray data in most cases. Limitations to this method are present because only genes showing a differential regulation after herbivore attack could be identified. Constitutively expressed proteins were not detectable. The genome-wide mapping of a maize

population would be the method of choice to detect genes that do not undergo changes in gene expression. Although, the microarray was generated from sequences of the maize line B73, Delprim was chosen as target maize line because of its ability to produce high levels of volatiles including (*E*)- β -caryophyllene. Still, only slight limitation should result from this due to the high genetic similarity between Delprim and B73. B73 is a homozygous inbred line while Delprim is a heterozygous hybrid line. Considering an allelic polymorphism of about 3 % between maize lines in general, there is a high probability that at least one of the alleles from Delprim can hybridize with the allele of B73. The herbivore-induced genes of Delprim belonged to many classes including transcription factors, kinases and phosphates, metabolic enzymes, developments genes, and several more. Several genes, including FPP synthases, terpene synthases, and methyl transferases, have also been found in other plant species after herbivory (Yuan *et al.* 2008). Thus, this method was appropriate to identify temporal changes in herbivore-induced maize.

4.2 The regulation of herbivore-induced transcription factors

4.2.1 Herbivore feeding influences the expression of transcription factors

Transcription factors act as switches of regulatory cascades (Scott 2000) and a combination of *cis*-acting elements are needed for the appropriate responses to stimuli (El-Shehawi *et al.* 2011). In Arabidopsis, up-regulation of transcriptions factors was demonstrated to cause a general shift in transcriptional regulation in response to insect damage (Reymond *et al.* 2004). In maize, we found a total number of 90 differentially regulated transcription factors in the leaves of herbivore-induced maize within 0.5 h and 4 h after damage. Out of that number, 12 genes had multiple hits. The majority of herbivore-regulated transcription factors were found at 0.5 h and 1 h while the number of differentially regulated metabolic enzymes started to increase after 1 h.

After an 18 h treatment with *S. littoralis*, only 7 transcription factors were identified in the leaves. The discrepancies in these observations are due to the sustained time of damage by the herbivore. Some of the early induced transcription factors were already down-regulated after 18 h. A distinct picture was observed in roots of maize. Many transcription factors

displayed a differential regulation pattern independently of the induction type (*D. virgifera* herbivory, mechanical damage, and systemic induction). A difference in the total number of transcription factors could be observed between *D. virgifera* feeding and mechanical damage in roots, indicating that some transcription factors function in an herbivore-specific pathway. Transcription factors induced by herbivory and mechanical damage might mediate a general wound-response of the roots. Roots of maize react strongly in response to above-ground herbivory. Still, the function and relevance of this type of response remains to be resolved. The results from these data implicate that there are similarities as well as differences in the signal transduction pathways between a systemic and a local damage reaction to herbivore attack (Erb *et al.* 2009).

The transcription factors identified in this study belong to several classes that are linked to plant stress responses. These include AP2/EREBP transcription factors, WRKY transcription factors, MYB transcription factors, and bZIP factors (Stracke *et al.* 2001; Singh *et al.* 2002). AP2/EREBPs, for example, were found in insect-induced defense in poplar (Ralph *et al.* 2006). In *Catharanthus roseus*, members of the AP2/EREBP family responded to jasmonic acid (Menke *et al.* 1999). A homolog of the maize transcription factor, *tf1*, was first identified in Arabidopsis (ATAF1/2). It shares a 74 % homology (amino acid level) to the ARAF1/2 in Arabidopsis and 94 % homology to rice. Both proteins were found to be expressed after herbivore attack (Delessert *et al.* 2005; Yuan *et al.* 2008). A similar expression pattern for *tf1* and its homologs was found in herbivore-challenged species like Arabidopsis (Reymond *et al.* 2004) and poplar (Ralph *et al.* 2006). Binding to a drought-responsive element, the DRE transcription factors were first related to the response to cold stress and salt stress (Zhao *et al.* 2006). Newer studies placed the DRE transcription factors into the group of AP2/EREB proteins, connecting them to the ethylene-dependent signaling pathway (Sun *et al.* 2008). One DRE transcription factor found in this study is *tf40* which showed a strong early induction after continuous herbivore feeding. A 53 % homolog from *Aloe vera* was found to be induced 12 h after cold stress (Wang and He 2007). It is therefore unlikely that the Aloe DREB1 and maize *tf40* have similar functions.

Also, maize CAF1-like protein *tf2*, is involved in defense responses in Arabidopsis and rice (Walley *et al.* 2007; Yuan *et al.* 2008). The AtCAF1a/b factor responds to wounding and biotic stresses within 5 min after induction stimuli and is under a circadian control (Walley *et*

al. 2007; Walley *et al.* 2010). The homology to *tf2* is 51 % based on amino acid level. The family of CCR4 associated factors (CAF) are factors involved in the post transcriptional regulation of genes. In yeast, they are part of the major cytoplasmic deadenylase, initiating mRNA turnover by removing the poly(A)-tail (Tucker *et al.* 2001). Recent studies indicate that CAF1 proteins might degrade repressors of the pathogen-related genes PR1 and PR2, assuming that this factor plays a role in the plant defense response (Liang *et al.* 2009). In this work, *tf2* showed an early induction in response to herbivore feeding and mechanical damage, suggesting a general role in maize defense responses.

MYB-transcription factors are involved in several developmental steps of the plant. It has been demonstrated that the transcription factor ODORANT1, a R2R3 MYB-transcription factor, controls the synthesis of volatile benzenoids present in the floral scent of petunia (Verdonk *et al.* 2005). We also identified two MYB-(like) factors in maize, *tf12* and *tf28*. While *tf12* was suppressed after herbivore feeding, *tf28* showed an induced expression. A 48 % amino acid identity was found between *tf12* and the R2R3 MYB-transcription factors that play a role in abiotic stress responses in *Triticum aestivum* (Zhang *et al.* 2011). But the low amino acid identity to bread wheat raises the question about the specific function of *tf12* in maize. Only 17 % sequence similarity of the maize *tf28* and TaMYB13 of *Triticum aestivum* was predicted by NCBI. TaMYB13 functions as an activator of fructosyltransferase genes (Xue *et al.* 2011), but due to the low similarity a functional consensus can not be assumed.

An I-box binding factor has first been described in tomato and was associated to the class of MYB-like proteins (Rose *et al.* 1999). The transcription factor *tf35* from maize was annotated as putative I-box binding factor and can therefore be grouped to the class of MYB transcription factors. *Tf35* was highly induced by herbivory and its homolog in bread wheat is implicated in abiotic stress responses (Zhang *et al.* 2011). Due to the low amino acid sequence similarity of 24 %, a prediction about the specific role of *tf35* in maize cannot be made.

One zinc-finger transcription factor of maize, *tf29*, showed a negative regulation of gene expression after herbivore attack. This factor shares a 95% amino acid sequence identity to OsLDS1 from *Oryza sativa*. Here, it has been described as a negative regulator of programmed cell death in plants (Wang *et al.* 2005). Programmed cell death is often associated with a hypersensitive response of plants to pathogen attack (Greenberg *et al.*

1994). The down-regulation of *tf29* after herbivory could be a precautionary step to increase resistance towards a subsequent pathogen attack.

The class of bHLH proteins is represented by *tf8* in maize. This transcription factor is strongly induced after herbivore attack, but no homolog of *tf8* has been described in other plant species yet.

An example of WRKY transcription factors induced by herbivory is the maize transcription factor *tf34*. WRKY transcription factors are unique to the plant kingdom (Eulgem *et al.* 1999) and have been identified to bind to W-boxes (Chen *et al.* 2002) which are found in the promoters of many plant defense genes (Kalde *et al.* 2003). A homolog of maize *tf34*, OsWRKY71 (90 % amino acid identity), encodes a transcriptional repressor of the gibberellin signaling pathway in rice (Zhang *et al.* 2004).

Further factors include an ETTIN-like auxin response factor (maize *tf20*) and putative phi-1-proteins (maize *tf42*) which respond both with an induced expression after herbivore attack. Characterization of *tf20* or putative homologs has not been conducted so far. As a homolog to the transcription factor *tf42*, phi-1 has only been implemented in the process of phosphorylation (Sano *et al.* 1999).

Although the maize transcription factors found in this study are regulated by herbivory on the level of expression, they might not necessarily be directly involved in terpene production or plant defenses. Generally, specific functions or putative targets cannot be predicted based on the low sequence similarities to other species. To unravel the functionality of these factors, further studies have to be conducted.

4.2.2 Specific induction stimuli lead to explicit expression patterns

Production of defense compounds like volatiles is cost-intensive and therefore often activated when needed (Wright *et al.* 1979). To orchestrate these specific responses, multiple signaling pathways regulate the stress response of plants (Glazebrook 2001; Knight and Knight 2001). However, gene expression patterns share significant similarities after different abiotic or biotic stresses (Durrant *et al.* 2000; Schenk *et al.* 2000; Seki *et al.* 2001; Chen *et al.* 2002; Erb *et al.* 2009).

The results in this work showed that maize discriminates between a short herbivore attack, long damage by herbivore feeding, and mechanical wounding by itself. *Tf1* and *tf35* are

maize transcription factors which are specifically induced in response to these different cues (Fig.4.1).

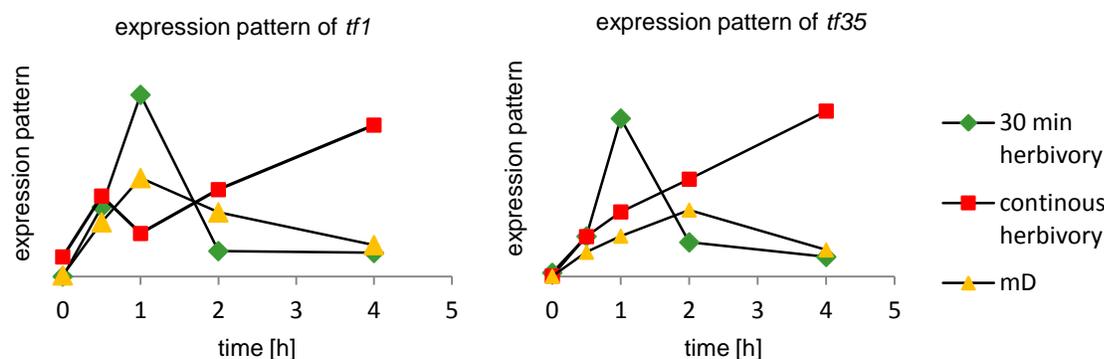


Fig.4.1: *Tf1* and *tf35* have a different expression pattern after diverse induction types. Maize plants were either treated with 30 min herbivore feeding, continuous feeding until harvesting, and continuous mechanical damage until harvesting (mD). Induction patterns are combined from independent experiments which were all standardized by the housekeeping genes. Therefore only the expression patterns are displayed without a relative expression fold.

The NAC-like transcription factor *tf1* reached maximum expression within an hour in mechanically damaged plants and in plants treated with 30 min of herbivory. The continuous herbivory of *S. littoralis* resulted in a steady increase of expression after 1 h of treatment. This indicated that maize plants recognize the different types of wounding. The same induction pattern could be observed for transcription factor *tf35*, a putative I-box binding factor.

In order to elucidate the function of transcription factors, maize plants with transposon knockouts were investigated. There was only one transposon-insertion line available for all of the identified transcription factors, with the transposon inserted in the 3' region of *tf1*. Unfortunately, this line could not be used to investigate the role of *tf1* since the background line, W22, is unable to produce herbivore-induced VOCs.

To be a reliable cue for herbivore enemies, the volatile signals emitted by the plant need to start quickly after the onset of feeding and stop when the herbivore moves on. Specific volatile profiles and kinetics were found for *tps23*. Gene expression was significantly up-regulated at 2 h and 4 h after mechanical damage with and without addition of caterpillar

regurgitate. Both kinds of treatments induced the transcript level of *tps23* to an equal amount. This suggests that wounding already fully induces *tps23* expression and regurgitate application does not provide a further increase. The emission of (*E*)- β -caryophyllene, formed by TPS23, was detected at 4 h after induction but a significant difference was found between the treatments. The volatile sesquiterpene was emitted after plants were treated with mechanical damage. However, addition of caterpillar regurgitate enhanced the level of this volatile compound significantly.

The difference in time lag between induction of transcript accumulation and volatile production can be explained by the period it takes to translate *tps23* mRNA into a sufficient amount of TPS23 enzyme. An adequate amount of TPS23 must be present to produce enough volatiles that can be measured by SPME. Still, this does not explain the discrepancy in the concentration of (*E*)- β -caryophyllene measured after mechanical damage and damage with regurgitate. The results indicate a posttranslational control point for TPS23 expression or product formation. Another regulatory step responsible for the increase of terpene production after regurgitate application could be the expression of FPP-synthases which synthesize the substrate for terpene synthases (Köllner *et al.* 2008). Putative FPP-synthases were identified with the microarray and showed an increased expression level at 2 h and 4 h after herbivore attack. Unfortunately, the influence of mechanical damage on the expression pattern of FPP-synthases has not been investigated yet. A similar mechanism of regurgitate-specific regulation might also control the emission of the volatile compounds (*E*)- β -farnesene, (*E*)- α -bergamotene, linalool, and DMNT. The responsible terpene synthases have not been investigated in this study, but the same explanation could apply.

On the other hand, the aromatic heterocyclic compound indole and the green leaf volatile hexenal were emitted in similar amounts after mechanical damage with and without caterpillar regurgitate. Putative indole synthases were found induced in the microarray data at 2 h after herbivore induction and have also been described to be induced after volicitin treatment (Frey *et al.* 2000). However, the data in this study indicate that mechanical damage is sufficient for the induced emission of indole and hexenal and does not require an additional elicitor.

Differential expression patterns of transcription factors can not only be found after different induction stimuli, but they also vary between maize lines. The variety Delprim is able to

produce (*E*)- β -caryophyllene above- and belowground after herbivore attack (Rasmann *et al.* 2005). The American inbred line B73 lost this ability and lacks a potential resistance mechanism against herbivore attackers (Köllner *et al.* 2008). Both maize lines express TPS10 after leaf attack by *S. littoralis*, which forms the major sesquiterpenes (*E*)- β -farnesene and (*E*)- α -bergamotene (Schnee *et al.* 2006). The *tps23* structural gene is intact in several American maize lines, but it is not transcribed after herbivore treatment (Köllner *et al.* 2008). The loss of *tps23* transcript accumulation might be the result of a disruption in the signaling cascade. To test the involvement of nine transcription factors into the regulation of TPS23, we tested their expression in B73 and Delprim. All factors were induced after herbivory in B73 and Delprim. Only three factors, *tf23*, *tf34*, and *tf42*, displayed a significantly higher transcript level in Delprim than in B73. Most likely, the difference in TPS23 volatile production cannot be explained by those factors.

4.2.3 Herbivore-induced transcription factors are localized throughout the genome

Transcription factors with altered expression level after herbivore feeding were mapped against the genome of *Zea mays* variety B73 in order to investigate their spatial distribution. The sesquiterpene synthases *tps10* and *tps23* were also added to this map (Fig.4.2). The differentially expressed transcription factors were evenly distributed among the chromosomes in maize and no clear pattern could be observed. Also, the transcription factors did not seem to cluster specifically to one region.

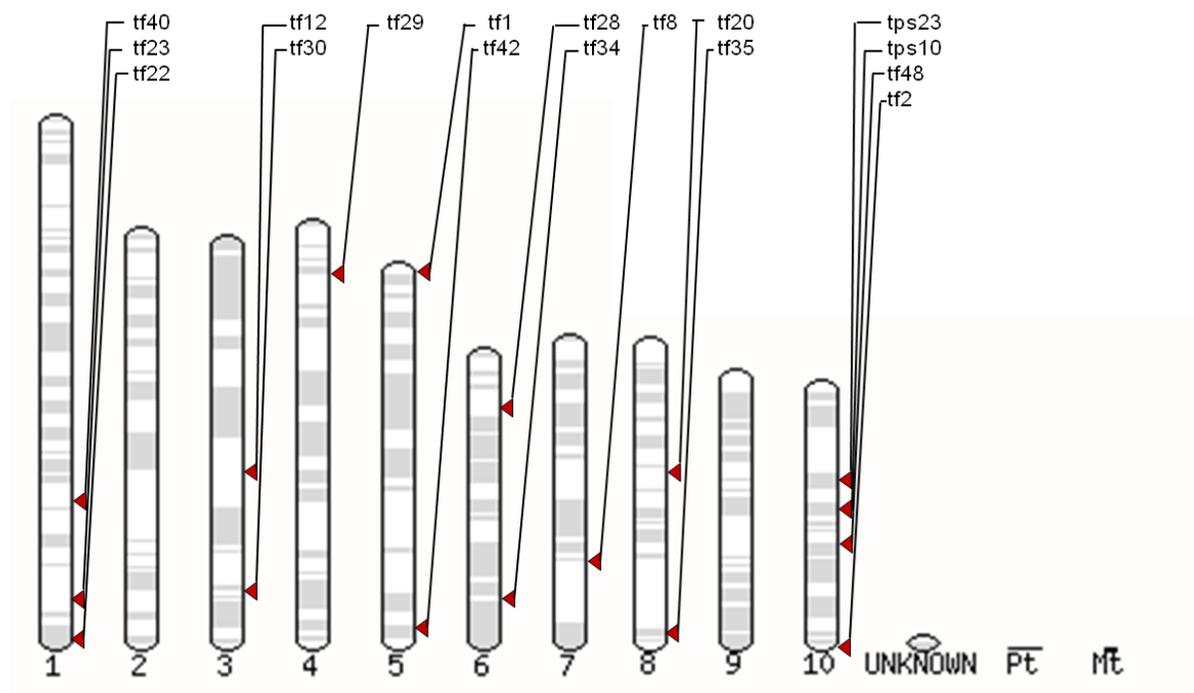


Fig.4.2: Approximate localization of the differentially expressed transcription factors among the maize genome. Sequences of transcription factors were blasted using maizesequence.org. In addition *tps10* and *tps23* are displayed. (maizesequence.org)

An operon-like gene cluster required for the triterpene synthesis (thalianol pathway) was reported for *Arabidopsis thaliana* (Field and Osbourn 2008). Also a cluster for the synthesis of antimicrobial terpenes (avenacins) was identified in oat (*Avena strigosa*). Avenacins accumulate in roots after the attack of pathogens (Wegel *et al.* 2009). However, results from this work indicate that herbivore-induced genes and their regulators are not organized in such a way.

4.3 The plant hormonal response is specific for the induction stimuli

The phytohormones JA, SA, and ET play an essential role in the regulation of induced defenses which are specific for the attacker (Reymond and Farmer 1998; Pieterse and van Loon 1999; Glazebrook 2001). Biotrophic pathogens are sensitive to SA-dependent responses, whereas JA/ET-dependent mechanisms are more effective against herbivore insects and necrotrophic pathogens (Thomma *et al.* 2001; Glazebrook 2005). De Vos and coworkers showed that herbivore feeding can induce cross-resistance against several

microbial pathogens. Also, ET can act synergistically on SA-inducible defenses and might therefore be responsible for an enhanced resistance against the biotrophic pathogen *Turnip crinkle virus* (De Vos *et al.* 2006).

In this work, the hormone level after mechanical wounding with or without the application of caterpillar regurgitate was investigated. Addition of caterpillar regurgitate to the mechanically damaged area imitated herbivore feeding. The results demonstrated that all measured phytohormones, JA, SA, JA-Ile, and ABA take part in the formation of the anti-herbivore responses. While jasmonic acid is highly induced when plants experience mechanical damage in connection with caterpillar regurgitate, the jasmonic acid-isoleucin derivate was increased only after half an hour to one hour after regurgitate treatment and declined afterwards. The level of both hormones was also significantly increased after mechanical damage only, but not as high.

Abscisic acid, an indicator for plant stress (Davies and Zhang 1991), was present at all times during the experiments. The levels of this hormone were significantly higher after induction with regurgitate compared to mechanical damage only. Since ABA is also involved in the regulation of stomata closure and water stress (Tardieu and Davies 1992), it is likely that this hormone is produced when the plants suffer from leaf damage connected with water loss (Erb *et al.* 2011).

The most intriguing observation was made for the plant hormone salicylic acid. SA is known to be induced after pathogen attack (Glazebrook 2005) and recent studies showed that there is synergistic cross talk between SA and JA, depending on the dose of SA (Mur *et al.* 2006). This is in contrast to observations where SA and JA acted antagonistically (Kazan and Manners 2008). To make the situation even more complex, SA was also found to be involved in plant defenses against herbivore attack (Zarate *et al.* 2007).

We observed that the level of SA increased similar to the level of JA after mechanical damage and caterpillar regurgitate. Mechanically damaged plants did not show an induction of SA level, except for the 4 h time point measurement. This indicates that the induction stimulus for SA is not mechanical damage by itself but rather the combination of damage and regurgitate. It was not possible to test whether this effect was due to regurgitate alone, since the development of induction methods of plants without harming the leaf surface were not successful. It is possible that maize reacts to herbivory with the production of SA to be

prepared for a subsequent pathogen attack at the site of herbivore damage. Herbivory does not alter SA level in infested maize roots (Erb *et al.* 2010), indicating that roots and leaves act differently upon attack. Caution has to be taken with this assumption, since the data from this work resemble time points within four hours after treatment. This differs from the *D. virgifera* infested roots used by Erb *et al.* (Erb *et al.* 2009) which were induced for 18 h. Also, the concentration of JA and JA-Ile in herbivore-induced roots differ from those of induced leaf material (Erb *et al.* 2009), indicating that hormone production is regulated differently in roots and leaves.

4.4 The transcription factors TF23 and TF30 are differentially regulated

Only two transcription factors, *tf23* and *tf30*, displayed an expression pattern specific for mechanical damage and caterpillar regurgitate, respectively. *Tf30*, annotated as ethylene responsive element, belongs to the class of AP2/EREBP transcription factors. This class of transcription factors is unique to plants (Riechmann *et al.* 2000) and binds two similar regulatory elements: the GCC-box and dehydration-responsive element (Singh *et al.* 2002). The large group of ethylene responsive factors has been implicated in regulating gene expression after several stresses like cold, drought, and pathogen attack, as well as responses to the hormones ET, SA, and JA (Onate-Sánchez and Singh 2002; Singh *et al.* 2002). Ethylene-responsive transcription factors that interact with the GCC-box were characterized in tobacco and Arabidopsis (Ohme-Takagi and Shinshi 1995; Solano *et al.* 1998; Fujimoto *et al.* 2000). Other studies showed that ERF are able to bind to the APETALA2 domain in the ORCA proteins (octadecanoid-responsive Catheranthus APETALA2) and therefore provide a link between jasmonic acid and the accumulation of terpenoid indole alkaloids (van der Fits and Memelink 2000; Memelink *et al.* 2001). An 84 % amino acid similarity was found between TF30 and the AP2/ERF9 protein in Arabidopsis. This gene was found induced after root colonization with the endophytic fungus *Piriformospora indica* (Camehl and Oelmüller 2010). Interestingly, homologues of *tf30* in tobacco and rice are known to induce hypersensitive response-like cell death after induction with *Tobacco mosaic virus* (Ogata *et al.* 2012) and are therefore implicated in defenses against pathogens.

Tf23 is a member of the bHLH transcription factor family. Only a weak similarity was found to a basic helix-loop-helix protein in *Arabidopsis lyrata* which has not been characterized yet. In tobacco, the homolog NtWIN4 is implicated in the hypersensitive defense and induced after wounding and pathogen attack (Kodama and Sano 2006). In maize, *tf23* seems to be involved in other plant responses. While *tf23* is expressed at a higher level after mechanical wounding and caterpillar regurgitate after half an hour and one hour, *tf30* showed an inverted expression pattern. This transcription factor reacted more strongly upon mechanical damage alone, indicating that there might be two different signaling pathways present (Fig.4.3).

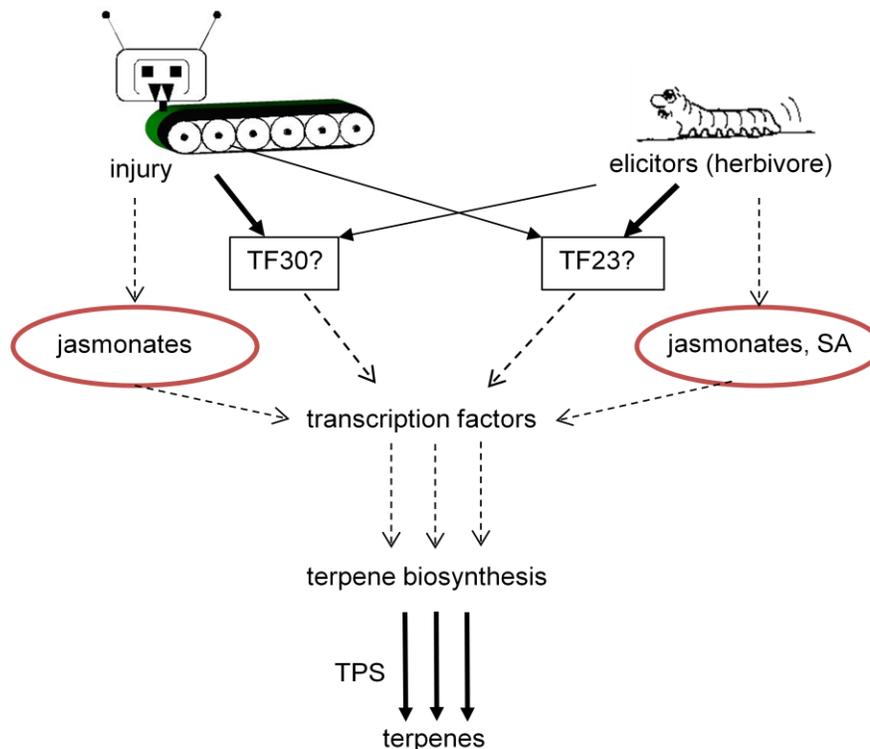


Fig.4.3: Schematic overview of the two hypothetical induction pathways in maize. Maize responds to mechanical damage and elicitors. The transcription factor TF30 is highly induced after mechanical damage, but only slightly after application of elicitors (caterpillar regurgitate). TF23, on the other hand, shows a higher expression level after induction with elicitors. The plant hormones react differently upon the two induction modes. While salicylate is only induced after elicitor treatment, jasmonates (JA and JA-Ile) are induced after both induction types. These hormones might act upon the regulation of transcription factors which in turn are responsible for the activation of terpene synthases (TPS). Dashed arrows represent multiple steps, bold arrows indicate a strong induction, and thin arrows indicate a weak induction.

In maize, it might be possible that transcription factor TF30 activates target genes after mechanical damage while TF23 is responsible for induction of target genes after herbivore attack. Still, it is possible that *tf23* might also reduce responses to herbivory, working in favor of the herbivore. This has been shown for *Nicotiana tabacum* and the herbivore *Helicoverpa zea*. In this example, the glucose oxidase in the caterpillar saliva suppresses the production of nicotine induced by caterpillar feeding (Musser *et al.* 2002).

Another indication for two different signaling cascades is the induction of SA after elicitor treatment only. The plant hormone jasmonic acid appears in both pathways.

4.5 Identification of regulatory sequences in the promoters of *tps10* and *tps23*

4.5.1 The binding ability of TF1, TF8, TF20, and TF22 to the promoters of *tps10* and *tps23*

In order to find transcription factors that bind to the promoter of *tps10* or *tps23*, four transcription factors were chosen for analysis. The transcription factors (TF1, TF8, TF20, and TF22) were selected because of their early induction after herbivore attack which was in contrast to the slower induction of *tps10* and *tps23*. The transcription factors *tf1* and *tf8* showed an increased expression level within half an hour after herbivore treatment. The remaining factors *tf20* and *tf22* displayed a negative regulation within the first half an hour. Both induction types were included in the band shift assays because it is not known whether *tps10* and *tps23* are subject to activation or de-repression.

Transcription factors TF1 and TF22 are annotated as NAC transcription factor (NO APICAL MERISTEM (NAM), ATAF1, 2 and CUP-SHAPED COTYLEDON (CUC2)) and have been described first by Aida *et al.* (Aida *et al.* 1997). More than 90 putative NAC-domain proteins in Arabidopsis and rice are implicated in plant development (Delessert *et al.* 2005) and some are induced by pathogen infection and/or mechanical wounding (Hegedus *et al.* 2003). With a domain similar to MYB-like transcription factors (Tran *et al.* 2004) and a binding overlapping the site of bZIP transcription factors (Duval *et al.* 2002), those factors seem to be a good candidate for binding studies. Nevertheless, the NAC transcription factors bind to the

CATGTG motif (Tran *et al.* 2004) that is present in the *tps10* and *tps23* promoters. In both promoters, this motif is found twice in the maize line Delprim. In the *tps10* promoter the binding motif is found at -22 bp and -488 bp upstream of the start codon, in the *tps23* promoter at -281 bp and -649 bp.

As member of the bHLH transcription factors, TF8 was chosen for analysis. This class of transcription factors recognizes G-box motifs in the promoter of target genes (Atchley and Fitch 1997). Analysis of the promoter sequences of *tps10* and *tps23* revealed several putative G-boxes over the whole promoter region.

Transcription factor TF20 was annotated as ETTIN-like auxin response factor. Auxin response factors were found to be induced in poplar after herbivore attack (Ralph *et al.* 2006). Those auxin response factors bind to a TGTCTC motif (Tiwari *et al.* 2003). This sequence motif was only found in the promoter of *tps10* at the locations -424 bp, -634 bp, and -1211 bp upstream of the start codon. Auxin levels were not investigated in this study, but this factor was included in the analysis because of three putative binding sites.

Unfortunately, no binding could be observed between the promoter fragments and the expressed transcription factors in EMSA experiments (3.3.1). Besides the absence of the proper binding site, lack of interaction could be due to improper assembly of the ORF of the factors or secondary structures of the promoter fragments used in the EMSA.

4.5.2 The promoters of *tps10* and *tps23* possess similar regulatory *cis*-acting elements

Each promoter has an exclusive organization of *cis*-acting sequences and therefore a unique platform for the binding of *trans*-acting factors. This arrangement is determined by the number of *cis*-acting elements, their distance to each other, their orientation, and their super-hierarchical order (Arguello-Astorga and Herrera-Estrella 1996; Acevedo-Hernández *et al.* 2005; Cazzonelli and Velten 2008).

Only small nucleotide differences were observed in the *tps23* promoter sequences of several maize lines and no clear pattern of differences was observed between a functional and a non-functional promoter. This suggests that the differences between the lines are due to *trans*-acting components in the signaling cascade (transcription factors, kinases, etc.). Still, the crucial regulatory elements could be located upstream of the assayed promoter sequences.

The promoter sequences of *tps10* differed more among (*E*)- β -farnesene-producing lines than those of *tps23*.

TPS10 and TPS23 are expressed in different organs (Köllner *et al.* 2004) and displayed varied terpene emission after herbivore feeding. Therefore, both promoter sequences were investigated to find specific elements that are responsible for the distinctive expression patterns. Truncation experiments with GUS-fusion constructs revealed an active 1.5 kb *tps10* promoter. The activity was lost in the 800 bp promoter fragment. In between those two fragments, only few *cis*-acting regulatory elements were identified: a WUN motif, 10 TATA-boxes, and four CAAT-boxes. TATA-boxes are common core promoter elements (Timko *et al.* 1985) while CAAT-boxes are common *cis*-acting elements and enhancer regions (Dorn *et al.* 1987). They respond to light, cytokinin and the functional development of plastids (Kusnetsov *et al.* 1999). Both types of regulatory sequences could be essential for *tps10* promoter activity, but are present throughout the whole promoter. The same was observed for G-boxes which are implicated in JA responsiveness and wounding (Menke *et al.* 1999; Delessert *et al.* 2004) and are distributed evenly in the promoter of *tps10*. An overview of the *cis*-acting elements that might be involved in the transcriptional regulation of *tps10* is shown in Fig.4.4.

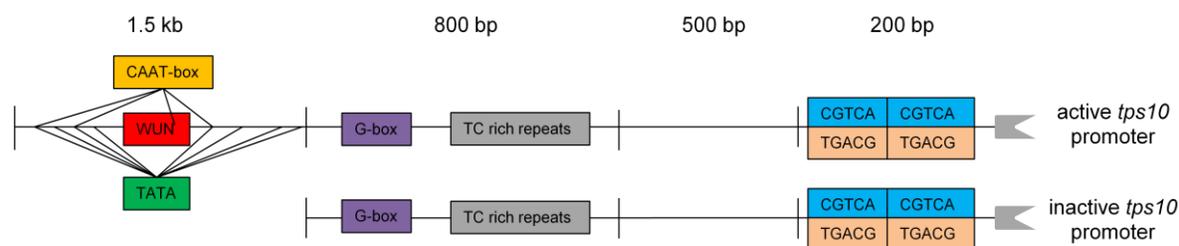


Fig.4.4: Schematical view of the *tps10* promoter. Truncation experiments were conducted using a 1.5 kb, 800 bp, 500 bp, and 200 bp fragment, respectively. Some elements with potential importance for regulation are displayed. Removal of the first fragment results in the loss of *tps10* promoter activity.

The results of the induction experiments revealed that *tps10* can only be activated when two induction stimuli are combined. Activity was only detected when Arabidopsis plants were induced by scratching the leaf and addition of jasmonic acid to the wounded site. Wounding

or jasmonic acid by itself were not able to induce *tps10* promoter activity. In order to get a more detailed view of the regulatory sequences of *tps10*, a promoter fragment 5' upstream of the 1.5 kb fragment should be investigated for additional elements.

For *tps23*, the mode of induction is different. While the 1.8 kb and 1.2 kb fragments were active upon JA treatment, the 600 bp fragment was active by mechanical wounding alone. The 400 bp fragment showed no promoter activity. Hence, the essential regulatory elements for *tps23* are between the 600 bp and the 400 bp fragment. Sequence analysis using PLACE and PlantCare showed several hypothetical regulatory elements (Fig.4.5).

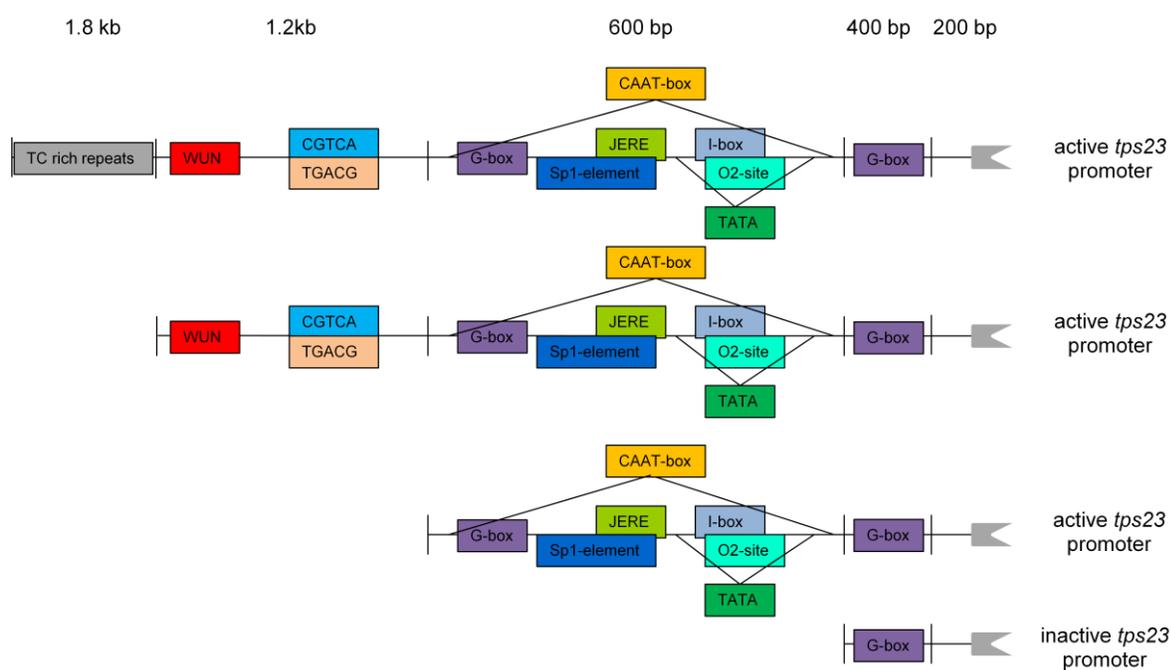


Fig.4.5: Analysis of hypothetical regulatory sequences in fragments of the *tps23* promoter. Deletion analysis was performed using promoter:: β -glucuronidase reporter gene constructs with promoter sizes 1.8 kb, 1.2 kb, 600 bp, 400 bp, and 200 bp. Potential regulatory elements are displayed. The 400 bp and 200 bp long promoter fragments were found to be inactive.

