Genetics and Molecular Biology, 30, 3 (suppl), 713-733 (2007)

www.sbg.org.br

Research Article

In silico analysis of phytohormone metabolism and communication pathways in citrus transcriptome

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Abstract

Plant hormones play a crucial role in integrating endogenous and exogenous signals and in determining developmental responses to form the plant body throughout its life cycle. In citrus species, several economically important processes are controlled by phytohormones, including seed germination, secondary growth, fruit abscission and ripening. Integrative genomics is a powerful tool for linking newly researched organisms, such as tropical woody species, to functional studies already carried out on established model organisms. Based on gene orthology analyses and expression patterns, we searched the *Citrus Genome Sequencing Consortium* (CitEST) database for Expressed Sequence Tags (EST) consensus sequences sharing similarity to known components of hormone metabolism and signaling pathways in model species. More than 600 homologs of functionally characterized hormone metabolism and signal transduction members from model species were identified in citrus, allowing us to propose a framework for phytohormone signaling mechanisms in citrus. A number of components from hormone-related metabolic pathways were absent in citrus, suggesting the presence of distinct metabolic pathways. Our results demonstrated the power of comparative genomics between model systems and economically important crop species to elucidate several aspects of plant physiology and metabolism.

Key words: defense responses, development, plant growth regulators.

Received: July 21, 2006; Accepted: March 6, 2007.

Introduction

Plant growth and development are controlled by the integration of several endogenous and environmental signals. Plant hormones play a crucial role in integrating endogenous and exogenous signals and in determining the final developmental responses to form the plant body. Hormones are molecules that are produced by one specific organ and conveyed to target tissues, where they elicit a physiological response at low concentration (Davies, 1995). This definition does not hold true for most of the plant hormones, which are synthesized by several different tissues or cell types, but can act locally, as well as at a distance. Moreover, the majority of the plant hormones are small, relatively simple molecules (Gray, 2004). Traditionally, plant hormones have been considered to be small lipophilic compounds, such as abscisic acid (ABA), auxin (IAA), brassinosteroids (BR), cytokinins (CK), ethylene (ET), gibberellin (GA), jasmonates (JA) and salicylic acid (SA) (Davies, 1995). Recent evidence from genetic and biochemical studies show the involvement of many secretory and non-secretory peptide signals in many aspects of plant growth regulation, including defense responses, callus growth, meristem organization, self-incompatibility, root growth, leaf-shape regulation, nodule development,

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and organ abscission, and comprise a newly-found class of peptide hormones in plants (Matsubayashi and Sakagami, 2006).

Virtually every aspect of plant growth and development is under hormonal control to some extent. A diverse array of cellular and developmental processes may be controlled by single or multiple hormones that may function in concert to control a single process. The application of exogenous hormone has been largely employed to study the function of its endogenous counterparts in plants. This leads to the attribution of general roles for each phytohormone such as the well-characterized function of ethylene in fruit ripening, the regulation of the cell cycle by auxin and CK, the induction of seed germination and stem elongation by GA, and the maintenance of seed dormancy by ABA. Forward genetic approaches have allowed the isolation of hormone biosynthetic and response mutants (Gazzarrini and McCourt, 2003). More recently, biochemical and *in silico* genome studies have demonstrated the hormonal role of previously unsuspected molecules (Matsubayashi and Sakagami, 2006). An increasing number of genomic tools are being used to probe hormone biosynthesis, transport and response; this integrated approach has contributed to a clearer picture of the mechanisms involved in plant developmental control by hormones.

Plant hormone biosynthesis is closely associated to primary and secondary metabolism. Auxins are tryptophan conjugates and distinct genetic pathways control their biosynthesis (Cohen *et al.*, 2003). CKs are adenine-related purines (Sakakibara, 2006); GAs are tetracyclic diterpenoids synthesized in a complex pathway involving plastids, endoplasmic reticulum and the cytosol (Fleet and Sun, 2005). ABA is synthesized either from MVA (mevalonic acid) or MEP (methylerythritol-phosphate) (Nambara and Marion-Poll, 2005), especially in vascular bundles and guard cells (Koiwai *et al.*, 2004). Ethylene is synthesized from methionine by the intermediate S-adenosyl-L-methionine (AdoMet) and 1 aminocyclopropane-1-carboxylate (ACC) (Chae and Kieber, 2005). BR biosynthesis is highly networked and consists of two parallel routes: an early and a late C-6 oxidation pathway, connected at multiple steps, and also linked to an early C-22 oxidation pathway (Fujioka and Yokota, 2003). JA is an oxylipin, consisting in a group of structurally diverse biologically active compounds, generated by the coordinated action of lipases, lipoxygenases, and a group of cytochrome P450 specialized in the metabolism of hydroperoxy fatty acids (Schilmiller and Howe, 2005). Peptide hormones are produced by the proteolytic processing of the C-terminus of a polypeptide precursor that may be or may be not synthesized through a secretory pathway as in the case of the RAPID ALKALINIZAITON FACTORS (RALF) and RALF like (RALFL) or systemin and phytosulfokine, respectively (Matsubayashi and Sakagami, 2006).

The extensive effects of even diminute concentrations of plant hormones have led to a tight control of function, not only by biosynthesis rate, but also by a plethora of factors, such as availability of receptors, catabolic rate, conversion into inert forms, translocation, and several interconnected steps of signal transduction. Surprisingly, hormone-mediated signal transduction appears to have evolved common themes for distinct phytohormones in plants, such as protein phosphorylation, G-protein and Calcium/calmodulin-mediated signal transduction, and bacterial two-component signaling system-like and regulated proteolysis (Serino and Deng, 2003; Assmann, 2004; Gray, 2004; Mizuno, 2005).

Ethylene and CK are both perceived by plasma membrane-associated receptors, similar to bacterial two-component regulators that contain an intracellular histidine kinase (HK) domain (Stepanova and Alonso, 2005; Sakakibara, 2006). The kinase activity is activated by ligand binding, resulting in self-phosphorylation and initiating a series of phospho-transfer reactions, which culminate in activation of a response regulator protein that functions as the effector component of the pathway (Guo and Ecker, 2004; Ferreira and Kieber, 2005). Ethylene is perceived by a family of five receptors: ETHYLENE RECEPTOR1 (ETR1) and ETHYLENE RESPONSIVE SENSOR1 (ERS1), containing a consensus HK domain, functioning as negative regulators of the pathway. The Raf-like Mitogen-Activated Protein (MAP) Kinase Kinase Kinase, CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) interacts with the receptors and also functions as a negative regulator. The integral membrane protein, ETHYLENE INSENSITIVE2 (EIN2), and the transcription factors EIN3 and EIN-LIKE1 (EIL1) are positive regulators of ethylene signaling, downstream of CTR1. The binding of ethylene to receptors inactivates them and results in down-regulation of CTR1 activity. In the absence of ethylene, transcription factors EIN3 and EIL1 are targeted for degradation by an SKP1/Cullin/F-box protein (SCF) complex (Chae and Kieber, 2005).

Similarly, BR is perceived by a receptor complex of two leucine-rich-repeat receptor-like kinases (LRR-RLKs) that interact with each other (Vert *et al.*, 2005). Activation of the receptor kinases by BR binding leads to the de-phosphorylation and accumulation of two nuclear proteins due to the inhibition of a negative regulator (Vert *et al.*, 2005). In the absence of BR, the negative regulator phosphorylates the nuclear proteins and targets them for degradation by the ubiquitin-dependent proteasome pathway. In the case of auxin, a large family of transcriptional repressors Aux/IAA dimerizes with members of the AUXIN RESPONSE FACTOR (ARF) family of transcription factors, preventing ARFs from activating auxin-responsive genes, reviewed in Woodward and Bartel (2005). Upon auxin stimuli, the receptor TRANSPORT INHIBITOR RESPONSE1 (TIR1), an SCF ubiquitin E3 ligase F-box protein, ubiquitinates Aux/IAA proteins marking them for degradation by the

26S proteasome, thereby de-repressing the response pathway (Parry and Estelle, 2006). In a similar manner, perception of active GAs leads to degradation of various transcriptional repressor proteins containing the DELLA-domain, via an SCF-E3 ubiquitin ligase-SLEEPY1 (SLY1) complex (McGinnis *et al.*, 2003). Interestingly, DELLA protein levels are also regulated by auxin and ethylene, indicating a function as a general regulator of plant growth mediated by several plant hormones (Fleet and Sun, 2005).

Perception and signal transduction mechanisms of plant hormones primarily involved in stress responses also share common themes, which are also shared with development regulatory hormones. The SCF complex mediates JA signaling; the F box protein COI1 (CORONATINE INSENSITIVE1) is part of an SCF complex that includes ARABIDOPSIS SKP1-LIKE1 (ASK1) or ASK2 and CULLIN1 (CUL1). However, the transcriptional regulator(s) affected by hormone interaction with SCF complexes remain unknown (Schilmiller and Howe, 2005). ABA transduction pathways are characterized by a plethora of intracellular messengers, reflecting its function in integrating several stress responses and antagonizing pathways via cross-talk (Himmelbach *et al.*, 2003). Accumulation of ABA is controlled by upstream signaling events, and plays a quantitative role in signal transduction (Verslues and Zhu, 2005). However, ABA accumulation can be controlled by several metabolic processes, such as ABA synthesis, catabolism or conjugation, and within each of these metabolic processes there are several genes that may act as rate-limiting factors. These genes may be subjected to feedback regulation by ABA (Verslues and Zhu, 2005). NCED (9-cis-epoxycarotenoid dioxygenase) catalyses the cleavage of the C25 carotenoids, 9-cis-neoxanthin or 9-cis-violaxanthin, to the C15 ABA-precursor xanthoxin; a reaction proposed to be rate limiting in ABA synthesis. NCED3 is the most strongly induced by dehydration, among the nine genes of NCED family in *Arabidopsis*; and thus, is a good candidate for direct regulation by upstream signaling (Verslues and Zhu, 2005). There are several possible candidates for modulators of ABA response. Recent evidence points to sugar sensing and reactive oxygen production as potential links between the metabolic status and ABA responses.

The precise signaling mechanism of peptide hormones in plants remains elusive, but biochemical evidence suggested that they translocate by vascular bundles, where they bind to the BR membrane receptor in a non-competitive manner. Subsequently, the peptide-receptor association triggers various events: the modulation of ion fluxes, increase in cytoplasmic calcium, up-regulation of calmodulin gene expression, inactivation of plasma membrane H^+-ATPase activity (via calcium-dependent phosphorylation), and activation of a MAPK leading to activation of phospholipase A and allene oxide cyclase which mediate the release of linolenic acid from membrane lipids and the formation of jasmonic acid-intermediate 12-*oxo*-phytodienoic acid (OPDA), respectively (Matsubayashi and Sakagami, 2006). Activating these signaling pathways in vascular bundles leads to increased production of jasmonic acid which further upregulates expression of the polypeptide precursor genes. This results in a further increase in the level of jasmonic acid in the bundles via a positive feedback loop. Finally, jasmonic acid or a related compound moves through the phloem and ultimately induces production of defensive proteins in the target leaves (Matsubayashi and Sakagami, 2006).

In this study, we have investigated the *Citrus Genome Sequencing Project Consortitum* (CitEST) database, employing bioinformatics tools and *in silico* expression analysis to dissect hormone metabolism and signal transduction pathways which regulate development and cellular communication. Expressed Sequence Tags (EST) contigs corresponding to genes involved in hormone biosynthesis, translocation, transport, and signaling were identified and the main components of phytohormone pathways which regulate citrus development were presented. We propose a framework for hormone-mediated signal transduction in citrus species based on comparative genomics analyses.

Material and Methods

Database searches and alignments

Homologs of functionally characterized genes involved in phytohormone metabolism and signaling were identified by BLAST (Altschul *et al.*, 1997) and keyword searches against citrus EST database (CitEST), consisting of approximately 176,200 EST sequences obtained from 53 libraries. Data validation was performed by local tBLASTx and tBLASTn searches of the retrieved sequences against the GenBank database. The resulting alignments were filtered by a threshold *e*-value of 10^{-15} and further analyzed according to functional domain description. Validated sequences were translated and the deduced protein alignments were performed using ClustalX (Thompson *et al.*, 1997). Whenever necessary, alignments were manually adjusted using Lasergene MegAlign (DNASTAR, Madison, WI, USA).

Motif analysis and *in silico* characterization

The identified citrus homologs were further investigated for recognizable functional domains described in several protein and gene function analysis databases built inside CitEST (European Bioinformatics Institute-European Molecular Biology Laboratory, EMBL-EBI, ExPaSy, Swiss Institute of Bioinformatics, SIB, Protein Families database and Pfam).

Phylogenetic analysis

The putative functionality of the deduced amino acid sequence of citrus transcripts, in comparison to their *Arabidopsis* and other model species homologs, was as-

sessed by genetic distance and phylogenetic studies. Phylogenetic analyses were performed using distance and parsimony methods with PAUP* 4.0b10, using default parameters. Re-sampling bootstrap trees containing 1,000 random samples were constructed using PSIGNFIT. Modular functional domains were employed for genetic distance studies with genes previously characterized as having divergent regions and conserved blocks.

In silico gene expression analysis

In silico qualitative gene expression profiling was performed using virtual northern blot (VNB) analyses of the citrus EST database. Frequency of reads of each EST contig for a given library was calculated and normalized to the total number of reads from the investigated library and the total number of reads in all libraries. A correlation matrix between EST contigs and libraries was then generated, and gene expression patterns among ESTs and libraries were obtained by hierarchical clustering based on Spearman Rank correlation matrix using Cluster v.2.11 (Eisen *et al.*, 1998), by substituting clusters by their average expression pattern. Graphic outputs were generated using Tree View v.1.6 software.

Results and Discussion

CitEST database survey

We have performed extensive BLAST and keyword searches of the citrus transcriptome to identify homologs of the genes involved in hormone biosynthesis and signal transduction in citrus. We have searched for genes related to auxin, cytokinin, ethylene, abscisic acid, gibberellic acid, jasmonic acid and peptide hormone biosynthesis, transport and translocation, catabolism and signal transduction pathways. In CitEST databases, 601 assembled sequences and EST singlets sharing significant sequence identity with functionally characterized proteins were identified and analyzed (Table 1, Figure 1). The functional characterization of citrus sequences is virtually equivalent to *Arabidopsis* in cellular component and molecular function (Figure 1, Figure S1 and Figure S2). However, transcripts with enzyme activity are more abundant in citrus whereas those showing transcription factor activity are more abundant in *Arabidopsis* proteome (Figure 1). The significance of these findings remains to be investigated in further functional studies.

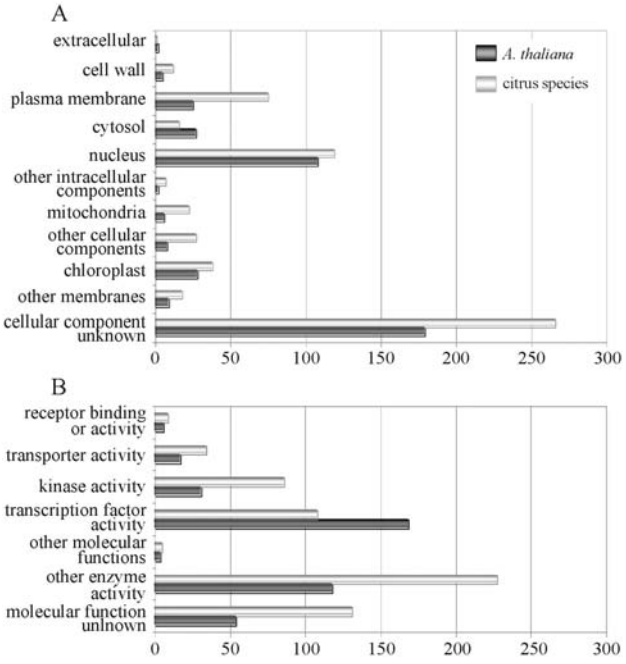

Figure 1 - Comparison between the functional classification of citrus transcripts and *Arabidopsis* proteome associated to phytohormone metabolism and signaling pathways using gene ontology. **A.** cellular component; **B.** molecular function. The normalized number of genes is represented on the x-axis. Assignments are based on the data available at the TIGR *Arabidopsis thaliana* Gene Index version 13.0.

Table 1 - Number of citrus transcripts identified by tBLASTn searches of CitEST databases showing sequence similarity to hormone metabolism and signaling components in model species.

	CitEST transcripts (%[a] - absent gene(s))		
Phytohormone	Biosynthesis, metabolism and transport	Signal transduction	Total
Abscisic acid	5 (100%)	24 - (92.3% - ABI1, ABI2)	29
Auxin	17 (94.1% - CYP707A)	36 (100%)	53
Brassinosteroids	33 (100%)	63 (96.8% - TTL, TRIP1)	96
Cytokinin	13 (100%)	31 (96.8% - CPC)	44
Ethylene	18 (100%)	35 (97.1% - EIN2)	53
Gibberellic acid	18 (100%)	14 (87.5% - PHOR1, miR159)	32
Jasmonic acid	87 (95.6% - *At*EXT family)	112 (100%)	199
Peptide hormones	0	15 (65.2% - TomSys, SCR/SP11, SCRL, IDA, IDL, PLS, CLV3, CLE)	15
Salicylic acid	33 (100%)	47 (97.9% - ACD6)	80
Total	224	377	601

[a]Percentage of identified sequences in comparison to the number of bait sequences searched.

Abscisic acid

Although leaf abscission is not primarily induced by ABA, but by ethylene, it has been shown that in `Cleopatra' mandarin (*Citrus reshni*) water-stressed seedlings required ABA accumulation in roots to induce ethylene synthesis (Gómez-Cadenas *et al.*, 1996). Similarly, fruitlet abscission in `Satsuma' mandarin (*Citrus unshiu*) has also been correlated to sugar shortage, leading to increased levels of ABA, which, in turn, triggered ethylene synthesis (Gómez-Cadenas *et al.*, 2000).

In plants, ABA plays major roles in environmental stress responses. ABA biosynthetic pathway has already been completely elucidated in plants, and shown to be highly conserved in angiosperms (Xiong and Zhu, 2003). ABA signaling and action are, however, much less understood and, so far, only fragmentary information is available. ABA is synthesized from a C_{40} carotenoid precursor. In fact, many ABA-deficient mutants showed impaired carotenoid biosynthesis, as exemplified by *Pinalate*, a sweet orange mutant which is defective in zetacarotene desaturase activity that leads to reduced ABA contents (Rodrigo *et al.*, 2003). The first step of the pathway is the conversion of zeaxanthin into violaxanthin catalyzed by zeaxanthin epoxidase (ZEP), followed by conversion to neoxanthin, a 9-cis-epoxycarotenoid, in plastids. Then, 9-cis-neoxanthin undergoes an oxidative cleavage by 9-cis-epoxycarotenoid dioxygenase (NCED) resulting in xanthoxin, a C_{15} intermediate that is exported to the cytosol. NCED is the first committed enzyme to an ABA pathway and a major regulatory point. Xanthoxin is then attacked by a short-chain alcohol dehydrogenase/reductase (SDR), producing ABA-aldehyde. The last step in the pathway is the ABA-aldehyde oxidation to abscisic acid (ABA) by ABA-aldehyde Oxidase (AAO). AAO requires molybdenum as cofactor (MoCo), which is sulfurylated by MoCo sulfurase (Seo *et al.*, 2000; Xiong *et al.*, 2001; Xiong *et al.*, 2002; Porch *et al.*, 2006).

Several CitEST reads were strongly related to genes involved in ABA biosynthesis: zeaxanthin epoxidase (ZEP/ABA1/LOS6); 9-cis-epoxicarotenoid dioxygenase (NCED/NOT/VP14); abscisic aldehyde oxidase (AAO/SIT/TAO3); and molybdenum cofactor sulfurase (ABA3/LOS5/FLC) (Table S1). Recently, two NCED genes from *Citrus sinensis* have been cloned and characterized as presenting differential expression patterns and distinct enzymatic properties concerning substrate recognition: *CsNCED1* was expressed in ripening fruits, whereas *CsNCED2* transcripts were found only in chromoplast-containing tissues, such as flavedo (Rodrigo *et al.*, 2006). ABA is catabolized by oxidation, reduction or conjugation (Cutler and Krochko, 1999). Phaseic acid (PA), dihydrophaseic acid (DPA), and glucose conjugates are the usual forms of inactivating ABA in plants. Recently, neophaseic acid (neoPA) was described as a novel ABA metabolite and detected in sweet orange fruits (Zhou *et al.*, 2004). No reads from CitEST database matched *Arabidopsis* ABA-8'-hydroxylase CYP707A, the main enzyme involved in ABA inactivation (Saito *et al.*, 2004).

ABA perception and signaling are still poorly understood. ROP10 is a small GTPase that participates in ABA signaling pathway as a negative regulator of ABA responses. It functions by modulating the expression of genes that respond to different ABA levels in *Arabidopsis* (Xin *et al.*, 2005). We were able to identify two contig transcripts showing sequence similarity to *ROP10* in the CitEST database.

ABA-responsive elements (ABRE) are present in promoters of ABA-regulated genes. ABRE-binding factors (ABF) activate transcription through the aforementioned *cis* elements by phosphorylation. TRAB1 from rice is a bZIP-domain transcription factor responsible for ABA regulation at ABRE (Kagaya *et al.*, 2002; Kobayashi *et al.*, 2005). CitEST database contained a single read sharing extensive sequence similarity to TRAB1. The role of TRAB1 in citrus remains unknown; however, the high level of sequence identity surpassed taxonomic classes (from a monocot to a dicot species), suggesting a conserved function.

OST1 (OPEN STOMATA1) is an ABA-activated serine-threonine protein kinase (AAPK) specifically implicated in the signaling pathway. Its expression is upregulated by ABA and osmotic stress. OST1 acts upstream to the production of reactive oxygen species (ROS), a key second messenger that induces cytosolic Ca^{2+} influx by activating plasma membrane Ca^{2+}-channels (Zhang *et al.*, 2001; Mustilli *et al.*, 2002). ABI1 and ABI2 are PP2C-type phosphatases that negatively regulate ABA activity in OST1-ROS cascade (Yoshida *et al.*, 2006). Citrus transcriptome analysis identified OST1-related cDNAs, but none was highly similar to ABI1 or ABI2, suggesting that the OST1-ROS pathway may be present in citrus and ABI negative regulators may be absent from the database due to their low transcriptional activity in comparison to OST1. Alternatively, ABI activities may have been replaced by other proteins.

RCN1 (ROOTS CURL IN NPA1) is a serine-threonine phosphatase type 2 regulatory subunit that functions as a general positive regulator during early stages of ABA signaling, upstream of the cytosolic Ca^{2+} sensing (Kwak *et al.*, 2002). Several transcripts sharing high sequence identity to RCN1 were identified in citrus. Seventeen reads were grouped into two EST contigs, demonstrating the extensive sequence conservation among citrus genomes.

Inactivation of RAC1 by ABA is critical for stomatal closure in *Arabidopsis*. RAC1/ROP6 is a small GTPase belonging to the *Rho* gene family, and associated to cytoskeleton regulation in yeast and animals (Lemichez *et al.*, 2001). Transcripts showing sequence similarity to RAC1 were the most abundant of ABA metabolism and signaling-related genes identified in citrus transcriptome: 31 reads assembled in five contigs. As a central element in

plant adaptation to osmotic stress given its involvement in stomatal closure, it is reasonable to assume that RAC1 may play a strategic role in drought tolerance in citrus. GPA1 is a canonical Gα subunit of a heterotrimeric G protein implicated in downstream ABA signaling events (Wang *et al.*, 2001). GCR1 is a G protein-coupled receptor, which physically interacts with GPA1, functioning as a negative regulator of GPA1-mediated ABA responses (Pandey and Assmann, 2004). In citrus transcriptome, we identified homologs of *Arabidopsis* GPA1 and GCR1, indicating a possible conserved mechanism in ABA signaling cascade involving these proteins. Since most investigations on ABA signaling have been carried out on model species, it is important to broaden these findings to other taxonomic groups to validate *bona fide* universal mechanisms of hormone action.

Auxin

We have identified 53 EST contigs showing similarity to proteins involved in auxin biosynthesis, metabolism, transport and signal transduction in the CitEST database. Results from comparisons against NCBI database can be found in Table S2. The longest contig consisted of 47 reads and showed similarity to a cullin-like protein. The contigs were assembled from sequences of almost all libraries but the most abundant were from leaf and fruit libraries (Table S2). The enzymes responsible for the biosynthesis of auxin are most active in young tissues: such as shoot apical meristems and in growing leaves and fruits. The same tissues are the locations where the highest concentrations of indole-3-acetic acid (IAA) are found. In fact, we have observed that the vast majority of sequences assembled in the contigs with similarity to proteins involved in IAA biosynthesis were from leaf (58.44%) and fruit (31.8%) libraries.

Auxins exert control over many important developmental processes in plants, including cell division and cell expansion, vascular tissue differentiation, root initiation, apical dominance, gravitropic and phototropic responses, flowering, fruit ripening, leaf senescence and abscission of leaves and fruit (Eckardt, 2001). Due to the importance of IAA in plant growth and development, extensive studies of the biosynthesis of this compound have been performed since its discovery as a plant hormone. The pathway for the biosynthesis of IAA in plants remains, however, to be elucidated, probably due to the existence of multiple pathways and possible functional redundancy among various participating enzymes. Indole-3-acetic acid is the most abundant naturally occurring auxin. Plants produce active IAA both by *de novo* synthesis and by releasing IAA from conjugates. This work emphasizes the analysis of the pathways involved in *de novo* IAA synthesis in citrus.