CAAT-boxes were also found throughout the *tps23* promoter. The Sp1 element resembles a GC box bound by the SP1 transcription factor (Letovsky and Dynan 1989). Also regulated by light and the circadian clock is the I-box element. This element was found in several promoters of *cab*-genes, encoding light-harvesting chlorophyll a/b binding proteins (Borello *et al.* 1993). Another potential regulatory element, the O2-site, plays a role in the zein

metabolism (Kemper *et al.* 1999) and therefore is unlikely to be involved in the regulation of *tps23*. TATA-boxes are also found in 600 bp fragment of the promoter. It has been reported that TATA-boxes are essential for transcription at positions up to -1598 bp from the start codon (Keddie *et al.* 1992) to -30 bp of the transcription start in tomato (*Lycopersicon esculentum*, (Cordes *et al.* 1989)). It is therefore not possible to say which TATA-box plays a crucial role for the regulation of *tps23*. The JERE motif responds to JA and MeJA (Menke *et al.* 1999; Chini *et al.* 2007) but cannot be an essential element regulating JA responses because the 600 bp promoter fragment containing the JERE is not active after JA treatment, but only inducible through mechanical damage. The other interesting *cis*-acting element is the G-box. Mainly involved in JA responses (Menke *et al.* 1999), it has also been found in large proportions of wound-inducible promoters (Delessert *et al.* 2004). This could be an indication for the essential role of the G-box in the *tps23* 600 bp::GUS promoter fragment. The class of basic Helix Loop Helix transcription factors are known to bind G-boxes (Toledo-Ortiz *et al.* 2003), whereas the JERE motif is recognized by AP2/ERF domain transcription factors (Menke *et al.* 1999).

Sequence analysis of the *tps23* promoter revealed two additional MeJA responsive elements in the 1.2 kb promoter fragment. The CGTCA motif is localized at -658 bp before the start codon, and the TGACG element (Rouster *et al.* 1997) is complementary to the first one, but localized in opposite direction on the minus strand. This sequence motif is missing in the 600 bp promoter fragment and could explain the loss of promoter activity after jasmonic acid treatment. Thus, there might be two distinct induction pathways for TPS23. The first pathway regulates via the induction of jasmonic acid and the second through mechanical wounding. This second pathway might be independent from JA. Nevertheless, it is still possible that the endogenous jasmonic acid induced in response to mechanical wounding of the plants activates the promoter of *tps23*. In addition, TPS10 is only found in induced leaves of *Zea mays* (Köllner *et al.* 2004) while TPS23 is synthesized in both herbivore-induced shoots and roots (Rasmann *et al.* 2005). The results confirm the idea of distinct signaling transduction pathways for *tps10* and *tps23* which utilize different cues for induction.

With the identification of crucial promoter elements, more transcription factor candidates could be tested in binding assays. So far, band shift assays revealed no binding of the G-box binding factor TF8 to the promoters of *tps10* and *tps23*. The transcription factor *tf23* is also

annotated as bHLH and is highly induced after herbivore attack. This factor would be an interesting candidate for further binding analysis because several G-boxes are present throughout the promoters of both terpene synthases. Annotated as an ethylene responsive element, the transcription factor *tf30* might serve as a binding protein for the JERE motif localized in the promoter of *tps23*. Although deletion analysis indicated that this element is not crucial for *tps23* expression, secondary effects with other binding factors or secondary structures cannot be excluded.

One has to keep in mind that the promoter deletion analyses were conducted with maize promoters in *Arabidopsis* plants. Conclusions have to be drawn carefully, because differences in the defense regulation of the two plant species cannot be ruled out.

4.5.3 The promoters of *tps10* and *tps23* respond to mechanical damage and herbivore attack

The sequence analysis revealed a WUN-motif in the promoter of *tps10* (-1097 bp and -1088 bp) and in the promoter of *tps23* (-692 – -684 bp). Deletion of a fragment containing the WUN-motif resulted in an inactive promoter of *tps10*. Because this motif was found to regulate the signaling pathway after wounding (Matton *et al.* 1993), the promoter sequences for this motif were compared between different maize lines.

The promoter sequences of *tps10* were compared between the maize lines Delprim, OH7B, MS71, and B73 (Fig.4.6). The lines Delprim, OH7B and B73 are able to produce TPS10 specific volatiles after herbivore damage, while MS71 does not. The WUN motif is present in all four lines regardless of the ability to produce TPS10-specific volatiles. Overall, the results suggest that the WUN motif is an important regulatory sequence in the promoter of *tps10* but not responsible for the ability to express TPS10 in the lines investigated here.

Delprim	ATC	TTCGTAT TGA	AATATTTT	} TPS10 volatiles
OH7B	ATC	TTCGTAT TGA	AATATTTT	
B73	ATC	TTCGTAT TGA	AATATTTT	
MS71	ATC	TTCGTAT TGA	AATATTTT	
				→ TPS10 volatiles

Fig.4.6: Sequence analysis of the WUN motif of *tps10* promoter in different maize lines. Sequence is shown from -1304 to -1296 upstream of the start codon. The WUN motif is highlighted in green. Maize line MS71 is not able to produce TPS10 specific volatile, although it possesses a complete WUN motif in its *tps10* promoter.

Promoter fragments of *tps23* are active up to -400 bp, indicating that the WUN-motif is not crucial for this promoter. It rather seems that the WUN-motif has a general activating role for *tps23*. The WUN-motif was compared between the maize lines to investigate potential correlations between the motif and gene activity (Fig.4.7). These maize lines were selected because they differ in their ability to produce (*E*)- β -caryophyllene. The European maize line Delprim is able to produce this volatile terpene, while the American maize line B73 seems to have lost this ability (Köllner *et al.* 2008). The lines NC358, Du101, and F476 possess a functional TPS23 enzyme while CML247, OH7B, and MS71 are not able to produce (*E*)- β -caryophyllene after herbivore attack (Degen *et al.* 2004). With the exception of Du101 and NC358, no differences in the WUN-motif between these maize lines were found. The single nucleotide exchange in those two lines does not seem to affect caryophyllene biosynthesis.

Du101	TTTGGCTTT G CCGTAATG AT	
NC358	TTTGGCTTT G CCGTAATG AT	
F476	TTTGGCTTT G TCGTAATG AT	
Delprim	TTTGGCTTT G TCGTAATG AT	
OH7B	TTTGGCTTT G TCGTAATG AT	
B73	TTTGGCTTT G TCGTAATG AT	
MS71	TTTGGCTTT G TCGTAATG AT	
Cml247	TTTGGCTTT G TCGTAATG AT	

Fig.4.7: Sequence analysis of the *tps23* promoter of different maize lines. Promoter sequences were amplified from genomic DNA, cloned and sequenced. The sequence comparison from -697 to -689 upstream of the start codon is shown. The WUN motif is marked in green, bold letters indicate a nucleotide exchange.

Sequence comparisons among the promoters of the lines also revealed the presence of the JERE, G-box, CGTCA, and CAAT-boxes in the same position range (data not shown). Therefore, loss of (*E*)- β -caryophyllene production is not likely due to differences in the *cis*-elements of this promoter fragment, but probably due to the loss of other factors in the signaling cascade.

5. Outlook

The aim of this project was to identify herbivore-regulated components of the cellular signal transduction network by microarray hybridization. This technique successfully identified a group of transcription factors with herbivore-induced alterations in gene expression. There are, however, some limitations associated with this technique since it does not identify constitutively expressed genes or factors that are activated or de-activated by mechanisms other than gene expression. An alternative approach is the identification of signal transduction components by molecular mapping. While these methods provide additional information about the function of the signal transduction components, they will not identify factors with redundant function. The combination of both methods might generate a complete overview of the plant responses after herbivory.

In continuation of this project, the investigation of transcription factors with overexpressing lines and knockout maize lines might elucidate their functions. Due to the apparent conservation of these pathways between maize and other plants, homologous transcription factors in *Arabidopsis thaliana* or *Nicotiana tabacum* could provide further information while making use of more accessible transformation systems. Another valuable resource are maize transposon knockout plants that are currently developed by several public outlets. We are constantly checking these resources for knockouts of the candidate genes identified in this study.

Further promoter deletion analysis will clarify the role of the *cis*-acting elements identified in the promoters of *tps10* and *tps23*. In the background of an *Arabidopsis* COI1 mutant, the effects of JA-mediated pathways can be evaluated. A transient expression system of transcription factors and promoter fragments which was established in tobacco could serve as an alternative method to identify potential targets (Van Der Linde *et al.* 2011).

6. References

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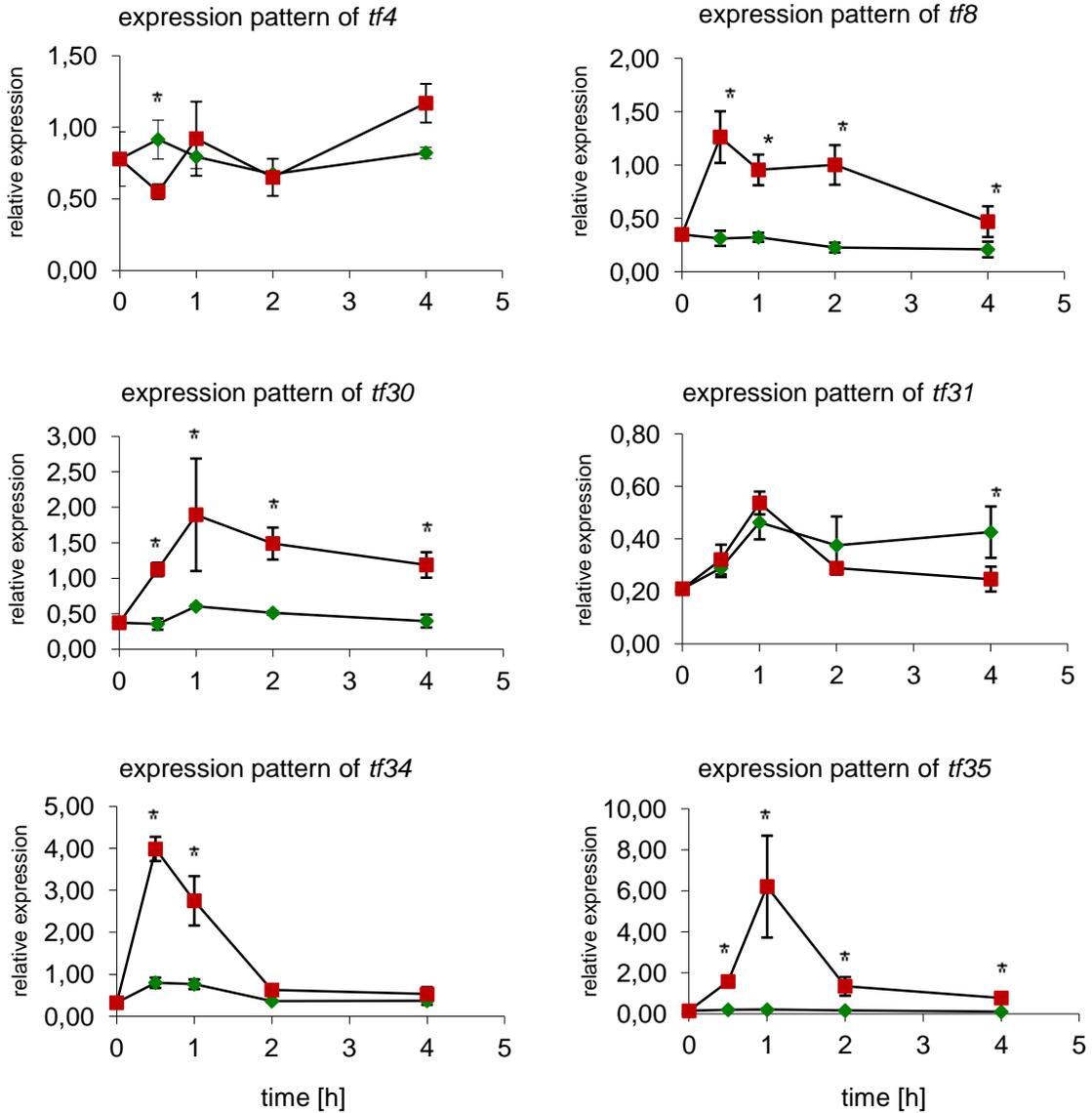
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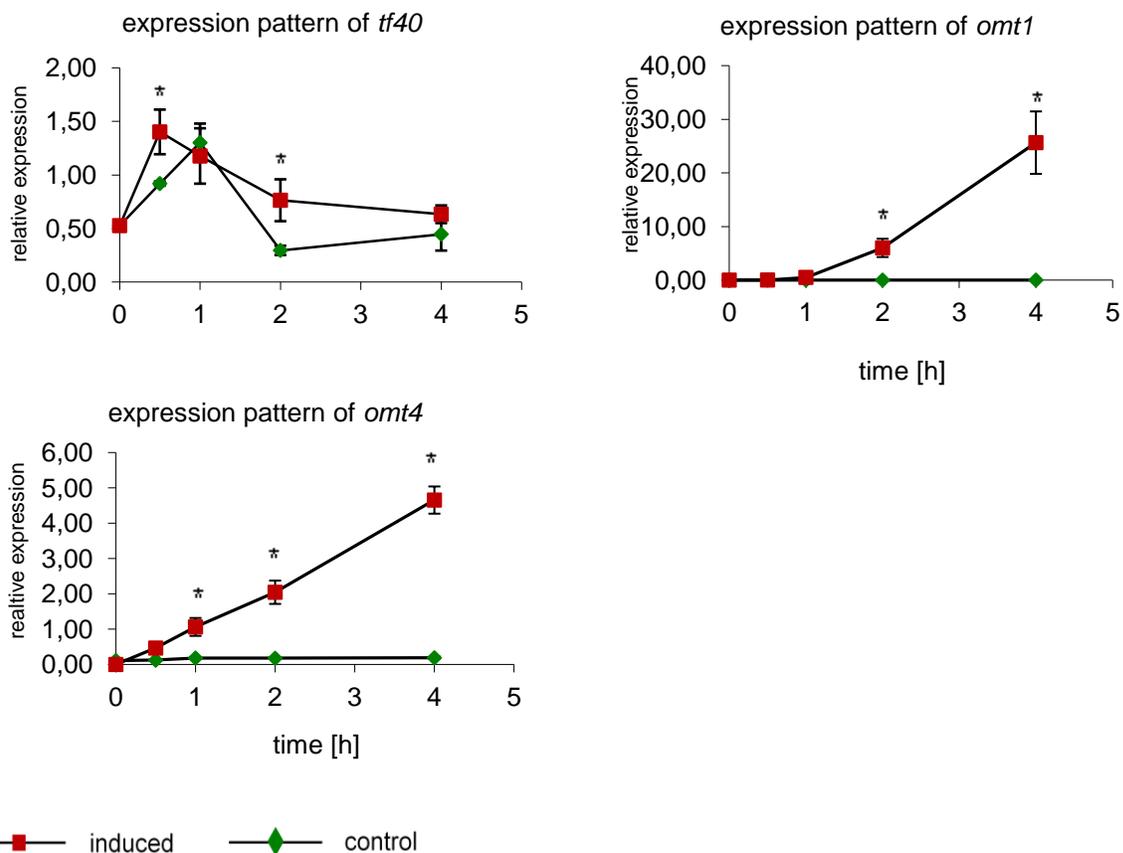
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7. Appendix

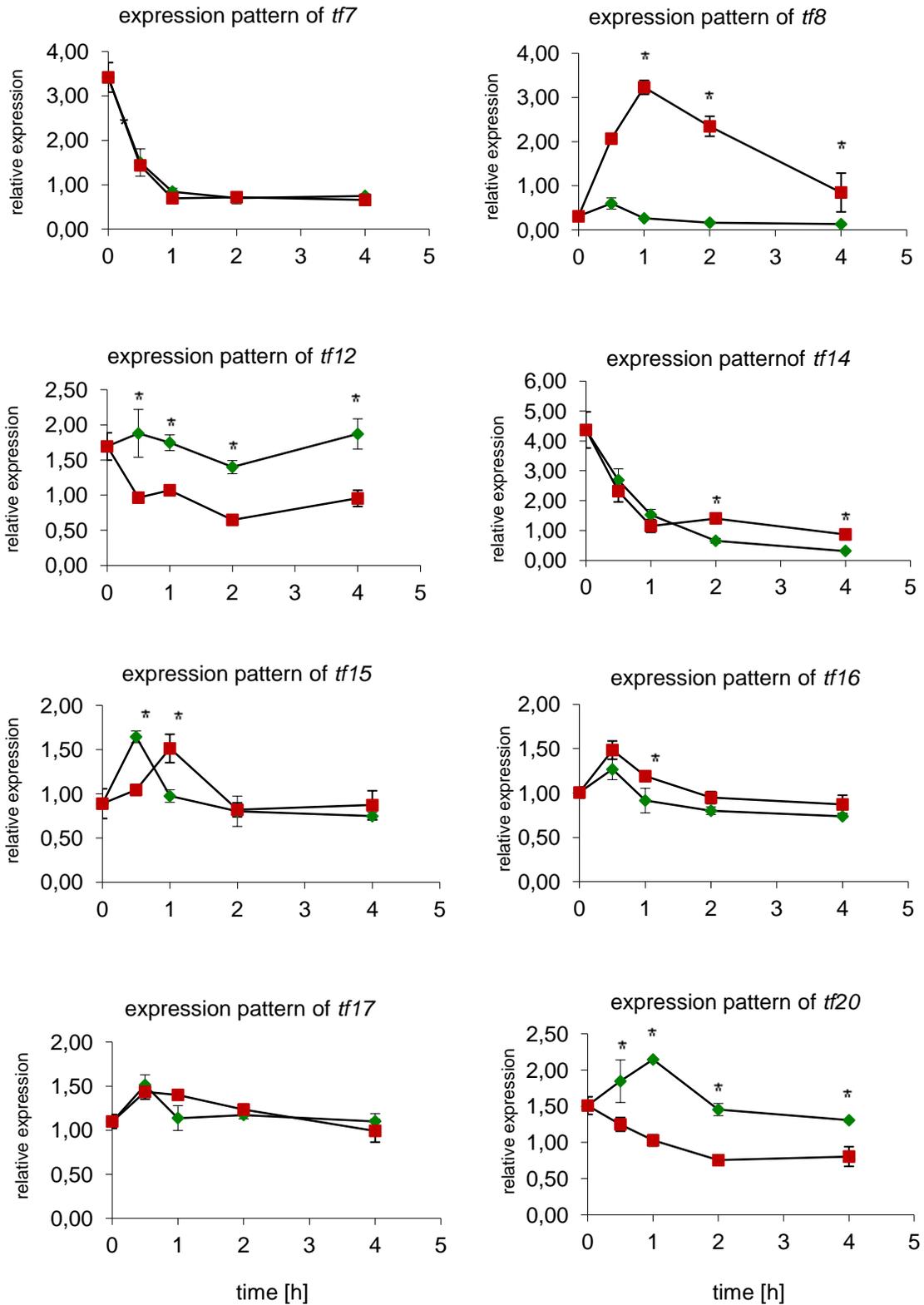
7.1 QRT-PCR of the plant material treated with 30 min herbivore feeding

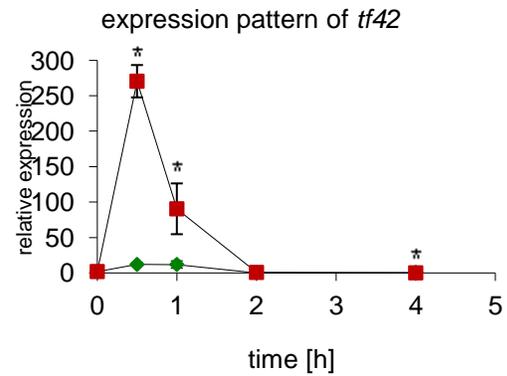
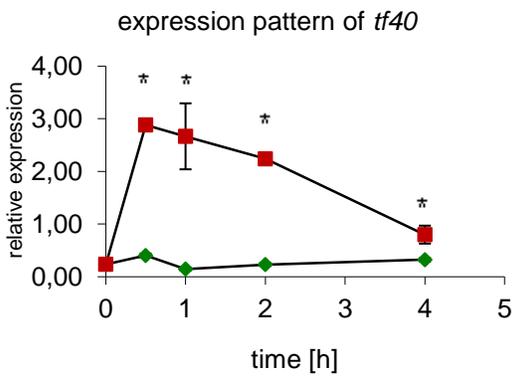
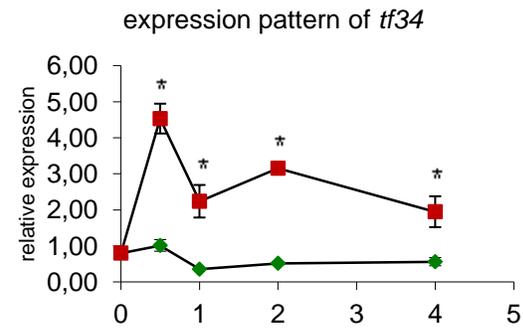
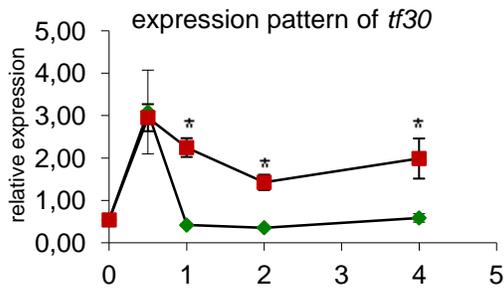
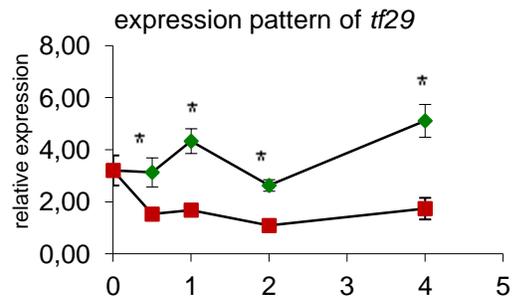
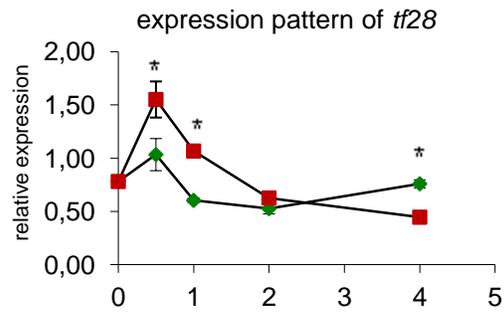
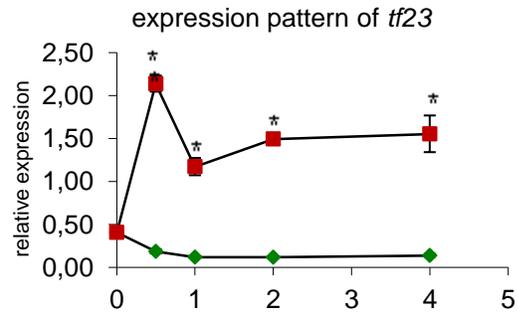
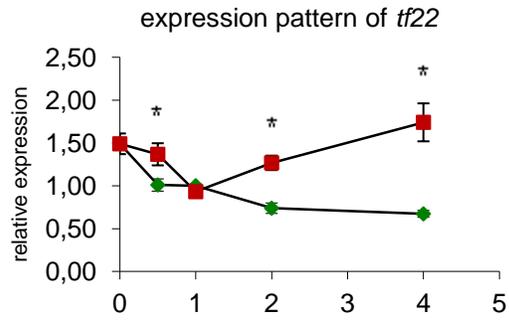


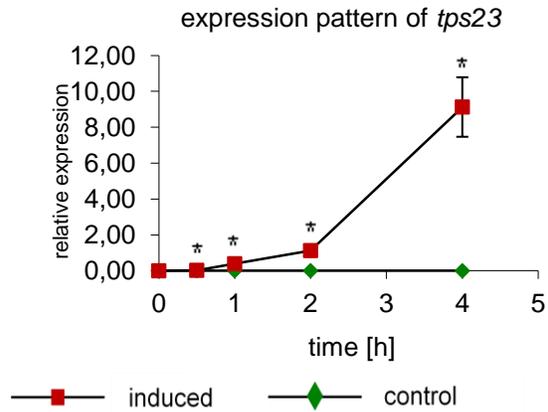


Relative expression pattern of further transcription factors and terpene synthases tested after 30 min herbivore feeding. Plants were treated with *S. littoralis* caged for 30 min on a single leaf. Material was harvested after: 0 h, 0,5 h, 1 h, 2 h, and 4 h. Means and standard error are shown (n=4). The relative expression levels were calculated as described in 2.4.9. Two-Way ANOVA was used to calculate statistical differences with time as factor one and treatment as second variable. * indicate significant differences between treatments within time points ($p < 0.05$).

7.2 QRT-PCR of plant material treated with continuous feeding

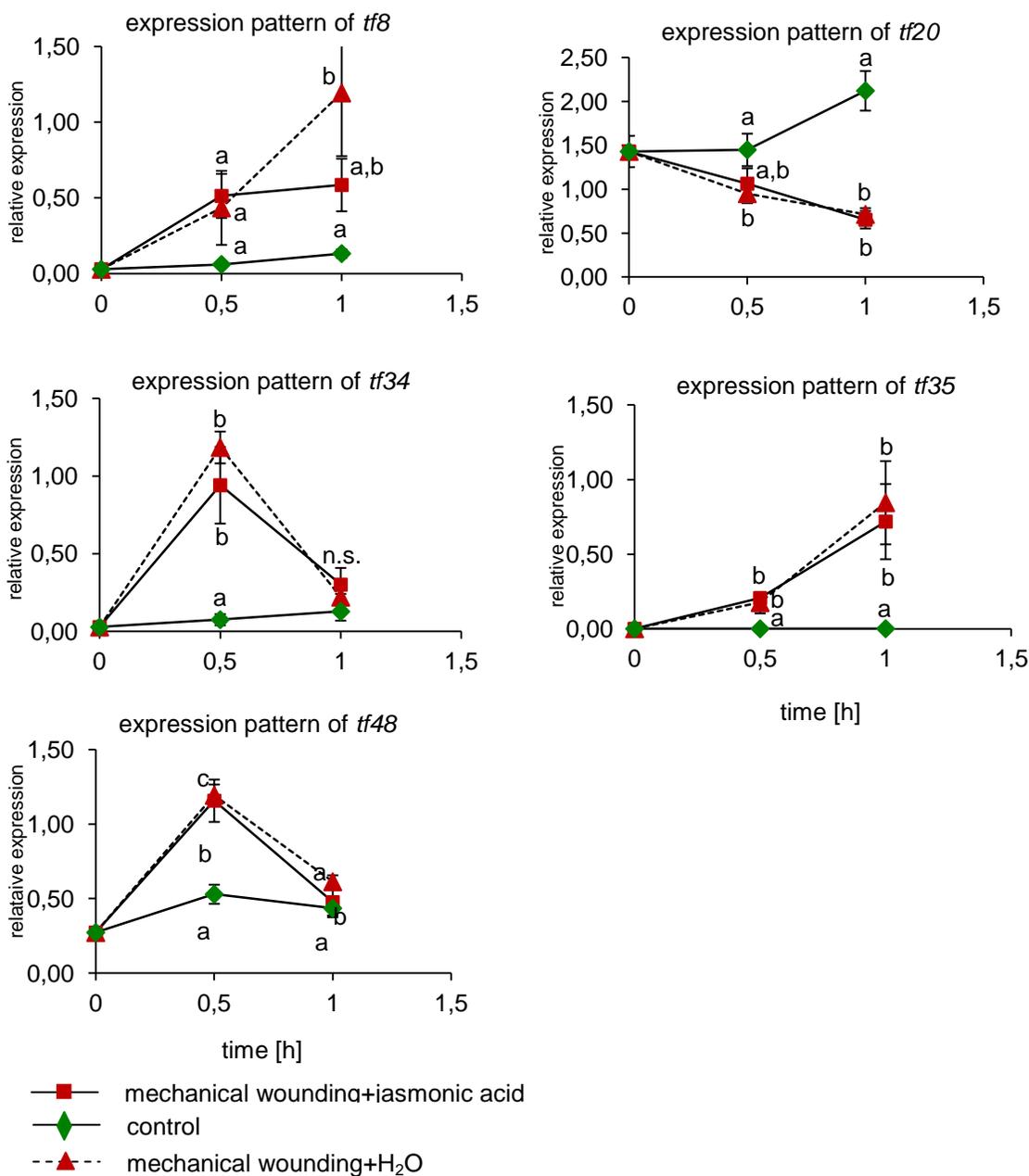






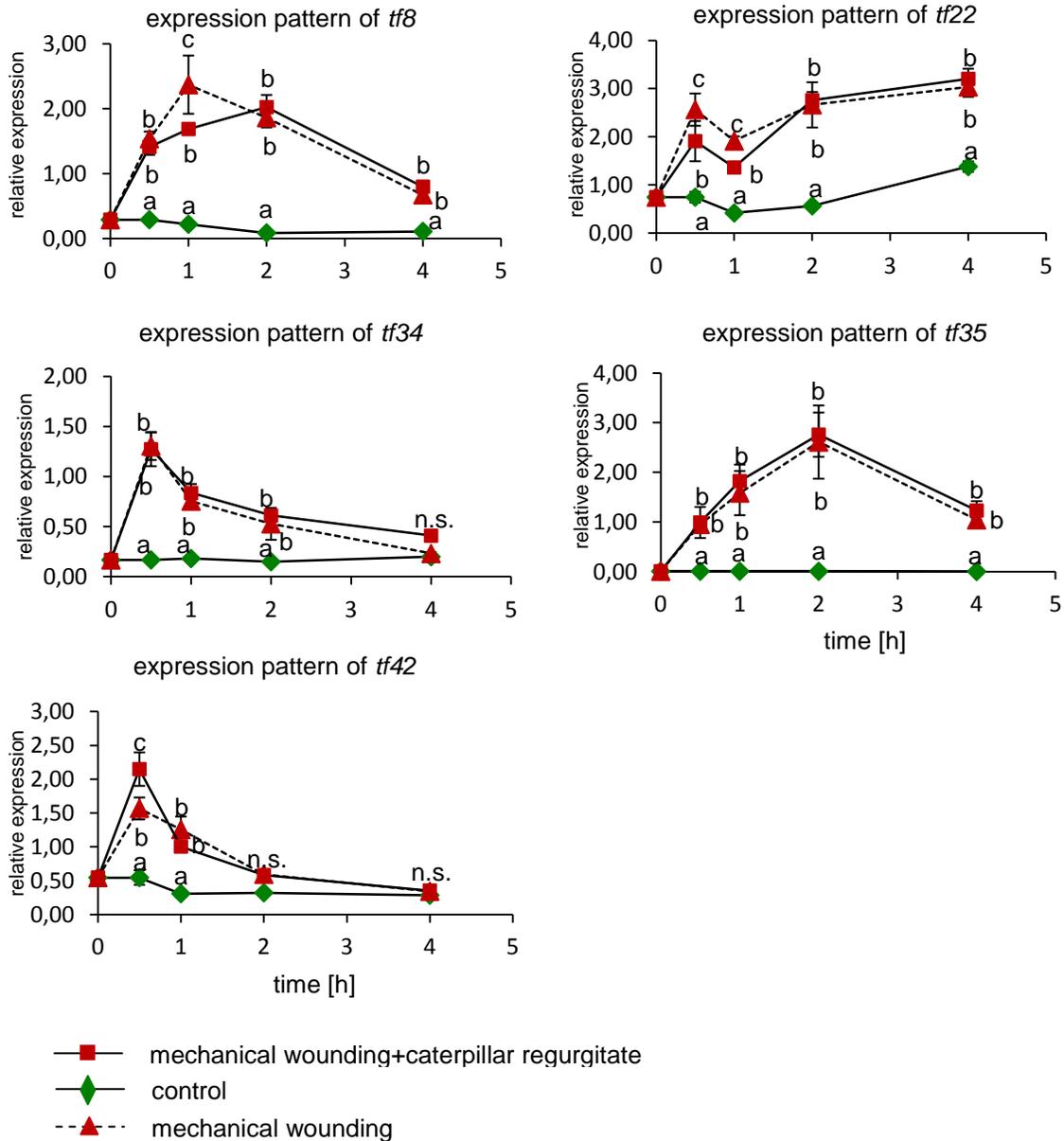
Relative expression pattern of further genes tested after continuous herbivore feeding. Expression pattern is shown over a time period of 4 h. Delprim maize leaves were treated with the caterpillar larvae *S. littoralis* until leaf material was harvested. Induced expression pattern is marked with a red square, control points with a green diamond. Expression was calculated against the housekeeping genes. Standard error (n=3) is shown for each measurement. Two-Way ANOVA was used to test significant differences with $p < 0.05$ defined as significant for the treatment within the time points (*).

7.3 QRT-PCR of plant material induced with jasmonic acid



Expression profile of further transcription factors and *tps23* after induction with jasmonic acid. Plants were cut and put into water or water containing jasmonic acid, respectively. The induction time was set to 1 hour to analyze early transcriptional changes. The relative expression was calculated as the expression of the housekeeping gene HG2. Means and standard errors are shown (n=3). Two-Way ANOVA was performed with time as the first variable and treatment as second factor. Different letters indicate significant differences between treatments within time points (p<0.05).

7.4 QRT-PCR of mechanical treated plant material (Mecworm)



QRT-PCR analysis of further transcription factors after mechanical induction with and without caterpillar regurgitate. Plants were treated with mechanical damage and mechanical damage plus the addition of caterpillar regurgitate. Means and standard errors are shown (n=4). The relative expression was calculated as described in 2.5.9. Two-Way ANOVA was performed with time as the first variable and treatment as second factor. Different letters indicate significant differences between treatments within time points ($p < 0.05$).

7.5 DNA sequences of expressed transcription factors

>*tf1*- Delprim

ATGACAGCGCAGAACCCGCCCACAGGACAGGACACACAGAGCCGAGCCACCCACCCA
CCATCGGCGACCAGCAGCCAGCCGCGGGAGCGAGCTGTTTAAAGACACCGAGTCGGAGT
CGGACGGAGGACTGGCAGGCACAACCGAAGCCACCGCTTCTAGTTCTCGGGTTCATCGC
CAGCAATCCAGACCACATAATGGGACTGCCGGTGACGAGGAGGAGGGAGAGGGACGCG
GAGGCGGAGCTGGACCTGCCGCCGGGGTCCGGTTCACCCACCGACGACGAGCTGGT
GGAGCACTACCTGTGCCGCAAGGCGGCGGGGCAGCGCCTCCCCGTGCCATCATCGCCG
AGGTGGACCTGTACAGTTTCGACCCCTGGGACCTGCCGGAGCGCGCGCTCTTCGGGGCC
CGGGAGTGGTACTTCTTCACGCCAGGGACCGCAAGTACCCCAACGGCTCCCGCCCCAA
CCGCGCCGCGGCGACGGATACTGGAAGGCCACCGGCGCCGACAAGCCCGTCGCGCCGC
GCGGCGCCCGCACGCTCGGGATCAAGAAGGCGCTCGTCTTCTACGCCGGCAAGGCGCCG
CGCGGGGTCAAGACGGACTGGATCATGCACGAGTACAGGCTCGCTGACGCCGGCCGCG
CGCCAAGAAAGGGTCGCTCAGGGTAAGTTTCTACACACACAAAAAAGAGGGGTGAA
CGAGGAATTTTTTAG

>*tf8*- Delprim

ATGAAACACCACCACCACCACCACCACGGTGGTCTGGTTCGCGTGGTTCATGGC
GGATCCGAATTCATGGAGTTTGACCTGCTGAATTACAGCCCGGAAGCGCAGCTTGAGCT
GATGACCACGATGCTCCAGCTGGAGCAGCTAACTGCGCTCGACGGCCATCAGTTTCCGA
CGATGGCGCCTGTCTCGCCGCGGATATCCCCGATGCAAACCCATCATGCAGGTCACAGCT
TGTCGCCTCCGCCACACATGTCGGCGACGACGACGACCACCACCACCGGGTACCCAGAG
CAGTACACGCCGGCGGGCGGCCGTGTACGGCGCCACCGCTACCGGCCTCGAGCACCTCCA
GGACTACGCGCTGCCCCACGGCGACATGTAA

>*tf20* - Delprim

ATGAAACACCACCACCACCACCACCACGGTGGTCTGGTTCGCGTGGTTCATGGC
GGATCCGAATTCATGAGTTTTAGGACTCGTCATGTTCCGTCTGCTCCTCAAACCTGTTGAG
GCTGCAAAACTTCAATCTTCTGGTGCTAGCTTCAATGAATCTATTGGATTCTCAGAGGTC
TTGCAAGGTCAAGAAATTTCTCGGGCAGTTCCTATGTTCCAAGGAATGATGTCTGAGGCT
TGTTCACTAAAAGGAGGATATGGGCTGCATAGTTATATGCGTACCCAGTTGCTGTTACT
GGATTGTCAGCCACAACCTCAAGAGTGTTCTCTCACACTATCTACTCCGCCAGGAGCACAA
GTTCCCTTCTGTCTACCCTGATAATATTTTAAACCGAACTGTGGTTCGACAGCTTGGACTGG
CAAGCAAGTTTGATGGTGGAGCTACAAATGCCAGCAGTCTGTCCATTTGATAGGCCG

AGGGAAATTTGGAGCAAGCCACAGCATGAAATATCTGATCAAACGAAAATGGATCACTT
TGAGACTAGAAGAGCTTCAGCACCTGGAGATGATGCTGCTAGGCATGGGTCTGGTGGAG
AGGTGGTTTCGCAAACTAGCTGCAGACTTTTTGGTTTTCTCGTTGACTGAGAAGATCTTGC
CAGCAGATGATGATGGCATCAAGGAAGTGACCTATGAGCCTGAGTGCCAGAATCCACGG
ATGCTGGACCTGTTCTGGGTACAACCTGCTCAGCCCCAAGTGCTGCTCTTCCAGCTCTGTGT
GCTGCCCCCTTTGGAATGTGA

>tf22- Delprim

ATGAAACACCACCACCACCACCACCACCACGGTGGTCTGGTTCGCGTGGTTCATGGC
GGATCCGAATTCATGCAGCTGGGGAAGACCGCCGTCGCCGGCGTCCGCGCCACCAAGGA
GGAGGCGATGGACATGGCCACCTCGCACACGCACTCCCACTCCCAATCACACTCGCACT
CGTGGGGCGAGACGCGCACGCCAGAGTCGGAGATCGTGGACAACGACCCGTTCCCGGA
GCTGGACTCGTTCGCGGTTCCAGGACCCGGCGATGATGATGACGGTGCCCAAGGAGG
AGCAGGTGGACGGCTGCAGCGCCAAGAGCGGCAACCTGTTTCGTGGACCTCAGCTACGAC
GACATCCAGGGCATGTACAGCGGCCTCGACATGCTGCCGCCGCCGGGGAGGACTTCTA
CTCCTCGCTCTTCGCGTCTCCAGGGTCAAGGGGAACCAGCCCGCCGGAGCCGCCGGGTT
GGGACAGTTCTGA

7.6 Maize genes involved in the metabolism regulated by herbivory

Tab.7. 1: 0.5 h after feeding induction

MZ-number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000384	NA	1.002215099	0.01782142	
MZ00000817	hypothetical protein {Arabidopsis thaliana}	1.225074771	0.01970904	
MZ00001133	putative LHY protein {Oryza sativa}	-1.138721789	0.00983245	TF41
MZ00001172	protein kinase domain containing protein {Zea mays}	1.750609659	0.0082661	
MZ00003452	putative glutathione transporter {Zea mays}	1.3412105	9.98E-06	
MZ00003946	Putative cytochrome P450 {Oryza sativa}	1.092928293	0.02645534	
MZ00004193	putative bZIP protein HY5 {Oryza sativa}	-1.058351385	0.0153649	TF44
MZ00004233	frataxin {Zea mays}	1.008195964	0.03720526	
MZ00004875	NA	1.432468124	0.00013116	
MZ00005052	hypothetical protein {Zea mays}	1.317170145	0.00350547	
MZ00005095	hypothetical protein {Zea mays}	1.125868895	0.00406344	
MZ00005101	PRAS-rich protein {Zea mays}	1.638643656	1.78E-06	
MZ00005245	NPK1-related protein kinase-like protein {Oryza sativa}	1.861273913	1.93E-07	TF9
MZ00005265	putative helix-loop-helix DNA-binding protein {Oryza sativa}	1.85600835	1.17E-05	TF23
MZ00006071	auxin Efflux Carrier family protein {Zea mays}	-1.020020493	0.00161051	
MZ00007257	OSJNBa0088A01.13 {Oryza sativa}	-1.15282516	0.00105839	
MZ00007425	unknown protein {Oryza sativa}	-1.076852341	0.00016534	
MZ00012919	bZIP transcription factor family protein {Zea mays}	1.34531873	0.00470068	
MZ00013307	hypothetical protein {Zea mays}	-1.427738471	0.00554345	
MZ00013411	ADP-ribosylation factor-like protein 8B {Zea mays}	1.821458597	6.69E-05	
MZ00014137	transcription factor-like {Oryza sativa}	1.066733794	0.02577085	TF45
MZ00014229	pathogenesis-related protein 10 {Zea mays}	-1.887100155	0.00376567	
MZ00014822	ribulosebiphosphate carboxylase {Zea	1.473387654	0.00313702	

	mays }			
MZ00015015	putative heat-shock protein {Oryza sativa }	1.38318122	0.00812735	
MZ00015033	senescence-associated protein DH {Zea mays }	1.103383772	0.00027801	
MZ00015377	beta-amylase {Zea mays }	1.244890875	0.01641968	
MZ00015673	DRE binding factor 1 {Zea mays }	1.015806977	0.0073984	TF40
MZ00015910	glycine-rich protein1 {Zea mays }	-1.026106562	0.01411353	
MZ00016732	plasma membrane intrinsic protein ZmPIP2-6 {Zea mays }	-1.623668308	1.57E-05	
MZ00016805	uroporphyrin-III C-methyltransferase {Zea mays }	-1.159692634	0.04434959	
MZ00016998	EF-hand Ca ²⁺ -binding protein CCD1 {Zea mays }	1.896118036	9.47E-06	
MZ00017211	hypothetical protein {Zea mays }	1.483682576	0.0250197	
MZ00017335	glutamine-fructose-6-phosphate transaminase 2 {Arabidopsis thaliana }	1.194048277	0.00062369	
MZ00017518	polcalcine Jun o 2 {Zea mays }	1.393254036	0.01588083	
MZ00018052	putative adenosine monophosphate binding protein 1 AMPBP1 {Oryza sativa }	1.101526338	0.00050198	
MZ00018113	protein kinase domain containing protein {Zea mays }	1.60640663	0.01505394	
MZ00018291	hypothetical protein {Zea mays }	-1.221127323	0.00023375	
MZ00018541	Putative EREBP-like protein {Oryza sativa }	-1.149462727	0.00017592	TF38
MZ00018568	hypothetical protein {Zea mays }	1.267529382	0.00927323	
MZ00018761	putative Myb-like DNA-binding protein {Oryza sativa }	-1.485146512	4.54E-06	TF7
MZ00018836	hypothetical protein {Zea mays }	1.126756936	0.00206509	
MZ00018917	iron-phytosiderophore transporter protein yellow stripe 1 {Zea mays }	3.392152206	0.00409997	
MZ00019090	aquaporin {Zea mays }	-1.023032661	0.00042113	
MZ00019110	hypothetical protein {Zea mays }	-1.221032441	2.69E-05	
MZ00019303	pyrimidine-specific ribonucleoside hydrolase {Zea mays }	-1.34740209	0.00665309	
MZ00019321	hypothetical protein {Sorghum bicolor }	-1.237836533	0.00245584	
MZ00019475	anthranilate N-benzoyltransferase protein 1	-1.042018924	0.04482982	

	{Zea mays}			
MZ00019555	putative 0-deacetylbaecatin III-10-O-acetyl transferase {Oryza sativa}	1.261594002	0.00010906	
MZ00019908	putative acid phosphatase unknown protein {Oryza sativa}	-1.530816677	0.00931408	
MZ00019970	unknown protein {Zea mays}	1.257489938	3.62E-05	
MZ00020525	hypothetical protein {Zea mays}	1.212590669	0.0083607	
MZ00020536	LHY protein {Zea mays}	-1.021075306	5.31E-06	
MZ00020619	transcription factor WRKY12 {Oryza sativa}	1.561235153	0.00013374	TF34
MZ00020668	hypothetical protein {Zea mays}	1.007334146	0.00577678	
MZ00020958	NA	1.585036469	0.01654144	
MZ00021033	trithorax-like {Oryza sativa}	-1.250706715	0.0105825	
MZ00021339	NA	1.020539002	0.00251806	
MZ00021743	hypothetical protein {Sorghum bicolor}	1.027060954	0.00435665	
MZ00021951	adenosine 5'-phosphosulfate reductase-like2 {Zea mays}	-1.29807553	0.00647109	
MZ00021963	lethal(2)denticleless-like protein {Oryza sativa}	-1.03714112	0.00079035	
MZ00022163	putative 10-deacetylbaecatin III-10-O-acetyl transferase {Oryza sativa}	1.654173807	0.04682672	
MZ00022180	hypothetical protein {Zea mays}	1.768363096	8.24E-06	
MZ00022294	putative esterase {Oryza sativa}	1.069100268	0.00137146	
MZ00022466	OSJNBa0088H09.11 {Oryza sativa}	1.271769412	9.46E-06	
MZ00022797	NA	1.027799084	6.31E-05	
MZ00023298	hypothetical protein {Zea mays}	1.261401763	4.88E-06	
MZ00023353	calmodulin-related protein 2 {Zea mays}	1.055719312	0.00169022	
MZ00023386	60S ribosomal protein L23 {Zea mays}	-1.292527637	0.00613443	
MZ00023975	unknown protein {Oryza sativa}	1.375785473	0.00621716	
MZ00023983	ribonuclease S homolog {Zea mays}	1.091699664	0.0020865	
MZ00024496	hypothetical protein {Zea mays}	1.026059166	0.02300198	
MZ00024615	unknown protein {Oryza sativa}	-1.168857089	0.00031705	
MZ00025206	O-methyltransferase BX7 {Zea mays}	1.418920537	0.00028954	
MZ00025556	unknown protein {Oryza sativa}	-1.067536688	0.00704987	
MZ00025614	hypothetical protein {Zea mays}	1.10482634	0.00815805	
MZ00025768	glutathione S-transferase GST 20 {Zea	1.384472158	0.02387482	

	mays }			
MZ00025832	putative bHLH transcription factor {Arabidopsis thaliana}	-1.341431929	0.00089563	TF32
MZ00025850	putative protein kinase {Oryza sativa}	1.016202897	0.03443971	
MZ00025868	putative katanin {Oryza sativa}	1.126236573	0.04800696	
MZ00026418	putative neutral invertase {Oryza sativa}	1.219947423	0.00180269	
MZ00026538	ZIM-motif family protein {Zea mays}	1.917098985	0.002039	
MZ00026596	ethylene responsive element binding factor3 {Oryza sativa}	1.15306929	8.59E-05	TF30
MZ0002666	putative NAC-domain protein {Oryza sativa}	1.155732123	0.02909881	TF1
MZ00026682	putative alkaline/neutral invertase {Oryza sativa}	1.091851629	0.00755716	
MZ00027468	DNA binding protein {Zea mays}	1.048388472	0.01397215	
MZ00027872	proline oxidase {Zea mays}	1.806996704	0.00015112	
MZ00028262	NA	1.633725077	0.00983358	
MZ00028561	hypothetical protein {Zea mays}	-1.146562714	0.00532343	
MZ00028681	alcohol dehydrogenase 1 {Zea mays}	1.570977449	0.01461834	
MZ00029025	hypothetical protein {Zea mays}	1.203301308	0.00884145	
MZ00029057	FIERG2 protein {Oryza sativa}	1.131378278	0.00321772	
MZ00029180	OSJNBa0088A01.13 {Oryza sativa}	-1.23416586	0.00046556	
MZ00029487	hypothetical protein {Zea mays}	1.161214665	6.01E-05	
MZ00029551	Zinc-finger protein 1 (WZF1) {Triticum aestivum}	1.052090047	0.01293518	TF43
MZ00029722	physical impedance induced protein2 {Zea mays}	1.315623111	0.00248652	
MZ00029740	hypothetical protein {Arabidopsis thaliana}	1.152982708	0.00308533	
MZ00029892	putative MtN21 {Oryza sativa}	-1.31039007	0.00015174	
MZ00030027	putative trehalose-6-phosphate synthase {Oryza sativa}	1.028281634	0.00231345	
MZ00030030	3-oxoacyl-synthase III {Zea mays}	1.015110039	0.04052413	
MZ00030212	kinesin like protein {Zea mays}	-1.174172327	0.00042761	
MZ00030358	hypothetical protein {Oryza sativa}	-1.167560121	0.00247419	
MZ00030756	Centromere/kinetochore protein zw10 homolog {Arabidopsis thaliana}	-1.581729142	0.00048224	
MZ00031007	NA	1.69416465	1.78E-08	

MZ00031212	unknown protein {Oryza sativa}	1.119784234	0.00265804	
MZ00031407	hypothetical protein {Sorghum bicolor}	-1.822478579	0.00420278	
MZ00031793	AP2 domain containing protein {Zea mays}	1.230839803	5.86E-06	
MZ00031823	NPK1-related protein kinase-like protein {Zea mays}	1.086242091	1.81E-05	
MZ00031906	calmodulin-related protein 2 {Zea mays}	1.33262819	0.00210969	
MZ00032136	1-deoxy-D-xylulose 5-phosphate synthase 2 (dxs2) {Zea mays}	1.131918466	0.00263036	
MZ00032666	OSJNBa0088A01.13 {Oryza sativa}	-1.305099542	0.01337902	
MZ00032725	hypothetical protein {Zea mays}	-1.103184019	0.00729237	
MZ00032877	putative phi-1 {Oryza sativa}	1.159345203	0.0026771	TF39
MZ00033310	MPI {Tripsacum dactyloides}	1.100159165	0.04799923	
MZ00033435	putative ethylene-responsive elongation factor EF-Ts precursor {Oryza sativa}	1.418271131	0.01793575	
MZ00033470	hypothetical protein {Zea mays}	1.154101928	0.00466612	
MZ00033517	putative progesterone 5-beta-reductase {Oryza sativa}	1.176457328	0.04221815	
MZ00033521	hypothetical protein {Zea mays}	1.002698778	0.0032547	
MZ00033715	ZIM-motif family protein {Zea mays}	2.156032	2.92E-05	
MZ00034421	Glucan endo-1,3-beta-glucosidase {Zea mays}	-1.136946409	0.0177255	
MZ00035024	hypothetical protein {Zea mays}	-1.079513099	0.04420718	
MZ00035095	putative nucleoid DNA-binding protein cnd41 {Oryza sativa}	1.078103935	0.04323517	TF47
MZ00035642	alcohol dehydrogenase 1 {Zea mays}	-1.119794958	0.01046085	
MZ00035750	adhesive/proline-rich protein {Zea mays}	1.026133492	0.00095019	
MZ00036129	NA	-1.028370541	0.0104233	
MZ00036398	xyloglucan endotransglycosylase homolog {Zea mays}	1.220899721	0.00052941	
MZ00036475	secondary cell wall-related glycosyltransferase {Zea mays}	1.051556788	0.02014676	
MZ00036572	NA	1.354812122	0.03661717	
MZ00036593	putative Formate dehydrogenase {Oryza sativa}	-1.445495348	0.00308184	
MZ00036791	hypothetical protein {Zea mays}	1.562801382	0.00374966	
MZ00036920	hypothetical protein {Zea mays}	1.249703526	0.00021124	

MZ00036928	hypothetical protein {Zea mays}	1.195779048	0.019425	
MZ00036929	hypothetical protein {Zea mays}	1.223658453	0.0245274	
MZ00037013	putative Bowman-Birk serine protease inhibitor {Zea mays}	-1.841815786	0.00037619	
MZ00037020	NA	1.864973044	0.03151926	
MZ00037034	hypothetical protein {Oryza sativa}	1.229175526	4.45E-05	
MZ00037045	NA	-1.25345424	0.00528173	
MZ00037085	Bowman-Birk type wound-induced proteinase inhibitor WIP1 {Zea mays}	1.005014501	0.01083856	
MZ00037126	ZIM-motif family protein {Zea mays}	1.493327424	0.02917107	
MZ00037480	putative acid phosphatase unknown protein {Oryza sativa}	-1.641308245	0.00428676	
MZ00037820	NA	-1.198127036	0.00342806	
MZ00038115	INDETERMINATE-related protein {Zea mays}	-1.220321247	0.00148355	
MZ00038372	fatty acid desaturase {Zea mays}	1.081983612	0.04941197	
MZ00038498	NA	1.086691517	0.04581955	
MZ00038664	histone H1-like protein {Zea mays}	-1.243520794	0.00520754	
MZ00039123	hypothetical protein {Zea mays}	-1.093423245	0.00270615	
MZ00039124	hypothetical protein {Zea mays}	-1.0824228	0.00356507	
MZ00039194	hypothetical protein {Zea mays}	-1.060646094	0.00979065	
MZ00039195	40S ribosomal protein S4 {Zea mays}	-1.124162616	0.00763094	
MZ00039280	cytokinin oxidase 3 {Zea mays}	1.011312915	0.02978865	
MZ00039375	NA	-1.02744543	0.00097929	
MZ00040302	B73 pathogenesis-related protein 2 and GASA-like protein {Zea mays}	1.084389599	0.03956753	
MZ00040935	hypothetical protein {Zea mays}	-1.147147725	0.03264779	
MZ00040938	hypothetical protein {Zea mays}	-1.147922347	0.01300695	
MZ00041038	histone H1-like protein {Zea mays}	-1.014901845	0.01065092	
MZ00041326	putative Bowman-Birk serine protease inhibitor {Oryza sativa}	-1.919720012	0.00482553	
MZ00041327	putative Bowman-Birk serine protease inhibitor {Zea mays}	-2.468488509	0.00039523	
MZ00041500	water stress inducible protein {Oryza sativa}	-1.108968869	5.15E-05	
MZ00041802	zinc finger protein {Oryza sativa}	-2.032905738	0.0001848	TF37

MZ00041804	zinc finger protein {Oryza sativa}	-1.389607547	2.05E-05	TF29
MZ00042096	adenosine 5'-phosphosulfate reductase-like1 {Zea mays}	-1.011458317	0.01433219	
MZ00042109	O-methyltransferase BX7 {Zea mays}	1.167581162	0.03113949	
MZ00042137	phi-1-like phosphate-induced protein {Zea mays}	1.244230396	7.09E-05	
MZ00042142	NA	1.582124932	0.00388343	
MZ00042242	adhesive/proline-rich protein {Zea mays}	1.41680588	0.00142954	
MZ00042304	protein kinase domain containing protein {Zea mays}	1.096680047	0.0186087	
MZ00042553	N-acetyltransferase {Zea mays}	1.202222391	0.01039437	
MZ00042739	WRKY transcription factor {Oryza sativa}	1.015746416	0.00129574	TF26
MZ00042797	putative glutathione transporter {Zea mays}	1.413729425	0.00476571	
MZ00042953	chlorophyll a/b binding protein {Oryza sativa}	1.428389379	0.0358288	
MZ00043103	IAA24 - auxin-responsive Aux/IAA family member {Zea mays}	1.365004205	6.46E-06	
MZ00043117	adhesive/proline-rich protein {Zea mays}	1.098964733	0.00040617	
MZ00043359	hypothetical protein {Zea mays}	1.239677815	0.04264502	
MZ00043640	cytochrome b561 {Zea mays}	-1.083928526	0.03009207	
MZ00043658	pathogenesis-related protein 4 {Triticum monococcum}	-1.345400846	0.00200967	
MZ00043659	defence-related protein {Zea mays}	-1.516810411	0.01026011	
MZ00043777	hypothetical protein {Oryza sativa}	-1.279854642	0.0031413	
MZ00043886	chitinase {Zea mays}	-1.222589817	0.00363474	
MZ00044190	allene oxide synthase 1 {Zea mays}	1.613732798	0.00444464	
MZ00044271	proline oxidase {Zea mays}	1.596989502	0.00111567	
MZ00044780	NA	-1.140434047	0.02047268	
MZ00045574	NA	1.140244664	0.02483458	
MZ00048730	putative immediate-early fungal elicitor protein {Oryza sativa}	1.10710032	0.0101808	
MZ00052490	pathogenesis-related protein 10 {Zea mays}	-1.259092359	0.00355347	
MZ00054907	putative cytochrome P450 {Oryza sativa}	1.173847891	0.00965439	
MZ00055183	alpha-expansin {Oryza sativa}	1.176149036	0.01950251	
MZ00055932	Bowman-Birk type trypsin inhibitor {Zea mays}	1.095542955	0.00885029	

MZ00056440	hypothetical protein {Zea mays}	1.125029317	0.00991602	
MZ00056523	putative phi-1 {Oryza sativa}	1.370339469	0.01270272	TF42
MZ00056629	OSJNBa0088A01.13 {Oryza sativa}	-1.247555126	0.04295964	
MZ00056647	hypothetical protein {Zea mays}	2.082786391	3.15E-05	
MZ00056690	glucan endo-1,3-beta-glucosidase homolog1 {Zea mays}	-1.565978075	0.00243899	
MZ00056902	unknown protein {Oryza sativa}	1.311417811	0.03255265	
MZ00056923	myb-like protein {Oryza sativa}	-1.08667495	0.01856965	TF12
MZ00057095	putative UVB-resistance protein UVR8 {Oryza sativa}	-1.299218025	0.01303813	
MZ00057372	hypothetical protein {Sorghum bicolor}	2.21483029	0.01145302	
MZ00057436	putative ZEITLUPE {Oryza sativa}	1.870414143	0.00705078	

Tab.7. 2: 1 h after feeding induction

MZ-number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000390	NA	1.283945388	0.02054797	
MZ00000666	lipoygenase (LOX4) {Zea mays}	1.371594955	7.07E-05	
MZ00001172	protein kinase domain containing protein {Zea mays}	1.612111385	0.00795182	
MZ00001288	unknown protein {Oryza sativa}	1.034227483	2.61E-05	
MZ00001464	NA	1.577397957	0.00642841	
MZ00003802	FIP1 {Zea mays}	1.124553927	0.00063602	
MZ00003819	putative ethylene-responsive transcriptional coactivator {Oryza sativa}	-1.217149183	6.06E-05	TF24
MZ00003857	splicing factor U2af 38 kDa subunit {Zea mays}	1.030400938	0.00290021	
MZ00003861	hypothetical protein {Zea mays}	1.345203399	0.03933033	
MZ00003912	OSJNBa0008M17.5 {Oryza sativa}	1.909888997	0.0031783	
MZ00003954	probable senescence-related protein {Arabidopsis thaliana}	1.185543681	0.01077074	
MZ00003984	POZ domain protein family-like {Oryza sativa}	1.036304983	0.02070894	
MZ00004356	metal ion binding protein {Zea mays}	1.058634555	0.00601811	
MZ00004452	NA	1.967087637	1.55E-05	

MZ00005052	hypothetical protein {Zea mays}	1.373818608	0.00135476	
MZ00005095	hypothetical protein {Zea mays}	1.672661508	7.66E-05	
MZ00005245	NPK1-related protein kinase-like protein {Oryza sativa}	1.308123996	3.86E-06	TF9
MZ00005265	putative helix-loop-helix DNA-binding protein {Oryza sativa}	1.559098533	2.82E-05	TF23
MZ00005899	terpene synthase 2 {Zea mays}	1.108941326	0.00158642	
MZ00006068	glucose-6-phosphate/phosphate translocator 2 {Zea mays}	1.200192939	0.03239775	
MZ00007376	CSLC9 {Oryza sativa}	1.024910794	0.02431587	
MZ00012674	zinc finger protein 3 {Zea mays}	2.082671995	2.25E-06	
MZ00012715	OSJNBa0016O02.24 {Oryza sativa}	1.192491009	8.16E-06	
MZ00013127	putative polyprenyl diphosphate synthase {Oryza sativa}	1.425803569	0.02905149	
MZ00013368	OSJNBb0059K02.3 {Oryza sativa}	-1.004493294	0.01509806	
MZ00013411	ADP-ribosylation factor-like protein 8B {Zea mays}	2.452564965	1.15E-06	
MZ00013555	hypothetical protein {Zea mays}	1.113295208	0.02862942	
MZ00013669	hypothetical protein {Zea mays}	1.121622079	0.01288449	
MZ00013836	S-adenosylmethionine decarboxylase {Oryza sativa}	2.823038792	1.28E-05	
MZ00014129	annexin P35 {Zea mays}	1.112943221	0.00303161	
MZ00014229	pathogenesis-related protein 10 {Zea mays}	-1.384732145	0.01446172	
MZ00014287	hypothetical protein {Zea mays}	1.206862355	0.00013999	
MZ00014350	pnFL-2 {Zea mays}	1.062725415	0.00298286	
MZ00014372	MTN 3 {Zea mays}	1.976927034	0.00690417	
MZ00014377	nodulation homolog 1 {Zea mays}	-1.261717145	0.01724428	
MZ00014737	UDP-glucose-4-epimerase {Zea mays}	1.22993013	0.00029907	
MZ00014772	alcohol dehydrogenase 1 {Zea mays}	2.037565425	2.49E-06	
MZ00014943	anthranilate synthase alpha 2 subunit {Oryza sativa}	1.234507649	0.00923884	
MZ00015033	senescence-associated protein DH {Zea mays}	1.152786027	8.22E-05	
MZ00015176	unknown protein {Oryza sativa}	1.434562773	1.72E-05	
MZ00015354	putative protein kinase {Oryza sativa}	1.266495161	0.04028384	
MZ00015377	beta-amylase {Zea mays}	1.262178892	0.00925194	

MZ00015378	putative chloroplast-targeted beta-amylase { <i>Oryza sativa</i> }	1.132113694	0.03585699	
MZ00015582	NA	-1.092305784	0.01211844	
MZ00015918	MTD1 { <i>Zea mays</i> }	1.607148225	9.52E-06	
MZ00016344	membrane related protein { <i>Oryza sativa</i> }	1.166804609	0.00378905	
MZ00016498	disease resistance response protein-like { <i>Oryza sativa</i> }	-1.384778856	0.00944444	
MZ00016555	unknown { <i>Arabidopsis thaliana</i> }	-1.401387587	0.03947377	
MZ00016666	hypothetical protein { <i>Zea mays</i> }	1.90760065	2.94E-05	
MZ00016732	plasma membrane intrinsic protein 2 ZmPIP2-6 { <i>Zea mays</i> }	-1.180412691	0.00014935	
MZ00016998	EF-hand Ca ²⁺ -binding protein CCD1 { <i>Zea mays</i> }	1.262560009	0.00021398	
MZ00017265	MtN3-like protein { <i>Arabidopsis thaliana</i> }	1.221856304	0.00151593	
MZ00017300	hypothetical protein { <i>Zea mays</i> }	1.752330241	1.09E-05	
MZ00017310	zinc finger protein-like { <i>Oryza sativa</i> }	1.028489903	0.00021037	TF27
MZ00017335	glutamine-fructose-6-phosphate transaminase 2 { <i>Arabidopsis thaliana</i> }	1.475060334	4.04E-05	
MZ00017432	ULT transcription factor { <i>Zea mays</i> }	1.195600214	1.14E-06	
MZ00017456	CBL-interacting protein kinase { <i>Zea mays</i> }	1.46120518	7.19E-06	
MZ00017547	putative HAK2 { <i>Oryza sativa</i> }	1.017351697	0.00783223	
MZ00017664	putative heterogeneous nuclear ribonucleoprotein A1 { <i>Oryza sativa</i> }	1.085573828	0.04407853	
MZ00017966	hypothetical protein { <i>Zea mays</i> }	1.045616267	0.0392025	
MZ00018113	protein kinase domain containing protein { <i>Zea mays</i> }	1.315325787	0.02639512	
MZ00018241	ribosome-inactivating protein { <i>Zea mays</i> }	1.469590655	0.01597708	
MZ00018300	OJ990528_30.9 { <i>Oryza sativa</i> }	-1.217754385	9.31E-05	
MZ00018541	Putative EREBP-like protein { <i>Oryza sativa</i> }	1.311906007	2.07E-05	TF38
MZ00018568	hypothetical protein { <i>Zea mays</i> }	2.057352534	0.00011444	
MZ00018741	putative cytochrome P450 reductase { <i>Oryza sativa</i> }	1.500747678	0.03564088	
MZ00018761	putative Myb-like DNA-binding protein { <i>Oryza sativa</i> }	1.04066563	7.01E-05	TF7
MZ00018825	putative transcription factor { <i>Oryza sativa</i> }	1.186320278	0.0136534	
MZ00018836	hypothetical protein { <i>Zea mays</i> }	2.717729317	1.42E-07	

MZ00018837	MAPK6 {Oryza sativa}	1.236381319	0.01265904	
MZ00019122	deoxyribodipyrimidine photolyase family protein-like {Oryza sativa}	1.007329577	0.01298166	
MZ00019182	hypothetical protein {Oryza sativa}	1.175688268	8.60E-05	
MZ00019244	putative l-asparaginase {Oryza sativa}	-1.64736429	0.0425309	
MZ00019278	hypothetical protein {Zea mays}	1.281599732	0.00042927	
MZ00019292	hypothetical protein {Zea mays}	1.222861576	0.00041217	
MZ00019416	hypothetical protein {Zea mays}	-1.126745108	0.04888914	
MZ00019442	riboflavin biosynthesis protein ribAB {Zea mays}	1.364170942	0.00525107	
MZ00019616	FIP1 {Zea mays}	1.119114173	0.00024829	
MZ00019632	calmodulin-like protein {Pennisetum ciliare}	1.483912516	0.00038138	
MZ00019729	terpene synthase 2 {Zea mays}	2.110081091	0.00039675	
MZ00019752	hypothetical protein {Zea mays}	-1.012347052	0.00919395	
MZ00019821	long cell-linked locus protein {Zea mays}	1.10562925	0.00960834	
MZ00019869	putative phospholipid cytidylyltransferase {Oryza sativa}	1.102652854	0.00316015	
MZ00019880	NA	-1.365101661	0.00882452	
MZ00019894	stem 28 kDa glycoprotein {Zea mays}	2.152361363	0.00252506	
MZ00019908	unknown protein {Oryza sativa}	-1.14481807	0.02708139	
MZ00019970	hypothetical protein {Zea mays}	1.643084916	7.92E-07	
MZ00020105	hypothetical protein {Zea mays}	1.280143412	0.00370592	
MZ00020210	hypothetical protein {Zea mays}	1.972645072	3.63E-05	
MZ00020230	epoxide hydrolase 2 {Zea mays}	1.804424994	0.00386692	
MZ00020243	AT3g52870/F8J2_40 {Arabidopsis thaliana}	1.035209885	0.0334502	
MZ00020448	OSJNBa0072K14.9 {Oryza sativa}	1.403011903	0.01409396	
MZ00020466	ATSWI3A {Zea mays}	1.108646789	0.00938197	
MZ00020525	hypothetical protein {Zea mays}	1.400286469	0.00174502	
MZ00020668	hypothetical protein {Zea mays}	1.496873511	0.00012698	
MZ00020968	putative lipase class 3 family protein {Oryza sativa}	1.508826516	0.00343915	
MZ00021254	OSJNBa0065B15.8 {Oryza sativa}	1.510637757	0.04090632	
MZ00021379	putative centromere protein {Oryza sativa}	1.214169153	9.16E-05	
MZ00021385	putative cytochrome P450 reductase {Oryza sativa}	1.287843366	0.00198669	
MZ00021537	hypothetical protein {Zea mays}	1.063738542	0.00974371	

MZ00021587	cell wall invertase {Zea mays}	1.512509917	0.01280814	
MZ00021860	hypothetical protein {Zea mays}	1.047594001	0.00651296	
MZ00021878	putative transposase {Zea mays}	1.102355582	0.00892014	
MZ00022163	putative 10-deacetylbaecatin III-10-O-acetyl transferase {Oryza sativa}	2.718399527	0.00164784	
MZ00022294	putative esterase {Oryza sativa}	1.152777866	0.00035921	
MZ00022380	unknown protein {Oryza sativa}	1.56001253	0.03149321	
MZ00022582	peroxidase 1 {Zea mays}	1.115187766	0.00077015	
MZ00022690	hypothetical protein {Zea mays}	1.195249585	0.00263689	
MZ00022994	allene oxide synthase 1 {Zea mays}	1.403238039	3.53E-06	
MZ00023013	unknown protein {Oryza sativa}	1.042821796	0.00490058	
MZ00023027	starch phosphorylase 2 precursor {Zea mays}	1.108487039	0.01001447	
MZ00023228	dihydroflavonol-4-reductase {Zea mays}	1.088088549	0.01178162	
MZ00023318	putative prephenate dehydratase {Oryza sativa}	1.438070495	6.39E-06	
MZ00023327	putative sialin {Oryza sativa}	1.643781238	0.04215142	
MZ00023353	calmodulin-related protein 2 {Zea mays}	1.054071243	0.0008588	
MZ00023367	Metallothionein-like protein type 2 {Zea mays}	-1.154877051	0.00818746	
MZ00023404	S-adenosylmethionine decarboxylase proenzyme {Zea mays}	1.629622925	0.04374811	
MZ00023441	S-adenosylmethionine synthetase 1 {Zea mays}	1.270714834	0.00636249	
MZ00023501	pnFL-2 {Zea mays}	1.222604249	0.02241659	
MZ00023529	hypothetical protein {Zea mays}	-1.011090527	0.0291208	
MZ00023975	unknown protein {Oryza sativa}	1.357257807	0.00380023	
MZ00023983	ribonuclease S homolog {Zea mays}	1.348549183	0.00017676	
MZ00024219	expressed protein {Oryza sativa}	1.217143239	0.01751258	
MZ00024302	hypothetical protein {Asparagus officinalis}	1.994609619	0.00186067	
MZ00024626	ETTIN-like auxin response factor {Triticum aestivum}	1.273105681	7.67E-06	TF20
MZ00024934	beta-glucosidase {Musa acuminata}	1.281155629	0.0285818	
MZ00025068	hypothetical protein {Zea mays}	1.036389328	0.01519457	
MZ00025407	polyamine oxidase1 {Zea mays}	1.287782744	0.00324827	
MZ00025614	hypothetical protein {Zea mays}	2.226268668	1.07E-05	
MZ00025698	MATE family protein {Zea mays}	1.143548408	0.0066219	

MZ00025768	glutathione S-transferase GST 20 {Zea mays}	1.835870959	0.00260985	
MZ00025872	hypothetical protein {Zea mays}	1.461184682	0.00127812	
MZ00026064	hypothetical protein {Zea mays}	1.33130622	0.00212193	
MZ00026277	hypothetical protein {Zea mays}	1.137972273	0.00147281	
MZ00026471	anthranilate synthase alpha 1 subunit {Oryza sativa}	2.021441239	0.00038029	
MZ00026538	ZIM-motif family protein {Zea mays}	2.940955093	2.11E-05	
MZ00026661	putative NAC-domain protein {Oryza sativa}	1.388006535	0.00667882	TF1
MZ00026936	kelch repeat-containing F-box-like {Oryza sativa}	1.843083594	0.00010782	
MZ00027105	putative Bowman-Birk serine protease inhibitor {Oryza sativa}	2.417900238	9.50E-07	
MZ00027775	putative branched-chain alpha-keto acid decarboxylase E1 beta subunit {Oryza sativa}	1.482827774	0.00065103	
MZ00028024	phosphatase subunit g4-1 {Zea mays}	1.0955425	0.04574018	
MZ00028203	hypothetical protein {Zea mays}	1.267265703	0.00069567	
MZ00028357	CBL-interacting protein kinase {Zea mays}	1.92912251	1.96E-07	TF14
MZ00028712	putative thioredoxin {Oryza sativa}	1.145237639	2.24E-05	
MZ00028745	surfeit locus protein 2 containing protein {Zea mays}	1.041294892	0.00316561	
MZ00028821	putative plastidic phosphoglucomutase {Oryza sativa}	1.366750839	0.03769162	
MZ00028873	putative mitochondrial carrier protein {Oryza sativa}	1.036433503	3.19E-06	
MZ00028934	heavy-metal associated domain containing protein {Zea mays}	-1.125135879	0.00036736	
MZ00029020	aldose reductase {Zea mays}	1.00128915	0.01748418	
MZ00029084	hypothetical protein {Zea mays}	-1.454682251	0.01461623	
MZ00029120	hypothetical protein {Zea mays}	-1.223869948	0.03174235	
MZ00029180	OSJNBa0088A01.13 {Oryza sativa}	2.055566334	1.16E-06	
MZ00029195	putative phosphoribosyl pyrophosphate synthase {Oryza sativa}	1.121328523	0.00837507	
MZ00029209	putative molybdenum cofactor biosynthesis protein A {Oryza sativa}	1.069362721	0.03204676	
MZ00029217	hypothetical protein {Zea mays}	1.205969411	0.00166739	
MZ00029560	phytoalexin {Actinidia deliciosa;}	2.15324457	0.00082834	