Genetic and biochemical experiments have demonstrated that two routes are responsible for IAA biosynthesis: a tryptophan-dependent and a tryptophan-independent one (Bartel, 1997). The starting point for IAA synthesis is in the tryptophan (Trp) biosynthetic pathway (Eckardt, 2001). Two routes from Trp to IAA are generally accepted as occurring in plants: one from Trp through indole-3-acetaldoxime (IAOx) and indole-3-acetonitrile (IAN) to IAA. Two *Arabidopsis* cytochrome P450 proteins, CYP79B2 and CYP79B3, can catalyse the conversion of Trp to IAOx *in vitro* (Hull *et al.*, 2000). In addition to cytochrome P450s, several *Arabidopsis* proteins with similarity to flavin monoxygenases (FMOs) have been found to increase IAA production via a probable IAOx intermediate in superexpression mutants. The FMO encoded by *YUCCA* gene converts tryptamine to N-hydroxyl tryptamine (Zhao *et al.*, 2001). Once YUCCA has converted tryptamine to N-hydroxyl tryptamine, another hydroxylation step is necessary to synthesize IAOx. This is presumably carried out by another FMO-like protein or a cythochrome P450. We have found five FMO contigs, from the largest number of reads (75). Six contigs of CYP83B1 were found and, according to Eckardt (2001), it is evidence that P450 CYP83B1 converts IAOx to its corresponding acinitro compound: the first step in indole-glucosinole biosynthesis. This represents a metabolic branch point between IAA and indole-glucosinolaste biosynthesis in *Arabidopsis* and CYP83B1 is found to play a role in regulating auxin homeostasis. One contig of CYP79B2 was found. In model systems, it catalyzes the formation of IAOx from Trp (Hull *et al.*, 2000).

As mentioned earlier, it is often assumed that the conversion of IAOx to IAA proceeds through IAN. IAN can be converted to IAA through the action of nitrilases. The distribution appears to be limited to three families (Cruciferae, Graminae and Musaceae), although it is possible that similar enzymes catalyze this reaction in other plants. Three nitrilases from *Arabidopsis* (NIT1 to NIT3) are capable of converting IAN to IAA (Eckardt, 2001). In the present work, we have found two contigs of NIT4 that probably do not play a role in IAA biosynthesis, according to Piotrowski *et al.* (2000). We have also found one contig with 12 reads showing sequence similarity to NITRILASE1-like protein of *Arabidopsis*. In maize, two nitrilases *Zm*NIT1 and *Zm*NIT2 are expressed in seeds. *Zm*NIT2 efficiently hydrolyzes indole-3-acetonitrile to IAA and could thus be involved in auxin biosynthesis. However, some studies using *nit1-1* mutants suggested that IAOx may be converted to IAA via a distinct route (Eckardt, 2001).

The alternative route proceeds from Trp via indole acetaldehyde (IAAld) to IAA. IAAld may be a good candidate for an intermediate between TAOx and IAA: instead of conversion to IAN, IAOx could be reduced to an imine followed by pH-dependent hydrolysis to IAAld, which can be converted to IAA via aldehyde oxidase (AO) (Normanly and Bartel, 1999). In fact, some investigations have shown that an AO, tentatively called IAAld oxidase, may be involved in IAA synthesis. In the present survey, we have

found two contigs with similarity to AO. According to our data, it seems that in citrus the same two vias are involved in the Trp-dependent IAA biosynthesis.

The versatility of auxins as signaling molecules is illustrated by the fact that a number of messages can be communicated simultaneously to different target cells. Precisely regulated auxin transport and redistribution have been implicated in some of these responses. Auxins are the only class of polarly transported phytohormones and their polar flow has been linked to many aspects of development, including establishment of the embryonic axis, continuous differentiation of vascular tissues and tropic growth responses, such as photo- and gravitropism. In higher plants, IAA is synthesized in shoot apices and transported towards the root tip, where it probably enters an opposite flow in the epidermis. The basipetal transport of IAA appears to be essential for the formation of continuous vascular strands and other aspects of cell polarity, while the opposite movement in the root epidermis is required for root gravitropism. Polar auxin transport proceeds in a cell-to-cell fashion involving influx and efflux through cell membranes. The directionality of the transport is thought to result from the polar distribution of specialized carrier molecules in the plasma membrane (Leyser and Berleth, 1999). Members of the *Arabidopsis PIN-FORMED1* (*PIN1*) and *AUXIN-RESISTANT1* (*AUX1*) gene families are suspected to constitute components of the auxin efflux and influx machinery, respectively. The PIN1 protein has been implicated in general auxin transport along the apical-basal axis, evidenced by the impaired auxin transport in *pin1* mutant stem segments. The recent identification of *PIN1* gene product as a basically membrane-localized protein with structural similarities to bacterial membrane transporters strongly suggests a role in auxin efflux (Leyser and Berleth, 1999). In citrus, we have identified three contigs corresponding to PIN family members.

Auxin causes rapid changes in gene expression, and two families of proteins have been identified in this response: auxin response factors (ARFs) and Aux/IAA proteins (Callis, 2005). Auxin regulates a broad spectrum of developmental processes, mediating transcriptional regulation via protein degradation (Weijers and Jürgens, 2004). Regulation by proteolysis has emerged as a resounding theme in plant hormone signaling. The ubiquitin-mediated degradation of key regulatory proteins has been demonstrated, or is at least likely, for all of the phytohormone response pathways (Smalle and Vierstra, 2004). In the case of auxin, the response pathway is normally subject to repression by a large family of transcriptional regulators called Aux/IAA proteins (Gray, 2004). These proteins dimerize with members of the AUXIN RESPONSE FACTOR (ARF) family of transcription factors, thus, preventing ARFs from activating auxin-responsive genes (Tiwari *et al.*, 2004). Upon an auxin stimulus, an SCF ubiquitin ligase containing the TIR1 F-box protein ubiquitinates the Aux/IAA proteins, marking them for degradation by the 26S proteasome, thereby de-repressing the response pathway (Gray *et al.*, 2001). Auxin de-represses the pathway by promoting AUX/IAA binding to SCF^{TIR1} and leading to their degradation. SCF^{TIR1} function requires AXR1-dependent RUB1 modification of an *At*CUL1 subunit of the SCF. In citrus, we have identified three contigs corresponding to a putative CULLIN1 from *A. thaliana*. One is similar to a putative CULLIN3 from *Oryza sativa* (japonica cultivar-group), four contigs have similarity to TRANSPORT INHIBITOR RESPONSE1 (TIR1) from *A. thaliana* (Table S3). Seven ARF7, one ARF2 and one ARF1-like transcripts were also identified (Table S3). Eight Aux/IAA transcriptional regulators, four with similarity with auxin responsive family in *Arabidopsis* and four closer to *Populus tremula*, were identified in citrus transcriptome.

One transcript corresponding to ABP1 (AUXIN BINDING PROTEIN1) was identified in citrus species. The identification of a plant auxin-binding protein 20 years ago marked a major advance in understanding auxin perception in plants. Developing plants lacking ABP1 show defective cell elongation, fail to organize the basic plant body plan, and subsequently degenerate (Callis, 2005). But, it was demonstrated that the auxin-dependent SCF^{TIR1}-Aux/IAA interaction requires neither integral membrane proteins nor the candidate auxin receptor, auxin binding protein 1 (ABP1) (Weijers and Jürgens, 2004). Recently, TIR1 has been demonstrated to function as the auxin receptor in plants (Dharmasiri *et al.*, 2005).

Brassinosteroids

We have identified 96 transcripts from citrus species that share sequence similarity to BR metabolism and signal transduction components from model species (Table S4, Table S5). Thirty-three are related to several steps of BR biosynthesis and processing, and the vast majority of them (20) are similar to BR oxidation P450 cytochrome proteins (Table S4). In citrus species, 63 ESTs are similar to BR signal perception and transduction: including 30 sequences homologous to receptor and receptor-like proteins, 25 similar to phosphorelay cascade and cross-talk intermediates and 7 whose deduced amino acid sequence is homologous to BR-induced transcriptional regulators (Table S5).

Brassinosteroids are C_{27}, C_{28}, and C_{29} steroids depending on their C-24 alkyl substituents, reviewed in Fujioka and Yokota (2003). In plants, they are present in a wide array of free, naturally occurring BRs. Brassinolide (BL) is the most biologically active C_{28} BR and, together with its C_{28} congeners, is widely distributed in the plant kingdom. BR biosynthetic pathway is highly networked and consists of several paralleled BL pathways that branch and interact with each other (Noguchi *et al.*, 1999). Initially, campesterol is converted to campestanol in an early C-22 oxidation pathway. Campestanol, in turn, is converted to castasterone (CS) through either early C-6 oxidation or late

C-6 oxidation, after which CS is further converted into BL. In citrus, we have identified homologs of the enzymes involved in the early C-22 oxidation pathway, such as DET2 and DWF4 and in both parallel C-6 oxidation pathways, including several CDP family members and BR6OX (Table S4). Thus, as demonstrated for rice, *Catharanthus roseus*, tobacco and *Arabidopsis* (Suzuki *et al.*, 1995; Choi *et al.*, 1996), BR biosynthetic pathways appear to be highly conserved in citrus species.

In plants, BRs are perceived by the transmembrane leucine-rich repeat (LRR) serine/threonine kinase protein BRI1 (BRASSINOSTEROID INSENSITIVE 1) that interacts with another LRR receptor kinase, BAK1 (BRI-*A*SSOCIATED RECEPTOR KINASE 1) (Li *et al.*, 2002; Nam and Li, 2002). Thus, BAK1 serves as a co-receptor for BRI1 to perceive the BR signal at the cell surface. The immediate targets for signal transmission from the receptor complex remain elusive, but several candidate signaling substrates have been identified for BRI1, including a transthyretin-like protein (TTL) and a TGF-β-receptor-interacting protein (TRIP1) (Nam and Li, 2004; Ehsan *et al.*, 2005). TTL has been demonstrated to function as a negative regulator of BR-induced plant growth, although it fails to affect BR-induced transcriptional changes (Sablowski and Harberd, 2005). Alternatively, it is likely to function by locally regulating BR-induced responses, such as cell expansion (Nam and Li, 2004). The receptor kinase TRIP1 has been hypothesized to function as a general transcriptional regulator, although it remains to be demonstrated (Ehsan *et al.*, 2005). The family of receptor kinase BRI1 and BRI-like (BRL) is represented by 30 transcripts identified in *C. sinensis*, *C. reticulata* and *P. trifoliata* transcriptomes (Table S5). Similarly, 9 cDNAs correspond to the co-receptor BAK1 in citrus species (Table S5). We were unable to identify TTL and TRIP1 homologs in citrus using bioinformatic tools.

Cytoplasmic protein kinase BIN2 (BRASSINOSTEROID INSENSITIVE 2) functions downstream of the receptor complex to negatively regulate BR-initiated signal transduction and shares sequence homology to *Drosophila* SHAGGY kinase and mammalian glycogen synthase kinase 3 (GSK3) (He *et al.*, 2002; Li and Nam, 2002). Three BIN2 homologs are present in citrus transcriptome and the deduced amino acid sequence of two of them is more than 50% identical to the sequence of *Arabidopsis* BIN2 (Table S5). BR-induced changes in gene expression have been demonstrated to involve BZR1 and BES1; there are two closely related nuclear proteins that function as positive transcriptional regulators (review in Li and Deng, 2005). In the absence of BR signaling, BZR1 and BES1 are present in phosphorylated forms, resultant from BIN2 kinase activity (Wang *et al.*, 2002; Yin *et al.*, 2005). Phosphorylated BZR1 has been hypothesized to be degraded by the 26S proteasome (Wang *et al.*, 2002). BZR1 and BZL are members of a novel sub-family of bHLH transcription factors (He *et al.*, 2005; Yin *et al.*, 2005). The BZR1 binding site, CGTG(T/C)G, is found in four BR biosynthetic genes that are feedback regulated, including *CPD* and *DWF4* (He *et al.*, 2005). In citrus, BZR family is represented by 2 cDNAs containing the characteristic domain of unknown function found in the *Arabidopsis* family (Table S5). The phosphorylation status of BES1 is modulated by the nuclear serine/threonine protein phosphatase BSU1, which is also likely to be involved in the proteolytic degradation of BES1 via the Kelch-repeat domain at the N-terminus of the phosphatase (Mora-Garcia *et al.*, 2004). *C. sinensis* and *P. trifoliata* BSU1 homologs show high sequence conservation at the kinase domain, although the identity at the Kelch repeats is less significant (data not shown). Citrus species have two BES-like proteins, sharing up to 40% of sequence identity between each other. The conserved domain of unknown function at the N-terminus of *Arabidopsis* BES1 is present in all identified citrus homologs. Thus, BR-regulated gene expression in citrus is hypothesized to be functionally equivalent to the *Arabidopsis* pathway.