MZ00029568	ZIM-motif family protein {Zea mays}	1.236126614	0.00034215	
MZ00029614	NA	1.1828159	0.00033281	
MZ00030493	ethylene-responsive transcription factor 3 {Zea mays}	1.0290928	0.00055539	
MZ00030505	putative GTP-binding protein ERG {Oryza sativa}	1.522171526	0.00361779	
MZ00030552	catalytic/hydrolase {Zea mays}	1.58166467	0.0038112	
MZ00030705	putative protein {Arabidopsis thaliana}	-1.060547377	0.01990202	
MZ00030776	calcium binding EF-hand protein {Zea mays}	-1.005686155	0.02811677	
MZ00030874	receptor-like kinase {Zea mays}	1.118536292	0.0030828	
MZ00030879	chaperone protein dnaJ 11 {Zea mays}	1.993290211	7.95E-05	
MZ00030891	VQ motif family protein {Zea mays}	-1.15284514	0.03456738	
MZ00030973	putative NAC-domain protein {Oryza sativa}	1.228642896	0.00054843	
MZ00031002	NA	1.081530113	0.00127147	
MZ00031039	hypothetical protein {Zea mays}	1.031826621	0.00112108	
MZ00031103	probable integral membrane protein {Oryza sativa}	1.014615406	0.02251413	
MZ00031189	putative genetic modifier {Oryza sativa}	1.208632916	0.00664902	
MZ00031225	At3g54650/T5N23_10 {Arabidopsis thaliana}	1.204296929	0.00773626	
MZ00031356	unknown protein {Oryza sativa}	1.658705941	7.41E-05	
MZ00031823	NPK1-related protein kinase-like protein {Zea mays}	1.361725316	5.83E-07	
MZ00031832	glutathione transferase {Hordeum vulgare subsp. vulgare}	1.680984351	4.92E-05	
MZ00031906	calmodulin-related protein 2 {Zea mays}	1.550046777	0.0003066	
MZ00032043	anthranilic acid methyltransferase 3 {Zea mays}	1.32976498	0.00210084	
MZ00032061	ADP-glucose pyrophosphorylase large subunit {Zea mays}	1.465354027	0.00175227	
MZ00032136	1-deoxy-D-xylulose 5-phosphate synthase 2 (dxs2) {Zea mays}	2.440654849	7.70E-07	
MZ00032170	putative centromere protein {Oryza sativa}	1.985916131	0.00130496	
MZ00032240	indole-3-glycerol phosphate synthase {Zea mays}	1.672229808	0.00023867	
MZ00032294	transferase, transferring glykosyl groups {Zea mays}	1.043999485	5.68E-05	

MZ00032358	hypothetical protein {Zea mays}	1.222266689	0.01935675	
MZ00032391	putative tocopherol polyprenyltransferase {Oryza sativa}	1.329069463	0.00689361	
MZ00032415	putative RNA recognition motif (RRM)-containing protein {Oryza sativa}	1.073694857	0.00030667	
MZ00032666	OSJNBa0088A01.13 {Oryza sativa}	2.047911387	0.00027915	
MZ00032709	hydrolase, alpha/beta fold family-like {Oryza sativa}	1.499271698	0.03191198	
MZ00032756	DEAD/DEAH box helicase-like {Oryza sativa}	1.164009341	0.01197905	
MZ00032999	indole-3-glycerol phosphate lyase {Zea mays}	1.215371277	0.01163255	
MZ00033042	hypothetical protein {Zea mays}	1.38304137	0.00011616	
MZ00033225	hypothetical protein {Zea mays}	-1.047186897	0.03503323	
MZ00033310	MPI {Tripsacum dactyloides}	2.352504023	0.00019555	
MZ00033313	histone H2A {Saccharomyces cerevisiae}	-1.145249522	0.01776911	
MZ00033383	hypothetical protein {Zea mays}	1.075793107	0.0040306	
MZ00033390	DNA binding protein {Zea mays}	1.96697149	1.57E-07	
MZ00033551	Chloroplast 30S ribosomal protein S12 {Zea mays}	1.510108354	0.0229258	
MZ00033552	psaC gene {Zea mays}	1.175663107	0.00223934	
MZ00033637	3-N-debenzoyl-2-deoxytaxol N-benzoyltransferase {Zea mays}	2.037004599	0.00012992	
MZ00033715	hypothetical protein {Oryza sativa}	2.525061974	2.14E-06	
MZ00034220	p53 binding protein-like {Oryza sativa}	1.053557067	0.00381158	
MZ00034292	S-adenosylmethionine synthetase 1 {Oryza sativa}	1.171875086	0.03257128	
MZ00034308	natterin 3 precursor {Thalassophryne nattereri}	1.788825543	0.00369945	
MZ00034328	putative branched-chain alpha keto-acid dehydrogenase E1 alpha subunit {Arabidopsis thaliana}	1.453173361	0.00088584	
MZ00034331	2-oxoglutarate-dependent oxygenase {Zea mays}	1.039943233	0.00087375	
MZ00034354	putative RNA-binding protein {Oryza sativa}	1.237627075	0.0198947	
MZ00034379	unknown protein {Oryza sativa}	1.228736771	0.03987185	
MZ00034402	allene oxide synthase 1 {Zea mays}	1.517704882	9.24E-05	

MZ00034412	xyloglucan endotransglycosylase homolog {Zea mays}	1.314451747	0.01393762	
MZ00034828	NAD(P)H-quinone oxidoreductase chain 5 {Zea mays}	1.440580128	0.00391932	
MZ00034831	NAD(P)H-quinone oxidoreductase chain I {Zea mays}	1.474552195	0.00813793	
MZ00034833	NAD(P)H-quinone oxidoreductase chain K {Zea mays}	1.424660392	0.02963362	
MZ00034834	Apocytochrome f precursor {Zea mays} ^ GB CAA60298.1 902234 ZMA86563 cyt- TRUNCATED-	1.67830938	0.00412018	
MZ00034838	PSI P700 apoprotein A1 {Zea mays}	1.107333632	0.00408091	
MZ00034839	Photosystem I P700 chlorophyll A apoprotein A2 (PsaB) (PSI-B) {Zea mays}	1.293290796	0.00485007	
MZ00034840	psaC gene {Zea mays}	1.055290493	0.00423838	
MZ00034841	psaI gene {Zea mays}	1.722333399	0.0009648	
MZ00034843	Photosystem Q(B) protein {Zea mays}	1.311383428	0.02994731	
MZ00034845	Photosystem II 44 kDa reaction center protein {Zea mays}	2.698661677	9.57E-06	
MZ00034846	Photosystem II D2 protein {Zea mays}	1.011636158	0.00437641	
MZ00034847	cytochrome b559 alpha chain {Oryza sativa}	1.161035732	0.031754	
MZ00034848	cytochrome b559 beta chain {Oryza sativa}	1.388158936	0.00833179	
MZ00034849	photosystem II phosphoprotein psbH {Zea mays}	1.313356163	0.03218212	
MZ00034851	Photosystem II reaction center J protein {Zea mays}	1.790096826	0.00083462	
MZ00034856	Ribulose biphosphate carboxylase large chain precursor {Zea mays}	2.873669082	0.00012187	
MZ00034865	ribosomal protein L33 {Zea mays}	1.145953078	0.02387387	
MZ00034866	ribosomal protein L36 {Oryza sativa}	1.187284713	0.0102146	
MZ00034867	DNA-directed RNA polymerase alpha chain {Zea mays}	1.672580203	0.00543532	
MZ00034871	Chloroplast 30S ribosomal protein S11 {Zea mays}	1.78226719	0.00127735	
MZ00034872	photosystem II subunit T {Narcissus elegans}	2.262354172	0.0002624	
MZ00034873	chloroplast 30S ribosomal protein S14 {Zea	1.1616309	0.00066217	

	mays}			
MZ00034874	ribosomal protein S15 {Saccharum officinarum}	1.285204464	0.00033142	
MZ00034881	Chloroplast 30S ribosomal protein S4 {Zea mays}	1.313624596	0.00896407	
MZ00034884	NA	1.158495359	0.01454772	
MZ00035054	putative zinc finger protein {Oryza sativa}	1.48720279	0.03209545	
MZ00035057	NA	1.133754204	0.00435236	
MZ00035071	NA	1.050473757	0.01624994	
MZ00035114	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.16485242	0.00012713	
MZ00035366	Alanine aminotransferase 2 {Panicum miliaceum}	1.087174227	0.03855972	
MZ00035453	hypothetical protein {Zea mays}	1.257113946	0.00022239	
MZ00035506	hypothetical protein {Zea mays}	2.400747194	0.00109718	
MZ00035543	hypothetical protein {Zea mays}	-1.49922424	0.02218476	
MZ00035555	putative senescence-associated protein {Pisum sativum}	1.564696139	0.00468348	
MZ00035606	phosphoglycerate mutase {Zea mays}	1.21897855	0.01659736	
MZ00035637	NA	1.515364506	0.03228395	
MZ00035640	non-photosynthetic NADP-malic enzyme {Zea mays}	1.440170074	0.01796981	
MZ00035685	NA	1.015336271	0.04670126	
MZ00035703	BLE1 protein {Oryza sativa}	-2.299274466	0.01335693	
MZ00035708	putative senescence-associated protein {Pisum sativum}	1.752899753	0.02869505	
MZ00035715	putative senescence-associated protein {Pisum sativum}	1.248414714	0.02925646	
MZ00035731	putative senescence-associated protein {Pisum sativum}	2.117231965	0.00162205	
MZ00035752	probable cytochrome P450 monooxygenase {Zea mays}	1.106080406	0.03558492	
MZ00035753	hypothetical protein {Zea mays}	2.44659364	0.00022346	
MZ00035770	putative senescence-associated protein {Pisum sativum}	1.58566971	0.019657	
MZ00035787	unnamed protein product {Kluyveromyces	2.344711357	0.01282742	

	lactis NRRL Y-1140}			
MZ00035813	putative zinc finger protein	1.053917829	0.02734094	
MZ00035825	unnamed protein product	2.112581184	0.01905008	
MZ00035833	unnamed protein product	1.972376292	0.00081464	
MZ00035834	unnamed protein product	1.942359748	0.00364203	
MZ00035840	putative senescence-associated protein {Pisum sativum}	1.662696715	0.01051349	
MZ00035844	unnamed protein product	2.238306542	0.00061687	
MZ00035848	hypothetical protein {Oryza sativa}	1.395434545	0.00154134	
MZ00035860	GRAB1 protein {Triticum sp.}	1.024494015	0.01022381	
MZ00035947	putative NAC-domain protein {Oryza sativa}	1.60998083	9.97E-06	TF22
MZ00035980	NAC-like protein {Zea mays}	1.135388343	0.00118448	
MZ00036019	putative NAC-domain protein {Oryza sativa}	1.595205937	5.76E-07	TF51
MZ00036046	triacylglycerol lipase like protein {Zea mays}	1.113995786	0.00047973	
MZ00036073	lichenase-2 {Zea mays}	1.174966934	0.01802089	
MZ00036084	mitochondrial 2-oxoglutarate/malate carrier {Zea mays}	1.052232967	0.01485252	
MZ00036109	VTC2 {Zea mays}	1.273763672	0.01041465	
MZ00036162	rRNA promoter binding protein {Rattus norvegicus}	1.46208632	0.01325462	
MZ00036167	hypothetical protein {Sorghum bicolor}	1.375199353	0.03189078	
MZ00036200	NA	1.050032387	0.04696438	
MZ00036203	histone one (H1) 101 {Zea mays}	2.081158338	0.00056666	
MZ00036205	putative senescence-associated protein {Pisum sativum}	1.656698754	0.00773435	
MZ00036243	granule bound starch synthase 2a precursor {Zea mays}	1.496744711	0.00659298	
MZ00036263	ribosomal protein L3 {Triticum aestivum}	1.08633943	0.00230476	
MZ00036264	putative senescence-associated protein {Pisum sativum}	1.822211512	0.00884855	
MZ00036265	unnamed protein product	2.057588987	0.04180248	
MZ00036295	unnamed protein product	2.731276682	0.00017084	
MZ00036308	unnamed protein product	2.067413012	0.02079413	
MZ00036332	Sucrose-Phosphate Synthase {Zea mays}	-1.071567807	0.03737248	
MZ00036448	NA	1.0929174	0.00495925	
MZ00036449	S-adenosylmethionine decarboxylase 2 {Zea	1.979322835	0.00638583	

	mays}			
MZ00036454	putative auxin efflux carrier (PIN1c) {Zea mays}	2.045271689	0.0014535	
MZ00036455	hypothetical protein {Zea mays}	1.97766129	0.01019146	
MZ00036457	alanine aminotransferase 2 {Zea mays}	1.197484877	0.04131038	
MZ00036460	LOL3 {Zea mays}	1.972315037	0.00538262	
MZ00036485	nicotianamine synthase 3 {Zea mays}	2.123606012	0.00044426	
MZ00036498	hypothetical protein {Sorghum bicolor}	1.522914588	0.0259925	
MZ00036513	putative senescence-associated protein {Pisum sativum}	1.862190258	0.00706775	
MZ00036519	putative fructose-bisphosphate aldolase {Oryza sativa}	1.751127213	0.04497906	
MZ00036530	unnamed protein product	1.571360192	0.04585194	
MZ00036531	hypothetical protein {Oryza sativa}	1.032160685	0.00956812	
MZ00036584	NA	2.337368374	1.31E-07	
MZ00036593	putative Formate dehydrogenase, mitochondrial precursor {Oryza sativa}	-1.760714531	0.00032591	
MZ00036758	hypothetical protein {Zea mays}	1.365868883	1.79E-05	
MZ00036778	putative chloroplast-targeted beta-amylase {Oryza sativa}	2.200249039	0.0002554	
MZ00036791	OSJNBa0079A21.19 {Oryza sativa}	2.023074532	0.00024511	
MZ00036819	putative senescence-associated protein {Pisum sativum}	1.816533685	0.00371414	
MZ00036829	putative senescence-associated protein {Pisum sativum}	1.448834749	0.01487503	
MZ00036845	unnamed protein product	1.718543049	0.02186395	
MZ00036847	Ac1147 {Rattus norvegicus}	1.589239801	0.01601009	
MZ00036849	zinc finger protein 7 {Zea mays}	1.074150196	0.03513485	
MZ00036918	CDH1-D {Gallus gallus}	1.837356235	0.02726923	
MZ00036934	unnamed protein product	2.551219889	0.00051638	
MZ00037011	NA	1.009418981	0.01122824	
MZ00037037	unnamed protein product	1.586973978	0.02819656	
MZ00037085	Bowman-Birk type wound-induced proteinase inhibitor WIP1 {Zea mays}	2.449247951	2.32E-06	
MZ00037115	OSJNBb0059K02.4 {Oryza sativa}	1.016379381	3.52E-05	
MZ00037253	subtilisin/chymotrypsin inhibitor {Zea mays}	1.002151325	0.04928815	

MZ00037281	lichenase 2 {Zea mays}	1.071845496	0.02822171	
MZ00037304	terpene synthase 2 {Zea mays}	2.047985944	7.84E-05	
MZ00037403	alanine aminotransferase {Zea mays}	1.17656431	0.04541594	
MZ00037469	dehydrin {Zea mays}	1.173895068	0.00718839	
MZ00037470	glutathione S-transferase:ISOTYPE=IV {Zea mays}	-1.013658351	0.01327121	
MZ00037600	hypothetical protein {Zea mays}	2.958693279	1.37E-07	
MZ00037902	UDP-glucose-4-epimerase {Zea mays}	1.00335287	0.00679671	
MZ00038047	adenine nucleotide translocator {Zea mays}	1.215160816	0.04819532	
MZ00038068	plasma membrane intrinsic protein2 {Zea mays}	1.520175078	0.01824837	
MZ00038276	catalytic/hydrolase {Zea mays}	1.23163918	0.00024122	
MZ00038457	ferredoxin-1 {Zea mays}	-1.106050548	0.03001737	
MZ00038646	ubiquitin-like protein 5 {Zea mays}	1.189879144	0.03871616	
MZ00038776	hypothetical protein {Zea mays}	1.024193973	0.01034773	
MZ00038794	elongation factor 1alpha {Zea mays}	1.183934964	0.0020084	
MZ00039156	hypothetical protein {Zea mays}	1.053561032	0.01248237	
MZ00039234	glyceraldehyde-3-phosphate dehydrogenase {Zea mays}	1.0047173	0.0240705	
MZ00039285	alpha-zein protein {Zea mays}	2.081110772	0.00350721	
MZ00039367	hypothetical protein {Zea mays}	1.379724837	0.0495681	
MZ00039711	phosphoglucosmutase 2 {Zea mays}	1.714834307	0.03606689	
MZ00039722	hypothetical protein {Zea mays}	1.071785258	0.01239221	
MZ00039734	beta-glucosidase {Zea mays}	1.161789806	0.0019116	
MZ00039764	Bowman-Birk type wound-induced proteinase inhibitor WIP1 {Zea mays}	1.689514344	0.00071951	
MZ00039805	hypothetical protein {Zea mays}	1.648433435	0.00014518	
MZ00039846	NAC1 transcription factor {Zea mays}	1.4951973	0.00021314	
MZ00040036	pyruvate,orthophosphate dikinase {Zea mays}	1.121827126	0.03904921	
MZ00040075	Granule-bound starch synthase precursor {Zea mays}	1.418209135	0.03455696	
MZ00040271	S-adenosylmethionine decarboxylase 2 {Zea mays}	1.756260576	0.02526966	
MZ00040277	putative cytochrome B5 {Oryza sativa}	1.500549536	3.95E-05	
MZ00040289	putative casein kinase I {Oryza sativa}	1.146119451	0.02344848	
MZ00040294	alcohol dehydrogenase 1 (adh1) {Zea mays}	-1.256672256	0.01302296	

MZ00040327	amino acid carrier {Zea mays}	1.052384531	0.03160973	
MZ00040437	MYB transcription factor {Zea mays}	1.076011466	0.00349677	
MZ00040479	ATP synthase beta subunit {Saccharum officinarum}	4.544642147	1.53E-05	
MZ00040486	Ribulose biphosphate carboxylase large chain precursor {Zea mays}	1.908224477	0.00157497	
MZ00040525	putative ATP synthase beta chain {Oryza sativa}	3.993841648	4.35E-05	
MZ00040654	glutathione S-transferase GST 25 {Zea mays}	1.133561637	0.00079982	
MZ00040799	psaI gene {Zea mays}	1.691581287	0.01875791	
MZ00040800	Photosystem II reaction center I protein {Triticum aestivum}	1.275611	0.04520172	
MZ00040806	Chloroplast 50S ribosomal protein L23 {Zea mays}	2.540319317	0.04844961	
MZ00040807	ribosomal protein L33 {Zea mays}	1.033648273	0.02523624	
MZ00040842	strongly similar to NP_195589.2 splicing factor	1.673406712	0.02429822	
MZ00041005	subtilisin-chymotrypsin inhibitor homolog1 {Zea mays}	1.071580564	0.03621874	
MZ00041163	hypothetical protein {Zea mays}	1.037888725	3.28E-05	
MZ00041326	putative Bowman-Birk serine protease inhibitor {Zea mays}	-1.494711493	0.01281047	
MZ00041327	putative Bowman-Birk serine protease inhibitor {Zea mays}	-1.835149134	0.00206329	
MZ00041372	xyloglucan endo-transglycosylase/hydrolase (xth1) {Zea mays}	1.395480882	0.00184888	
MZ00041665	UDP-glucose-4-epimerase {Zea mays}	1.227136386	0.00259668	
MZ00041671	60S ribosomal protein L13a {Zea mays}	1.553188404	3.60E-05	
MZ00041708	unknown protein {Oryza sativa}	-1.298762583	0.0041991	
MZ00041802	zinc finger protein {Oryza sativa}	-1.388768717	0.00204068	TF37
MZ00041945	translational initiation factor eIF-4A {Zea mays}	1.476509001	0.00859072	
MZ00042040	small multi-drug export protein {Zea mays}	1.42644296	3.00E-05	
MZ00042137	phi-1-like phosphate-induced protein {Zea mays}	1.431577512	6.89E-06	
MZ00042324	ribosome-inactivating protein {Zea mays}	1.314396472	0.00546222	

MZ00042472	unknown protein { <i>Oryza sativa</i> }	1.231514922	0.00542327	
MZ00042545	amino acid transport protein { <i>Zea mays</i> }	1.257388074	0.00221837	
MZ00042564	transducin { <i>Oryza sativa</i> }	1.209766028	0.0414948	
MZ00042678	ribosome-inactivating protein { <i>Zea mays</i> }	1.687783842	0.01531937	
MZ00042739	WRKY transcription factor { <i>Oryza sativa</i> }	1.287648034	7.67E-05	TF26
MZ00042841	Histone H2A { <i>Zea mays</i> }	1.752637372	0.01824991	
MZ00042953	chlorophyll a/b binding protein { <i>Oryza sativa</i> }	1.474493885	0.02031815	
MZ00043103	IAA24 - auxin-responsive Aux/IAA family member { <i>Zea mays</i> }	1.346996393	2.84E-06	
MZ00043393	2-oxoglutarate-dependent oxygenase { <i>Zea mays</i> }	1.639466238	1.45E-05	
MZ00043400	hypothetical protein { <i>Asparagus officinalis</i> }	1.857301079	2.63E-05	
MZ00043484	cysteine protease 1 { <i>Zea mays</i> }	1.19870677	0.03749325	
MZ00043526	unknown protein { <i>Oryza sativa</i> }	1.459866856	0.00030876	
MZ00043539	transmembrane 9 superfamily protein member 4 { <i>Zea mays</i> }	2.012963009	0.00048569	
MZ00043988	hypothetical protein { <i>Zea mays</i> }	-1.330528902	0.00185394	
MZ00044104	unknown protein { <i>Oryza sativa</i> }	1.393725382	0.00053211	
MZ00044126	hypothetical protein { <i>Zea mays</i> }	1.861114031	0.00038173	
MZ00044135	hypothetical protein { <i>Zea mays</i> }	1.106562177	1.63E-05	
MZ00044190	allene oxide synthase 1 { <i>Zea mays</i> }	2.40482948	8.46E-05	
MZ00044413	benzoxazinone synthesis BX9 { <i>Zea mays</i> }	1.084818665	0.04468022	
MZ00044555	putative phosphoribosyl pyrophosphate synthase { <i>Oryza sativa</i> }	1.273373219	0.00105759	
MZ00046581	beta-amylase { <i>Zea mays</i> }	1.172624746	6.33E-06	
MZ00046685	unknown protein { <i>Oryza sativa</i> }	1.200878543	0.00058811	
MZ00046697	putative peptidyl-prolycis-trans isomerase protein { <i>Oryza sativa</i> }	1.07355831	0.0056298	
MZ00046814	zeaxanthin epoxidase { <i>Zea mays</i> }	1.160342049	0.006663	
MZ00046924	mitochondrial transcription termination factor-like { <i>Oryza sativa</i> }	1.06888442	0.04287812	
MZ00046986	unknown protein { <i>Oryza sativa</i> }	1.256543668	0.00076368	
MZ00047586	putative root hairless 1 { <i>Oryza sativa</i> }	1.589256026	0.00272862	
MZ00047697	unknown protein { <i>Oryza sativa</i> }	1.098830369	0.0001109	
MZ00047756	IrgB-like family protein { <i>Zea mays</i> }	1.87547375	0.03497623	

MZ00047903	hypothetical protein {Zea mays}	1.017055501	0.00240156	
MZ00048363	putative sialin {Oryza sativa}	1.424518949	0.00333418	
MZ00048364	beta-amylase {Zea mays}	2.077666631	2.88E-05	
MZ00048444	Myb factor protein {Oryza sativa}	1.931871565	0.00022486	TF28
MZ00048483	ZIM-motif family protein {Zea mays}	2.028045873	0.00082304	
MZ00048497	putative ATP synthase gamma chain 1, chloroplast (H(+)-transporting two-sector ATPase/F(1)-ATPase/ATPC1) {Oryza sativa}	1.104324531	0.02589263	
MZ00048814	putative nucleoid DNA-binding protein cnd41 {Oryza sativa}	1.630870608	0.00547833	
MZ00049082	von Willebrand factor type A domain containing protein {Zea mays}	1.081249464	0.00012647	
MZ00050411	LOB domain protein 11 {Zea mays}	1.202533332	0.00274949	
MZ00052267	putative senescence-associated protein {Pisum sativum}	2.305749457	0.00274858	
MZ00052283	hypothetical protein {Zea mays}	1.664694247	0.00076009	
MZ00054822	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.037114826	0.00034495	
MZ00054907	putative cytochrome P450 {Oryza sativa}	1.444210825	0.00129733	
MZ00054995	hypothetical protein {Zea mays}	1.129921377	0.00028563	
MZ00055184	putative senescence-associated protein {Pisum sativum}	1.370242333	0.00824871	
MZ00055463	esterase {Zea mays}	1.874727155	4.29E-07	
MZ00055884	hypothetical protein {Zea mays}	1.314444877	7.05E-08	
MZ00055932	Bowman-Birk type trypsin inhibitor {Zea mays}	1.232359054	0.00226953	
MZ00056629	OSJNBa0088A01.13 {Oryza sativa}	1.871600301	0.00274666	
MZ00056644	disease resistance response protein-like {Oryza sativa}	-1.10469911	0.00763107	
MZ00056647	hypothetical protein {Zea mays}	2.395220087	2.83E-06	
MZ00056690	glucan endo-1,3-beta-glucosidase homolog1 {Zea mays}	-1.020114018	0.01949473	
MZ00056859	myb-related protein-like {Oryza sativa}	1.01087786	0.02310255	
MZ00057095	putative UVB-resistance protein UVR8 {Oryza sativa}	-1.057543077	0.02392993	
MZ00057165	OSJNBa0039C07.4 {Oryza sativa}	1.239777368	0.02144499	

MZ00057252	probable ring finger protein - barley {Hordeum vulgare}	1.180631224	0.02183747	
MZ00057436	putative ZEITLUPE {Oryza sativa}	3.060342811	6.89E-05	

Tab.7. 3: 2 h after feeding induction

MZ-number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000390	NA	1.03023686	0.04988142	
MZ00000418	transferase {Zea mays}	1.373511474	0.00068188	
MZ00001464	NA	2.361058379	0.0002537	
MZ00003912	OSJNBa0008M17.5 {Oryza sativa}	1.858015327	0.00329458	
MZ00003937	putative UOS1 {Oryza sativa}	1.286657337	0.03894326	
MZ00004418	TGF-beta receptor-interacting protein 1 {Phaseolus vulgaris}	1.091491534	0.16466971	
MZ00004465	anthranilic acid methyltransferase 1 {Zea mays}	1.953334566	0.09519435	
MZ00004614	glucose-6-phosphate/phosphate translocator 2 {Zea mays}	1.014436622	0.00134676	
MZ00005352	NA	1.023726633	0.06403457	
MZ00005405	farnesyl pyrophosphate synthase1 {Zea mays}	1.87367602	0.00037421	
MZ00005471	putative tyrosine/dopa decarboxylase {Oryza sativa}	1.277068191	0.00077685	
MZ00005511	hypothetical protein {Zea mays}	1.19741694	0.00066932	
MZ00005899	terpene synthase 2 {Zea mays}	2.445964131	5.77E-07	
MZ00005958	indole-3-glycerol phosphate lyase {Zea mays}	2.144543334	0.00237136	
MZ00012674	zinc finger protein 3 {Zea mays}	1.302183953	0.00020717	
MZ00012854	putative zinc transporter {Oryza sativa}	1.009181101	0.27292594	
MZ00013037	pyruvate,orthophosphate dikinase {Zea mays}	1.054684327	0.09088338	
MZ00013112	Phospholipid-transporting ATPase 1 {Arabidopsis thaliana}	1.066196225	0.00066572	
MZ00013321	hypothetical protein {Sorghum bicolor}	1.078840592	5.34E-05	
MZ00013411	ADP-ribosylation factor-like protein 8B {Zea mays}	1.772779	2.92E-05	
MZ00013555	hypothetical protein {Zea mays}	1.387785261	0.00782457	
MZ00013725	DRE-binding protein 4 {Zea mays}	1.431889465	0.00355558	

MZ00013773	UDP-glucose 6-dehydrogenase {Zea mays}	1.410818771	0.00070133	
MZ00013836	S-adenosylmethionine decarboxylase {Oryza sativa}	2.668290682	1.82E-05	
MZ00013840	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.798847947	0.00022038	
MZ00013841	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.354861127	0.01272741	
MZ00013846	lipoxygenase11 {Zea mays}	1.665138179	0.15612374	
MZ00013941	profilin A {Zea mays}	-1.123587087	0.0146436	
MZ00014008	Farnesyl pyrophosphate synthetase {Zea mays}	1.159666733	0.01844409	
MZ00014027	glycine hydroxymethyltransferase {Arabidopsis thaliana}	1.374526933	0.00021777	
MZ00014072	light-inducible protein ATLS1 {Arabidopsis thaliana}	-1.095767662	0.05327683	
MZ00014307	Cytochrome P450 71C2 {Zea mays}	1.70239388	0.00474709	
MZ00014377	nodulation homolog 1 {Zea mays}	-1.303107325	0.01284684	
MZ00014943	anthranilate synthase alpha 2 subunit {Oryza sativa}	1.209071744	0.00915593	
MZ00015021	polyphenol oxidase {Saccharum hybrid cultivar}	1.15465864	0.00250256	
MZ00015105	indole synthase {Zea mays}	1.030830422	0.01214453	
MZ00015138	UDP-glucuronic acid decarboxylase {Oryza sativa}	1.201321097	0.0011263	
MZ00015154	cylicin-1 {Zea mays}	1.131634848	0.00588923	
MZ00015218	hypothetical protein {Zea mays}	1.113226298	0.00315424	
MZ00015279	macrophage migration inhibitory factor {Zea mays}	-1.013855835	0.14618563	
MZ00015502	vacuolar sorting receptor homolog {Zea mays}	1.352260391	0.00260728	
MZ00015504	blue fluorescent1 {Zea mays}	1.428410541	0.00079352	
MZ00015505	putative fruit protein {Oryza sativa}	1.630659685	0.03466756	
MZ00015701	lipoxygenase {Zea mays}	2.581695148	0.00051602	
MZ00016249	probable hydroxymethylglutaryl-CoA reductase (NADPH2) {Oryza sativa}	1.539376405	0.00299231	
MZ00016498	disease resistance response protein-like	-1.251673213	0.01472235	

	{Oryza sativa}			
MZ00016835	nucleotide pyrophosphatase/phosphodiesterase {Zea mays}	1.492979993	0.00034351	
MZ00016876	putative cell division protein FtsH3 {Oryza sativa}	1.067788439	0.00805575	
MZ00016940	unknown protein {Oryza sativa}	1.002783573	0.35891415	
MZ00017092	hypothetical protein {Zea mays}	1.617888668	0.05015276	
MZ00017211	hypothetical protein {Zea mays}	1.550151642	0.01108802	
MZ00017245	ABC family1 {Zea mays}	1.230093808	0.17593178	
MZ00017265	MtN3-like protein {Arabidopsis thaliana}	1.387075724	0.00045726	
MZ00017300	hypothetical protein {Zea mays}	1.045359846	0.00106156	
MZ00017335	glutamine-fructose-6-phosphate transaminase 2 {Arabidopsis thaliana}	1.106680174	0.00046455	
MZ00017386	hypothetical protein {Oryza sativa}	-1.127725041	0.00470713	
MZ00017392	flavonoid 3-monooxygenase {Zea mays}	1.368355694	0.03466701	
MZ00017522	Putative serine/threonine kinase {Oryza sativa}	1.131462373	0.12184173	
MZ00017601	hydroperoxide lyase {Zea mays}	1.215532646	2.08E-05	
MZ00017814	putative alcohol dehydrogenase {Oryza sativa}	-1.017998925	0.03574206	
MZ00017989	hypothetical protein {Sorghum bicolor}	1.423193812	0.1475606	
MZ00018016	Putative transporter {Oryza sativa}	1.008044236	0.01600165	
MZ00018189	hypothetical protein {Zea mays}	1.060194828	0.22352922	
MZ00018193	beta-expansin 1a {Zea mays}	-1.059161246	0.00060302	
MZ00018234	OSJNBb0085F13.14 {Oryza sativa}	1.020119199	0.03142899	
MZ00018241	ribosome-inactivating protein {Zea mays}	2.566665061	0.00026059	
MZ00018258	hypothetical protein {Zea mays}	1.145522492	0.0719273	
MZ00018280	OSJNBa0019K04.6 {Oryza sativa}	1.092957667	0.07619123	
MZ00018568	hypothetical protein {Zea mays}	2.336558636	2.66E-05	
MZ00018741	putative cytochrome P450 reductase {Oryza sativa}	2.154914042	0.00433498	
MZ00018836	hypothetical protein {Zea mays}	1.26210382	0.00034168	
MZ00018952	hypothetical protein {Oryza sativa}	-1.108451485	0.01277162	
MZ00019196	senescence-associated protein DH {Zea mays}	1.3692118	5.90E-05	
MZ00019277	unknown protein {Oryza sativa}	-1.01309242	0.02812032	
MZ00019475	anthranilate N-benzoyltransferase protein 1	1.307470694	0.00817859	

	{Zea mays}			
MZ00019560	putative AMP deaminase {Oryza sativa}	1.843768023	7.88E-05	
MZ00019701	hypothetical protein {Oryza sativa}	2.278043903	0.0001613	
MZ00019729	terpene synthase 2 {Zea mays}	2.546889179	5.76E-05	
MZ00019894	stem 28 kDa glycoprotein {Zea mays}	2.410031229	0.00090632	
MZ00019908	hypothetical protein {Zea mays}	-1.045638506	0.03687567	
MZ00020111	4-coumarate--CoA ligase 4CL1 {Lolium perenne}	1.006469385	0.00983614	
MZ00020230	epoxide hydrolase 2 {Zea mays}	1.715044275	0.0046751	
MZ00020495	Probable disease resistance protein {Arabidopsis thaliana} ^ GB CAB38788.1 4490297 ATF17M5 putat-TRUNCATED-	1.026662725	0.00333622	
MZ00020968	putative lipase class 3 family protein {Oryza sativa}	2.090846466	0.00021577	
MZ00021254	OSJNBa0065B15.8 {Oryza sativa}	1.636843291	0.02582368	
MZ00021385	putative cytochrome P450 reductase {Oryza sativa}	1.026286532	0.00784386	
MZ00021997	putative auxin-regulated protein {Oryza sativa}	1.166719003	0.00416705	
MZ00022163	putative 10-deacetylbaocatin III-10-O-acetyl transferase {Oryza sativa}	2.795284145	0.00112124	
MZ00022180	hypothetical protein {Zea mays}	1.461594647	1.89E-05	
MZ00022380	hypothetical protein {Sorghum bicolor}	1.66586002	0.02069314	
MZ00022582	peroxidase 1 {Zea mays}	1.047218026	0.00106451	
MZ00022994	allene oxide synthase 1 {Zea mays}	1.865077821	1.06E-07	
MZ00023013	unknown protein {Oryza sativa}	1.171634486	0.00186209	
MZ00023027	starch phosphorylase 2 precursor {Zea mays}	1.048303355	0.01212634	
MZ00023087	hypothetical protein {Zea mays}	1.26569152	0.00012539	
MZ00023228	dihydroflavonol-4-reductase {Zea mays}	1.056075907	0.01229024	
MZ00023293	MCB2 protein {Hordeum vulgare subsp. vulgare}	-1.045847437	0.00349516	
MZ00023318	putative prephenate dehydratase {Oryza sativa}	1.483733745	3.58E-06	
MZ00023441	S-adenosylmethionine synthetase 1 {Zea mays}	1.567118066	0.00125196	

MZ00023501	pnFL-2 {Zea mays}	1.33327923	0.01265398	
MZ00023547	putative aminotransferase class-III {Oryza sativa}	1.742252391	0.0123728	
MZ00023952	vacuolar proton pump homolog1 {Zea mays}	1.009359434	0.34530679	
MZ00023983	ribonuclease S homolog {Zea mays}	1.145738995	0.00060099	
MZ00024039	photosystem I reaction center subunit III {Zea mays}	1.616016643	0.22302104	
MZ00024196	superoxide dismutase {Zea mays}	1.47353444	9.49E-05	
MZ00024204	putative C-4 sterol methyl oxidase {Oryza sativa}	1.257850194	0.04459984	
MZ00024350	anthranilate synthase component II {Zea mays}	2.327282998	0.00358114	
MZ00024351	anthranilate synthase component II {Zea mays}	1.465293952	0.00089003	
MZ00024600	S-like RNase {Triticum aestivum}	1.005051476	0.00013909	
MZ00024662	putative indole-3-glycerol phosphate synthase {Oryza sativa}	1.176295043	0.00089011	
MZ00024753	putative angio-associated migratory cell protein {Oryza sativa}	1.194788081	0.16552594	
MZ00024904	OSJNBa0058K23.19 {Oryza sativa}	1.397660148	0.00151183	
MZ00025169	DEAD box RNA helicase {Zea mays}	1.227198799	0.03618211	
MZ00025171	DEAD box RNA helicase1 {Zea mays}	1.157403615	0.00051684	
MZ00025289	cytidine deaminase2 {Zea mays}	1.790277357	0.02487384	
MZ00025407	polyamine oxidase1 (pao1) {Zea mays}	1.664314353	0.00037069	
MZ00025580	OSJNBa0058G03.5 {Oryza sativa}	1.080026156	0.07692816	
MZ00025708	ZIM-motif family protein {Zea mays}	1.131402858	0.00286847	
MZ00025768	glutathione S-transferase GST 20 {Zea mays}	2.1001018	0.00079211	
MZ00025895	farnesyl-pyrophosphate synthetase {Oryza sativa}	1.906928244	0.00476959	
MZ00025944	glutathione S-transferase GST 10 {Zea mays}	-1.008378342	0.07490153	
MZ00026004	glucose-6-phosphate/phosphate translocator 2 {Zea mays}	1.042467121	0.23435668	
MZ00026277	hypothetical protein {Zea mays}	1.519029522	0.00010354	
MZ00026471	anthranilate synthase alpha 1 subunit {Oryza sativa}	2.859966877	1.10E-05	
MZ00026538	ZIM-motif family protein {Zea mays}	1.380001549	0.00865806	

MZ00026661	putative NAC-domain protein { <i>Oryza sativa</i> }	1.269517381	0.00999369	TF1
MZ00026684	hypothetical protein { <i>Zea mays</i> }	1.077745834	0.00159198	
MZ00026739	putative lipase { <i>Arabidopsis thaliana</i> }	2.26447486	0.03384457	
MZ00027030	12-oxo-phytodienoic acid reductase8 { <i>Zea mays</i> }	1.342701039	0.02357186	
MZ00027037	OSJNBa0008M17.5 { <i>Oryza sativa</i> }	2.694023971	0.01666961	
MZ00027073	hypothetical protein { <i>Zea mays</i> }	1.286337591	0.08322685	
MZ00027387	cinnamyl alcohol dehydrogenase1 { <i>Zea mays</i> }	1.543096669	0.00444697	
MZ00027416	autophagy-related 12 { <i>Zea mays</i> }	1.26397244	0.05916287	
MZ00027491	Cytochrome P450 71C4 { <i>Zea mays</i> }	1.234304683	0.02707684	
MZ00027637	chloride channel { <i>Zea mays</i> }	1.12853167	0.00081646	
MZ00028221	putative phosphoethanolamine methyltransferase { <i>Oryza sativa</i> }	1.114589694	0.02651546	
MZ00028790	hypothetical protein { <i>Zea mays</i> }	1.043993089	0.00318289	
MZ00028898	hypothetical protein { <i>Zea mays</i> }	1.391299979	0.0006231	
MZ00029195	putative phosphoribosyl pyrophosphate synthase { <i>Oryza sativa</i> }	2.560977534	6.02E-06	
MZ00029519	OSJNBa0008M17.5 { <i>Oryza sativa</i> }	1.558536958	0.00333745	
MZ00029560	phytocystatin { <i>Actinidia deliciosa</i> }	2.808226321	6.41E-05	
MZ00029584	arogenate dehydrogenase { <i>Zea mays</i> }	1.296479229	1.36E-05	
MZ00029594	sesquiterpene cyclase { <i>Zea mays</i> }	1.605835591	9.10E-06	
MZ00029629	histone deacetylase { <i>Zea mays</i> }	1.773627052	0.00677565	
MZ00029916	hypothetical protein { <i>Zea mays</i> }	1.119383857	0.00074939	
MZ00029921	anthranilic acid methyltransferase 1 { <i>Zea mays</i> }	1.27577375	0.07055959	
MZ00030298	sucrose-phosphatase { <i>Zea mays</i> }	1.285520188	2.22E-05	
MZ00030501	terpene synthase { <i>Zea mays</i> }	1.316340614	0.00018751	
MZ00030642	chemocyanin { <i>Zea mays</i> }	1.114132022	0.00269918	
MZ00030744	integral membrane protein DUF6 containing protein { <i>Zea mays</i> }	1.175017063	0.01859008	
MZ00030829	aldo-keto reductase/ oxidoreductase { <i>Zea mays</i> }	1.631060743	0.00398554	
MZ00031225	At3g54650/T5N23_10 { <i>Arabidopsis thaliana</i> }	1.130579468	0.00986646	
MZ00031356	unknown protein { <i>Oryza sativa</i> }	1.590638565	8.93E-05	
MZ00031477	hypothetical protein { <i>Zea mays</i> }	1.036987982	0.00035747	
MZ00031624	putative prephenate dehydratase { <i>Oryza</i> }	1.124857295	0.00043053	

	sativa}			
MZ00031736	terpene synthase10 {Zea mays}	2.478956434	0.00139642	
MZ00031748	hypothetical protein {Zea mays}	1.100082514	0.01356034	
MZ00031832	glutathione transferase {Hordeum vulgare subsp. vulgare}	1.578120101	7.31E-05	
MZ00031957	unknown protein {Oryza sativa}	1.004907218	0.00168799	
MZ00032043	anthranilic acid methyltransferase 3 {Zea mays}	1.33487533	0.00173148	
MZ00032136	1-deoxy-D-xylulose 5-phosphate synthase 2 (dxs2) {Zea mays}	3.227763457	2.18E-08	
MZ00032170	putative centromere protein {Oryza sativa}	1.944509146	0.00129221	
MZ00032240	indole-3-glycerol phosphate synthase {Zea mays}	2.034400307	3.00E-05	
MZ00032999	indole-3-glycerol phosphate lyase {Zea mays}	1.563299031	0.0019546	
MZ00033310	MPI {Tripsacum dactyloides}	3.01765026	1.39E-05	
MZ00033551	Chloroplast 30S ribosomal protein S12 {Zea mays}	2.293633708	0.00147481	
MZ00033552	psaC gene {Zea mays}	1.427344874	0.00040398	
MZ00033637	3-N-debenzoyl-2-deoxytaxol N-benzoyltransferase {Zea mays}	1.974223767	0.00014116	
MZ00033688	pyruvate, orthophosphate dikinase {Zea mays}	1.556751962	0.17929047	
MZ00034236	vacuolar sorting receptor homolog1 {Zea mays}	1.272306222	0.00144763	
MZ00034292	S-adenosylmethionine synthetase 1 {Oryza sativa}	1.101136222	0.0387964	
MZ00034295	methionine synthase {Zea mays}	2.307220428	3.28E-06	
MZ00034298	seed specific protein of balanced nutritional quality {Amaranthus hypochondriacus}	1.362589081	0.00856829	
MZ00034308	natterin 3 precursor {Thalassophryne nattereri}	1.723248995	0.00409385	
MZ00034331	2-oxoglutarate-dependent oxygenase {Zea mays}	1.03500533	0.00075819	
MZ00034402	allene oxide synthase {Zea mays}	1.671836552	2.88E-05	
MZ00034828	NAD(P)H-quinone oxidoreductase chain 5 {Zea mays}	1.508385378	0.00243565	
MZ00034831	NAD(P)H-quinone oxidoreductase chain I	1.188448296	0.02334139	

	{Zea mays}			
MZ00034832	NAD(P)H-quinone oxidoreductase chain J {Zea mays}	1.312867972	0.02494666	
MZ00034833	NAD(P)H-quinone oxidoreductase chain K {Zea mays}	2.179723534	0.00208898	
MZ00034834	Apocytochrome f precursor {Zea mays}^ ^GB CAA60298.1 902234 ZMA86563 cyt-TRUNCATED-	1.704495188	0.00318348	
MZ00034838	PSI P700 apoprotein A1 {Zea mays}	2.784546574	5.88E-07	
MZ00034839	Photosystem I P700 chlorophyll A apoprotein A2 {Zea mays}	2.086225666	8.50E-05	
MZ00034840	psaC gene {Zea mays}	1.48064752	0.00025438	
MZ00034841	psaI gene {Zea mays}	2.201212435	9.34E-05	
MZ00034842	photosystem I subunit IX {Oryza nivara}	2.27378194	0.00955403	
MZ00034843	Photosystem Q(B) protein {Zea mays}	1.633766858	0.00832835	
MZ00034845	Photosystem II 44 kDa reaction center protein {Zea mays}	2.854597088	4.13E-06	
MZ00034846	Photosystem II D2 protein {Zea mays}	1.818045758	2.52E-05	
MZ00034847	cytochrome b559 alpha chain {Oryza sativa}	1.775936741	0.00233617	
MZ00034848	cytochrome b559 beta chain {Oryza sativa}	1.606566149	0.00277161	
MZ00034849	photosystem II phosphoprotein psbH {Zea mays}	2.438270573	0.00052747	
MZ00034850	Photosystem II reaction center I protein {Triticum aestivum}	2.054908102	0.00190159	
MZ00034851	Photosystem II reaction center J protein {Zea mays}	2.142328229	0.00014564	
MZ00034853	photosystem II L protein {Oryza sativa}	1.187725187	0.00697087	
MZ00034854	NA	2.050272742	0.00272595	
MZ00034855	photosystem II protein N {Oryza sativa}	2.02867509	0.00540431	
MZ00034856	Ribulose biphosphate carboxylase large chain precursor {Zea mays}	3.708092123	7.59E-06	
MZ00034857	Chloroplast 30S ribosomal protein S12 {Zea mays}	2.38414342	0.00187299	
MZ00034858	Chloroplast 50S ribosomal protein L14 {Zea mays}ribosomal protein L14 {Zea mays} ^-TRUNCATED-	1.865715686	0.02070231	

MZ00034859	Chloroplast 50S ribosomal protein L16 {Zea mays}	2.252885787	0.03994905	
MZ00034860	ribosomal protein L2 {Zea mays}	2.099443546	0.11249719	
MZ00034862	Chloroplast 50S ribosomal protein L22 {Zea mays}	2.981569473	0.0411925	
MZ00034863	Chloroplast 50S ribosomal protein L23. {Zea mays} ^ ^PIR S01396 R5ZM23 ribosomal protein L23 - maize chloroplast {Zea mays} ^ ^GB BAD27383.1 49659602 AP006714 ribosomal protein L23 {Saccharum officinarum;}-TRUNCATED-	2.426050952	0.05597254	
MZ00034865	ribosomal protein L33 {Zea mays}	1.326255597	0.00969839	
MZ00034866	ribosomal protein L36 {Oryza sativa}	2.30907383	4.47E-05	
MZ00034867	DNA-directed RNA polymerase alpha chain {Zea mays}	2.178472896	0.00066476	
MZ00034871	Chloroplast 30S ribosomal protein S11 {Zea mays}	2.053424775	0.00033218	
MZ00034872	photosystem II subunit T {Narcissus elegans}	2.705007934	3.96E-05	
MZ00034873	Chloroplast 30S ribosomal protein S14 {Zea mays}	1.156380639	0.00057142	
MZ00034874	ribosomal protein S15 {Saccharum officinarum}	2.058579357	2.42E-06	
MZ00034877	Chloroplast 30S ribosomal protein S18 {Zea mays}	2.002648333	0.00181047	
MZ00034878	ribosomal protein S19 {Zea mays}	1.988382211	0.03254884	
MZ00034879	Chloroplast 30S ribosomal protein S2 {Zea mays}	1.997499065	0.01274782	
MZ00034880	Chloroplast 30S ribosomal protein S3 {Zea mays}	2.834132874	0.00551188	
MZ00034881	Chloroplast 30S ribosomal protein S4 {Zea mays}	2.792795774	1.45E-05	
MZ00034882	Chloroplast 30S ribosomal protein S7 {Zea mays}	2.44499997	0.08955369	
MZ00034883	Chloroplast 30S ribosomal protein S8 {Zea mays}	1.834970735	0.08675822	
MZ00034895	hypothetical protein {Zea mays}	2.155866354	0.13762338	

MZ00034901	NA	1.041764824	0.02735304	
MZ00034902	NA	1.310040741	0.01478759	
MZ00034904	NA	1.458630883	0.00261811	
MZ00034910	NA	1.489061889	0.0431608	
MZ00034930	lipoxygenase10 {Zea mays}	1.124129713	0.00242893	
MZ00035055	lipoxygenase6 {Zea mays}	1.049963052	0.00588982	
MZ00035057	hypothetical protein {Sorghum bicolor}	1.703244145	0.00014203	
MZ00035114	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.112711835	0.00015787	
MZ00035119	putative senescence-associated protein {Pisum sativum}	1.078421948	0.06953704	
MZ00035213	prpol {Zea mays}	1.057014741	0.01829625	
MZ00035259	putative senescence-associated protein {Pisum sativum}	1.506943662	0.00363069	
MZ00035364	ABA- and ripening-inducible-like protein {Zea mays}	1.090715747	0.25096348	
MZ00035373	AER {Zea mays}	1.296724162	0.00016459	
MZ00035374	methionine synthase {Zea mays}	1.973657913	1.84E-06	
MZ00035394	putative senescence-associated protein {Pisum sativum}	1.735292879	0.00095714	
MZ00035414	synthase proteolipid subunit,ATP {Spinacia sp.}	2.105032338	0.2767487	
MZ00035506	hypothetical protein {Zea mays}	2.610852895	0.00046109	
MZ00035555	putative senescence-associated protein {Pisum sativum}	2.013258584	0.00060866	
MZ00035616	pyruvate,orthophosphate dikinase {Zea mays}	1.105201782	0.55002025	
MZ00035637	NA	2.674661284	0.00080415	
MZ00035685	NA	2.082256523	0.00047532	
MZ00035702	NA	1.927259588	0.00308432	
MZ00035704	NA	1.093259361	0.01590859	
MZ00035708	putative senescence-associated protein {Pisum sativum}	1.638762142	0.03516077	
MZ00035715	putative senescence-associated protein {Pisum sativum}	2.149019892	0.00082668	
MZ00035720	hypothetical protein {Zea mays}	1.550246251	0.00015237	
MZ00035731	putative senescence-associated protein {Pisum	1.862421144	0.00347306	

	sativum}			
MZ00035752	probable cytochrome P450 monooxygenase {Zea mays}	1.776277849	0.00197458	
MZ00035753	hypothetical protein {Zea mays}	2.362359758	0.00025024	
MZ00035770	putative senescence-associated protein {Pisum sativum}	2.265850802	0.00184994	
MZ00035787	unnamed protein product	2.602140335	0.00605042	
MZ00035813	putative zinc finger protein	1.22852734	0.01096484	
MZ00035825	unnamed protein product	2.523691105	0.00607011	
MZ00035828	putative senescence-associated protein {Pisum sativum}	1.685101641	0.00102647	
MZ00035833	unnamed protein product	2.277198937	0.00019668	
MZ00035834	unnamed protein product	2.105099096	0.00174862	
MZ00035835	unnamed protein product	1.466083263	0.00612952	
MZ00035840	putative senescence-associated protein {Pisum sativum}	1.906894424	0.00387732	
MZ00035842	carbonic anhydrase {Zea mays}	1.119621929	0.33733191	
MZ00035844	unnamed protein product	2.010759187	0.00121774	
MZ00035848	Hypothetical protein {Oryza sativa Zea mays}	1.183254307	0.00425262	
MZ00035981	NAD-dependent epimerase/dehydratase family protein {Zea mays}	1.43966079	0.00323953	
MZ00035987	hypothetical protein {Oryza sativa}	1.483413721	0.00506518	
MZ00035997	lipoygenase6 {Zea mays}	1.529627126	0.00345538	
MZ00036078	hypothetical protein {Zea mays}	1.091621379	1.19E-05	
MZ00036098	metallothionein-like protein {Zea mays}	-1.013899956	0.32926996	
MZ00036151	putative senescence-associated protein {Pisum sativum}	1.254159615	0.00567947	
MZ00036162	rRNA promoter binding protein {Rattus norvegicus}	1.843663789	0.00267149	
MZ00036167	hypothetical protein {Sorghum bicolor}	2.258290515	0.00138824	
MZ00036171	UDP-glucose 6-dehydrogenase {Zea mays}	1.363534355	0.00056812	
MZ00036200	NA	1.835061841	0.0017198	
MZ00036203	histone one (H1) 101 {Zea mays}	2.101125169	0.00043311	
MZ00036205	putative senescence-associated protein {Pisum sativum}	1.89849751	0.00272458	
MZ00036223	putative senescence-associated protein {Pisum	1.57220368	0.00046902	

	sativum}			
MZ00036242	putative senescence-associated protein {Pisum sativum}	1.478318745	0.03563985	
MZ00036243	granule bound starch synthase 2a precursor (GBSS2a) {Zea mays}	1.094694143	0.03137115	
MZ00036244	unnamed protein product	1.004911815	0.00479287	
MZ00036264	putative senescence-associated protein {Pisum sativum}	1.873646486	0.0065148	
MZ00036265	unnamed protein product	1.966699518	0.04593108	
MZ00036281	NA	1.506155728	0.00426289	
MZ00036282	putative senescence-associated protein {Pisum sativum}	1.592934397	0.00868318	
MZ00036291	putative senescence-associated protein {Pisum sativum}	1.586983509	8.55E-05	
MZ00036295	unnamed protein product	2.422442263	0.00040533	
MZ00036305	putative senescence-associated protein {Pisum sativum}	1.788881631	0.00604369	
MZ00036306	putative senescence-associated protein {Pisum sativum}	1.179195899	0.01808718	
MZ00036308	unnamed protein product	2.337353837	0.00944129	
MZ00036310	putative senescence-associated protein {Pisum sativum}	1.524420782	0.00140849	
MZ00036349	hypothetical protein {Zea mays}	-1.462407949	0.22449326	
MZ00036382	putative senescence-associated protein {Pisum sativum}	1.641639011	0.01431298	
MZ00036442	GR1a protein {Zea mays}	-1.136685438	0.04741771	
MZ00036454	putative auxin efflux carrier (PIN1c) {Zea mays}	1.575126155	0.00748912	
MZ00036455	hypothetical protein {Zea mays}	1.858269238	0.01278453	
MZ00036485	nicotianamine synthase 3 {Zea mays}	1.840102697	0.00118929	
MZ00036498	hypothetical protein {Sorghum bicolor}	1.675552124	0.01425941	
MZ00036513	putative senescence-associated protein {Pisum sativum}	2.190952853	0.00202693	
MZ00036530	unnamed protein product	2.901123628	0.00107926	
MZ00036531	hypothetical protein {Oryza sativa}	1.008471784	0.00961719	
MZ00036538	subtilisin/chymotrypsin inhibitor {Zea mays}	1.195270515	0.00786864	

MZ00036544	probable cytochrome P450 monooxygenase {Zea mays}	1.395566362	0.00077366	
MZ00036635	hypothetical protein {Zea mays}	1.345974808	0.02378928	
MZ00036754	hypothetical protein {Zea mays}	2.521828061	0.00346663	
MZ00036758	hypothetical protein {Zea mays}	1.14114505	8.51E-05	
MZ00036768	putative senescence-associated protein {Pisum sativum}	1.713283174	0.00029757	
MZ00036791	hypothetical protein {Zea mays}	1.425780091	0.00328259	
MZ00036819	putative senescence-associated protein {Pisum sativum}	1.76285171	0.00391167	
MZ00036829	putative senescence-associated protein {Pisum sativum}	1.829644036	0.00306785	
MZ00036845	unnamed protein product	2.677675051	0.00112489	
MZ00036847	Ac1147 {Rattus norvegicus}	2.273354409	0.00136262	
MZ00036916	NA	2.712528549	0.00075519	
MZ00036918	CDH1-D {Gallus gallus}	2.635988843	0.00291371	
MZ00036934	unnamed protein product	2.459725572	0.00058132	
MZ00036948	NA	-1.092872771	0.10983161	
MZ00036957	protein phosphatase 2C isoform gamma {Zea mays}	1.000833318	0.45243199	
MZ00037011	hypothetical protein {Sorghum bicolor}	1.648072715	0.00026833	
MZ00037032	beta-glucosidase aggregating factor precursor {Zea mays}	1.042936523	0.52779442	
MZ00037037	unnamed protein product	2.508856965	0.00151149	
MZ00037070	polyamine oxidase1 {Zea mays}	1.882864467	0.00893681	
MZ00037083	metallothionein-like protein {Zea mays}	-1.30986033	0.26800897	
MZ00037085	Bowman-Birk type wound-induced proteinase inhibitor WIP1 {Zea mays}	3.145357742	1.01E-07	
MZ00037127	hypothetical protein {Zea mays}	1.283188616	0.00871686	
MZ00037226	putative Regulatory protein NPR1 {Oryza sativa}	1.158551478	0.02019668	
MZ00037253	subtilisin/chymotrypsin inhibitor {Zea mays}	1.341218552	0.01083495	
MZ00037304	terpene synthase 2 {Zea mays}	2.478170865	9.15E-06	
MZ00037445	NA	2.012765459	0.06455987	
MZ00037482	putative senescence-associated protein {Pisum sativum}	1.196111214	0.03836735	

MZ00037636	2-oxoglutarate-dependent oxygenase {Zea mays}	1.300747818	0.05385622	
MZ00038613	PseudARR-B transcription factor {Zea mays}	1.010471674	0.00796261	
MZ00038794	elongation factor 1alpha {Zea mays}	1.880044664	2.85E-05	
MZ00038859	hypothetical protein {Zea mays}	1.393594349	0.00110408	
MZ00039037	plastid-specific 30S ribosomal protein 3 {Zea mays}	-1.109421309	0.08519377	
MZ00039051	putative potassium transporter {Oryza sativa}	1.141033632	0.10016461	
MZ00039088	1,4-alpha-glucan branching enzyme {Zea mays}	1.272853793	0.00250618	
MZ00039164	inositol phosphatase-like protein {Oryza sativa}	1.203209221	0.20528438	
MZ00039285	alpha-zein protein {Zea mays}	2.06370811	0.00318572	
MZ00039489	Bowman-Birk type wound-induced proteinase inhibitor {Zea mays}	1.171174207	0.07264903	
MZ00039593	NA	1.299125433	0.19897827	
MZ00039598	blue fluorescent1 {Zea mays}	1.02023615	0.00446823	
MZ00039734	beta-glucosidase {Zea mays}	1.539320025	0.00015339	
MZ00039764	Bowman-Birk type wound-induced proteinase inhibitor WIP1 {Zea mays}	2.785896639	4.82E-06	
MZ00039805	hypothetical protein {Zea mays}	1.044672424	0.00455325	
MZ00039806	anthranilate synthase component II {Zea mays}	1.372195418	0.10902343	
MZ00039973	ribosomal protein L2 {Saccharum officinarum}	1.281628918	0.28746312	
MZ00040003	NA	1.743584142	0.00545019	
MZ00040009	W22 ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit {Zea mays}	1.17171518	0.33207763	
MZ00040034	germin-like protein {Zea mays}	-1.11057651	0.12083606	
MZ00040075	Granule-bound starch synthase precursor {Zea mays}	1.125875028	0.07774499	
MZ00040188	photosystem II protein K {Oryza sativa}	1.188031134	0.18562822	
MZ00040243	hypothetical protein {Zea mays}	1.106538341	0.14931719	
MZ00040343	prephenate dehydratase {Ipomoea trifida}	1.112203562	0.00216773	

MZ00040479	coupling factor beta-subunit and coupling factor epsilon subunit {Zea mays}	3.822815669	6.92E-05	
MZ00040486	Ribulose biphosphate carboxylase large chain precursor {Zea mays}	2.733285584	5.74E-05	
MZ00040508	NA	1.554799983	0.00531562	
MZ00040525	coupling factor beta-subunit and coupling factor epsilon subunit {Zea mays}	3.671220342	7.94E-05	
MZ00040527	ribosomal protein L2 {Zea mays}	2.540899268	0.03867241	
MZ00040628	myb-related gene Zm1 {Zea mays}	-1.27109151	0.01837966	
MZ00040654	glutathione S-transferase GST 25 {Zea mays}	1.854784425	6.03E-06	
MZ00040686	sesquiterpene cyclase {Zea mays}	1.145045375	0.00142632	
MZ00040778	Late embryogenesis abundant protein {Zea mays}	-1.139368541	0.01569762	
MZ00040799	psaI gene {Zea mays}	2.230967347	0.00308213	
MZ00040800	Photosystem II reaction center I protein {Triticum aestivum}	1.942170688	0.00426513	
MZ00040803	NA	1.964366004	0.00591802	
MZ00040806	Chloroplast 50S ribosomal protein L23 {Zea mays}	2.11583341	0.08656297	
MZ00040807	ribosomal protein L33, chloroplast {Zea mays}	1.4412571	0.00317229	
MZ00040840	hypothetical protein {Oryza sativa}	1.502598241	0.00429305	
MZ00040842	strongly similar to NP_195589.2 splicing factor	2.401907649	0.00244596	
MZ00041005	subtilisin/chymotrypsin inhibitor {Zea mays}	1.923266389	0.00086624	
MZ00041019	gibberellin responsive2 {Zea mays}	-1.838835982	0.08294999	
MZ00041271	lipoygenase {Zea mays}	2.505159931	1.66E-06	
MZ00041304	glutaredoxin homolog1 {Zea mays}	2.024433968	0.15610566	
MZ00041326	putative Bowman-Birk serine protease inhibitor {Zea mays}	-1.011123658	0.0677727	
MZ00041462	putative LHY protein {Oryza sativa}	1.173702164	0.09398768	
MZ00041612	Nonspecific lipid-transfer protein precursor {Zea mays}	-1.010754305	0.12150455	
MZ00041682	pyruvate, orthophosphate dikinase1 {Zea mays}	1.461197038	0.00067066	
MZ00041721	ADP-ribosylation factor GTPase-activating	1.021027131	0.00450159	

	protein {Zea mays}			
MZ00041757	early light-induced protein {Zea mays}	-1.326550517	0.01909108	
MZ00041768	putative zinc finger protein {Zea mays}	1.876859616	0.00674281	
MZ00042158	ripening-related protein {Zea mays}	1.288863788	1.26E-05	
MZ00042245	O-succinylhomoserine sulfhydrylase {Zea mays}	1.348953347	0.00021578	
MZ00042324	ribosome-inactivating protein {Zea mays}	2.192584792	7.35E-05	
MZ00042472	unknown protein {Oryza sativa}	1.399418899	0.00192199	
MZ00042564	transducin {Oryza sativa}	1.243124141	0.03349451	
MZ00042637	phosphoenolpyruvate carboxylase {Zea mays}	1.425763064	0.00813485	
MZ00042678	ribosome-inactivating protein {Zea mays}	1.898939795	0.00680879	
MZ00042841	Histone H2A {Zea mays}	2.564840296	0.00138606	
MZ00042873	S-adenosylmethionine synthetase 1 {Zea mays}	1.229118789	0.01383571	
MZ00042935	Bowman-Birk type wound-induced proteinase inhibitor {Zea mays}	1.693144719	0.00062321	
MZ00042953	chlorophyll a/b binding protein {Oryza sativa}	2.437134414	0.00062186	
MZ00042981	hypothetical protein {Zea mays}	1.210135134	0.06125176	
MZ00043035	chitinase {Zea mays}	1.08489003	0.01839433	
MZ00043120	glycogen synthase kinase-3 MsK-3 {Zea mays}	1.224967912	0.20131319	
MZ00043160	hypothetical protein {Zea mays}	1.269621672	0.1367847	
MZ00043232	putative glycine-rich protein {Oryza sativa}	1.290025949	0.00063507	
MZ00043311	patatin-like protein {Sorghum bicolor}	1.006969191	0.05401471	
MZ00043393	2-oxoglutarate-dependent oxygenase {Zea mays}	1.900113408	2.34E-06	
MZ00043436	hypothetical protein {Zea mays}	-1.516879775	0.09696187	
MZ00043437	36.4 kDa proline-rich protein {Zea mays}	-1.270344591	0.39887341	
MZ00043517	allene oxide cyclase {Zea mays}	1.031104822	0.04933524	
MZ00043695	hypothetical protein {Zea mays}	1.076869941	0.28161283	
MZ00043885	farnesyl-pyrophosphate synthetase {Oryza sativa}	1.333004762	0.00146245	
MZ00044190	allene oxide synthase {Zea mays}	2.077496591	0.00026439	
MZ00044245	lipxygenase6 {Zea mays}	1.112313827	0.00120756	
MZ00044256	naringenin 3-dioxygenase {Zea mays}	-1.028395083	0.04002628	
MZ00044555	putative phosphoribosyl pyrophosphate	2.84328689	2.73E-07	

	synthase {Oryza sativa}			
MZ00045590	putative senescence-associated protein {Pisum sativum}	1.764488313	0.00297732	
MZ00046685	unknown protein {Oryza sativa}	1.21984965	0.00042702	
MZ00046774	alpha expansin1 {Zea mays}	1.235942194	0.00035693	
MZ00046814	zeaxanthin epoxidase {Zea mays}	1.07631936	0.00918206	
MZ00047625	hypothetical protein {Arabidopsis thaliana}	1.027418035	0.33318035	
MZ00047697	unknown protein {Oryza sativa}	1.166447195	5.05E-05	
MZ00048483	ZIM-motif family protein {Zea mays}	1.160538777	0.0253863	
MZ00048932	putative PPR protein {Oryza sativa}	1.039192736	0.00870651	
MZ00049082	von Willebrand factor type A domain containing protein {Zea mays}	1.05442141	0.00012978	
MZ00049092	G-box binding protein {Oryza sativa}	1.030464571	2.35E-05	TF21
MZ00052267	putative senescence-associated protein {Pisum sativum}	2.69681953	0.00070173	
MZ00054821	putative cellulose synthase, catalytic subunit {Oryza sativa}	1.021784979	0.10730215	
MZ00054822	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.179987378	8.69E-05	
MZ00054897	glutathione S-transferase GST 25 {Zea mays}	1.022009763	0.0029306	
MZ00055184	putative senescence-associated protein {Pisum sativum}	1.627309067	0.00227724	
MZ00055270	ripening-related protein {Zea mays}	1.134046361	0.00333059	
MZ00055448	hypothetical protein {Zea mays}	1.429817444	0.00181569	
MZ00055463	esterase {Zea mays}	1.628271524	1.65E-06	
MZ00055632	NA	1.13429863	0.00111829	
MZ00055932	Bowman-Birk type trypsin inhibitor {Zea mays}	1.858499428	5.62E-05	
MZ00056320	NA	1.108260008	0.12288358	
MZ00056687	farnesyl pyrophosphate synthase1 {Zea mays}	1.368180368	0.00848017	
MZ00056914	lipid binding protein {Zea mays}	-1.396168321	0.00961975	
MZ00056987	acyl-desaturase {Zea mays}	1.49581173	0.0024615	
MZ00057163	putative dehydration-induced myb-related protein Cpm7 {Oryza sativa}	1.191934847	0.14664984	
MZ00057436	putative ZEITLUPE {Oryza sativa}	1.271767928	0.03065219	

Tab.7. 4: 4 h after feeding induction

MZ-number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000418	transferase {Zea mays}	1.647718896	0.00010682	
MZ00000664	RING finger-like {Oryza sativa}	1.029575535	0.00119236	TF50
MZ00001096	putative multidrug resistance protein {Oryza sativa}	1.206465531	0.01457681	
MZ00001464	NA	1.296340485	0.01500009	
MZ00003835	nonspecific lipid-transfer protein AKCS9 {Zea mays}	2.412798892	7.43E-06	
MZ00004337	O-methyltransferase BX7 {Zea mays}	1.014204268	1.56E-05	
MZ00004436	acidic class I chitinase {Zea mays}	1.437908238	6.15E-07	
MZ00004614	glucose-6-phosphate/phosphate translocator 2 {Zea mays}	1.086569521	0.00062124	
MZ00005060	putative tyrosine/dopa decarboxylase {Oryza sativa}	1.060656784	8.12E-05	
MZ00005219	Bowman-Birk type trypsin inhibitor {Zea mays}	2.100082667	2.45E-08	
MZ00005242	4-methyl-5-thiazole monophosphate biosynthesis protein {Zea mays}	1.085640116	0.00042067	
MZ00005352	NA	1.097040333	0.04385328	
MZ00005405	farnesyl pyrophosphate synthase1 {Zea mays}	3.009614571	2.57E-06	
MZ00005471	putative tyrosine/dopa decarboxylase {Oryza sativa}	2.251851334	2.40E-06	
MZ00005511	hypothetical protein {Zea mays}	1.894304158	6.41E-06	
MZ00005543	lingual lipase-like {Oryza sativa}	1.147904357	0.03654641	
MZ00005899	terpene synthase 2 {Zea mays}	3.07901935	2.72E-08	
MZ00005958	indole-3-glycerol phosphate lyase {Zea mays}	2.278405955	0.00121973	
MZ00012674	zinc finger protein 3 {Zea mays}	2.013439353	1.86E-06	
MZ00012818	NA	1.087796896	5.94E-05	
MZ00013037	pyruvate,orthophosphate dikinase {Zea mays}	1.244240049	0.04504177	
MZ00013321	hypothetical protein {Sorghum bicolor}	1.225292226	1.10E-05	

MZ00013555	hypothetical protein {Zea mays}	2.137020135	0.00024802	
MZ00013773	UDP-glucose 6-dehydrogenase {Zea mays}	1.785566767	6.63E-05	
MZ00013816	adenosine kinase {Zea mays}	1.476870418	1.23E-06	
MZ00013836	S-adenosylmethionine decarboxylase {Oryza sativa}	1.090388287	0.01679961	
MZ00013840	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	2.616442997	3.94E-06	
MZ00013846	lipoxygenase11 {Zea mays}	2.774923944	0.02267559	
MZ00013941	profilin A {Zea mays}	-1.040261611	0.01892245	
MZ00014008	farnesyl pyrophosphate synthase1 {Zea mays}	1.416681809	0.00485544	
MZ00014027	glycine hydroxymethyltransferase {Arabidopsis thaliana}	2.336426886	6.81E-07	
MZ00014197	ribonucleoprotein A {Zea mays}	-1.773472715	0.03465986	
MZ00014212	putative thioredoxin {Oryza sativa}	1.477468485	0.01147305	
MZ00014229	pathogenesis-related protein 10 {Zea mays}	1.304638089	0.01517031	
MZ00014307	Cytochrome P450 71C2 {Zea mays}^GB CAA72208.1 1870201 ZMCI31 AC2 cytoc-TRUNCATED-	2.792136642	6.63E-05	
MZ00014361	NADP-dependent malic enzyme {Zea mays}	1.02233304	0.02336121	
MZ00014377	nodulation homolog 1 {Zea mays}	-1.379055539	0.00783907	
MZ00014624	hypothetical protein {Zea mays}	1.480555719	1.71E-05	
MZ00014883	beta-D-glucan exohydrolase {Zea mays}	-1.286702844	0.0052577	
MZ00014943	anthranilate synthase alpha 2 subunit {Oryza sativa}	1.521101771	0.00161973	
MZ00015021	polyphenol oxidase {Saccharum hybrid cultivar}	2.698095179	5.66E-07	
MZ00015105	indole synthase {Zea mays}	2.898369593	1.16E-06	
MZ00015138	UDP-glucuronic acid decarboxylase {Oryza sativa}	1.310454838	0.00043916	
MZ00015154	cylicin-1 {Zea mays}	1.035090828	0.0085689	
MZ00015227	putative Magnaporthe grisea pathogenicity protein {Oryza sativa}	1.048192452	0.00019924	
MZ00015397	hypothetical protein {Oryza sativa}	-1.057378026	0.01781058	
MZ00015502	vacuolar sorting receptor homolog {Zea mays}	2.340645738	1.61E-05	