Cytokinin

We have identified 44 ESTs corresponding to CK-related metabolism in citrus. From the total, 13 correspond to genes involved in the hormone biosynthesis, transport and catabolism (Table S6) and the remaining 31 are involved in CK-mediated signal transduction (Table S7).

The enzymes responsible for the first step in CK biosynthesis, the modification of the adenine moiety, are represented by one *C. reticulata* transcript highly similar to IPT3, IPT4, IPT7 and IPT8 from *A. thaliana* and several other species, and two *C. sinensis* cDNAs: one similar to IPT1 and one closer to IPT5 (Table S6). In general, IPT coding sequences are conserved throughout evolution, especially at the ATP/GTP binding motif (Kakimoto, 2001), thus the biological meaning of the family divergence in citrus species is likely to reflect functional redundancy. The enzymes modifying the adenine side-chain, the second step of CK biosynthesis, share extensive sequence similarity to several UDP-glycosyl transferases and corresponded to one EST singlet and one contig in citrus (Table S6). The purine permease family consists of several small hydrophobic polytopic membrane proteins involved in translocation of adenine-related compounds; however only two members, *At*PUP1 and *At*PUP2, have been demonstrated to translocate CK (Burkle *et al.*, 2003). The family is represented in *C. sinensis* by two EST contigs that share sequence homology to both *At*PUP1 and *At*PUP2. In *Arabidopsis*, CK degradation is mediated by a five-gene family of cytokinin oxydases (*CKX*). Similarly in citrus, *CKX*-related transcripts were identified (Table S6).

Genetic and biochemical evidence has demonstrated that cytokinin signal transduction is primarily dependent on the bacterial and yeast two-component signal transduction pathways where stimuli-binding specifically leads to histi-

dine-asparagine multi-step phosphorelays, which in turn induce changes in gene expression (Grefen and Harter, 2004). Two-component signaling systems consist of sensor kinases, histidine phosphotransfer proteins and response regulators (Mizuno, 2005). The *Arabidopsis* cytokinin receptor kinases (*Arabidopsis* HISTIDINE KINASE2 (AHK2), AHK3, AHK4/CYTOKININ RESPONSE 1 (CRE1)/WOODENLEG (WOL)) contain a conserved extra-cellular cytokinin-binding domain called CHASE (cyclases/histidine kinases associated sensory extracellular), a histidine kinase and a receiver domain (Ferreira and Kieber, 2005; Riefler *et al.*, 2006). In citrus, we have identified 5 EST contigs sharing extensive sequence conservation with *Arabidopsis* AHKs, including at the functional receiver domain (Table S7, Figure 2). Five *Arabidopsis* histidine-phosphotransfer proteins (AHPs) encode small proteins (of about 150 amino acids) mediating the phosphotransfer from the receptor kinases to the response regulators (Ferreira and Kieber, 2005).

The *Arabidopsis* genome has another 23 genes that are similar in sequence and domain structure to bacterial response regulators, and these encode both positive and negative elements in cytokinin signaling. In citrus, we have identified 3 transcripts corresponding to AHP genes from *A. thaliana* and from the woody model species *Populus tremuloides* (Table S7). Highest sequence homology is observed at the His-Asp phosphotransfer region, suggesting that these transcripts correspond to functionally active proteins in phosphorelay-mediated signal transduction (Figure 2). *Arabidopsis* RR genes belong to two main groups based on their sequence similarities: domain structure and transcriptional response to cytokinin (Mizuno, 2005). The type-A ARRs consist of a receiver domain and a short carboxyl terminus and their transcription is rapidly elevated in response to exogenous cytokinin; these are considered to be primary response genes (Liebfried *et al.*, 2005; Kim *et al.*, 2006). The type-B ARRs have a carboxy-terminal output domain that has a DNA-binding glutamic acid-rich protein (GARP) domain and a transcriptional activation domain in addition to the receiver domain, and in contrast, its transcription is not altered by cytokinin (Ferreira and Kieber, 2005; Mason *et al.*, 2006). In citrus, we have identified 13 ARR genes: 7 corresponding to B-type and 6 to A-type response regulators.

Ethylene

The structural simplicity of the plant hormone ethylene contrasts with its dramatic effects on various developmental processes. These range from seed germination to senescence and organ abscission, and in the cellular processes that ethylene initiates in response to a diversity of environmental signals (Stepanova and Alonso, 2005).

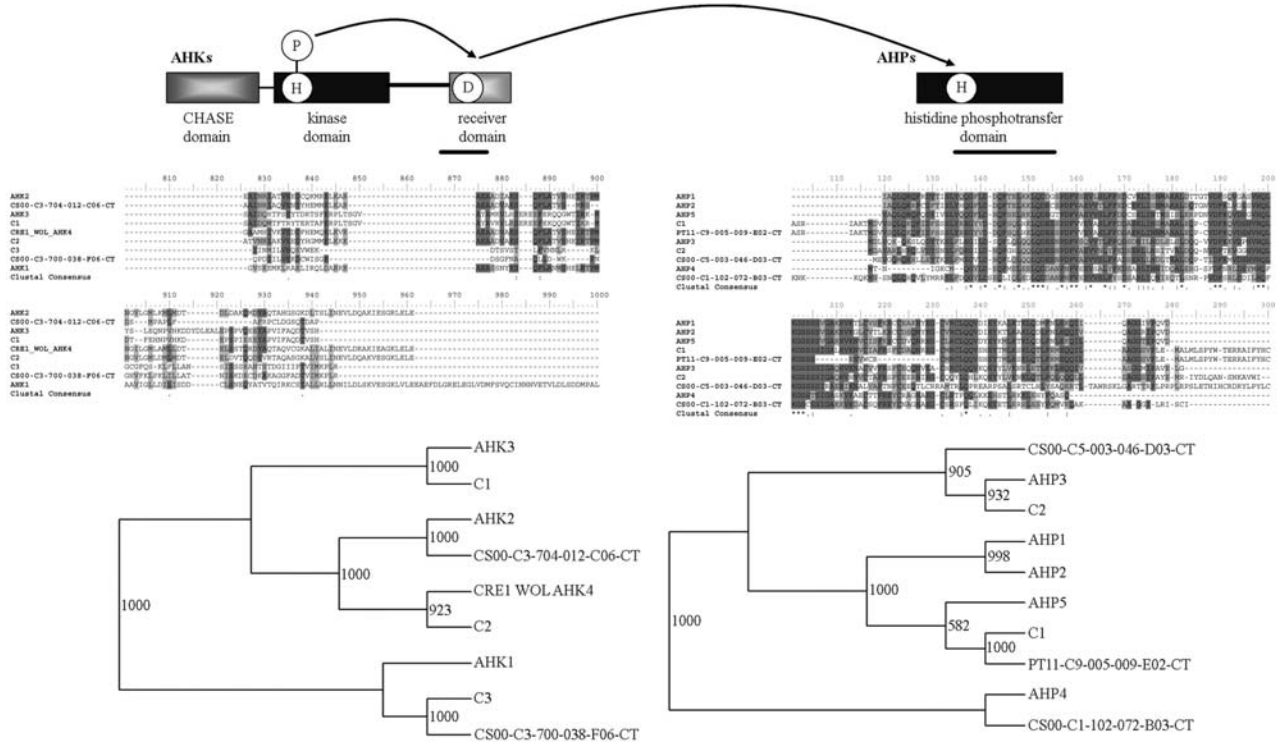

Figure 2 - Cytokinin-mediated signal transduction in citrus. Schematic representation of AHK to AHP phosphorelay. Alignment of the receiver domain from *A. thaliana* and citrus AHK proteins and phylogenetic analysis of citrus AHKs. Alignment of the conserved histidine phosphotransfer domain from *A. thaliana* and citrus AHP proteins and phylogenetic analysis of the deduced amino acid sequence of citrus and *Arabidopsis* full-length proteins. Phylogenetic analysis was performed using full-length *Arabidopsis* proteins and the deduced amino acid sequence of citrus transcripts as described. Black and gray shading of amino acid residues represents sequence identity and similarity, respectively.

Ethylene is synthesized from the amino acid methionine via the intermediates S-adenosyl-L-methionine (AdoMet) and 1-aminocyclopropane-1-carboxylate (ACC). The conversion of AdoMet to ACC is the first committed, and generally, rate-limiting step in ethylene biosynthesis and is catalyzed by the enzyme ACC synthase (ACS). Ethylene is then made from ACC by the enzyme ACC oxidase (ACO). Both ACS and ACO are encoded by multigene families in most plant species and these genes are regulated differently at the transcriptional level (Chae and Kieber, 2005). The *Arabidopsis* genome contains nine ACS genes that encode eight functional and one non-functional ACS protein. In *C. sinensis* transcriptome, we have identified three transcripts showing sequence similarity to *Arabidopsis* ACS enzymes (Table S8). Similarly, in *P. trifoliata, the* ACS family is represented by three transcripts (Table S8). Molecular genetic studies in *Arabidopsis* have provided evidence that ACS protein stability is regulated by the ubiquitin-26S proteasome, reviewed in Chae and Kieber (2005). In this species, the gene *ETO1* encodes a BTB (Broad-complex, Tramtrack, Bric-à-brac) domain-containing protein, a class that has been shown to link CUL3-based ubiquitin ligase to substrate proteins (Pintard *et al.*, 2004). ETO1 also contains six predicted tetratricopeptide repeat motifs, which are involved in diverse protein - protein interactions and can serve as a scaffold for the assembly of multiprotein complexes. In citrus, we have identified four ETO1 homologs (Table S8), including a transcript sharing more than 70% of sequence identity to the *Arabidopsis* protein.

Ethylene is sensed by a family of endoplasmic reticulum (ER)-localized membrane-bound receptors (Chen *et al.*, 2002) that share sequence similarity with bacterial two-component histidine kinases (Stepanova and Alonso, 2005). The functional role of the kinase activity of ET receptors is not clear (Chen *et al.*, 2005). The citrus ETR family consists of six transcripts, whose deduced amino acid sequence is highly similar to ETR proteins from model species, especially at the kinase domain (Table S9). The receptors function as negative regulators of the signaling partner CTR1 (Hua and Meyerowitz, 1998). CTR1 is a Raf-like protein kinase (Kieber *et al.*, 1993) that has a role as a second negative regulator of the pathway that co-localizes and directly interacts with the receptors (Gao *et al.*, 2003). We have identified several citrus cDNAs showing sequence similarity to CTR1 (Table S9). However, the sequence identity is mostly localized at the conserved Raf kinase domain. Thus, at this point, we cannot rule out a role for citrus CTR1-like transcripts in ET-independent signaling pathways.

EIN2, a novel plant-specific protein, is the downstream signaling partner of CTR1. EIN2 has two well-defined domains: (i) an N terminus with similarity to the NRAMP family of metal ion transporters and (ii) a unique hydrophilic C terminus (Alonso *et al.*, 1999). Interestingly, we were unable to find EIN2 homologs in citrus transcriptome databases. In contrast, nine homologs of EIN3/EIN3-like proteins, a family of plant-specific transcription factors that are structurally and functionally conserved among several plant species (Stepanova and Alonso, 2005), were identified (Table S9). The activation of this family by ET is, at least in part, mediated by the regulation of their protein abundance through an ubiquitin-mediated proteasomal pathway. Two F-box proteins (EBF1 and EBF2) that form part of an SCF complex are involved in the regulation of EIN3 levels by ET in *Arabidopsis* (Guo and Ecker, 2003; Potuschak *et al.*, 2003). Functional studies of EIN3 have demonstrated that it binds to the promoter sequences of an ethylene-inducible transcription factor gene *ERF1*, a member of the ethylene response element binding protein (EREBP) family of genes, reviewed in Chen *et al.* (2005). ET-triggered signal transduction controls the expression levels of a large number of target genes through what seems to be a transcriptional cascade (Alonso *et al.*, 2003).