MZ00015504	blue fluorescent1 {Zea mays}	3.294858187	1.13E-07	
MZ00015505	putative fruit protein {Oryza sativa}	2.464343265	0.00280619	
MZ00015562	putative C-4 sterol methyl oxidase {Oryza sativa}	1.146349495	5.17E-05	
MZ00015582	NA	-1.382495022	0.0018843	
MZ00015701	lipoxygenase {Zea mays}	3.426651786	2.87E-05	
MZ00015910	glycine-rich protein1 {Zea mays}	1.608130556	0.0001958	
MZ00016101	putative RAB7A protein (GTP-binding protein) {Oryza sativa}	1.214100915	0.00070002	TF49
MZ00016102	UDP-glucosyltransferase BX8 {Zea mays}	1.217485779	0.00011047	
MZ00016249	probable hydroxymethylglutaryl-CoA reductase {Oryza sativa}	1.055182242	0.0236022	
MZ00016502	spermidine synthase {Zea mays}	1.244468942	6.64E-06	
MZ00016732	plasma membrane intrinsic protein ZmPIP2-6 {Zea mays}	1.462920291	1.09E-05	
MZ00016835	nucleotide pyrophosphatase/phosphodiesterase {Zea mays}	2.171743586	6.76E-06	
MZ00016891	desiccation-related protein {Zea mays}	1.00284981	0.01941399	
MZ00017092	hypothetical protein {Zea mays}	3.40528207	0.00042136	
MZ00017211	hypothetical protein {Zea mays}	2.041112354	0.00147243	
MZ00017265	MtN3-like protein {Arabidopsis thaliana}	1.787407692	3.35E-05	
MZ00017300	hypothetical protein {Zea mays}	1.519836765	2.81E-05	
MZ00017392	flavonoid 3-monooxygenase {Zea mays}	2.3033303	0.00126532	
MZ00017601	hydroperoxide lyase {Zea mays}	1.507149055	1.52E-06	
MZ00017669	ATP-dependent Clp protease ATP-binding subunit precursor {Oryza sativa}	1.023755915	0.00594516	
MZ00018225	branched-chain-amino-acid aminotransferase {Zea mays}	1.61386825	5.11E-05	
MZ00018234	OSJNBb0085F13.14 {Oryza sativa}	2.238448389	0.00010782	
MZ00018241	ribosome-inactivating protein {Zea mays}	4.00774054	2.22E-06	
MZ00018568	hypothetical protein {Zea mays}	4.382704752	1.57E-08	
MZ00018837	MAPK6 {Oryza sativa}	1.050730695	0.02275596	
MZ00019117	chitinase {Zea mays}	1.46420269	1.87E-09	
MZ00019196	senescence-associated protein DH {Zea mays}	1.46074733	2.34E-05	

MZ00019212	Cytochrome P450 98A1 {Sorghum bicolor}	1.271706542	5.22E-06	
MZ00019387	unknown protein {Oryza sativa}	1.357516679	0.02117362	
MZ00019475	anthranilate N-benzoyltransferase protein 1 {Zea mays}	2.448152777	4.23E-05	
MZ00019533	putative class I chitinase {Cryptomeria japonica}	2.474647715	1.60E-05	
MZ00019560	putative AMP deaminase {Oryza sativa}	1.853404456	5.69E-05	
MZ00019701	hypothetical protein {Oryza sativa}	4.787255698	3.80E-08	
MZ00019729	terpene synthase 2 {Zea mays}	3.979099203	3.50E-07	
MZ00019785	hypothetical protein {Zea mays}	1.211209862	0.01378212	
MZ00019894	stem 28 kDa glycoprotein {Zea mays}	3.881688222	7.90E-06	
MZ00019895	putative casein kinase {Oryza sativa}	1.135924211	6.10E-06	
MZ00020111	4-coumarate--CoA ligase 4CL1 {Lolium perenne}	1.771570295	0.00010365	
MZ00020230	epoxide hydrolase 2 {Zea mays}	2.455054342	0.00023007	
MZ00020437	O-methyltransferase ZRP4 {Zea mays}	1.482863701	1.22E-05	
MZ00020525	hypothetical protein {Zea mays}	1.156131212	0.00469091	
MZ00020592	aluminum-induced protein {Zea mays}	1.09956014	0.01715516	
MZ00020968	putative lipase class 3 family protein {Oryza sativa}	2.882426198	6.84E-06	
MZ00021254	OSJNBa0065B15.8 {Oryza sativa}	2.323154923	0.0027783	
MZ00021663	dehydration-responsive element-binding protein {Zea mays}	1.151011291	0.02685228	
MZ00021682	unknown {Oryza sativa}	-1.381465127	0.02418672	
MZ00021951	adenosine 5'-phosphosulfate reductase-like2 {Zea mays}	1.05629426	0.00968449	
MZ00021997	putative auxin-regulated protein {Oryza sativa}	1.067903291	0.00615988	
MZ00022083	1-deoxy-D-xylulose 5-phosphate synthase {Zea mays}	1.053043025	3.49E-05	
MZ00022163	putative 10-deacetylbaocatin III-10-O-acetyl transferase {Oryza sativa}	4.899689512	4.25E-06	
MZ00022180	hypothetical protein {Zea mays}	1.764031312	1.85E-06	
MZ00022800	serine carboxypeptidase K10B2.2 {Zea mays}	2.031921506	6.62E-06	
MZ00022994	allene oxide synthase {Zea mays}	2.643815963	1.04E-09	

MZ00023013	unknown protein {Oryza sativa}	1.776995487	3.84E-05	
MZ00023087	hypothetical protein {Zea mays}	1.027214959	0.00061337	
MZ00023228	dihydroflavonol-4-reductase {Zea mays}	1.402008269	0.00158823	
MZ00023318	putative prephenate dehydratase {Oryza sativa}	1.547390935	1.64E-06	
MZ00023438	adenosylhomocysteinase {Zea mays}	2.425651631	4.44E-07	
MZ00023441	S-adenosylmethionine synthetase 1 {Zea mays}	2.898485381	2.76E-06	
MZ00023501	pnFL-2 {Zea mays}	1.734685259	0.00191934	
MZ00023547	putative aminotransferase class-III {Oryza sativa}	1.647437738	0.01440386	
MZ00023983	ribonuclease S homolog {Zea mays}	2.668811706	6.74E-08	
MZ00024058	cortical cell-delineating protein {Zea mays}	1.350617385	5.68E-06	
MZ00024099	hypothetical protein {Zea mays}	1.008994697	0.00604603	
MZ00024166	Cytochrome P450 71C1 {Zea mays}	1.190936906	6.00E-05	
MZ00024196	superoxide dismutase {Zea mays}	2.573824329	1.75E-07	
MZ00024204	putative C-4 sterol methyl oxidase {Oryza sativa}	1.801911567	0.00596782	
MZ00024232	vacuolar ATP synthase 16 kDa proteolipid subunit {Zea mays}	1.072665632	3.39E-05	
MZ00024287	monodehydroascorbate reductase {Oryza sativa}	1.0840865	7.41E-05	
MZ00024296	2-oxoglutarate-dependent oxygenase {Zea mays}	1.10563189	0.00255405	
MZ00024350	anthranilate synthase component II {Zea mays}	2.409350416	0.00229962	
MZ00024351	anthranilate synthase component II {Zea mays}	2.01451089	3.93E-05	
MZ00024544	cold shock protein-1 {Triticum aestivum;}	1.499160326	5.69E-06	
MZ00024574	NADH dependent Glutamate Synthase {Oryza sativa}	1.127233867	0.00792172	
MZ00024618	putative chorismate mutase precursor {Oryza sativa}	1.29047541	0.00498575	
MZ00024662	putative indole-3-glycerol phosphate synthase {Oryza sativa}	1.389109439	0.00016764	
MZ00024672	methylenetetrahydrofolate reductase {Zea	1.728822638	7.12E-05	

	mays}			
MZ00025068	hypothetical protein {Zea mays}	1.685936725	0.00034882	
MZ00025169	DEAD box RNA helicase {Zea mays}	1.183698296	0.03741489	
MZ00025206	O-methyltransferase BX7 {Zea mays}	2.134200036	1.11E-06	
MZ00025407	polyamine oxidase1 (pao1) {Zea mays}	3.881562411	3.34E-08	
MZ00025508	lipoxygenase {Zea mays}	1.010782356	0.00575184	
MZ00025768	glutathione S-transferase GST 20 {Zea mays}	3.77124288	2.02E-06	
MZ00025815	1,4-alpha-glucan branching enzyme {Zea mays}	1.036859589	0.0149282	
MZ00025895	farnesyl-pyrophosphate synthetase {Oryza sativa}	2.579127864	0.00038868	
MZ00026064	hypothetical protein {Zea mays}	1.184553754	0.00338943	
MZ00026065	Uroporphyrinogen decarboxylase {Zea mays}	-1.106241231	0.03380446	
MZ00026277	hypothetical protein {Zea mays}	1.286843129	0.00035883	
MZ00026471	anthranilate synthase alpha 1 subunit {Oryza sativa}	4.106031991	1.38E-07	
MZ00026538	ZIM-motif family protein {Zea mays}	1.577377563	0.00303287	
MZ00026684	hypothetical protein {Zea mays}	1.017309026	0.00198065	
MZ00026717	membrane protein-like {Oryza sativa}	1.309573377	2.98E-05	
MZ00026739	putative lipase {Arabidopsis thaliana}	3.788609788	0.00127186	
MZ00026889	unknown protein {Oryza sativa}	1.050254905	4.16E-05	
MZ00027029	12-oxo-phytodienoic acid reductase8 {Zea mays}	1.760037257	2.78E-05	
MZ00027030	12-oxo-phytodienoic acid reductase8 {Zea mays}	1.677728673	0.00575783	
MZ00027105	putative Bowman-Birk serine protease inhibitor {Oryza sativa}	1.080427659	0.00141663	
MZ00027113	cell death suppressor protein lls1 {Zea mays}	1.013428417	0.03161038	
MZ00027387	cinnamyl alcohol dehydrogenase1 {Zea mays}	3.455374242	2.35E-06	
MZ00027491	Cytochrome P450 71C4 {Zea mays}	3.489589549	6.03E-06	
MZ00027637	chloride channel {Zea mays}	1.657696416	1.81E-05	
MZ00028098	NADP-dependent oxidoreductase P1 {Zea mays}	1.104943201	0.0008107	
MZ00028511	ferredoxin-1 {Zea mays}	1.777191667	0.00174606	
MZ00028617	putative cytochrome P450 {Oryza sativa}	-1.069192553	0.03567487	

MZ00028806	OSJNBb0039L24.4 {Oryza sativa}	1.008487868	0.00260146	
MZ00028898	hypothetical protein {Zea mays}	1.162896807	0.00202356	
MZ00029141	hypothetical protein {Zea mays}	1.492809751	0.0151496	
MZ00029195	putative phosphoribosyl pyrophosphate synthase {Oryza sativa}	2.45294004	7.05E-06	
MZ00029386	glycoside hydrolase {Zea mays}	1.954809177	7.81E-06	
MZ00029560	phytocystatin {Actinidia deliciosa}	5.654218225	1.95E-08	
MZ00029584	arogenate dehydrogenase {Zea mays}	2.620787512	2.78E-09	
MZ00029594	sesquiterpene cyclase {Zea mays}	2.273228904	1.33E-07	
MZ00029921	anthranilic acid methyltransferase 1 {Zea mays}	1.812580743	0.0128333	
MZ00030045	elicitor inducible beta-1,3-glucanase-like {Oryza sativa}	1.20452908	0.01864414	
MZ00030298	sucrose-phosphatase {Zea mays}	2.016399959	1.09E-07	
MZ00030501	terpene synthase {Zea mays}	1.104756847	0.00066751	
MZ00030570	hypothetical protein {Zea mays}	1.123078132	0.00010771	
MZ00030642	chemocyanin {Zea mays}	2.322197319	2.32E-06	
MZ00030744	integral membrane protein DUF6 containing protein {Zea mays}	1.241663347	0.01185062	
MZ00030829	aldo-keto reductase/ oxidoreductase {Zea mays}	2.130167564	0.00041614	
MZ00031013	PDR-like ABC transporter {Oryza sativa}	1.035294458	0.00529047	
MZ00031018	ethylene-responsive transcription factor 4 {Zea mays}	1.168680405	0.00140573	
MZ00031067	RING zinc finger protein-like {Zea mays}	1.020444315	9.18E-05	
MZ00031356	unknown protein {Oryza sativa}	1.843466329	1.55E-05	
MZ00031407	hypothetical protein {Sorghum bicolor}	1.603643997	0.00388671	
MZ00031476	putative oxysterol-binding protein {Zea mays}	1.263785725	0.00838334	
MZ00031477	hypothetical protein {Zea mays}	1.328280615	2.67E-05	
MZ00031481	NA	1.001796222	0.0002705	
MZ00031624	putative prephenate dehydratase {Oryza sativa}	1.191331874	0.00020282	
MZ00031651	hypothetical protein {Zea mays}	1.128068735	0.00060626	
MZ00031721	hypothetical protein {Zea mays}	1.014749425	0.00123222	
MZ00031736	terpene synthase10 {Zea mays}	4.13524189	9.78E-06	

MZ00031748	hypothetical protein {Zea mays}	1.784642927	0.00036155	
MZ00031957	unknown protein {Oryza sativa}	2.794228564	3.87E-08	
MZ00031982	putative naphthoate synthase {Oryza sativa}	-1.157700769	0.0022313	
MZ00032043	anthranilic acid methyltransferase 3 {Zea mays}	2.514674154	3.60E-06	
MZ00032136	1-deoxy-D-xylulose 5-phosphate synthase 2 (dxs2) {Zea mays}	3.509334029	5.53E-09	
MZ00032240	indole-3-glycerol phosphate synthase {Zea mays}	2.924372824	4.27E-07	
MZ00032974	putative protein {Oryza sativa}	1.463064686	0.00012928	
MZ00032999	indole-3-glycerol phosphate lyase {Zea mays}	2.095648312	0.00013394	
MZ00033132	putative pirin {Oryza sativa}	1.766897353	0.00064096	
MZ00033298	OSJNBa0094O15.5 {Oryza sativa}	1.258139749	2.42E-05	
MZ00033310	MPI {Tripsacum dactyloides}	4.942795839	3.76E-08	
MZ00033339	Histidine-rich glycoprotein precursor {Dictyostelium discoideum}	1.298137318	0.01295126	
MZ00033428	putative chitinase {Musa acuminata}	1.032715744	0.00026745	
MZ00033723	hypothetical protein {Zea mays}	1.277967278	4.51E-06	
MZ00034292	S-adenosylmethionine synthetase 1 {Oryza sativa}	1.731836302	0.00254092	
MZ00034295	methionine synthase {Zea mays}	3.685154095	9.85E-09	
MZ00034298	seed specific protein of balanced nutritional quality {Amaranthus hypochondriacus}	2.735807896	2.27E-05	
MZ00034300	chitinase {Zea mays}	1.672698603	0.01447863	
MZ00034308	natterin 3 precursor {Thalassophryne nattereri}	2.736152141	7.31E-05	
MZ00034328	putative branched-chain alpha keto-acid dehydrogenase E1 alpha subunit {Arabidopsis thaliana}	1.096548339	0.0047625	
MZ00034331	2-oxoglutarate-dependent oxygenase {Zea mays}	1.895001827	1.53E-06	
MZ00034402	allene oxide synthase 1 {Zea mays}	2.203460979	1.10E-06	
MZ00034408	UDP-glucosyltransferase BX8 {Zea mays}	1.770840426	3.67E-06	
MZ00034412	xyloglucan endotransglycosylase homolog {Zea mays}	-1.079653967	0.02909091	

MZ00034841	psaI gene {Zea mays}	1.435163876	0.00260033	
MZ00034843	Photosystem Q(B) protein {Zea mays}	1.299706548	0.02460417	
MZ00034849	photosystem II phosphoprotein psbH {Zea mays}	1.359253167	0.02150718	
MZ00034856	Ribulose biphosphate carboxylase large chain precursor {Zea mays}	1.509317075	0.01083472	
MZ00034872	photosystem II subunit T {Narcissus elegans}	1.066671967	0.02925808	
MZ00034930	lipoxygenase10 {Zea mays}	1.821197884	2.86E-05	
MZ00034968	hypothetical protein {Zea mays}	1.076881855	7.99E-05	
MZ00035055	lipoxygenase6 {Zea mays}	1.975575199	2.36E-05	
MZ00035114	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.53183477	4.80E-06	
MZ00035120	adenosine kinase {Zea mays}	1.206479286	0.00125126	
MZ00035243	adenosylhomocysteinase {Zea mays}	1.310404132	0.00024358	
MZ00035273	nonspecific lipid-transfer protein AKCS9 {Zea mays}	1.5566982	0.00115757	
MZ00035373	AER {Zea mays}	1.454416719	4.21E-05	
MZ00035374	methionine synthase {Zea mays}	4.150849163	1.63E-10	
MZ00035604	12-oxo-phytodienoic acid reductase8 {Zea mays}	1.552010275	5.98E-07	
MZ00035632	adenosylhomocysteinase {Zea mays}	1.833118349	1.35E-05	
MZ00035637	NA	1.38553636	0.03829489	
MZ00035698	Thiazole biosynthetic enzyme 1-2 {Zea mays}	-1.238592469	0.00420821	
MZ00035833	unnamed protein product	1.043803947	0.03181342	
MZ00035997	lipoxygenase6 {Zea mays}	2.361100536	7.54E-05	
MZ00036024	AER {Zea mays}	1.148408817	5.67E-05	
MZ00036078	hypothetical protein {Zea mays}	1.924555595	1.29E-08	
MZ00036171	UDP-glucose 6-dehydrogenase {Zea mays}	2.229600337	3.62E-06	
MZ00036200	NA	1.771636514	0.00180923	
MZ00036295	unnamed protein product	1.335942472	0.01941835	
MZ00036303	beta-expansin 1 protein {Zea mays}	1.025577602	0.01427073	
MZ00036398	xyloglucan endotransglycosylase homolog {Zea mays}	-1.106608878	0.00037272	
MZ00036485	nicotianamine synthase 3 {Zea mays}	1.084554877	0.02674138	
MZ00036530	unnamed protein product	1.813587954	0.01882393	

MZ00036538	subtilisin/chymotrypsin inhibitor {Zea mays}	3.650827443	1.89E-07	
MZ00036614	methionine synthase {Coffea arabica}	3.005417872	3.22E-05	
MZ00036626	Asr protein {Zea mays}	-1.051647385	0.00972258	
MZ00036710	NA	1.412688237	0.00041375	
MZ00036717	abscisic stress protein homolog {Zea mays}	1.020777793	0.02372581	
MZ00036758	hypothetical protein {Zea mays}	1.425188233	6.72E-06	
MZ00036774	O-succinylhomoserine sulfhydrylase {Zea mays}	1.703850736	4.54E-05	
MZ00036847	Ac1147 {Rattus norvegicus}	1.258534232	0.03839008	
MZ00036884	disease resistance-like protein {Arabidopsis thaliana}	1.235296364	0.00026378	
MZ00036916	NA	1.423860238	0.03495645	
MZ00036918	CDH1-D {Gallus gallus;}	1.539952036	0.0472115	
MZ00036940	tonoplast water channel {Zea mays}	1.133447104	0.00061518	
MZ00036975	Asr protein {Zea mays}	-1.186471142	0.01189242	
MZ00037045	pathogenesis related protein-5 {Zea mays}	1.297543025	0.00155856	
MZ00037070	polyamine oxidase1 {Zea mays}	3.088978537	0.00017111	
MZ00037085	Bowman-Birk type wound-induced proteinase inhibitor WIP1 {Zea mays}	5.65932748	4.83E-11	
MZ00037092	homeobox transcription factor KNOTTED1 (kn1) {Zea mays}	1.29106848	0.00046329	
MZ00037106	hypothetical protein {Zea mays}	-1.720936039	0.02571698	
MZ00037127	hypothetical protein {Zea mays}	2.554537218	2.54E-05	
MZ00037158	adenosylhomocysteinase {Zea mays}	1.639577332	6.10E-07	
MZ00037240	putative phosphoethanolamine methyltransferase {Oryza sativa}	1.130183996	0.00849243	
MZ00037253	subtilisin/chymotrypsin inhibitor {Zea mays}	3.467529924	2.36E-06	
MZ00037256	cytochrome P450 monooxygenase CYP71C3v2 {Zea mays}	1.330953174	0.00484051	
MZ00037263	annexin P35 {Zea mays}	1.02662211	0.00260273	
MZ00037304	terpene synthase 2 {Zea mays}	3.84179118	4.51E-08	
MZ00037357	safener induced1 {Zea mays}	1.560353844	0.00061984	
MZ00037429	adenosine kinase {Zea mays}	1.237351476	0.00181642	
MZ00037482	putative senescence-associated protein {Pisum sativum}	1.215580094	0.0315315	
MZ00037574	NA	1.31540595	1.21E-06	

MZ00037615	putative NLI interacting factor { <i>Oryza sativa</i> }	1.736868267	7.84E-07	TF48
MZ00037636	2-oxoglutarate-dependent oxygenase { <i>Zea mays</i> }	1.363467578	0.03965708	
MZ00037735	lipoxygenase11 { <i>Zea mays</i> }	1.302532528	0.00132902	
MZ00037858	UDP-glucuronic acid decarboxylase { <i>Oryza sativa</i> }	1.124100904	4.50E-05	
MZ00038365	beta-glucosidase aggregating factor precursor { <i>Zea mays</i> }	1.239380265	0.00036756	
MZ00038776	hypothetical protein { <i>Zea mays</i> }	1.302419117	0.00147753	
MZ00038794	elongation factor 1alpha { <i>Zea mays</i> }	3.339536678	3.30E-08	
MZ00038859	hypothetical protein { <i>Zea mays</i> }	2.213988295	1.19E-05	
MZ00039167	Putative C-4 sterol methyl oxidase { <i>Oryza sativa</i> }	2.164065924	0.00011494	
MZ00039459	putative thioredoxin { <i>Oryza sativa</i> }	1.213779261	2.82E-05	
MZ00039489	Bowman-Birk type wound-induced proteinase inhibitor { <i>Zea mays</i> }	3.342140742	6.52E-05	
MZ00039589	cytochrome b5 { <i>Oryza sativa</i> }	1.177676906	4.15E-05	
MZ00039598	blue fluorescent1 { <i>Zea mays</i> }	1.642364948	7.26E-05	
MZ00039734	beta-glucosidase { <i>Zea mays</i> }	2.191760902	3.21E-06	
MZ00039764	Bowman-Birk type wound-induced proteinase inhibitor WIP1 { <i>Zea mays</i> }	4.946218601	4.13E-09	
MZ00039782	cystatin II { <i>Zea mays</i> }	1.754947344	0.00388596	
MZ00039805	hypothetical protein { <i>Zea mays</i> }	1.460139962	0.0002745	
MZ00039806	anthranilate synthase component II { <i>Zea mays</i> }	1.924034011	0.0273911	
MZ00039818	cytochrome P450 monooxygenase CYP71C3v2 { <i>Zea mays</i> }	2.06096152	0.00574876	
MZ00040006	hypothetical protein { <i>Zea mays</i> }	1.377795851	0.01050715	
MZ00040123	HSP protein (HSP90-2) { <i>Zea mays</i> }	1.463121515	0.02353243	
MZ00040243	hypothetical protein { <i>Zea mays</i> }	2.677106357	0.00209871	
MZ00040386	Serine/threonine kinase protein	1.115811329	0.00495188	
MZ00040420	CSLE6 - cellulose synthase-like family E { <i>Zea mays</i> }	-1.000659501	0.00384163	
MZ00040469	hypothetical protein { <i>Sorghum bicolor</i> }	1.311579979	2.22E-05	
MZ00040479	coupling factor beta-subunit and coupling factor epsilon subunit { <i>Zea mays</i> }	1.764906451	0.01787083	

MZ00040486	Ribulose biphosphate carboxylase large chain precursor {Zea mays}	1.009652592	0.04574858	
MZ00040525	coupling factor beta-subunit and coupling factor epsilon subunit {Zea mays}	1.759964033	0.01579834	
MZ00040651	ribosome-inactivating protein {Zea mays}	1.16169119	0.00034695	
MZ00040654	glutathione S-transferase GST 25 {Zea mays}	4.008690578	4.57E-10	
MZ00040686	sesquiterpene cyclase {Zea mays}	1.185829618	0.00086881	
MZ00040837	dnaK-type molecular chaperone hsc70-2 {Arabidopsis thaliana}	1.014991123	0.04735592	
MZ00040937	hypothetical protein {Sorghum bicolor}	1.147020541	0.00025899	
MZ00040980	dnaK-type molecular chaperone hsp70 {Oryza sativa}	1.375815251	0.01261521	
MZ00041005	subtilisin-chymotrypsin inhibitor homolog1 {Zea mays}	3.959773934	4.86E-07	
MZ00041019	gibberellin responsive2 {Zea mays}	-2.716600508	0.01344002	
MZ00041129	tonoplast water channel {Zea mays}	1.381561891	0.00098186	
MZ00041213	putative UDP-glucose dehydrogenase {Sorghum bicolor}	1.973709909	0.0001496	
MZ00041214	UDP-glucose 6-dehydrogenase {Zea mays}	2.421688461	9.53E-07	
MZ00041254	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.224644729	1.63E-05	
MZ00041260	putative 70 kDa peptidylprolyl isomerase {Oryza sativa}	1.039472436	0.0113347	
MZ00041271	lipoygenase {Zea mays}	4.521047126	1.01E-09	
MZ00041287	heat shock protein hsp70 {Zea mays}	1.465548359	0.00039756	
MZ00041335	legumain-like protease {Zea mays}	-1.198148554	0.00116106	
MZ00041405	putative phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Oryza sativa}	1.595995236	0.00390226	
MZ00041439	dehydrin (dhn2) {Zea mays}	1.002675913	0.0039044	
MZ00041513	wound-induced protease inhibitor {Zea mays subsp. parviglumis}	1.665990925	8.15E-06	
MZ00041530	IN2-1 protein {Zea mays}	1.034567658	0.04443869	
MZ00041596	ZIM-motif family protein {Zea mays}	1.027619472	0.01852564	
MZ00041601	beta-glucosidase aggregating factor precursor {Zea mays}	1.356055442	0.02873466	
MZ00041768	putative zinc finger protein {Zea mays}	3.616692105	2.31E-05	

MZ00041827	3-dehydroquinate synthase {Zea mays}	1.108372983	0.04699278	
MZ00042000	At5g04080 {Arabidopsis thaliana}	1.725110889	0.00119655	
MZ00042109	O-methyltransferase BX7 {Zea mays}	2.061277542	0.00027866	
MZ00042158	ripening-related protein {Zea mays}	1.766710856	2.79E-07	
MZ00042217	hypothetical protein {Zea mays}	1.017663782	0.00029728	
MZ00042245	O-succinylhomoserine sulfhydrylase {Zea mays}	1.975941173	3.55E-06	
MZ00042324	ribosome-inactivating protein. {Zea mays}	3.633688185	2.36E-07	
MZ00042637	phosphoenolpyruvate carboxylase {Zea mays}	1.272955991	0.01328751	
MZ00042678	ribosome-inactivating protein {Zea mays}	3.638665168	2.48E-05	
MZ00042841	Histone H2A {Zea mays}	1.347556855	0.0481309	
MZ00042873	S-adenosylmethionine synthetase 1 {Zea mays}	2.254520198	0.00012398	
MZ00042904	HSP protein (HSP90-2) {Zea mays}	1.347214915	0.00085693	
MZ00042921	methionine synthase {Zea mays}	1.942360533	7.22E-06	
MZ00042935	Bowman-Birk type wound-induced proteinase inhibitor {Zea mays}	2.806477643	3.53E-06	
MZ00042940	hypothetical protein {Zea mays}	-1.038946349	0.00095885	
MZ00042953	chlorophyll a/b binding protein {Oryza sativa}	3.703542259	8.95E-06	
MZ00042980	hypothetical protein {Zea mays}	2.718403746	0.00028633	
MZ00042981	hypothetical protein {Zea mays}	2.869766039	0.00023453	
MZ00043004	cystatin {Zea mays}	2.877029847	0.00272396	
MZ00043035	chitinase {Zea mays}	2.304194705	5.10E-05	
MZ00043160	hypothetical protein {Zea mays}	2.515494427	0.00645937	
MZ00043179	subtilisin/chymotrypsin inhibitor {Zea mays}	1.389935228	8.58E-05	
MZ00043232	putative glycine-rich protein {Oryza sativa}	2.724001999	2.33E-07	
MZ00043240	ribonuclease S homolog {Zea mays}	1.936173351	0.00034383	
MZ00043311	patatin-like protein {Sorghum bicolor}	1.086772039	0.03507248	
MZ00043339	Thiazole biosynthetic enzyme 1-2 {Zea mays}	-1.110096582	0.00364068	
MZ00043393	2-oxoglutarate-dependent oxygenase {Zea mays}	2.480265011	7.84E-08	
MZ00043517	allene oxide cyclase {Zea mays}	1.61478242	0.00392188	
MZ00043518	allene oxide cyclase {Zea mays}	1.276933426	0.000768	

MZ00043781	lipoxygenase {Zea mays}	1.19008889	0.00380615	
MZ00043784	trans-cinnamate 4-monooxygenase {Zea mays}	1.042706134	0.00329744	
MZ00043885	farnesyl-pyrophosphate synthetase {Oryza sativa}	1.630982419	0.00021638	
MZ00044135	hypothetical protein {Zea mays}	1.187979142	4.50E-06	
MZ00044190	allene oxide synthase 1 {Zea mays}	4.078079317	1.65E-07	
MZ00044245	lipoxygenase6 {Zea mays}	2.141181888	1.68E-06	
MZ00044413	benzoxazinone synthesis BX9 {Zea mays}	1.235658497	0.01949316	
MZ00044555	putative phosphoribosyl pyrophosphate synthase {Oryza sativa}	3.80066505	5.90E-09	
MZ00044562	NA	1.490490377	3.22E-06	
MZ00044642	hypothetical protein {Oryza sativa}	2.051187735	5.80E-08	
MZ00046552	hypothetical protein {Zea mays}	1.026512391	0.00813629	
MZ00046592	glutathione transferase40 {Zea mays}	1.272607956	2.08E-05	
MZ00047034	putative arginine methyltransferase {Oryza sativa}	1.112998783	0.03587827	
MZ00047153	methylenetetrahydrofolate reductase {Zea mays}	1.718731981	2.64E-05	
MZ00048571	acc oxidase {Zea mays}	1.4146259	1.44E-05	
MZ00048905	hypothetical protein {Zea mays}	1.01234542	0.00030394	
MZ00049092	G-box binding protein {Oryza sativa}	1.200285945	3.47E-06	TF21
MZ00049506	hypothetical protein {Oryza sativa}	1.554045332	0.0130741	
MZ00052059	flowering promoting factor-like 1 {Zea mays}	1.211568258	0.00772696	
MZ00052490	pathogenesis-related protein 10 {Zea mays}	1.415478947	0.00050066	
MZ00054816	adenosylhomocysteinase {Zea mays}	1.650873179	1.74E-06	
MZ00054822	phospho-2-dehydro-3-deoxyheptonate aldolase 1 {Zea mays}	1.041476239	0.00021236	
MZ00054897	glutathione S-transferase GST 25 {Zea mays}	3.109945306	3.26E-08	
MZ00054995	hypothetical protein {Zea mays}	1.678339411	3.37E-06	
MZ00055013	methylenetetrahydrofolate reductase {Zea mays}	1.538239481	1.13E-06	
MZ00055270	ripening-related protein {Zea mays}	1.7991641	5.49E-05	
MZ00055448	hypothetical protein {Zea mays}	1.782120094	0.00023721	
MZ00055463	esterase {Zea mays}	1.661780979	9.55E-07	

MZ00055932	Bowman-Birk type trypsin inhibitor {Zea mays}	2.569479125	1.37E-06	
MZ00056269	hypothetical protein {Sorghum bicolor}	1.004733332	2.81E-05	
MZ00056664	hypothetical protein {Sorghum bicolor}	1.306314787	0.01244828	
MZ00056687	farnesyl pyrophosphate synthase1 {Zea mays}	2.021782258	0.00039941	
MZ00056765	prefoldin subunit 2 {Zea mays}	-1.179215429	0.01330582	
MZ00056914	lipid binding protein {Zea mays}	-1.007349915	0.04252574	
MZ00056987	acyl-desaturase {Zea mays}	1.264800142	0.00628795	
MZ00057095	putative UVB-resistance protein UVR8 {Oryza sativa}	1.035194979	0.02070113	
MZ00057294	putative dehydration-responsive protein RD22 precursor {Oryza sativa}	1.907172925	0.00151744	
MZ00057421	Glyceraldehyde 3-phosphate dehydrogenase {Zea mays}	1.078073788	0.04255359	
MZ00057436	putative ZEITLUPE {Oryza sativa}	1.740651364	0.0045763	

7.7 Transcription factors regulated by herbivory, mechanical damage, and systemic induction in leaves and roots of maize

Tab.7. 5: Transcription factors identified in roots. Comparison between systemic induction and mechanical damage

MZ-number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000664	RING finger-like {Oryza sativa}	-1.17311677	0.00256481	TF50
MZ00000942	putative Myb-related transcription factor {Oryza sativa}	-1.11768355	0.013915	
MZ00005189	RING-H2 zinc finger protein ATL6-like {Oryza sativa}	-1.08860955	0.0003481	
MZ00012674	zinc finger protein 3 {Zea mays}	-1.07583988	0.01514441	
MZ00013314	myb-related gene Zm1. {Zea mays}	-1.2089436	0.00156514	
MZ00013458	putative zinc finger protein ZmZf {Zea mays}	1.45477385	0.00073486	
MZ00015793	putative DNA-binding protein PD3 {Oryza sativa}	1.62217624	0.01486775	
MZ00018661	F-box protein family {Arabidopsis thaliana}	1.02444407	0.00162727	
MZ00018825	putative transcription factor {Oryza sativa}	1.12985982	0.00129396	
MZ00019229	twin LOV protein 1 {Arabidopsis thaliana}	1.0775719	0.00291805	
MZ00020499	development regulation gene OsNAC4 {Oryza sativa}	-1.22221271	0.01218443	
MZ00021114	putative F-box protein {Oryza sativa}	2.00276908	0.00586368	
MZ00022225	zinc finger (AN1-like)-like protein {Oryza sativa}	-1.19631869	0.04278423	
MZ00022460	putative Ras-GTPase-activating protein binding protein 1 {Oryza sativa}	-1.20398419	0.00162251	
MZ00024843	putative zinc finger transcription factor {Oryza sativa}	1.20573495	5.73E-05	
MZ00025533	myb-related transcription factor MYB59 {Arabidopsis thaliana}	1.03666135	0.00476553	
MZ00026127	development regulation gene OsNAC4	-1.02172616	0.02978254	

	{ <i>Oryza sativa</i> }			
MZ00026538	ZIM-motif family protein { <i>Zea mays</i> }	-1.41036951	0.00419174	
MZ00026661	putative NAC-domain protein { <i>Oryza sativa</i> }	-2.38974895	0.00013525	TF1
MZ00028419	bZIP protein { <i>Oryza sativa</i> }	1.37423647	0.00066664	
MZ00028904	myb-related gene Zm38. { <i>Zea mays</i> }	1.02765764	0.00930875	
MZ00030906	transcription factor Rap212 { <i>Oryza sativa</i> }	-1.13699108	0.00591033	
MZ00030973	putative NAC-domain protein { <i>Oryza sativa</i> }	-1.289499	9.73E-05	
MZ00031271	transcription factor { <i>Oryza sativa</i> }	-1.04920935	0.03016695	
MZ00032440	putative leucine zipper-containing protein { <i>Oryza sativa</i> }	-1.03465161	0.04385328	
MZ00033157	Myb-related transcription factor-like protein { <i>Oryza sativa</i> }	1.00375338	0.00733829	
MZ00033341	transcription factor Myb3 { <i>Triticum aestivum</i> }	-1.19466639	0.00259737	
MZ00033711	putative NAC-domain protein { <i>Oryza sativa</i> }	-1.07117436	0.00300881	
MZ00033715	ZIM-motif family protein { <i>Zea mays</i> }	-1.39199293	0.03460359	
MZ00035860	GRAB1 protein { <i>Triticum sp.</i> }	-1.0185033	0.01178006	
MZ00035947	putative NAC-domain protein { <i>Oryza sativa</i> }	-1.01956023	0.00138845	TF22
MZ00036019	putative NAC-domain protein { <i>Oryza sativa</i> }	-2.44954618	0.00014679	TF51
MZ00037166	putative zinc finger transcription factor { <i>Oryza sativa</i> }	1.42192839	5.77E-05	
MZ00038922	WRKY transcription factor { <i>Oryza sativa</i> }	-1.01094044	0.00583476	
MZ00039225	putative zinc finger protein ZmZf { <i>Zea mays</i> }	1.3916815	0.00070862	
MZ00040842	strongly similar to NP_195589.2 splicing factor	-1.82242211	0.01655065	
MZ00041306	putative ASR2 { <i>Oryza sativa</i> }	-1.2099269	0.00401326	
MZ00041768	putative zinc finger protein { <i>Zea mays</i> }	-1.87712778	1.24E-05	
MZ00042353	R2R3 Myb transcription factor MYB-	-1.43425611	0.00450982	

	IF35 {Zea mays}			
MZ00042676	smr domain-containing protein -like {Oryza sativa}	-1.38823076	0.00880997	
MZ00042739	WRKY transcription factor {Oryza sativa}	-1.20980279	0.00117118	TF26
MZ00046918	putative homeodomain protein {Oryza sativa}	-1.05545756	0.00014435	
MZ00049598	putative bZIP protein {Oryza sativa}	1.17967009	0.00020976	
MZ00056649	putative SWIb domain-containing protein {Oryza sativa}	-1.04308183	0.01821648	
MZ00057150	putative FKBP12 interacting protein {Oryza sativa}	-1.02718293	0.00011043	
MZ00057284	zinc finger protein family-like {Oryza sativa}	1.01316243	0.03649266	

Tab.7. 6: Transcription factors identified in roots. Comparison between local herbivore damage and mechanical damage

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000937	putative bHLH084 transcription factor {Arabidopsis thaliana}	-1.45459357	0.00104639	
MZ00000942	putative Myb-related transcription factor {Oryza sativa}	-1.23641091	0.00973813	
MZ00005265	putative helix-loop-helix DNA-binding protein {Oryza sativa (japonica cultivar-group);}	1.76936392	0.00118141	TF23
MZ00012587	putative ethylene response factor 1 {Oryza sativa}	-1.13552342	0.00507723	
MZ00012674	zinc finger protein 3 {Zea mays}	1.10422733	0.0154617	
MZ00013750	LIM transcription factor homolog {Zea mays}	-1.07140769	0.00381153	
MZ00015793	putative DNA-binding protein PD3 {Oryza sativa}	2.10213416	0.00439977	
MZ00018321	AP2 domain transcription factor EREBP {Oryza sativa}	2.03570049	0.03399623	
MZ00018515	putative zinc finger and C2 domain	-1.12721725	7.15E-05	

	protein {Oryza sativa}			
MZ00019724	zinc-finger protein S3574 {Oryza sativa}	1.06297339	0.00287096	
MZ00022534	FKF1 {Mesembryanthemum crystallinum}	1.1901183	0.00033774	
MZ00025599	putative zinc finger protein {Oryza sativa}	-1.15153203	4.81E-05	
MZ00026726	similar to nucleoid DNA-binding-like protein {Oryza sativa}	-1.21283829	0.00106192	
MZ00029568	ZIM-motif family protein {Zea mays}	1.07886517	0.00027794	
MZ00031271	transcription factor {Oryza sativa}	2.30031778	0.00040686	
MZ00033715	ZIM-motif family protein {Zea mays}	1.74012163	0.01462193	
MZ00037906	Radc1 {Oryza sativa}	-1.23534247	0.0042087	
MZ00040702	R2R3 Myb protein {Zea mays}	-1.54732424	0.00029875	
MZ00041718	floral organ regulator 2 {Oryza sativa}	-1.07285153	0.00272305	
MZ00043644	putative EREBP-type transcription factor {Oryza sativa}	2.02737794	0.00339868	
MZ00043824	putative WD-40 repeat protein {Oryza sativa}	-1.04729989	0.00152939	
MZ00044166	floral organ regulator 2 {Oryza sativa}	-1.20193017	0.0049003	

Tab.7. 7: Transcription factors identified in systemic induced roots

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00004381	bZIP transcription factor-like protein {Arabidopsis thaliana}	-1.14356897	0.0116739	
MZ00014951	transcriptional activator DEMETER {Arabidopsis thaliana}	1.03194135	0.00033156	
MZ00017495	OSE3 {Oryza sativa}	1.35581577	0.00678932	
MZ00019076	Hox2b {Zea mays}	1.04260847	0.0003865	
MZ00021809	zwh21.1 {Oryza sativa}	-1.09485472	0.00644913	
MZ00022377	ZIGA2 protein-like {Oryza sativa}	-1.03227658	0.00182741	
MZ00022460	putative Ras-GTPase-activating protein binding protein 1 {Oryza sativa}	-1.34135467	0.0006585	
MZ00023293	MCB2 protein {Hordeum vulgare subsp.}	1.1090304	0.0012409	

	Vulgare}			
MZ00026127	development regulation gene OsNAC4 {Oryza sativa}	1.11467291	0.01781558	
MZ00026870	RISBZ5 {Oryza sativa}	1.10551394	0.02792086	
MZ00027941	putative ATP-binding-cassette protein {Oryza sativa}	2.30432218	0.01084393	
MZ00028351	zinc-finger protein S3574 {Oryza sativa}	-1.52392556	0.01036772	
MZ00036053	SAR DNA binding protein {Oryza sativa}	-1.05992594	0.00028416	
MZ00036067	OSE3 {Oryza sativa}	1.13275655	0.00188415	
MZ00036162	rRNA promoter binding protein {Rattus norvegicus}	-1.87284556	0.00771251	
MZ00039225	putative zinc finger protein ZmZf {Zea mays}	1.02017357	0.0042214	
MZ00040842	strongly similar to NP_195589.2 splicing factor	-2.34866516	0.00367576	
MZ00048943	putative transcription factor {Oryza sativa}	-1.11201395	0.00033161	
MZ00054846	WRKY transcription factor 70 {Oryza sativa}	2.58060619	0.00271994	

Tab.7. 8: Transcription factors identified in herbivore-attacked roots

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000937	putative bHLH084 transcription factor {Arabidopsis thaliana}	-1.44819319	0.00132125	
MZ00001769	myb transcription factor {Hordeum vulgare subsp. Vulgare}	1.21169365	0.00085054	
MZ00004381	bZIP transcription factor-like protein {Arabidopsis thaliana}	-1.03848128	0.0278384	
MZ00005189	RING-H2 zinc finger protein ATL6-like {Oryza sativa}	1.05349043	0.00067154	
MZ00005265	putative helix-loop-helix DNA-binding protein {Oryza sativa}	1.61261607	0.00259158	TF23
MZ00012674	zinc finger protein 3 {Zea mays}	1.99250531	0.00056628	
MZ00015793	putative DNA-binding protein PD3	1.44270659	0.03312226	

	{Oryza sativa}			
MZ00017226	DNA-binding protein RAV2-like {Oryza sativa}	1.03257487	0.00075845	
MZ00017495	OSE3 {Oryza sativa}	1.60672272	0.00432566	
MZ00018321	AP2 domain transcription factor EREBP {Oryza sativa}	3.09154154	0.0051438	
MZ00019886	transcription factor MYC7E {Zea mays}	1.21347465	0.00012983	
MZ00020499	development regulation gene OsNAC4 {Oryza sativa}	1.67398892	0.00300613	
MZ00026127	development regulation gene OsNAC4 {Oryza sativa}	1.73215694	0.00266655	
MZ00026538	ZIM-motif family protein {Zea mays}	2.31151939	0.0002444	
MZ00026661	putative NAC-domain protein {Oryza sativa}	2.55609061	0.00012955	TF2
MZ00028044	WRKY-type DNA binding protein {Solanum tuberosum}	1.57561401	0.00536121	
MZ00028131	kelch repeat-containing F-box-like {Oryza sativa}	1.12681359	0.00016689	
MZ00028389	putative GAMYB-binding protein {Oryza sativa}	1.09379614	0.02579671	
MZ00031271	Transcription Factor {Oryza sativa}	3.21045114	4.31E-05	
MZ00033341	transcription factor Myb3 {Triticum aestivum}	1.23358786	0.00310482	
MZ00033715	ZIM-motif family protein {Zea mays}	2.66226357	0.00157363	
MZ00035959	putative homeodomain protein {Oryza sativa}	1.54528552	9.28E-05	
MZ00035991	putative chloroplast nucleoid DNA-binding protein cnd41 {Oryza sativa}	1.05895222	0.00751199	
MZ00036019	putative NAC-domain protein {Oryza sativa}	2.14675134	0.00058666	TF51
MZ00036067	OSE3 {Oryza sativa} leuzin zipper	1.49577716	0.00054186	
MZ00040702	R2R3 Myb protein {Zea mays}	-1.16150608	0.00249669	
MZ00041768	putative zinc finger protein {Zea mays}	2.29463039	4.00E-06	
MZ00042353	R2R3 Myb transcription factor MYB-IF35 {Zea mays}	1.88808181	0.00121086	
MZ00042739	WRKY transcription factor {Oryza	1.18263124	0.00201012	TF26

	sativa}			
MZ00043644	putative EREBP-type transcription factor {Oryza sativa;}	1.98563475	0.00460597	
MZ00046918	putative homeodomain protein {Oryza sativa}	1.19531442	9.01E-05	
MZ00056566	zinc finger transcription factor ZF1 {Oryza sativa}	1.42091009	0.00147646	TF46

Tab.7. 9: Transcription factors identified in mechanical wounded roots

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000942	putative Myb-related transcription factor {Oryza sativa}	1.23401259	0.01833295	
MZ00004235	PRLI-interacting factor G-like protein {Oryza sativa }	1.01257862	0.02626641	
MZ00004381	bZIP transcription factor-like protein {Arabidopsis thaliana}	-1.52370818	0.00681936	
MZ00013767	initiation factor (iso)4f p82 subunit {Triticum aestivum}	1.14695383	0.00075967	
MZ00020499	development regulation gene OsNAC4 {Oryza sativ}	2.04519041	0.00156283	
MZ00021114	putative F-box protein {Oryza sativa}	-2.22404443	0.0077869	
MZ00023973	putative NAC-domain protein {Oryza sativa}	1.04455787	0.00244106	
MZ00026127	development regulation gene OsNAC4 {Oryza sativa}	2.13639907	0.00129392	
MZ00026538	ZIM-motif family protein {Zea mays}	1.38545928	0.01087381	
MZ00026661	putative NAC-domain protein {Oryza sativa}	2.48132865	0.00032211	TF1
MZ00028044	WRKY-type DNA binding protein {Solanum tuberosum}	1.46023276	0.01327617	
MZ00028351	zinc-finger protein S3574 {Oryza sativa}	-1.73644816	0.01363055	
MZ00028419	bZIP protein {Oryza sativa}	-1.65777426	0.00053869	
MZ00031018	ethylene-responsive transcription factor 4 {Zea mays}	1.00709987	0.02262177	
MZ00035959	putative homeodomain protein {Oryza	1.49720919	0.00023715	

	sativa}			
MZ00036019	putative NAC-domain protein {Oryza sativa}	1.93159471	0.00218935	TF51
MZ00041306	putative ASR2 {Oryza sativa}	1.38555034	0.00453449	
MZ00041768	putative zinc finger protein {Zea mays}	2.11656395	1.67E-05	
MZ00042353	R2R3 Myb transcription factor MYB-IF35 {Zea mays}	1.19742317	0.02499708	
MZ00046918	putative homeodomain protein {Oryza sativa}	1.38046222	6.20E-05	
MZ00054846	WRKY transcription factor 70 {Oryza sativa}	1.81331771	0.04033333	
MZ00055542	zinc-finger protein R2931 {Oryza sativa}	1.09809491	0.0006885	
MZ00056523	putative phi-1 {Oryza sativa}	1.49562379	0.01697013	TF42
MZ00057404	PHD finger protein-like {Oryza sativa}	1.02584234	0.01008488	

Tab.7. 10: Transcription factors in leaves. Comparison between systemic induction and mechanical damage

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00000664	RING finger-like {Oryza sativa}	-1.17021127	0.00069659	TF50
MZ00001133	putative LHY protein {Oryza sativa}	-1.54479187	0.00029076	TF41
MZ00014272	putative LHY protein {Oryza sativa}	-2.08412602	0.02891213	
MZ00021611	NAM-like protein {Oryza sativa}	-1.19152825	0.0197212	
MZ00023293	MCB2 protein {Hordeum vulgare subsp. Vulgare}	-1.10641059	0.00319869	
MZ00030973	putative NAC-domain protein {Oryza sativa}	-1.14540025	0.00049405	
MZ00032849	putative phi-1 {Oryza sativa}	1.09935974	0.00190247	
MZ00041462	putative LHY protein {Oryza sativa}	-2.00264664	0.01398821	
MZ00056649	putative SWIb domain-containing protein {Oryza sativa}	-1.20443432	0.02147772	

Tab.7. 11: Transcription factors in leaves. Comparison between herbivore induction and mechanical damage

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00001821	zinc finger (C3HC4-type RING finger)	-1.0821601	0.02187925	

	protein-like {Oryza sativa}			
MZ00012674	zinc finger protein 3 {Zea mays}	2.32013829	4.13E-04	
MZ00017617	putative WD-repeat protein 12 {Oryza sativa}	-1.25582415	0.00183641	
MZ00017641	DNA binding protein-like {Oryza sativa}	-1.07749508	0.00586116	
MZ00018435	CDC5 protein {Zea mays}	2.03854817	0.00041144	
MZ00019167	DNA-binding protein-like {Oryza sativa}	-1.12845165	0.0162055	
MZ00020099	putative zinc finger (C3HC4-type RING finger) protein {Oryza sativa}	-1.20289123	0.00629871	
MZ00025599	putative zinc finger protein {Oryza sativa}	-1.06110654	0.03758546	
MZ00026538	ZIM-motif family protein {Zea mays}	1.80812953	0.01490771	
MZ00028904	myb-related gene Zm38. {Zea mays}	-1.14581193	0.01165362	
MZ00031176	DNA-binding protein-like protein {Oryza sativa}	-1.15092799	0.03580132	
MZ00031553	putative HAC13 protein {Oryza sativa}	-1.30267487	0.00238518	
MZ00032076	putative ZR1 protein {Oryza sativa}	-2.20207462	0.00859237	
MZ00035947	putative NAC-domain protein {Oryza sativa}	1.27497329	0.01101208	TF22
MZ00036019	putative NAC-domain protein {Oryza sativa}	1.22392712	0.00352567	TF51
MZ00037092	homeobox transcription factor KNOTTED1 (kn1) {Zea mays}	2.54708663	0.01426112	
MZ00040600	SBP-domain protein 7 {Zea mays}	-1.00525421	0.02227148	
MZ00041768	putative zinc finger protein {Zea mays}	5.08405164	2.65E-03	
MZ00044274	putative finger transcription factor {Oryza sativa}	-1.25357837	0.03216688	
MZ00044596	R2R3MYB-domain protein {Zea mays}	-1.05460042	0.0039064	

Tab.7. 12: Transcription factors regulated in mechanical damaged leaves

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00021611	NAM-like protein {Oryza sativa}	1.18	0.03	
MZ00028419	bZIP protein {Oryza sativa}	-1.11	0.02	
MZ00028904	myb-related gene Zm38. {Zea mays}	1.15	0.02	

Tab.7. 13: Transcription factors regulated in herbivore-damaged leaves

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00012674	zinc finger protein 3 {Zea mays}	2.23317188	0.00032993	
MZ00016101	putative RAB7A protein (GTP-binding protein) {Oryza sativa}	1.18925891	0.00021631	TF49
MZ00017495	OSE3 {Oryza sativa}	1.1681705	2.05E-02	
MZ00024412	EREBP-like protein {Oryza sativa}	-1.31531362	0.02761637	
MZ00026538	ZIM-motif family protein {Zea mays}	1.72623912	0.01317232	
MZ00036019	putative NAC-domain protein {Oryza sativa}	1.19363088	0.00269634	TF51
MZ00037092	homeobox transcription factor KNOTTED1 (kn1) {Zea mays}	2.37885291	0.01395867	
MZ00041768	putative zinc finger protein {Zea mays}	5.04517754	0.0018165	

Tab.7. 14: Transcription factors in systemic induced leaves

MZ number	Annotation	fold expression (log10)	p-value	Abbreviation
MZ00001373	putative F-box protein Fbl2 {Oryza sativa}	-1.93695949	0.03356703	
MZ00016166	putative transcription factor EREBP1 {Oryza sativa}	-1.0654935	0.02284059	
MZ00018541	Putative EREBP-like protein {Oryza sativa}	-1.6199081	0.03713096	TF38
MZ00020231	zinc finger protein family-like {Oryza sativa}	-1.12971082	0.03208903	
MZ00030973	putative NAC-domain protein {Oryza sativa}	-1.18177136	0.00056905	
MZ00032849	putative phi-1 {Oryza sativa}	1.30492278	0.00093883	
MZ00035947	putative NAC-domain protein {Oryza sativa}	-1.44313555	1.05E-02	TF22
MZ00036608	putative transcription factor EREBP1 {Oryza sativa}	-1.9900955	0.01739547	
MZ00056649	putative SWIb domain-containing protein {Oryza sativa}	-1.51655072	0.00947039	

7.8 Regulatory cis-elements of the 1.5 kb promoter fragment of *tps23* in *Zea mays* var. Delprim

Tab.7. 15

tps23 1500 bp	Organism	Position	Strand	Sequence	Database
-300ELEMENT		377	-	TGHAAARK	PLACE
AACACOREOSGLUB1		443	+	AACAAAC	PLACE
AAGAA-motif	<i>Avena sativa</i>	729	-	GAAAGAA	PlantCARE
AAGAA-motif	<i>Avena sativa</i>	1205	-	GAAAGAA	PlantCARE
AAGAA-motif	<i>Avena sativa</i>	978	-	GAAAGAA	PlantCARE
A-box	<i>Petroselinum crispum</i>	327	-	CCGTCC	PlantCARE
ABRE	<i>Hordeum vulgare</i>	41	+	CGTACGTGCA	PlantCARE
ABRE	<i>Oryza sativa</i>	44	+	GACACGTACGT	PlantCARE
ABRE	<i>Hordeum vulgare</i>	163	+	CGTACGTGCA	PlantCARE
ABRE	<i>Arabidopsis thaliana</i>	43	+	TACGTG	PlantCARE
ABRE	<i>Arabidopsis thaliana</i>	165	+	TACGTG	PlantCARE
ABRE	<i>Arabidopsis thaliana</i>	47	-	TACGTG	PlantCARE
ABRE	<i>Arabidopsis thaliana</i>	157	-	CACGTG	PlantCARE
ABRE	<i>Hordeum vulgare</i>	45	-	CGTACGTGCA	PlantCARE
ABRELATERD1		163	+	ACGTG	PLACE
ABRELATERD1		53	+	ACGTG	PLACE
ABRELATERD1		170	-	ACGTG	PLACE
ABRELATERD1		162	-	ACGTG	PLACE
ABRELATERD1		48	-	ACGTG	PLACE
ABREOSRAB21		166	+	ACGTSSSC	PLACE
ABREOSRAB21		56	+	ACGTSSSC	PLACE
ABREOSRAB21		170	-	ACGTSSSC	PLACE
ABRERATCAL		164	+	MACGYGB	PLACE
ABRERATCAL		163	-	MACGYGB	PLACE
ACA-motif	<i>Arabidopsis</i>	661	+	AATCACAACCA	PlantCARE

	<i>thaliana</i>			TA	
ACGTABOX		175	+	TACGTA	PLACE
ACGTABOX		175	-	TACGTA	PLACE
ACGTATERD1		1488	+	ACGT	PLACE
ACGTATERD1		933	+	ACGT	PLACE
ACGTATERD1		174	+	ACGT	PLACE
ACGTATERD1		170	+	ACGT	PLACE
ACGTATERD1		162	+	ACGT	PLACE
ACGTATERD1		52	+	ACGT	PLACE
ACGTATERD1		48	+	ACGT	PLACE
ACGTATERD1		1488	-	ACGT	PLACE
ACGTATERD1		933	-	ACGT	PLACE
ACGTATERD1		174	-	ACGT	PLACE
ACGTATERD1		170	-	ACGT	PLACE
ACGTATERD1		162	-	ACGT	PLACE
ACGTATERD1		52	-	ACGT	PLACE
ACGTATERD1		48	-	ACGT	PLACE
ACGTOSGLUB1		53	+	GTACGTG	PLACE
ACGTOSGLUB1		172	-	GTACGTG	PLACE
ACGTOSGLUB1		50	-	GTACGTG	PLACE
ACGTTBOX		1489	+	AACGTT	PLACE
ACGTTBOX		1489	-	AACGTT	PLACE
AE-box	<i>Arabidopsis thaliana</i>	1470	+	AGAAACAA	PlantCARE
ANAERO1CONSENSUS		1543	+	AAACAAA	PLACE
ANAERO1CONSENSUS		717	+	AAACAAA	PLACE
ANAERO1CONSENSUS		442	+	AAACAAA	PLACE
ANAERO1CONSENSUS		1476	-	AAACAAA	PLACE
ANAERO2CONSENSUS		741	+	AGCAGC	PLACE
ARE	<i>Zea mays</i>	319	-	TGGTTT	PlantCARE
ARE	<i>Zea mays</i>	1427	-	TGGTTT	PlantCARE

ARR1AT		1704	+	NGATT	PLACE
ARR1AT		1562	+	NGATT	PLACE
ARR1AT		1456	+	NGATT	PLACE
ARR1AT		1223	+	NGATT	PLACE
ARR1AT		1139	+	NGATT	PLACE
ARR1AT		1037	+	NGATT	PLACE
ARR1AT		990	+	NGATT	PLACE
ARR1AT		896	+	NGATT	PLACE
ARR1AT		791	+	NGATT	PLACE
ARR1AT		769	+	NGATT	PLACE
ARR1AT		674	+	NGATT	PLACE
ARR1AT		577	+	NGATT	PLACE
ARR1AT		1661	-	NGATT	PLACE
ARR1AT		1337	-	NGATT	PLACE
ARR1AT		706	-	NGATT	PLACE
ARR1AT		777	-	NGATT	PLACE
ARR1AT		1425	-	NGATT	PLACE
ARR1AT		864	-	NGATT	PLACE
ASF1MOTIFCAMV		658	+	TGACG	PLACE
BIHD1OS		1557	+	TGTCA	PLACE
BIHD1OS		1440	-	TGTCA	PLACE
BIHD1OS		1234	-	TGTCA	PLACE
Box 4	<i>Petroselinum crispum</i>	788	-	ATTAAT	PlantCARE
Box I	<i>Pisum sativum</i>	344	+	TTTCAA	PlantCARE
CAAT-box	<i>Brassica rapa</i>	179	+	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	190	+	CAAT	PlantCARE
CAAT-box	<i>Glycine max</i>	280	+	CAATT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	281	+	CAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	574	+	CAAAT	PlantCARE
CAAT-box	<i>Glycine max</i>	940	+	CAATT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	941	+	CAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	1259	+	CAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	1381	+	CAAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	1468	+	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	79	-	CAAT	PlantCARE

CAAT-box	<i>Glycine max</i>	177	-	CAATT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	313	-	CAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	402	-	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	621	-	CAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	699	-	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	771	-	CAAT	PlantCARE
CAAT-box	<i>Glycine max</i>	1010	-	CAATT	PlantCARE
CAATBOX1		1014	+	CAAT	PLACE
CAATBOX1		775	+	CAAT	PLACE
CAATBOX1		625	+	CAAT	PLACE
CAATBOX1		317	+	CAAT	PLACE
CAATBOX1		181	+	CAAT	PLACE
CAATBOX1		83	+	CAAT	PLACE
CAATBOX1		1509	-	CAAT	PLACE
CAATBOX1		1263	-	CAAT	PLACE
CAATBOX1		945	-	CAAT	PLACE
CAATBOX1		285	-	CAAT	PLACE
CAATBOX1		194	-	CAAT	PLACE
CACGTGMOTIF		163	+	CACGTG	PLACE
CACGTGMOTIF		163	-	CACGTG	PLACE
CACTFTPPCA1		1375	+	YACT	PLACE
CACTFTPPCA1		1294	+	YACT	PLACE
CACTFTPPCA1		1269	+	YACT	PLACE
CACTFTPPCA1		1239	+	YACT	PLACE
CACTFTPPCA1		1052	+	YACT	PLACE
CACTFTPPCA1		910	+	YACT	PLACE
CACTFTPPCA1		485	+	YACT	PLACE
CACTFTPPCA1		310	+	YACT	PLACE
CACTFTPPCA1		232	+	YACT	PLACE
CACTFTPPCA1		177	+	YACT	PLACE
CACTFTPPCA1		150	+	YACT	PLACE
CACTFTPPCA1		92	+	YACT	PLACE
CACTFTPPCA1		16	+	YACT	PLACE
CACTFTPPCA1		1157	-	YACT	PLACE
CACTFTPPCA1		1119	-	YACT	PLACE
CACTFTPPCA1		145	-	YACT	PLACE

CACTFTPPCA1		71	-	YACT	PLACE
CACTFTPPCA1		1772	-	YACT	PLACE
CACTFTPPCA1		1690	-	YACT	PLACE
CACTFTPPCA1		1537	-	YACT	PLACE
CACTFTPPCA1		1513	-	YACT	PLACE
CACTFTPPCA1		1469	-	YACT	PLACE
CACTFTPPCA1		1153	-	YACT	PLACE
CACTFTPPCA1		1067	-	YACT	PLACE
CACTFTPPCA1		860	-	YACT	PLACE
CACTFTPPCA1		822	-	YACT	PLACE
CACTFTPPCA1		337	-	YACT	PLACE
CACTFTPPCA1		12	-	YACT	PLACE
CACTFTPPCA1		7	-	YACT	PLACE
CANBNNAPA		1451	-	CNAACAC	PLACE
CARGCW8GAT		788	+	CWWWWWWW WG	PLACE
CARGCW8GAT		194	+	CWWWWWWW WG	PLACE
CARGCW8GAT		128	+	CWWWWWWW WG	PLACE
CARGCW8GAT		788	-	CWWWWWWW WG	PLACE
CARGCW8GAT		194	-	CWWWWWWW WG	PLACE
CARGCW8GAT		128	-	CWWWWWWW WG	PLACE
CBFHV		708	+	RYCGAC	PLACE
CBFHV		261	+	RYCGAC	PLACE
CCGTCC-box	<i>Arabidopsis thaliana</i>	327	-	CCGTCC	PlantCARE
CGACGOSAMY3		223	-	CGACG	PLACE
CGTCA-motif	<i>Hordeum vulgare</i>	653	+	CGTCA	PlantCARE
CIACADIANLELHC		1660	+	CAANNNNATC	PLACE
CIACADIANLELHC		1081	-	CAANNNNATC	PLACE
circadian	<i>Lycopersicon esculentum</i>	1071	+	CAANNNNATC	PlantCARE

CMSRE1IBSPOA		334	-	TGGACGG	PLACE
CURECORECR		1691	+	GTAC	PLACE
CURECORECR		1068	+	GTAC	PLACE
CURECORECR		978	+	GTAC	PLACE
CURECORECR		631	+	GTAC	PLACE
CURECORECR		176	+	GTAC	PLACE
CURECORECR		172	+	GTAC	PLACE
CURECORECR		149	+	GTAC	PLACE
CURECORECR		50	+	GTAC	PLACE
CURECORECR		1691	-	GTAC	PLACE
CURECORECR		1068	-	GTAC	PLACE
CURECORECR		978	-	GTAC	PLACE
CURECORECR		631	-	GTAC	PLACE
CURECORECR		176	-	GTAC	PLACE
CURECORECR		172	-	GTAC	PLACE
CURECORECR		149	-	GTAC	PLACE
CURECORECR		50	-	GTAC	PLACE
DOFCOREZM		1745	+	AAAG	PLACE
DOFCOREZM		1656	+	AAAG	PLACE
DOFCOREZM		1523	+	AAAG	PLACE
DOFCOREZM		1280	+	AAAG	PLACE
DOFCOREZM		1214	+	AAAG	PLACE
DOFCOREZM		1210	+	AAAG	PLACE
DOFCOREZM		1117	+	AAAG	PLACE
DOFCOREZM		1087	+	AAAG	PLACE
DOFCOREZM		1022	+	AAAG	PLACE
DOFCOREZM		987	+	AAAG	PLACE
DOFCOREZM		983	+	AAAG	PLACE
DOFCOREZM		867	+	AAAG	PLACE
DOFCOREZM		820	+	AAAG	PLACE
DOFCOREZM		734	+	AAAG	PLACE
DOFCOREZM		718	+	AAAG	PLACE
DOFCOREZM		698	+	AAAG	PLACE
DOFCOREZM		639	+	AAAG	PLACE
DOFCOREZM		557	+	AAAG	PLACE
DOFCOREZM		473	+	AAAG	PLACE

DOFCOREZM		5	+	AAAG	PLACE
DOFCOREZM		1764	-	AAAG	PLACE
DOFCOREZM		1589	-	AAAG	PLACE
DOFCOREZM		1551	-	AAAG	PLACE
DOFCOREZM		950	-	AAAG	PLACE
DOFCOREZM		761	-	AAAG	PLACE
DOFCOREZM		745	-	AAAG	PLACE
DOFCOREZM		373	-	AAAG	PLACE
DPBFCOREDCDC3		279	+	ACACNNG	PLACE
DPBFCOREDCDC3		1448	-	ACACNNG	PLACE
DRECRTCOREAT		261	+	RCCGAC	PLACE
E2FCONSENSUS		330	+	WTTSSCSS	PLACE
EBOXBNNAPA		1614	+	CANNTG	PLACE
EBOXBNNAPA		1602	+	CANNTG	PLACE
EBOXBNNAPA		1545	+	CANNTG	PLACE
EBOXBNNAPA		1429	+	CANNTG	PLACE
EBOXBNNAPA		1377	+	CANNTG	PLACE
EBOXBNNAPA		1231	+	CANNTG	PLACE
EBOXBNNAPA		647	+	CANNTG	PLACE
EBOXBNNAPA		600	+	CANNTG	PLACE
EBOXBNNAPA		408	+	CANNTG	PLACE
EBOXBNNAPA		279	+	CANNTG	PLACE
EBOXBNNAPA		270	+	CANNTG	PLACE
EBOXBNNAPA		163	+	CANNTG	PLACE
EBOXBNNAPA		104	+	CANNTG	PLACE
EBOXBNNAPA		1614	-	CANNTG	PLACE
EBOXBNNAPA		1602	-	CANNTG	PLACE
EBOXBNNAPA		1545	-	CANNTG	PLACE
EBOXBNNAPA		1429	-	CANNTG	PLACE
EBOXBNNAPA		1377	-	CANNTG	PLACE
EBOXBNNAPA		1231	-	CANNTG	PLACE
EBOXBNNAPA		647	-	CANNTG	PLACE
EBOXBNNAPA		600	-	CANNTG	PLACE
EBOXBNNAPA		408	-	CANNTG	PLACE
EBOXBNNAPA		279	-	CANNTG	PLACE
EBOXBNNAPA		270	-	CANNTG	PLACE

EBOXBNNAPA		163	-	CANNTG	PLACE
EBOXBNNAPA		104	-	CANNTG	PLACE
EECCRCAH1		1608	+	GANTTNC	PLACE
EECCRCAH1		1293	+	GANTTNC	PLACE
EECCRCAH1		1226	+	GANTTNC	PLACE
EECCRCAH1		920	+	GANTTNC	PLACE
EECCRCAH1		580	+	GANTTNC	PLACE
EECCRCAH1		428	-	GANTTNC	PLACE
ERE	<i>Dianthus caryophyllus</i>	344	+	ATTTCAAA	PLACE; PlantCARE
GAG-motif	<i>Arabidopsis thaliana</i>	1291	+	AGAGAGT	PlantCARE
GATABOX		1421	+	GATA	PLACE
GATABOX		1050	+	GATA	PLACE
GATABOX		564	+	GATA	PLACE
GATABOX		462	+	GATA	PLACE
GATABOX		1369	-	GATA	PLACE
GATABOX		1325	-	GATA	PLACE
GATABOX		1161	-	GATA	PLACE
GATABOX		361	-	GATA	PLACE
GATABOX		296	-	GATA	PLACE
G-Box	<i>Antirrhinum majus</i>	47	+	CACGTA	PlantCARE
G-box	<i>Daucus carota</i>	43	+	TACGTG	PlantCARE
G-box	<i>Daucus carota</i>	165	+	TACGTG	PlantCARE
G-Box	<i>Antirrhinum majus</i>	43	-	CACGTA	PlantCARE
G-Box	<i>Pisum sativum</i>	271	-	CACACATGGAA	PlantCARE
G-Box	<i>Pisum sativum</i>	157	-	CACGTG	PlantCARE
G-Box	<i>Antirrhinum majus</i>	165	-	CACGTA	PlantCARE
G-box	<i>Daucus carota</i>	47	-	TACGTG	PlantCARE
G-box	<i>Zea mays</i>	564	-	CACGAC	PlantCARE
G-box	<i>Oryza sativa</i>	46	-	GTACGTG	PlantCARE
G-box	<i>Solanum tuberosum</i>	273	-	CACATGG	PlantCARE
GT1CONSENSUS		1751	+	GRWAAW	PLACE
GT1CONSENSUS		1727	+	GRWAAW	PLACE
GT1CONSENSUS		1423	+	GRWAAW	PLACE
GT1CONSENSUS		1008	+	GRWAAW	PLACE

GT1CONSENSUS		358	+	GRWAAW	PLACE
GT1CONSENSUS		872	+	GRWAAW	PLACE
GT1CONSENSUS		1767	-	GRWAAW	PLACE
GT1CONSENSUS		1325	-	GRWAAW	PLACE
GT1CONSENSUS		1226	-	GRWAAW	PLACE
GT1CONSENSUS		1018	-	GRWAAW	PLACE
GT1CONSENSUS		382	-	GRWAAW	PLACE
GT1CONSENSUS		204	-	GRWAAW	PLACE
GT1CONSENSUS		203	-	GRWAAW	PLACE
GT1CORE		1759	+	GGTTAA	PLACE
GT1CORE		1761	-	GGTTAA	PLACE
GT1CORE		1143	-	GGTTAA	PLACE
GT1GMSCAM4		872	+	GAAAAA	PLACE
GT1GMSCAM4		203	-	GAAAAA	PLACE
GT1-motif	<i>Avena sativa</i>	1136	+	GGTTAAT	PlantCARE
GT1-motif	<i>Arabidopsis thaliana</i>	1137	+	GGTTAA	PlantCARE
GTGANTG10		1232	+	GTGA	PLACE
GTGANTG10		806	+	GTGA	PLACE
GTGANTG10		620	+	GTGA	PLACE
GTGANTG10		530	+	GTGA	PLACE
GTGANTG10		523	+	GTGA	PLACE
GTGANTG10		155	+	GTGA	PLACE
GTGANTG10		1426	-	GTGA	PLACE
GTGANTG10		1127	-	GTGA	PLACE
GTGANTG10		962	-	GTGA	PLACE
GTGANTG10		644	-	GTGA	PLACE
I-box	<i>Flaveria trinervia</i>	458	-	GATATGG	PlantCARE
IBOXCORE		1422	+	GATAA	PLACE
IBOXCORE		1325	-	GATAA	PLACE
INRNTPSADB		1016	+	YTCANTYY	PLACE
INRNTPSADB		183	+	YTCANTYY	PLACE
INRNTPSADB		777	+	YTCANTYY	PLACE
INTRONLOWER		604	+	TGCAGG	PLACE
IRO2OS		164	+	CACGTGG	PLACE
JERE	<i>Arabidopsis</i>	507	-	AGACCGCC	PlantCARE

	<i>thaliana</i>				
LECPLEACS2		1368	+	TAAAATAT	PLACE
LTRECOREATCOR15		261	+	CCGAC	PLACE
MNF1	<i>Zea mays</i>	160	+	GTGCCC(A/T)(A/T)	PlantCARE
MNF1	<i>Zea mays</i>	50	-	GTGCCC(A/T)(A/T)	PlantCARE
MYB1AT		1433	-	WAACCA	PLACE
MYB1AT		325	-	WAACCA	PLACE
MYB1AT		955	-	WAACCA	PLACE
MYB1AT		669	-	WAACCA	PLACE
MYB2CONSENSUSAT		802	+	YAACKG	PLACE
MYB2CONSENSUSAT		1032	-	YAACKG	PLACE
MYBATRD22		956	-	CTAACCA	PLACE
MYBCORE		585	+	CNGTTR	PLACE
MYBCORE		43	+	CNGTTR	PLACE
MYBCORE		1032	+	CNGTTR	PLACE
MYBCORE		1144	-	CNGTTR	PLACE
MYBCORE		802	-	CNGTTR	PLACE
MYBCOREATCYCB1		802	+	AACGG	PLACE
MYBCOREATCYCB1		491	+	AACGG	PLACE
MYBCOREATCYCB1		1352	-	AACGG	PLACE
MYBCOREATCYCB1		1031	-	AACGG	PLACE
MYBPZM		1194	+	CCWACC	PLACE
MYBST1		1050	+	GGATA	PLACE
MYCATERD1		1231	+	CATGTG	PLACE
MYCATERD1		270	+	CATGTG	PLACE
MYCATERD1		647	-	CATGTG	PLACE
MYCATERD1		279	-	CATGTG	PLACE
MYCATRD22		647	+	CACATG	PLACE
MYCATRD22		279	+	CACATG	PLACE
MYCATRD22		1231	-	CACATG	PLACE
MYCATRD22		270	-	CACATG	PLACE
MYCCONSensusAT		1614	+	CANNTG	PLACE
MYCCONSensusAT		1602	+	CANNTG	PLACE
MYCCONSensusAT		1545	+	CANNTG	PLACE