Gibberellin

The biosynthesis of the diterpene carboxylate hormone gibberellin (GA) has been well-characterized (Hedden and Phillips, 2000). We searched for genes involved in biosynthesis and signaling of GA in *Citrus sinensis*, and we were able to identify homologs to all biosynthesis components, and several transcripts shared sequence identity to genes involved in signaling (Table S10). At the first stage of GA biosynthesis, geranyl-geranyl diphosphate is cyclized to *ent*-kaurene by copalyl diphosphate synthase (CPS) and/or *ent*-kaurene synthase in the chloroplasts. Genes coding for these two enzymes share a high level of similarity, hindering identification. In the CitEST database, one homologous sequence to the genes coding for these enzymes was present as a singlet. In *Arabidopsis*, extremely low amounts of CPS mRNA were detected during plant development, with cell-specific expression (Olszewski *et al.*, 2002). In the second step, *ent*-kaurene is oxidized by *ent*-kaurene oxidase (KO) to *ent*-kaurenoic acid, which is then oxidized by *ent*-kaurenoic acid oxidase (KAO) to GA_{12}-aldehyde by cytochrome P450 monooxygenases in the endoplasmic reticulum (Helliwell *et al.*, 1998, Helliwell *et al.*, 2001). Three EST-contigs similar to KO were identified, whereas three contigs and two singlets resembling KAO were identified. The conversion of GA_{12}-aldehyde to C19-GA proceeds via the 13-hydroxylation pathway, resulting in GA_{20} and GA_1, or alternatively by the non-13 hydroxylation pathway, which produces GA_9 and GA_4. These enzymes are encoded by a small multigene family in *Arabidopsis* (Olszewski *et al.*, 2002). Transcripts for both enzymes were present in the citrus transcriptome: one contig showed low similarity to GA_3 hydroxilase, and another to GA_{20} oxidase.

Gibberellins are synthesized in apical meristematic tissues and immature seeds, and have been detected in phloem translocation streams and in root exudates (Huntley *et al.*, 2002; Lough and Lucas, 2006). Little is known about the regulation of GA catabolism, but the first step of GA degradation involves GA_2-oxidases (GAOX) that hydroxylate C-2 of active GAs (Martin *et al.*, 1999; Thomas *et al.*, 1999; Sakamoto *et al.*, 2001). Three contigs (two from *C. sinensis*) and one singlet showing homology to genes encoding for the GAOX group of enzymes were identified. GA_2 oxidase, the enzyme involved in the first step of GA degradation, displayed high expression levels in all analyzed libraries (data not shown). GA_2-oxidase genes have been cloned from several species, and in *Arabidopsis* they are encoded by a five-gene family, two genes associated with C19-GAs hydroxylation, and the remaining three coding for enzymes capable of hydroxylating C20 (Schomburg *et al.*, 2003). The identified reads (26) were grouped into three distinct expression clusters with one containing 21 reads.

Regulation of GA biosynthesis is complex and it is likely to be feedback-regulated. Several important positive and negative regulators of GA signaling have been identified in model plants. GA is believed to bind a receptor, activating G-proteins, which in turn, enhance GA signaling pathways. GID1 was identified and characterized as a soluble receptor of GA in rice (Ueguchi-Tanaka *et al.*, 2005). In citrus, two contigs with high similarity to GID1 were identified. The gene *PHOR1* was described in *Solanum tuberosum* (Amador *et al.*, 2001), and it has been shown that, upon GA binding, PHOR1 was translocated into the nucleus, where it acts as a positive regulator. No *PHOR1* homologous sequences were found in the CitEST database.

The GA signal also activates protein kinase and GID2/SLY1-(F-box)-mediated degradation of DELLA proteins (Nambara *et al.*, 1998). DELLA proteins function as negative regulators of GA signaling, and their degradation through the ubiquitin/proteasome pathway is considered a key event in the regulation of GA-initiated processes (Peng *et al.*, 1997; Richards *et al.*, 2001). One contig with high similarity to SLEEPY (SLY) protein was found in our analysis (Table S10). The expression pattern of the putative DELLA contigs in citrus displayed one read expressed in flowers, and all the others present in juvenile leaves. The observed profile of DELLA transcripts suggested that the genes present a tissue- and treatment-specific expression pattern. The limited number of reads prevented us from performing expression analysis in other citrus species. DELLA is a sub-family within the GRAS family of plant regulatory proteins involved in several aspects of plant development (Bolle, 2004). In rice, wheat, barley and maize, there is one homolog of *Arabidopsis* RGA/GAI coding for a DELLA protein repressor. However, in the dicot *A. thaliana*, five DELLA proteins were identified: RGA (REPRESSOR OF *ga1-3*) and GAI (GA INSENSITIVE) and three RGA-LIKE proteins: RGL1, RGL2, and RGL3. Recent evidence suggests that RGA, RGL1, and RGL2 are involved in modulating floral development in a tissue-specific manner (Lee *et al.*, 2002; Wen and Chang, 2002; Tyler *et al.*, 2004). In contrast, *Lycopersicum esculentum* genome appears to contain a single DELLA protein, named *Le*GAI (Bassel *et al.*; 2004). Six DELLA-related contigs were identified in citrus. Four contigs were identified containing domains that allow the identification of GRAS sequences from the DELLA subfamily (Tian *et al.*, 2004), suggesting that *Citrus sinensis* contains at least four DELLA genes. Incomplete consensus sequence in the majority of the contigs carrying the DELLA domain prevented us from performing a comprehensive phylogenetic analysis of the citrus DELLA family. Interestingly, three contigs presented *C. sinensis* reads matching contigs with reads from other species or genera (data not shown). Furthermore, the *C. sinensis* genome contains genes highly similar to other *Citrus* and *Poncirus* species.

The expression of *Arabidopsis GAI* in rice caused a dwarf phenotype, suggesting that GAI is sufficiently conserved to allow it to function in the context of a heterologous genome (Peng *et al.*, 1999). Recent evidence demonstrates that GAI mRNA is translocated through the phloem (Haywood *et al.*, 2005). A specific population of RNA molecules was identified in the phloem translocation stream (Yoo *et al.*, 2004). Haywood *et al.* (2005) proposed that GAI mRNA delivery via phloem allows flexibility in fine-tuning developmental programs, allowing transcripts access to petal primordia and ground tissues.

DELLA proteins are targeted to *o*-GlcNAc modifications (Thornton *et al.*, 1999). In *Arabidopsis*, SPINDLY (SPY) is a negative regulator of GA signal transduction. Sequence analysis of SPY suggests that it encodes an *o*-glucosyl-N-acetyltransferase, which can activate DELLA proteins (Jacobsen *et al.*, 1996). In the citrus transcriptome, one contig and three singlets with high similarity to SPY were identified (Table S10). After degradation of DELLA proteins, positive transcription factors that were once blocked by DELLA, such as GAMYB, are free to activate the transcription of several genes (Gubler *et al.*, 2002). A single sequence sharing similarity with GAMYB was found in *Citrus reticulata*. Achard *et al.*(2004) suggested that microRNA (miR159) modulates GA-mediated development via its effects on GAMYB activity, thus acting as a homeostatic regulator. We were unable to identify miR159 homologs in the CitEST database.

GA-mediated signal transduction is likely to encompass other genes, but the involvement of GAR2, SHI, GAST and several SCARECROW-regulators awaits further functional evidence. We have identified CitEST sequences homologous to those GA-signaling candidates; however, they were not referred to in this study due to space constraints.

Jasmonate

The jasmonate family of molecules includes jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA) and is derived from oxylipins, a group of biologically active compounds synthesized from the oxidative metabolism of polyunsaturated fatty acids (Schilmiller and Howe, 2005). These compounds are involved in regulating stress-induced gene expression, mechanical responses such as tendril coiling, and reproductive development (Browse, 2005).

In a survey of citrus transcriptome, we have identified 199 homologs of model plant genes involved in the metabolism and functioning as signaling partners of the jasmonate family of plant hormones (Table S11, Table S12). From the total, 87 citrus transcripts share sequence homology to JA biosynthesis components (Table S11), whereas the remaining 112 are similar to JA-triggered signal transduction pathway members (Table S12). Interestingly,the family of extensins, a group of homologous hydroxyproline-rich glycoproteins responsible for plant cell wall self-assembly and cell extension (Ringli *et al.*, 2001) and JA-induced wounding and pathogen infection defense mechanisms (Shanmugam, 2005), is absent from citrus transcriptome databases. The crucial role of extensins in negative and positive regulation of cell expansion and elongation constitutes a major morphogenetic mechanism operating at all levels of plant growth and development, thus it is unlikely that this class of protein is absent from citrus species.

Most plant oxylipins are synthesized in a pathway initiated by lipoxygenase (LOX), a non-heme iron dioxygenase that adds molecular oxygen to either the 9 or the 13 position of the C_{18} chain of linoleic acid. Plants express numerous LOX isoforms with distinguishable expression pattern, subcellular location, and substrate utilization (Schilmiller and Howe, 2005). In citrus, we have identified 15 transcripts whose deduced amino acid sequences show the conserved lipoxygenase LH2 domain (Table S11). The conservation at the functional domain indicates that they are likely to represent functional LOX proteins. The hydroxyperoxy products of LOX are metabolized to an array of oxylipins by several enzymes, including closely related members of a cytochrome P450 family: allene oxide synthase (AOS), epoxy alcohol synthase, peroxygenase, alkyl hydroperoxide reductase, and LOX itself (Schilmiller and Howe, 2005).

The family of AOS enzymes transforms 13-hydroperoxy linolenic acid (13-HPOT) to the jasmonate family of compounds that includes JA/MeJA and their metabolic precursor, 12-oxo-phytodienoic acid (12-OPDA) (Schilmiller and Howe, 2005). Metabolism of 9-hydroperoxy fatty acids generates a group of oxylipins that are structurally related but distinct to the oxylipins generated by the 13-LOX pathway, the 9-hydroperoxy linolenic acid (9-HPOT). The 13- and 9-hydroxyperoxydes are metabolized by allene oxide cyclase (AOC) isoforms (Schilmiller and Howe, 2005). AOC and AOS family homologs were identified in citrus species (Table S11). The α,β-unsaturated carbonyl moiety that distinguishes cyclopentenone oxylipins (*e.g.* 12-OPDA) from cyclopentanone oxylipins (*e.g.* JA) functions as a negative regulator of novel signaling activity (Seo *et al.*, 2001).

The discovery of a gene encoding a JA carboxyl methyltransferase (JMT) indicates that MeJA is an important component of the mix of oxylipin signals that mediates defense responses (Seo *et al.*, 2001; Zubieta *et al.*, 2003). The deduced amino acid sequences of citrus JMT are moderately similar to the *Arabidopsis* protein with the highest similarity at the methyltransferase domain (Table S11). However, at this point a methyltransferase activity dissociated from JA metabolism cannot be ruled out. *DAD1* is a member of *DAD1*-like gene family in *Arabidopsis* and this family is hypothesized to regulate jasmonate production in response to other cues or to be involved in the biosynthesis of other classes of oxylipins (Matsui *et al.*, 2004). In citrus, the family is represented by five transcripts including three *C. reticulata*-specific mRNAs (Table S11).

Jasmonates function as cellular regulators in diverse developmental processes, such as seed germination, root growth, fertility, fruit ripening, and senescence. In addition, JA activate plant defense mechanisms in response to insect wounding, various pathogens, and environmental stresses such as drought, low temperature and salinity and are also involved in the regulation of some stages of secondary metabolism (Cheong and Choi, 2003). The leucine-rich repeats and F-box motif protein COI1 is required to degrade a repressor of the jasmonate signaling pathway (Liechti *et al.*, 2006). Signaling in the jasmonate pathway depends on at least two massive signaling machines that interact *in vivo* to form a complex of at least 0.7 mDa (Feng *et al.*, 2003). The first of these complexes is the SCF^{COI1} complex, which is an E3-ubiquitin ligase (Devoto *et al.*, 2002). In this complex, the F-box protein COI1 (Ren *et al.*, 2005) physically associates with Skp-like proteins cullin and *At*Rbx1 to form active SCF^{COI1}. The second multimolecular complex involved in JA signaling is the COP9 signalosome (CSN), which interacts *in vivo* with SCF^{COI1} (Feng *et al.*, 2003). We have identified citrus transcripts sharing extensive sequence conservation with COI1 (Table S12, Figure 3). The sequence identity is higher at the F-box domain (Figure 3). Interestingly, COI1-like transcripts are highly frequent in *C. latifolia* libraries.

Reversible protein phosphorylation has been demonstrated to be involved in JA signal transduction pathways leading to jasmonate-induced gene transcription (Jensen *et al.*, 2002). A transposon-inactivation study revealed that mitogen-activated protein kinase 4 (MPK4) is required for jasmonate-responsive gene expression in *Arabidopsis* (Petersen *et al.*, 2000). Interestingly, the deduced amino acid sequence of six citrus transcripts is more than 50% identical to *At*MPK4 (Table S12), suggesting a functional conservation between the proteins from citrus species and their

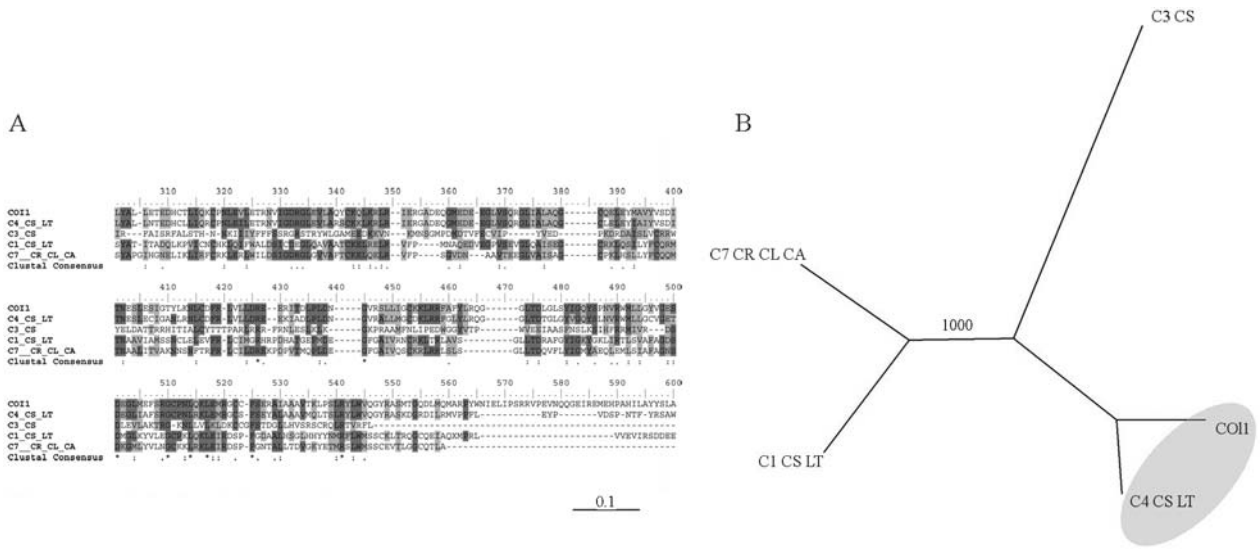

Figure 3 - Jasmonates-mediated signal transduction in citrus. Alignment of the degenerated F-box domain from *A. thaliana* and citrus COI1 proteins. Phylogenetic analyses of the deduced amion acid sequence of citrus COI1 homologs and full length *A. thaliana* counterpart. Phylogenetic analysis was performed as described. Shaded circle represents the AtCOI1 branch.

Arabidopsis counterpart. The other seven cDNAS from citrus present intermediate (from 44 to 28%) deduced amino acid identity to *At*MPK4 (Table S12).

The expression of some JA-responsive genes is controlled in part by AP2/ERF-domain transcription factors (Yanhui *et al.*, 2006). These proteins bind to "jasmonate- and elicitor-responsive elements" (JEREs) (van der Fits and Memelink, 2001). In citrus, several homologs of JA-induced transcriptional regulators were identified: including the development regulators AS1, ATAF2 and CPC (He and Gan, 2001; Delessert *et al.*, 2005; Kwak *et al.*, 2005); the basic-loop-helix JIN1 (Boter *et al.*, 2004); the homeobox OCP3 (Coego *et al.*, 2005); and the MYB telomere repeat-binding family of TRF1 and TRFL proteins (Yanhui *et al.*, 2006) (Table S12). In citrus, JA-induced transcriptional regulators are highly frequent in biotic stress- and senescence-associated libraries, although distinct classes of factors show non-overlapping expression patterns (Figure 4).

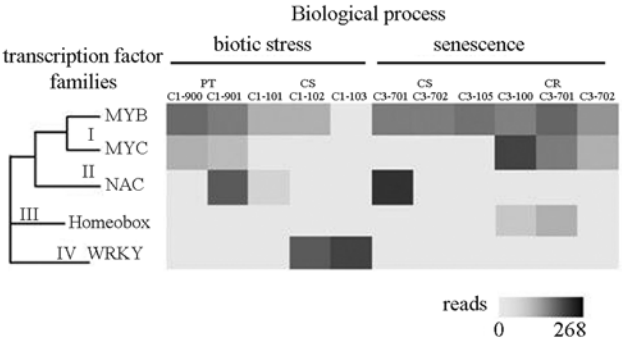

Figure 4 - Differential expression of JA-induced transcription factors in citrus. The normalized number of transcription factor homologous reads in each library is represented as grayscale. Citrus species libraries are represented by two-word abbreviations (CS, *C. sinensis*; CR, *C. reticulata*; CL, *C. limonia*; CG, *C. aurantifolia*; CA, *Citrus aurantium;* LT, *C. latifólia;* PT, *Poncirus trifoliata*). Hierarchical clustering of the expression patterns is represented by Roman numerals.

Peptide hormones

Recently, a novel class of non-lipophilic peptide hormones was identified in several plant species, including *A. thaliana*, *Solanum tuberosum* and *S. lycopersicum*. Several secretory and non-secretory peptide signals have been demonstrated to be involved in plant growth regulation: including defense responses, callus growth, meristem organization, self-incompatibility (SI), root growth, leaf-shape regulation, nodule development, and organ abscission (Matsubayashi and Sakagami, 2006). These peptides have been identified by biochemical purification, genetic studies and *in silico* genome analysis. In general, they consist of a great number of highly-homologous genes that encode short open reading frames that are transcribed and translated at very low levels but severely affect plant development (Wen *et al.*, 2004). In the CitEST database, we have identified 15 ESTs showing strong sequence similarity to previously characterized plant peptide hormones (Table S13). Interestingly, a large group of peptide hormones was absent from the screened citrus databases: including *Solanum lycopersicum* systemins (TomSys), *S. lycopersicum* and *A. thaliana* small, secreted, cysteine rich proteins (SCR/SP11, SCRL), *A. thaliana* inflorescence deficient in abscission (IDA) and IDA-like (IDL), *A. thaliana* POLARIS (PLS), *A. thaliana* CLAVATA3 and CLV3-like (CLV3, CLE and approximately another 100 similar sequences). Although this absence is unexpected, it might be attributed to the undetectable levels of expression of peptide hormone genes in wild type plants (Wen *et al.*, 2004). In addition, the expression pattern of PSK1 and BRI1

homologs was distinct from the profile obtained for RALFL homologs: the normalized frequency of RALFL-like reads was higher in all species, including in *C. limoni, C. aurantifolia*, *C. aurantium* and *C. latifolia* that presented no PSK1 and BRI1 homolog (Figure 5).

Salicylic acid

SA belongs to a group of plant phenolics that possesses an aromatic ring bearing a hydroxyl group or its functional derivative. It was called a plant hormone by Raskin (Raskin, 1992a; Raskin, 1992b). Recently, SA has been demonstrated to be involved in various physiological processes like stomatal closure, flowering induction, and heat production, besides playing a central role in defense responses against biotic and abiotic stresses (Raskin, 1992a; Lee *et al.*, 1995). *In planta* SA levels are probably the result of *de novo* synthesis (Yalpani *et al.*, 1993); thus biosynthesis and metabolism knowledge functions as control steps for manipulating disease resistance.

In plants, the biosynthesis of SA consists of two parallel routes (Wildermuth *et al.*, 2001). In citrus transcriptome analyses, we have found 33 sequences with high similarity to *Arabidopsis* components of SA metabolism: 26 for biosynthesis and seven for its catabolism. The first SA biosynthetic pathway is derived from shikimic acid. Firstly, chorismate is converted to prephenate, and prephenate to phenylpyruvate that is subsequently converted to phenylalanine by the action of the enzymes chorismate mutase (CM), prephenate dehydratase (PD) and phenylpyruvate amino transferase, respectively (Warpeha *et al.*, 2006). Trans-cinnamic acid (CA) is synthesized from Phe by the action of Phe ammonia lyase (PAL). The production of benzoic acid (BA) from CA is not well understood and it could be step-limiting in SA biosynthesis. BA2H, the monooxygenase responsible for the conversion of BA to SA conjugates, is constitutively expressed in tobacco and is highly induced by the TMV inoculation in this species, just before the onset of HR-cell death, as well as under UV irradiation, or exposure to O_3 (Leon *et al.*, 1995, Chong *et al.*, 2001). This step is also responsible for stress-induced flowering of *Pharbitis nil* (Hatayama and Takeno, 2003). BA2H is the first soluble cytochrome P450 identified in plants and animals, but no coding sequence is yet known in plants.

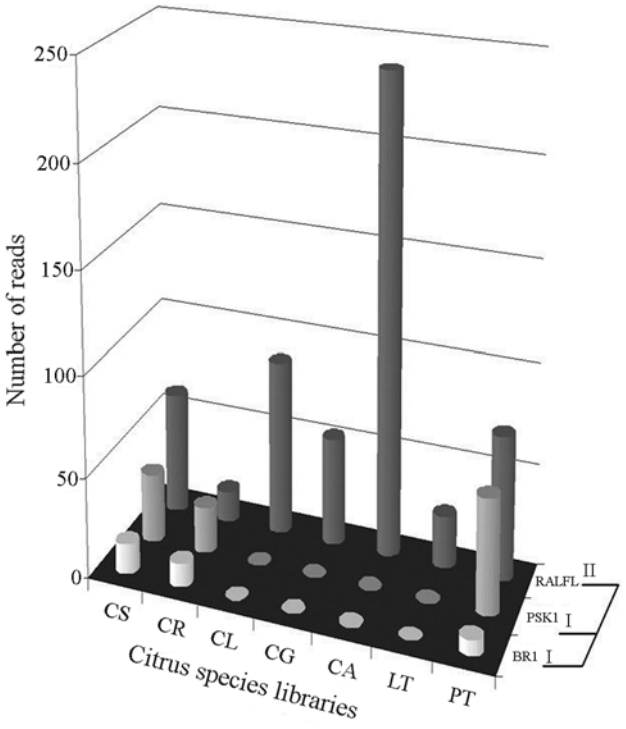

Figure 5 - Differential expression of peptide hormones in citrus species. The normalized number of reads for the transcripts in each library is represented in the y-axis. Citrus species libraries are represented by two-word abbreviations (CS, *C. sinensis*; CR, *C. reticulata*; CL, *C. limonia*; CG, *C. aurantifolia*; CA, *Citrus aurantium;* LT, *C. latifolia;* PT, *Poncirus trifoliata*). Hierarchical clustering of the expression patterns is represented by Roman numerals.

A second pathway for the synthesis of SA has been recently shown in plants (Wildermuth *et al.*, 2001). Chorismate is converted to isochorismate by isochorismate-synthase (ICS) and then to SA and pyruvate, probably due to the action of pyruvate lyase (IPL). In citrus transcriptome survey, we have identified one transcript homologous to *Arabidopsis ICS1* gene covering the chorismate-binding region (Table S14). In model species, SAR responses require SA synthesized through ICS1. Interestingly, three of the four *C. sinensis* ESTs forming the contig C1-CR/CS originated from libraries of infected leaves.

In plants, SA is present as a free acid and as conjugated metabolites: SA 2-*O*-β-D-glucoside (SAG), glucosyl salicylate (GS) and methylsalicylate (MeSA) (Lee and Raskin, 1999). GS formation is transiently induced under pathogen attack and serves as a protective mechanism from the phytotoxic effects of high concentrations of SA (Lee and Raskin, 1999). In citrus transcriptome, four ESTs presented relatively high similarity to tobacco SA GTase. The methylation of SA into methyl-salicylate (MeSA**)** is mediated by a carboxyl methyl transferase that has salicylic acid (SAMT) as specificity of substrate. This enzyme belongs to a recently described family named `SABATH' with reference to model proteins (D'Auria *et al.*, 2003). In citrus, we have identified four homologs to benzoic acid/salicylic acid:carboxyl methyl transferases (Table S14). The deduced amino acid sequences of these transcripts are highly conserved at the SA-binding critical residues in comparison to their tobacco counterparts (Zubieta *et al.*, 2003).

We have found 70 homologs of genes involved in SA signaling pathway (Table S15). Plant-pathogen interactions trigger a complex network of regulatory processes among phytohormone-controlled pathways, including the antagonism between SA and JA signaling. In *Arabidopsis*, *AtMPK4*, *WRKY70* and *SSI2* have been demonstrated to have cross-talk intermediates and are discussed in JA subsection. Interestingly, *ACD6* homologs were absent from citrus transciptome. They encode a novel ankyrin repeat-

containing protein that participates in cell death control and probably has overlapping and/or redundant functions with other proteins. NDR1, a protein of unknown function, hypothesized to be a transducer of pathogen signals and/or to interact directly with pathogens, is represented by two homologous sequences in citrus EST databases.