MYCCONSENSUSAT		1429	+	CANNTG	PLACE
MYCCONSENSUSAT		1377	+	CANNTG	PLACE
MYCCONSENSUSAT		1231	+	CANNTG	PLACE
MYCCONSENSUSAT		647	+	CANNTG	PLACE
MYCCONSENSUSAT		600	+	CANNTG	PLACE
MYCCONSENSUSAT		408	+	CANNTG	PLACE
MYCCONSENSUSAT		279	+	CANNTG	PLACE
MYCCONSENSUSAT		270	+	CANNTG	PLACE
MYCCONSENSUSAT		163	+	CANNTG	PLACE
MYCCONSENSUSAT		104	+	CANNTG	PLACE
MYCCONSENSUSAT		1614	-	CANNTG	PLACE
MYCCONSENSUSAT		1602	-	CANNTG	PLACE
MYCCONSENSUSAT		1545	-	CANNTG	PLACE
MYCCONSENSUSAT		1429	-	CANNTG	PLACE
MYCCONSENSUSAT		1377	-	CANNTG	PLACE
MYCCONSENSUSAT		1231	-	CANNTG	PLACE
MYCCONSENSUSAT		647	-	CANNTG	PLACE
MYCCONSENSUSAT		600	-	CANNTG	PLACE
MYCCONSENSUSAT		408	-	CANNTG	PLACE
MYCCONSENSUSAT		279	-	CANNTG	PLACE
MYCCONSENSUSAT		270	-	CANNTG	PLACE
MYCCONSENSUSAT		163	-	CANNTG	PLACE
MYCCONSENSUSAT		104	-	CANNTG	PLACE
NODCON1GM		989	+	AAAGAT	PLACE
NODCON1GM		641	+	AAAGAT	PLACE
NODCON1GM		475	+	AAAGAT	PLACE
NODCON2GM		1588	+	CTCTT	PLACE
NODCON2GM		1303	-	CTCTT	PLACE
NODCON2GM		1216	-	CTCTT	PLACE
NTBBF1ARROLB		821	-	ACTTTA	PLACE
O2-site	<i>Zea mays</i>	679	+	GATGATGTGG	PlantCARE
O2-site	<i>Zea mays</i>	455	-	GATGATATGG	PlantCARE
OSE1ROOTNODULE		989	+	AAAGAT	PLACE
OSE1ROOTNODULE		641	+	AAAGAT	PLACE
OSE1ROOTNODULE		475	+	AAAGAT	PLACE
OSE2ROOTNODULE		1588	+	CTCTT	PLACE

OSE2ROOTNODULE		1303	-	CTCTT	PLACE
OSE2ROOTNODULE		1216	-	CTCTT	PLACE
PALBOXAPC		333	+	CCGTCC	PLACE
P-box	<i>Oryza sativa</i>	740	-	CCTTTTG	PlantCARE
POLASIG1		1485	+	AATAAA	PLACE
POLASIG1		829	+	AATAAA	PLACE
POLASIG1		471	+	AATAAA	PLACE
POLASIG1		1631	-	AATAAA	PLACE
POLASIG2		1059	+	AATTA AA	PLACE
POLASIG3		833	+	AATAAT	PLACE
POLASIG3		293	+	AATAAT	PLACE
POLASIG3		1313	-	AATAAT	PLACE
POLLEN1LELAT52		1416	+	AGAAA	PLACE
POLLEN1LELAT52		1213	+	AGAAA	PLACE
POLLEN1LELAT52		1086	+	AGAAA	PLACE
POLLEN1LELAT52		986	+	AGAAA	PLACE
POLLEN1LELAT52		870	+	AGAAA	PLACE
POLLEN1LELAT52		733	+	AGAAA	PLACE
POLLEN1LELAT52		1478	-	AGAAA	PLACE
POLLEN1LELAT52		1436	-	AGAAA	PLACE
POLLEN1LELAT52		592	-	AGAAA	PLACE
PREATPRODH		1605	-	ACTCAT	PLACE
PYRIMIDINEBOXHVE PB1		204	+	TTTTTTCC	PLACE
PYRIMIDINEBOXOSR AMY1A		1765	+	CCTTTT	PLACE
PYRIMIDINEBOXOSR AMY1A		746	+	CCTTTT	PLACE
PYRIMIDINEBOXOSR AMY1A		1281	-	CCTTTT	PLACE
RAV1AAT		315	+	CAACA	PLACE
RAV1AAT		1342	-	CAACA	PLACE
RAV1AAT		878	-	CAACA	PLACE
RAV1AAT		765	-	CAACA	PLACE
RAV1AAT		585	-	CAACA	PLACE
RAV1AAT		321	-	CAACA	PLACE

RAVIAAT		43	-	CAACA	PLACE
RAVIBAT		1429	+	CACCTG	PLACE
RAVIBAT		104	+	CACCTG	PLACE
RBCSCONSENSUS		769	-	AATCCAA	PLACE
REALPHALGLHCB21		954	-	AACCAA	PLACE
REALPHALGLHCB21		324	-	AACCAA	PLACE
RHERPATEXPA7		170	+	KCACGW	PLACE
RHERPATEXPA7		162	+	KCACGW	PLACE
RHERPATEXPA7		48	+	KCACGW	PLACE
RHERPATEXPA7		54	-	KCACGW	PLACE
RHERPATEXPA7		530	-	KCACGW	PLACE
RHERPATEXPA7		155	-	KCACGW	PLACE
ROOTMOTIFTAPOX1		1627	+	ATATT	PLACE
ROOTMOTIFTAPOX1		1274	+	ATATT	PLACE
ROOTMOTIFTAPOX1		998	+	ATATT	PLACE
ROOTMOTIFTAPOX1		797	+	ATATT	PLACE
ROOTMOTIFTAPOX1		193	+	ATATT	PLACE
ROOTMOTIFTAPOX1		1368	-	ATATT	PLACE
ROOTMOTIFTAPOX1		997	-	ATATT	PLACE
ROOTMOTIFTAPOX1		796	-	ATATT	PLACE
ROOTMOTIFTAPOX1		787	-	ATATT	PLACE
ROOTMOTIFTAPOX1		627	-	ATATT	PLACE
ROOTMOTIFTAPOX1		360	-	ATATT	PLACE
ROOTMOTIFTAPOX1		295	-	ATATT	PLACE
ROOTMOTIFTAPOX1		249	-	ATATT	PLACE
ROOTMOTIFTAPOX1		126	-	ATATT	PLACE
RYREPEATBNNAPA		387	+	CATGCA	PLACE
RYREPEATBNNAPA		90	+	CATGCA	PLACE
RYREPEATBNNAPA		88	-	CATGCA	PLACE
RYREPEATGMGY2		388	+	CATGCAT	PLACE
RYREPEATGMGY2		88	-	CATGCAT	PLACE
RYREPEATLEGUMIN BOX		388	+	CATGCAY	PLACE
RYREPEATLEGUMIN BOX		91	+	CATGCAY	PLACE
RYREPEATLEGUMIN		88	-	CATGCAY	PLACE

BOX					
SEBFCONSSTPR10A		1235	-	YTGTCWC	PLACE
SEF1MOTIF		1631	+	ATATTTAWW	PLACE
SEF1MOTIF		249	-	ATATTTAWW	PLACE
SEF4MOTIFGM7S		1635	+	RTTTTTR	PLACE
SEF4MOTIFGM7S		817	+	RTTTTTR	PLACE
SEF4MOTIFGM7S		904	-	RTTTTTR	PLACE
SITEIIATCYTC		1412	+	TGGGCY	PLACE
SITEIIATCYTC		1578	-	TGGGCY	PLACE
SORLIP1AT		1494	+	GCCAC	PLACE
SORLIP1AT		517	+	GCCAC	PLACE
Sp1	<i>Zea mays</i>	513	-	CC(G/A)CCC	PlantCARE
SURECOREATSULTR1 1		1462	-	GAGAC	PLACE
SURECOREATSULTR1 1		610	-	GAGAC	PLACE
TAAAGSTKST1		820	+	TAAAG	PLACE
TAAAGSTKST1		639	+	TAAAG	PLACE
TAAAGSTKST1		1590	-	TAAAG	PLACE
TAAAGSTKST1		1552	-	TAAAG	PLACE
TATA-box	<i>Arabidopsis thaliana</i>	186	+	TATAA	PlantCARE
TATA-box	<i>Zea mays</i>	238	+	TTTAAAAA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	240	+	TATTTAAA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	450	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	467	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	610	+	TTTTA	PlantCARE
TATA-box	<i>Helianthus annuus</i>	645	+	TATACA	PlantCARE
TATA-box	<i>Glycine max</i>	793	+	TAATA	PlantCARE
TATA-box	<i>Zea mays</i>	811	+	TTTAAAAA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	820	+	TTTTA	PlantCARE

TATA-box	<i>Glycine max</i>	994	+	TAATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	1035	+	TATAA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	1059	+	TATAA	PlantCARE
TATA-box	<i>Glycine max</i>	1270	+	TAATA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1273	+	TTTTA	PlantCARE
TATA-box	<i>Glycine max</i>	1309	+	TAATA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1360	+	TTTTA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	5	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	10	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	75	-	TATA	PlantCARE
TATA-box	<i>Glycine max</i>	120	-	TAATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	123	-	TATA	PlantCARE
TATA-box	<i>Brassica napus</i>	185	-	ATTATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	187	-	TATA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	239	-	TTTTA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	246	-	TATA	PlantCARE
TATA-box	<i>Glycine max</i>	286	-	TAATA	PlantCARE
TATA-box	<i>Glycine max</i>	289	-	TAATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	647	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	659	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	779	-	TATA	PlantCARE
TATA-box	<i>Glycine max</i>	781	-	TAATA	PlantCARE

TATA-box	<i>Glycine max</i>	790	-	TAATA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	812	-	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	844	-	TTTTA	PlantCARE
TATA-box	<i>Oryza sativa</i>	895	-	TACAAAA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	988	-	TTTTA	PlantCARE
TATA-box	<i>Glycine max</i>	991	-	TAATA	PlantCARE
TATA-box	<i>Brassica napus</i>	1034	-	ATTATA	PlantCARE
TATA-box	<i>Ac</i>	1036	-	TATAAAT	PlantCARE
TATA-box	<i>Brassica napus</i>	1058	-	ATTATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	1060	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	1268	-	TATA	PlantCARE
TATA-box	<i>Glycine max</i>	1478	-	TAATA	PlantCARE
TATABOX2		1043	+	TATAAAT	PLACE
TATABOX3		1777	+	TATTAAT	PLACE
TATABOX3		795	-	TATTAAT	PLACE
TATABOX5		1632	+	TTATTT	PLACE
TATABOX5		1753	-	TTATTT	PLACE
TATABOX5		832	-	TTATTT	PLACE
TATABOX5		828	-	TTATTT	PLACE
TATABOXSPAL		248	-	TATTTAA	PLACE
TBOXATGAPB		558	-	ACTTTG	PLACE
TC-rich repeats	<i>Nicotiana tabacum</i>	1429	-	GTTTTCTTAC	PlantCARE
TGACG-motif	<i>Hordeum vulgare</i>	653	-	TGACG	PlantCARE
TGTCACACMCUCUM ISIN		1234	-	TGTCACA	PLACE
Unnamed_1	<i>Zea mays</i>	136	-	CGTGG	PlantCARE
Unnamed_1	<i>Zea mays</i>	159	-	CGTGG	PlantCARE
Unnamed_4	<i>Petroselinum hortense</i>	602	+	CTCC	PlantCARE
Unnamed_4	<i>Petroselinum hortense</i>	1302	+	CTCC	PlantCARE

Unnamed__4	<i>Petroselinum hortense</i>	1128	+	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	139	+	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	27	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	962	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	534	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	834	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	1459	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	1102	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	549	-	CTCC	PlantCARE
Unnamed__4	<i>Petroselinum hortense</i>	1200	-	CTCC	PlantCARE
WBOXATNPR1		657	+	TTGAC	PLACE
WBOXATNPR1		1558	-	TTGAC	PLACE
WBOXHVIS01		525	+	TGACT	PLACE
WBOXHVIS01		1135	-	TGACT	PLACE
WBOXNTCHN48		1136	-	CTGACY	PLACE
WBOXNTERF3		525	+	TGACY	PLACE
WBOXNTERF3		1135	-	TGACY	PLACE
WRKY71OS		1439	+	TGAC	PLACE
WRKY71OS		1233	+	TGAC	PLACE
WRKY71OS		657	+	TGAC	PLACE
WRKY71OS		524	+	TGAC	PLACE
WRKY71OS		1557	-	TGAC	PLACE
WRKY71OS		1135	-	TGAC	PLACE
WUN-motif	<i>Brassica oleracea</i>	684	-	TCATTACGAA	PlantCARE

7.9 Regulatory cis-elements in the 1.5 kb promoter fragment of *tps10* of *Zea mays* var. Delprim

Tab.7. 16

tps10 1300 bp	Organism	Position	Strand	Sequence	Database
-10PEHVPSBD		635	+	TATTCT	PLACE
A-box	<i>Petroselinum crispum</i>	303	-	CCGTCC	PlantCARE
ABRE	<i>Oryza sativa</i>	152	+	AGTACGTGGC	PlantCARE
ABRE	<i>Arabidopsis thaliana</i>	455	-	CACGTG	PlantCARE
ABRELATERD1		456	+	ACGTG	PLACE
ABRELATERD1		457	-	ACGTG	PLACE
ABRELATERD1		875	-	ACGTG	PLACE
ABRERATCAL		455	+	MACGYGB	PLACE
ABRERATCAL		844	-	MACGYGB	PLACE
ACGTATERD1		114	+	ACGT	PLACE
ACGTATERD1		281	+	ACGT	PLACE
ACGTATERD1		296	+	ACGT	PLACE
ACGTATERD1		457	+	ACGT	PLACE
ACGTATERD1		875	+	ACGT	PLACE
ACGTATERD1		114	-	ACGT	PLACE
ACGTATERD1		281	-	ACGT	PLACE
ACGTATERD1		296	-	ACGT	PLACE
ACGTATERD1		457	-	ACGT	PLACE
ACGTATERD1		875	-	ACGT	PLACE
ACGTTBOX		295	+	AACGTT	PLACE
ACGTTBOX		295	-	AACGTT	PLACE
ANAERO1CONSENSUS		87	+	AAACAAA	PLACE
ANAERO2CONSENSUS		5	-	AGCAGC	PLACE
ANAERO2CONSENSUS		185	-	AGCAGC	PLACE
ARE	<i>Zea mays</i>	350	-	TGGTTT	PlantCARE

ARFAT		410	+	TGTCTC	PLACE
ARFAT		617	+	TGTCTC	PLACE
ARFAT		988	+	TGTCTC	PLACE
ARR1AT		434	+	NGATT	PLACE
ARR1AT		763	+	NGATT	PLACE
ARR1AT		915	+	NGATT	PLACE
ARR1AT		948	+	NGATT	PLACE
ARR1AT		493	-	NGATT	PLACE
ARR1AT		952	-	NGATT	PLACE
ARR1AT		1098	-	NGATT	PLACE
ARR1AT		1143	-	NGATT	PLACE
ARR1AT		128	-	NGATT	PLACE
ARR1AT		592	-	NGATT	PLACE
ARR1AT		701	-	NGATT	PLACE
ASF1MOTIFCAMV		45	+	TGACG	PLACE
ASF1MOTIFCAMV		23	-	TGACG	PLACE
BIHD1OS		577	+	TGTCA	PLACE
BIHD1OS		1000	-	TGTCA	PLACE
BIHD1OS		1263	-	TGTCA	PLACE
Box 4	<i>Petroselinum crispum</i>	668	-	ATTAAT	PlantCARE
Box 4	<i>Petroselinum crispum</i>	944	-	ATTAAT	PlantCARE
Box 4	<i>Petroselinum crispum</i>	932	-	ATTAAT	PlantCARE
Box 4	<i>Petroselinum crispum</i>	1115	-	ATTAAT	PlantCARE
BOXIINTPATPB		710	+	ATAGAA	PLACE
BP5OSWX		873	-	CAACGTG	PLACE
CAAT-box	<i>Hordeum vulgare</i>	129	+	CAAT	PLACE; PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	147	+	CAAT	PLACE; PlantCARE
CAAT-box	<i>Glycine max</i>	319	+	CAATT	PlantCARE
CAAT-box	<i>Arabidopsis thaliana</i>	320	+	gGCAAT	PlantCARE

CAAT-box	<i>Hordeum vulgare</i>	446	+	CAAT	PLACE; PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	451	+	CAAT	PLACE; PlantCARE
CAAT-box	<i>Glycine max</i>	483	+	CAATT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	484	+	CAAT	PLACE; PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	494	+	CAAT	PLACE; PlantCARE
CAAT-box	<i>Brassica rapa</i>	724	+	CAAAT	PlantCARE
CAAT-box	<i>Arabidopsis thaliana</i>	893	+	CCAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	1060	+	CAAAT	PlantCARE
CAAT-box	<i>Brassica rapa</i>	1236	+	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	178	-	CAAT	PLACE; PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	368	-	CAAT	PLACE; PlantCARE
CAAT-box	<i>Brassica rapa</i>	426	-	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	554	-	CAAT	PLACE; PlantCARE
CAAT-box	<i>Brassica rapa</i>	811	-	CAAAT	PlantCARE
CAAT-box	<i>Hordeum vulgare</i>	1089	-	CAAT	PLACE; PlantCARE
CAAT-box	<i>Brassica rapa</i>	1183	-	CAAAT	PlantCARE
CAATBOX1		321	+	CAAT	PLACE
CAATBOX1		894	+	CAAT	PLACE
CACGTGMOTIF		456	+	CACGTG	PLACE
CACGTGMOTIF		456	-	CACGTG	PLACE
CACTFTPPCA1		111	+	YACT	PLACE
CACTFTPPCA1		138	+	YACT	PLACE
CACTFTPPCA1		210	+	YACT	PLACE
CACTFTPPCA1		394	+	YACT	PLACE
CACTFTPPCA1		1013	+	YACT	PLACE
CACTFTPPCA1		1162	+	YACT	PLACE
CACTFTPPCA1		1202	+	YACT	PLACE

CACTFTPPCA1		1224	+	YACT	PLACE
CACTFTPPCA1		1261	+	YACT	PLACE
CACTFTPPCA1		359	-	YACT	PLACE
CACTFTPPCA1		732	-	YACT	PLACE
CACTFTPPCA1		1177	-	YACT	PLACE
CACTFTPPCA1		1277	-	YACT	PLACE
CACTFTPPCA1		159	-	YACT	PLACE
CACTFTPPCA1		263	-	YACT	PLACE
CACTFTPPCA1		589	-	YACT	PLACE
CACTFTPPCA1		652	-	YACT	PLACE
CACTFTPPCA1		720	-	YACT	PLACE
CACTFTPPCA1		759	-	YACT	PLACE
CACTFTPPCA1		767	-	YACT	PLACE
CACTFTPPCA1		961	-	YACT	PLACE
CACTFTPPCA1		994	-	YACT	PLACE
CACTFTPPCA1		1121	-	YACT	PLACE
CANBNNAPA		1173	-	CNAACAC	PLACE
CAREOSREP1		421	+	CAACTC	PLACE
CARGCW8GAT		888	+	CWWWWWWW WG	PLACE
CARGCW8GAT		888	-	CWWWWWWW WG	PLACE
CCAATBOX1		894	+	CCAAT	PLACE
CCGTCC-box	<i>Arabidopsis thaliana</i>	303	-	CCGTCC	PlantCARE
CERGLUBOX2PSLE GA		1054	+	TGAAAAC	PLACE
CGACGOSAMY3		42	+	CGACG	PLACE
CGCGBOXAT		845	+	VCGCGB	PLACE
CGCGBOXAT		845	-	VCGCGB	PLACE
CGTCA-motif	<i>Hordeum vulgare</i>	22	+	CGTCA	PlantCARE
CGTCA-motif	<i>Hordeum vulgare</i>	44	-	CGTCA	PlantCARE
circadian	<i>Lycopersicon esculentum</i>	687	+	CAANNNNATC	PlantCARE; PLACE
circadian	<i>Lycopersicon esculentum</i>	872	-	CAANNNNATC	PlantCARE; PLACE

CMSRE1IBSPOA		304	+	TGGACGG	PLACE
CTRMCAV35S		612	+	TCTCTCTCT	PLACE
CURECORECR		112	+	GTAC	PLACE
CURECORECR		158	+	GTAC	PLACE
CURECORECR		249	+	GTAC	PLACE
CURECORECR		262	+	GTAC	PLACE
CURECORECR		476	+	GTAC	PLACE
CURECORECR		112	-	GTAC	PLACE
CURECORECR		158	-	GTAC	PLACE
CURECORECR		249	-	GTAC	PLACE
CURECORECR		262	-	GTAC	PLACE
CURECORECR		476	-	GTAC	PLACE
DOFCOREZM		85	+	AAAG	PLACE
DOFCOREZM		104	+	AAAG	PLACE
DOFCOREZM		515	+	AAAG	PLACE
DOFCOREZM		734	+	AAAG	PLACE
DOFCOREZM		1017	+	AAAG	PLACE
DOFCOREZM		1152	+	AAAG	PLACE
DOFCOREZM		1194	+	AAAG	PLACE
DOFCOREZM		1253	+	AAAG	PLACE
DOFCOREZM		1294	+	AAAG	PLACE
DOFCOREZM		1244	-	AAAG	PLACE
DOFCOREZM		1285	-	AAAG	PLACE
DPBFCOREDCDC3		1160	+	ACACNNG	PLACE
DPBFCOREDCDC3		1259	+	ACACNNG	PLACE
DPBFCOREDCDC3		251	-	ACACNNG	PLACE
DPBFCOREDCDC3		1176	-	ACACNNG	PLACE
DPBFCOREDCDC3		1276	-	ACACNNG	PLACE
E2FCONSENSUS		321	-	WTTSSCSS	PLACE
EBOXBNNAPA		16	+	CANNTG	PLACE
EBOXBNNAPA		456	+	CANNTG	PLACE
EBOXBNNAPA		471	+	CANNTG	PLACE
EBOXBNNAPA		623	+	CANNTG	PLACE
EBOXBNNAPA		812	+	CANNTG	PLACE
EBOXBNNAPA		836	+	CANNTG	PLACE
EBOXBNNAPA		908	+	CANNTG	PLACE

EBOXBNNAPA		1060	+	CANNTG	PLACE
EBOXBNNAPA		1236	+	CANNTG	PLACE
EBOXBNNAPA		16	-	CANNTG	PLACE
EBOXBNNAPA		456	-	CANNTG	PLACE
EBOXBNNAPA		471	-	CANNTG	PLACE
EBOXBNNAPA		623	-	CANNTG	PLACE
EBOXBNNAPA		812	-	CANNTG	PLACE
EBOXBNNAPA		836	-	CANNTG	PLACE
EBOXBNNAPA		908	-	CANNTG	PLACE
EBOXBNNAPA		1060	-	CANNTG	PLACE
EBOXBNNAPA		1236	-	CANNTG	PLACE
ECCRCAH1		319	-	GANTTNC	PLACE
ECCRCAH1		421	-	GANTTNC	PLACE
Gap-box	<i>Arabidopsis thaliana</i>	1055	+	CAAATGAA(A/G) A	PlantCARE
GATABOX		400	+	GATA	PLACE
GATABOX		639	+	GATA	PLACE
GATABOX		659	+	GATA	PLACE
GATABOX		883	+	GATA	PLACE
GATABOX		978	+	GATA	PLACE
GATABOX		233	-	GATA	PLACE
GATABOX		573	-	GATA	PLACE
GATABOX		959	-	GATA	PLACE
GATABOX		1021	-	GATA	PLACE
G-Box	<i>Pisum sativum</i>	873	+	CACGTT	PlantCARE
G-box	<i>Zea mays</i>	873	+	CACGTT	PlantCARE
G-Box	<i>Pisum sativum</i>	455	-	CACGTG	PlantCARE
G-box	<i>Arabidopsis thaliana</i>	455	-	CACGTG	PlantCARE
GCCCORE		324	-	GCCGCC	PLACE
GT1CONSENSUS		79	+	GRWAAW	PLACE
GT1CONSENSUS		976	+	GRWAAW	PLACE
GT1CONSENSUS		1129	+	GRWAAW	PLACE
GT1CONSENSUS		1137	+	GRWAAW	PLACE
GT1CONSENSUS		398	+	GRWAAW	PLACE
GT1CONSENSUS		707	+	GRWAAW	PLACE

GT1CONSENSUS		1168	+	GRWAAW	PLACE
GT1CONSENSUS		66	-	GRWAAW	PLACE
GT1CONSENSUS		346	-	GRWAAW	PLACE
GT1CONSENSUS		1241	-	GRWAAW	PLACE
GT1CORE		826	-	GGTTAA	PLACE
GT1GMSCAM4		707	+	GAAAAA	PLACE
GT1GMSCAM4		1168	+	GAAAAA	PLACE
GT1-motif	<i>Avena sativa</i>	825	-	GGTTAAT	PlantCARE
GTGANTG10		336	+	GTGA	PLACE
GTGANTG10		470	+	GTGA	PLACE
GTGANTG10		545	+	GTGA	PLACE
GTGANTG10		731	+	GTGA	PLACE
GTGANTG10		211	-	GTGA	PLACE
GTGANTG10		459	-	GTGA	PLACE
GTGANTG10		507	-	GTGA	PLACE
GTGANTG10		877	-	GTGA	PLACE
IBOX		1021	-	GATAAG	PLACE
IBOXCORE		399	+	GATAA	PLACE
IBOXCORE		977	+	GATAA	PLACE
IBOXCORE		1021	-	GATAA	PLACE
IBOXCORENT		1021	-	GATAAGR	PLACE
INRNTPSADB		780	-	YTCANTYY	PLACE
INRNTPSADB		730	-	YTCANTYY	PLACE
INTRONLOWER		12	+	TGCAGG	PLACE
INTRONLOWER		36	-	TGCAGG	PLACE
LECPLEACS2		1081	-	TAAAATAT	PLACE
MBS	<i>Arabidopsis thaliana</i>	622	+	CAACTG	PlantCARE
MNF1	<i>Zea mays</i>	627	-	GTGCCC(A/T)(A/T)	PlantCARE
MYBIAT		351	+	WAACCA	PLACE
MYB2CONSENSUSAT		623	+	YAACKG	PLACE
MYBCORE		623	-	CNGTTR	PLACE
MYBCORE		825	-	CNGTTR	PLACE
MYBST1		400	+	GGATA	PLACE
MYBST1		572	-	GGATA	PLACE

MYCATERD1		16	+	CATGTG	PLACE
MYCATERD1		471	+	CATGTG	PLACE
MYCATRD22		16	-	CACATG	PLACE
MYCATRD22		471	-	CACATG	PLACE
MYCCONSENSUSAT		16	+	CANNTG	PLACE
MYCCONSENSUSAT		456	+	CANNTG	PLACE
MYCCONSENSUSAT		471	+	CANNTG	PLACE
MYCCONSENSUSAT		623	+	CANNTG	PLACE
MYCCONSENSUSAT		812	+	CANNTG	PLACE
MYCCONSENSUSAT		836	+	CANNTG	PLACE
MYCCONSENSUSAT		908	+	CANNTG	PLACE
MYCCONSENSUSAT		1060	+	CANNTG	PLACE
MYCCONSENSUSAT		1236	+	CANNTG	PLACE
MYCCONSENSUSAT		16	-	CANNTG	PLACE
MYCCONSENSUSAT		456	-	CANNTG	PLACE
MYCCONSENSUSAT		471	-	CANNTG	PLACE
MYCCONSENSUSAT		623	-	CANNTG	PLACE
MYCCONSENSUSAT		812	-	CANNTG	PLACE
MYCCONSENSUSAT		836	-	CANNTG	PLACE
MYCCONSENSUSAT		908	-	CANNTG	PLACE
MYCCONSENSUSAT		1060	-	CANNTG	PLACE
MYCCONSENSUSAT		1236	-	CANNTG	PLACE
NODCON1GM		102	+	AAAGAT	PLACE
NODCON2GM		1286	+	CTCTT	PLACE
NODCON2GM		1192	-	CTCTT	PLACE
NODCON2GM		1251	-	CTCTT	PLACE
NTBBF1ARROLB		733	-	ACTTTA	PLACE
OSE1ROOTNODULE		102	+	AAAGAT	PLACE
OSE2ROOTNODULE		1286	+	CTCTT	PLACE
OSE2ROOTNODULE		1192	-	CTCTT	PLACE
OSE2ROOTNODULE		1251	-	CTCTT	PLACE
PALBOXAPC		304	-	CCGTCC	PLACE
P-box	<i>Oryza sativa</i>	83	-	CCTTTTG	PlantCARE
POLASIG1		1186	-	AATAAA	PLACE
POLASIG1		1229	-	AATAAA	PLACE

POLASIG2		429	-	AATTAAA	PLACE
POLASIG2		1269	-	AATTAAA	PLACE
POLLENILELAT52		709	+	AGAAA	PLACE
PREATPRODH		779	-	ACTCAT	PLACE
PYRIMIDINEBOXOSR AMY1A		84	-	CCTTTT	PLACE
RAV1AAT		20	+	CAACA	PLACE
RAV1AAT		142	+	CAACA	PLACE
RAV1AAT		684	+	CAACA	PLACE
REALPHALGLHCB21		532	+	AACCAA	PLACE
RGATAOS		228	-	CAGAAGATA	PLACE
RHERPATEXPA7		457	+	KCACGW	PLACE
RHERPATEXPA7		875	+	KCACGW	PLACE
RHERPATEXPA7		455	-	KCACGW	PLACE
ROOTMOTIFTAPOX1		637	+	ATATT	PLACE
ROOTMOTIFTAPOX1		930	+	ATATT	PLACE
ROOTMOTIFTAPOX1		1084	+	ATATT	PLACE
ROOTMOTIFTAPOX1		1113	+	ATATT	PLACE
ROOTMOTIFTAPOX1		77	-	ATATT	PLACE
ROOTMOTIFTAPOX1		854	-	ATATT	PLACE
ROOTMOTIFTAPOX1		892	-	ATATT	PLACE
ROOTMOTIFTAPOX1		931	-	ATATT	PLACE
ROOTMOTIFTAPOX1		1085	-	ATATT	PLACE
ROOTMOTIFTAPOX1		1114	-	ATATT	PLACE
RYREPEATBNNAPA		215	+	CATGCA	PLACE
RYREPEATBNNAPA		222	+	CATGCA	PLACE
RYREPEATBNNAPA		378	+	CATGCA	PLACE
RYREPEATBNNAPA		224	-	CATGCA	PLACE
RYREPEATGMGY2		214	+	CATGCAT	PLACE
RYREPEATGMGY2		221	+	CATGCAT	PLACE
RYREPEATGMGY2		377	+	CATGCAT	PLACE
RYREPEATLEGUMIN BOX		214	+	CATGCAY	PLACE
RYREPEATLEGUMIN BOX		221	+	CATGCAY	PLACE
RYREPEATLEGUMIN		377	+	CATGCAY	PLACE

BOX					
S1FBOXSORPS1L21		70	-	ATGGTA	PLACE
SEBFCONSSTPR10A		410	+	YTGTCWC	PLACE
SEF4MOTIFGM7S		783	-	RTTTTTR	PLACE
SEF4MOTIFGM7S		603	-	RTTTTTR	PLACE
IIATCYTC		300	-	TGGGCY	PLACE
IIATCYTC		382	-	TGGGCY	PLACE
Skn-1_motif	<i>Oryza sativa</i>	575	+	GTCAT	PlantCARE
SORLIP1AT		327	-	GCCAC	PLACE
SORLIP1AT		801	-	GCCAC	PLACE
SORLIP2AT		301	-	GGGCC	PLACE
SORLREP3AT		738	+	TGTATATAT	PLACE
SP8BFIBSP8BIB		767	-	TACTATT	PLACE
SREATMSD		399	-	TTATCC	PLACE
SURE1STPAT21		708	+	AATAGAAAA	PLACE
SURECOREATSULTR1 1		192	-	GAGAC	PLACE
SURECOREATSULTR1 1		237	-	GAGAC	PLACE
SURECOREATSULTR1 1		410	-	GAGAC	PLACE
SURECOREATSULTR1 1		617	-	GAGAC	PLACE
SURECOREATSULTR1 1		988	-	GAGAC	PLACE
T/GBOXATPIN2		874	-	AACGTG	PLACE
TAAAGSTKST1		515	+	TAAAG	PLACE
TAAAGSTKST1		734	+	TAAAG	PLACE
TAAAGSTKST1		1243	-	TAAAG	PLACE
TATA-box	<i>Glycine max</i>	77	+	TAATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	161	+	TATAA	PlantCARE
TATA-box	<i>Glycine max</i>	276	+	TAATA	PlantCARE
TATA-box	<i>Daucus carota</i>	671	+	ccTATAAATT	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	732	+	taTATAAagg	PlantCARE

TATA-box	<i>Lycopersicon esculentum</i>	733	+	taTATAAAg	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	734	+	TATAAA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	735	+	TATATAA	PlantCARE
TATA-box	<i>Glycine max</i>	769	+	TAATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	783	+	ccTATAAAaa	PlantCARE
TATA-box	<i>Helianthus annuus</i>	850	+	TATACA	PlantCARE
TATA-box	<i>Glycine max</i>	854	+	TAATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	889	+	TATAA	PlantCARE
TATA-box	<i>Glycine max</i>	931	+	TAATA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	971	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1033	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1047	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1080	+	TTTTA	PlantCARE
TATA-box	<i>Glycine max</i>	1114	+	TAATA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1225	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1230	+	TTTTA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	1271	+	TTTTA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	73	-	TATA	PlantCARE
TATA-box	<i>Brassica napus</i>	74	-	ATATAT	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	75	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	162	-	TATA	PlantCARE

TATA-box	<i>Arabidopsis thaliana</i>	736	-	TATATATA	PlantCARE
TATA-box	<i>Brassica napus</i>	737	-	ATATAT	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	738	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	740	-	TATA	PlantCARE
TATA-box	<i>Oryza sativa</i>	772	-	TACAAAA	PlantCARE
TATA-box	<i>Lycopersicon esculentum</i>	784	-	TTTTA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	852	-	TATA	PlantCARE
TATA-box	<i>Brassica napus</i>	888	-	ATTATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	890	-	TATA	PlantCARE
TATA-box	<i>Arabidopsis thaliana</i>	1041	-	TATA	PlantCARE
TATA-box	<i>Glycine max</i>	1111	-	TAATA	PlantCARE
TATA-box	<i>Glycine max</i>	1117	-	TAATA	PlantCARE
TATABOX3		1116	+	TATTAAT	PLACE
TATABOX3		932	-	TATTAAT	PLACE
TATABOX3		1115	-	TATTAAT	PLACE
TATABOX4		736	+	TATATAA	PLACE
TATABOX5		1185	+	TTATTT	PLACE
TATABOX5		1228	+	TTATTT	PLACE
TATAPVTRNALEU		735	-	TTTATATA	PLACE
TC-rich repeats	<i>Nicotiana tabacum</i>	648	-	ATTTTCTTCA	PlantCARE
TGACG-motif	<i>Hordeum vulgare</i>	44	+	TGACG	PlantCARE
TGACG-motif	<i>Hordeum vulgare</i>	22	-	TGACG	PlantCARE
TRANSINITDICOTS		444	+	AMNAUGGC	PLACE
TRANSINITMONOCOTS		444	+	RMNAUGGC	PLACE
Unnamed_4	<i>Petroselinum hortense</i>	26	+	CTCC	PlantCARE
Unnamed_4	<i>Petroselinum hortense</i>	1075	+	CTCC	PlantCARE

Unnamed_4	<i>Petroselinum hortense</i>	419	+	CTCC	PlantCARE
Unnamed_4	<i>Petroselinum hortense</i>	1246	+	CTCC	PlantCARE
Unnamed_4	<i>Petroselinum hortense</i>	315	+	CTCC	PlantCARE
Unnamed_4	<i>Petroselinum hortense</i>	540	+	CTCC	PlantCARE
WBOXPCWRKY1		201	+	TTTGACY	PLACE
WBOXATNPR1		202	+	TTGAC	PLACE
WBOXATNPR1		1001	+	TTGAC	PLACE
WBOXATNPR1		1264	+	TTGAC	PLACE
WBOXATNPR1		22	-	TTGAC	PLACE
WBOXHVIS01		201	+	TGACT	PLACE
WBOXHVIS01		543	+	TGACT	PLACE
WBOXNTERF3		201	+	TGACY	PLACE
WBOXNTERF3		543	+	TGACY	PLACE
WRKY71OS		46	+	TGAC	PLACE
WRKY71OS		202	+	TGAC	PLACE
WRKY71OS		544	+	TGAC	PLACE
WRKY71OS		1001	+	TGAC	PLACE
WRKY71OS		1264	+	TGAC	PLACE
WRKY71OS		23	-	TGAC	PLACE
WRKY71OS		577	-	TGAC	PLACE
WUN-motif	<i>Brassica oleracea</i>	1088	-	TCATTACGAA	PlantCARE

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Publications

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