Transcripts corresponding to the proteins forming the EDS1-PAD4-SAG101 complex were identified in citrus. These proteins present sequence similarity to acyl hydrolases, reinforcing the role of lipid signals in defense responses and indicating another probable cross-talk point in SA and JA/ET signaling (Wiermer *et al.*, 2005). NPR1 and its homologous NPR4 are represented by two and seven homologs in citrus species, respectively. NPR1 plays a central regulator role in SA signaling and plant defense responses and NPR4 is required for the basal defense against pathogens.

Remarkably, we have found four sequences with high sequence similarity to SABP2 in citrus transcriptome. In *Arabidopsis*, it displays SA-stimulated lipase activity essential for basal resistance (Kumar and Klessig, 2003; Wiermer *et al.*, 2005). It has been thought to be the long-sought-after SA receptor; however, recent evidence has implicated it in the hydrolysis of inactive MeSA to SA (Forouhar *et al.*, 2005). More interestingly, citrus SABP2 displays higher levels of sequence conservation to a *C. sinensis* transcript coding for an ethylene-induced esterase (Zhong *et al.*, 2001). This suggests the existence of a direct link between SA- and ET-mediated signaling pathways.

The SA-responsive genes are mainly regulated by a set of transcription factors that includes TGA, WRKY70 and WHIRLY1, which are present in citrus transcription databases. TGA constitutes a conserved plant subfamily of NPR1-regulated basic domain/Leu zipper (bZIP) transcription factors associated with detoxification and defense (Klinedinst *et al.*, 2000; Pontier *et al.*, 2001). The most important NPR1-interacting TGA factors are TGA2 and TGA3 that are represented by several transcripts in citrus. In *Arabidopsis*, WHY1 is dependent on SA but it functions in an NPR1-independent manner to induce plant defense gene expression and to mediate SAR (Desveaux *et al.*, 2004). This type of regulator response confirms that SA signaling has an NPR1-independent branch. The great similarity observed between the citrus and *Arabidopsis* transcripts probably reflects functional conservation between the species. Another interesting finding is the high sequence conservation observed between VAD1 and two citrus transcripts. VAD1 is hypothesized to represent a new function in cell-death control associated to cells in the vicinity of vascular bundles. It contains a GRAM domain that functions in membrane-associated processes to protein or lipid binding (Lorrain *et al.*, 2004).

Concluding Remarks

This preliminary survey of citrus components of hormone-associated pathway has provided useful information for further studies of developmental control in these spe-

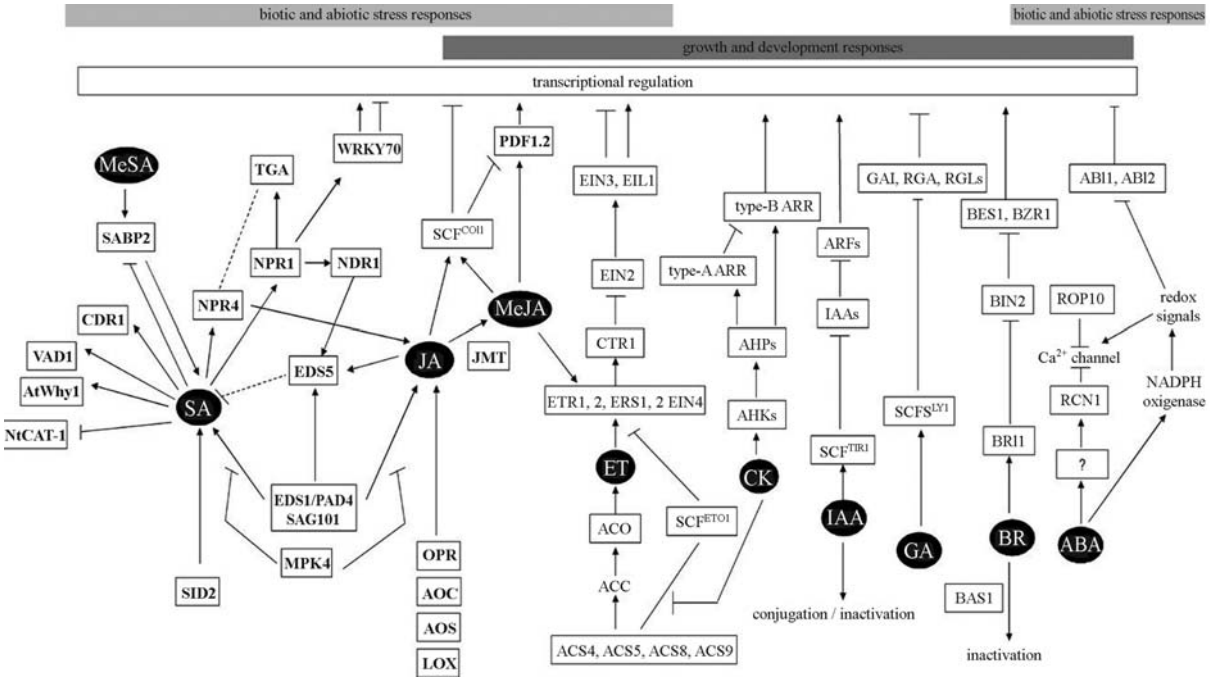

Figure 6 - Schematic representation of citrus hormone signaling pathways controlling stress and developmental responses. Phytohormones are represented by black circles and pathway components are shown in white boxes. Full lines represent genetic and direct interactions and dashed lines show hypothesized interactions. Arrowheads demonstrate positive interactions; lines without arrowheads are for interactions where directionality is unknown and blocked lines represent negative interactions. For clarity reasons, not all relevant genes or interactions have been shown.

cies. It has allowed the identification of conserved members of signaling pathways in a non-model species and the elaboration of a framework for future studies (Figure 6). The results obtained here indicate high conservation in hormone biosynthetic pathways between model species and citrus. Signaling pathways are generally less conserved, although the vast majority of investigated processes were identified in citrus species (Figure 6). These studies will help to elucidate the molecular basis of developmental control and to understand how environmental factors modulate plant development and the expression of phenotypic characters. The results obtained give a new perspective on several aspects of hormonal regulation of physiological processes in citrus.

Acknowledgments

The work in the authors' laboratories is supported by CNPq and FAPESP. J.B.M.M.is a recipient of a CAPES-PRODOC fellowship. The CITEST project was sponsored by grants from CNPq/Institutos do Milênio/Citrus 620054/01-8 to Marcos A. Machado.

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Internet Resources

Citrus Biotechnology Laboratory, http://citest.centrodecitricultura.br (September 13, 2006).

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PAUP* 4.0b10 Software, http://paup.csit.fsu.edu/.
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The Institute for Genomic Research (TIGR) *Arabidopsis thaliana* v.13.0 Gene Ontology Assignments, http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/GO_browser.pl?species = Arabidopsis&gi_dir = agi (October 23, 2006).
Tree View v.1.6 Software, http://rana.lbl.gov/EisenSoftware.htm.

Supplementary Material

The following online material is available for this article:

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Associate Editor: Marco Aurélio Takita

Table S1– Citrus ESTs homologous to *Arabidopsis* and *Oryza sativa* functionally-characterized ABA biosynthesis and signaling pathway genes.

Arabidopsis/Oryza sativa		**CitEST**			**Protein motifs and**	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**References**
AAO	AT5G20960	C1-CS (2)	70.0	5e-86	aldehyde oxidase; molybdopterin-binding domain; FAD-binding domain, ABA biosynthesis	Seo *et al.*, 2000
ABA1/LOS6/ ZEP	AT5G67030	C1-CS (4)	70.0	1e-146	zeaxanthin epoxidase,	Peeters *et al.*, 2002
		C2-PT (3)	55.0	1e-137	ABA biosynthesis	
ABA3	AT1G16540	C1-CS/PT (2)	65.0	3e-35	selenocysteine lyase,	Xiong *et al.*, 2001
		PT11-C1-900-046-C09-CT	65.0	3e-35	ABA biosynthesis	
ABF2/AREB1	AT1G45249	CS00-C3-705-096-D09-CT	50.0	3e-26	bZIP domain	Kobayashi *et al.*, 2005
GCR1	AT1G48270	C1-CS/CR (3)	84.0	1e-129	cAMP receptor	Pandey and Assmann, 2004

GPA1	AT2G26300	C1-CS/CR/PT/LT (8)	86.0	0	G protein α domain; ARF-like domain	Pandey and Assmann, 2004
		CS00-C3-704-024-D11-CT	86.0	0		
NCED	AT3G63520	PT11-C1-900-054-G12-CT	70.0	1e-111	9-cis-epoxicarotenoid dioxigenase, biosynthesis	Iuchi *et al.*, 2001
OST1	AT4G33950	PT11-C1-900-002-B02-CT	81.0	6e-125	serine/threonine protein kinase, signal transduction	Mustilli *et al.*, 2002
RCN1	AT3G25800	C2 – CS/CR/CA (10)	87.0	0	armadillo/beta-catenin-like repeats; HEAT repeats, serine/threonine phosphatase type 2A regulatory subunit, signal transduction	Kwak *et al.*, 2002
		C1- CS/CR (2)	84.0	7e-101		
		C3-CS/CG (2)	80.0	0		
		PT11-C1-900-014-E10-CT	30.5	1e-99		
		CA26-C1-002-092-H05-CT	15.2	2e-62		
ROP6/RAC1	AT3G51300	C2-CS/CR/CG (11)	91.0	2e-96	Ras-like small GTPase, signal transduction	Lemichez *et al.*, 2001
		C5-CS (3)	90.0	6e-96		
		C1-CS/CR/CA (6)	86.0	1e-95		
		C4-CS/CR/PT (6)	82.0	1e-87		

		C3-CR (2)	78.0	8e-74		
		CS00-C1-101-048-A07-CT	90.9	2e-94		
		CG32-C1-003-037-B10-CT	56.95	3e-48		
		PT11-C1-901-020-E06-CT	0.3	2e-84		
ROP10	AT3G48040	C1-CS/CA (3)	84.0	4e-90	ROP family	Xin *et al.*, 2005
		C2-CS (2)	71.0	2e-83		
OsTRAB1	CAB85632[c]	CS00-C3-705-096-D09-CT	48.0	1e-39	Zinc finger transcription factor, signal transduction	Hobo *et al.*, 1999
ZEP1	AT5G67030	CS00-C3-702-073-E06-CT	70.0	1e-146	mono-oxygenase domain	Xiong *et al.*, 2002
		PT11-C2-300-033-E11-CT	55.0	1e-137		

[a]Gene name abbreviations: *AAO*: ABA-aldehyde oxidase, *ABA*: ABA deficient, *ABF*: ABRE-binding bZIP factor, *ABRE*: ABA responsive element, *GCR*: G protein-coupled receptor, *GPA*: G protein α subunit, *LOS*: low expression of osmotic stress-responsive genes, *NCED*: 9-cis-epoxy-carotenoid dioxygenase, *Os*: *Oryza sativa*, *OST*: open stomata; *RCN1*: roots curl in NPA, *ROP*: RHO of plants, *TRAB*: transcription factor responsible for ABA regulation, *ZEP*: zeaxanthin epoxidase.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]GenBank accession number.

Table S2 – Citrus ESTs homologous to *Arabidopsis* and *Oryza sativa* functionally-characterized auxin metabolism genes.

Arabidopsis thaliana		**CitEST**			**Protein motifs and**	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**References**
AAO1	AT5G20960	C1-CS/CA (3)	70.0	1e-91	aldehyde oxidase and	Sekimoto *et al.*,
		C2-CA/CR/PT (5)	68.0	0.0	xanthine dehydrogenase	1998
					a/b domain, ferredoxin,	
					aldehyde oxidase activity	
CYP83B1	AT2G22330	C1-CS (5)	56.0	4e-74	cytochrome P450,	Barlier *et al.*, 2000
family[d]		C2-CS (3)	52.0	1e-78	converts tryptophan to	
		C3-CS (2)	51.0	1e-104	indo-3-acetaldoxime	
		C4-CS/CR/LT (12)	50.0	1e-145	(IAOx), precursos	
		C5-PT/CG (4)	48.0	1e-99	biosynthesis	
		C6-CS (2)	46.0	8e-59		
		C7-CS (7)	45.0	1e-86		
NIT family[e]	AT4G08790	C1-CS (9)	90.0	0.0	carbon-nitrogen	Vorwerk *et al.*,
		C2-CS (4)	82.0	1e-100	hydrolase, precursor	2001

		C3-CS (12)	67.0	8e-20	biosynthesis	
YUCCA family[f]	AT4G32540	C1-CR/CG/CS (9)	50.0	1e-108	flavin-containing	Zhao *et al.*, 2001
		C2-LT/CR/CG/CS (12)	50.0	1e-100	monooxygenase family,	
		C3-CS/LT/CR (21)	50.0	1e-88	disulfide, monooxygenase	
		C4-CG (3)	49.0	5e-92	and oxidoreductase	
		C5-CR/CS/PT/CA/LT (30)	46.0	1e-103	activity	

[a]Gene name abbreviations: *AAO*: aldehyde oxidase, *CYP*: cytochrome P450, *NIT*: nitrilase, *YUCCA*: yucca tree.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]CYP79B2 family in *A. thaliana*: AT4G31500, AT4G39950.

[e]NIT family in *A. thaliana*: AT3G44310, AT3G44300.

[f]YUCCA family in *A. thaliana*: AT1G04180, AT1G04610, AT1G21430, AT2G33230, AT4G13260, AT4G28720, AT5G11320, AT5G25620, AT5G43890.

Table S3 – Citrus ESTs homologous to *Arabidopsis* functionally-characterized auxin signaling pathway genes.

Arabidopsis thaliana		**CitEST**			**Protein motifs[d] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
ABP1	AT4G02980	CS00-C1-100-010-B05-CT	79.0	3e-78	unidimensional cell growth, auxin binding	Jones *et al.*, 1998; Shimomura, 2006
ARF family[e]	AT1G59750	C1-CG/CS/PT (7) ARF1	69.0	3e-75	transcriptional factor B3 domain, auxin-mediated transcriptional regulation	Ulmasov *et al.*, 1997b; Okushima *et al.*, 2005
		C2- PT/LT (3) ARF7	65.0	1e-151		
		C3-CR (4) ARF7	55.0	1e-99		
		C4-CS/CR (5) ARF7	54.0	1e-127		
		C5- CS/CR/CL (5) ARF2	53.0	4e-61		
		C6-CS (2)	49.0	3e-91		
		C7-CS/CR (4)	42.0	1e-85		
		C8-CS/CR (2)	41.0	3e-62		
		CS00-C3-705-071-D01-CT	48.0	5e-93		
AUX/IAA family[f]	AT2G38120	C1-CS/CR (4) AXR1	75.0	0.0	transcription regulator acting as repressor of	Overvoorde *et al.*, 2006; Ulmasov *et*

		C3-CG (3)	68.0	1e-71	auxin-inducible gene	*al*., 1997a
		C4- CS/CR/CA (12)	68.0	2e-87	expression	
		C5-CS/PT (7)	67.0	4e-87		
		C6-CS/CR (7)	64.0	6e-86		
		C7- CS/CA/LT (4)	64.0	6e-86		
		C8-CS/CG (12)	59.0	3e-89		
		C9-CS/PT (5)	51.0	1e-79		
BIG	AT3G02260	C1-CS/CR/CA/CG (47)	80.0	0.0	Zinc finger ZZ type,	Gil *et al.*, 2001
					polar auxin transport	
CUL family[g]	AT4G02570	C1-CS (9)	89.0	0.0	cullin domain, component	Gray *et al.*, 1999;
		C2-CS/CR (12) *At*CUL3	88.0	1e-101	of SCF ubiquitin ligase	Quint *et al.*, 2005
		C3- CS/CR/CL/PT/CA (5) *At*CUL1	83.0	0.0	complexes, protein	
		C4- CS/CR/PT (4) *At*CUL1			degradation	
			83.0	1e-113		
EIR1	AT5G57090	C2-CS/CA (2)	49.0	1e-158	auxin transport protein,	Luschnig *et al.*,
		C1-CS/CR/CG (8)	32.0	3e-109	auxin:hydrogen symporter	1998

		CS00-C3-700-106-C03-CT	35.0	3e-93	activity	
PIN / PID	AT1G73590	C2-CA/CS (2) PIN3	25.0	3e-89	auxin efflux carrier,	Petrasek *et al.*,
family[h]		C1- CG/CS (7) PIN1	24.0	2e-75	auxin:hydrogen symporter	2006; Vernoux *et*
		CS00-C3-700-106-C03-CT - PIN3	39.0	4e-97	activity	*al.*, 2000
TIR1	AT3G62980	C1-CS/CR (3)	63.0	0.0	leucine-rich repeat,	Ruegger *et al.*,
		C2-CR/CS/CA/PT/CL (13)	63.0	0.0	cysteine-containing,	1998; Dharmasiri
		C3-PT (2)	59.0	1e-93	cyclin-like F-box, E3	*et al.*, 2005
		C4-CS/LT (4)	52.0	4e-73	ubiquitin ligase SCF	
		PT11-C1-901-035-F11-CT	57.0	3e-84	complex, auxin receptor	

[a]Gene name abbreviations: *ABP*: auxin-binding protein, *ARF*: auxin-responsive factor, *AUX/IAA*: auxin-responsive protein /indoleacetic acid-induced protein, *AXR*: auxin resistant, *CUL*: cullin, *EIR*: ethylene insensitive root, *PID*: pinoid, *PIN*: pin-formed, *TIR*: transport inhibitor response.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Protein motif: SCF: SKP1/Cullin/F-box protein.

[e]ARF family in *A. thaliana*: AT1G19220, AT1G19850, AT1G30330, AT1G34170, AT1G34310, AT1G34390, AT1G34410, AT1G35240, AT1G35520, AT1G35540, AT1G43950, AT1G77850, AT2G24765, AT2G28350, AT2G33860, AT2G46530, AT2G47170, AT3G61830, AT4G23980, AT4G30080, AT5G20730, AT5G37020, AT5G60450, AT5G62000.

[f]AUX/IAA family in *A. thaliana*: AT5G65670, AT5G57420 , AT5G43700 , AT4G32280, AT4G29080 , AT4G28640 , AT4G14550, AT3G62100 , AT1G04100 , AT1G04240 , AT1G04250 , AT1G04550 , AT1G15050 , AT1G15580 , AT1G51950 , AT1G52830 , AT1G80390, AT2G01200 , AT2G22670 , AT2G33310 , AT2G46990 , AT3G04730 , AT3G15540 , AT3G16500 , AT3G17600 , AT3G23030 , AT3G23050.

[g]CULIIN family in *A. thaliana*: AT1G02980 , AT1G26830 , AT1G69670.

[h]PIN/PID family in *A. thaliana*: AT1G23080 , AT1G70940 , AT1G77110 , AT2G01420 , AT2G34650 , AT5G15100 , AT5G54490.

Table S4 – Citrus ESTs with homology to genes involved in brassinosteroid metabolism in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs[d] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
BR6OX	AT5G38970	C3-CS/CR (3)	68.2	2e-83	cytochrome P450,	Shimada *et*
		C1-CS (2)	42.0	4e-56	brassinosteroids C-6 oxidation,	*al.*, 2001
					BR biosynthesis	
CPD	AT5G05690	C3-CS/PT (6)	80.7	1e-138	cytochrome P450, member of	Wang *et al.*,
		C4-CS/CR (7)	64.9	5e-99	CP90A family, BR biosynthesis	2002
		C1-CS (2)	43.4	2e-95		
		C2-CS/CR (2)	29.7	1e-88		
CYP72C1	AT1G17060	C1- CS (4)	36.4	1e-51	cytochrome P450, BR	Bancos *et*
		C9-PT (2)	28.6	1e-54	biosynthesis	*al.*, 2002
		C4- CG/CS (4)	23.5	1e-44		
		CS00-C3-701-100-A11-UV	33.9	1e-72		
		CR05-C1-103-084-D10-CT	32.9	7e-45		
CYP90D1	AT3G13730	C4-CR (2)	32.0	1e-85	cytochrome P450 E-class,	Bancos *et*

		C3-CS/CR (2)	31.8	6e-61	member of CYP90D family, BR biosynthesis	*al.*, 2002; Kim *et al.*, 2005
DET2	AT2G38050	C1-CS (2)	28.8	1e-136	3-oxo-5-alpha-steroid 4-dehydrogenase, BR biosynthesis	Fujioka *et al.*, 1997
		C2-CS (2)	26.8	1e-131		
DWF1	AT3G19820	C1-CS/CR (25)	56.3	1e-110	Ca^{2+}-dependent calmodulin binding, BR biosynthesis	Choe *et al.*, 1999a
DWF4	AT3G50660	C4-CS/PT (7)	38.0	1e-104	C-22 hydroxylation of a variety of C27-, C28- and C29-sterols, BR biosynthesis	Choe *et al.*, 2001
		C3-CS/CR (3)	32.7	3e-98		
		C2-CS (2)	31.4	2e-95		
		C8-CR/PT (2)	26.2	7e-28		
		C5-CS/CR (7)	22.0	1e-34		
		CR05-C3-700-062-F07-CT	51.7	1e-75		
		CR05-C3-700-006-D11-EU	24.8	1e-26		
DWF5	AT1G50430	C2-CG (3)	74.3	1e-121	7-DHC reductase / sterol delta-	Choe *et al.*,

		C1-CR (1)	23.0	2e-25	7-reductase (ST7R), BR biosynthesis	2000
ROT3	AT4G36380	C4-CS/PT (6)	38.2	7e-83	cytochrome P450 class 90C1, BR biosynthesis	Kim *et al.*, 1998
		C3-CS/CR (3)	29.8	2e-50		
		C5-CSCR (7)	25.1	3e-45		
		C7-CR/PT (2)	23.6	2e-41		
		CG32-C1-003-089-B04-CT	23.7	5e-43		
		CR05-C3-700-006-D11-EU	25.2	2e-41		
		CR05-C3-700-062-F07-CT	19.9	5e-31		
STE1	AT3G02580	C1-CS (3)	68.1	1e-123	delta 7-sterol-C5-desaturase, BR biosynthesis	Choe *et al.*, 1999b

[a]Gene name abbreviations: *BR6OX*: brassinosteroid 6-oxidase, *CPD*: carboxypeptidase D precursor, *CYP*: cytochrome P450 precursor, *DET*: de-etiolated, *DWF:*dwaf , *ROT*: rotundifolia, *STE*: sterol desaturase.

[b]C: contig, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[c]Functional domain abbreviations: BR: brassinosteroid, DHC: delta-hydroxy-reductase.

Table S5 – Citrus ESTs with homology to genes involved in brassinosteroid-initiated signal transduction in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs[d] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
receptors and putative receptors						
BRI1	AT4G39400	CS00-C3-702-092-G08-CT	80.8	1e-133	PK, ATP-binding region	Clouse *et al.*,
		CS00-C1-650-008-E09-CT	70.9	1e-115	signature, LRR receptor kinase,	1996
		PT11-C1-900-089-E02-CT	63.9	1e-99	brassinosteroid receptor,	
		CR05-C3-702-027-G11-CT	63.1	5e-90	systemin receptor	
		CS00-C3-700-068-G06-CT	57.1	3e-88		
		CR05-C1-103-056-H03-CT	42.5	1e-73		
BRL1	AT1G55610	C12-CS (7)	58.9	1e-112	PK, ATP-binding region	Cano-Delgad
		C8-CR (3)	47.1	2e-88	signature, LRR receptor kinase,	o *et al.*, 2004
		C5-CS/CR (3)	41.0	4e-76	brassinosteroid receptor	
		C2-CS/PT (2)	35.4	2e-64		
		CR05-C3-702-017-C08-CT	38.4	7e-60		
		CS00-C3-702-076-D04-CT	38.2	8e-55		

		CG32-C1-003-035-B06-CT	35.3	4e-65		
		CR05-C3-701-030-G08-CT	31.9	2e-50		
		CR05-C1-100-070-E05-CT	31.7	7e-49		
		CR05-C3-702-017-B06-CT	31.6	3e-49		
		CS00-C3-703-071-D07-CT	30.5	2e-69		
BRL2	AT2G01950	C2-CS/PT (2)	35.4	5e-63	*BRL* family	Cano-Delgad
		CR05-C1-103-056-H03-CT	57.5	2e-103		o *et al.*, 2004
		CS00-C1-101-097-B06-EU	35.7	3e-71		
		PT11-C1-901-087-D01-CT	34.9	5e-63		
BRL3	AT3G13380	C3-CS/PT (2)	45.7	4e-77	*BRL* family	Cano-Delgad
		C4-CS/CR (3)	43.2	9e-65		o *et al.*, 2004
		C7-CS (2)	37.0	6e-55		
		CS00-C1-100-123-E06-CT	36.1	3e-61		
		CS00-C3-704-049-B04-CT	35.0	2e-67		
		CS00-C1-102-052-F12-CT	33.2	2e-58		
		CS00-C3-702-037-F05-CT	32.6	4e-65		

		CR05-C3-701-030-G08-CT	31.9	1e-49		
		CR05-C1-100-070-E05-CT	31.7	4e-47		
				signaling intermediates		
BAK1	AT4G33430	C9-CS/CR/LT (3)	64.6	1e-155	LRR, PK domain, identical to	Li *et al.*,
		C5-CS (4)	55.0	1e-123	somatic embryogenesis	2002a; Nam
		C3-CS (5)	48.5	1e-105	receptor-like kinase 3 (SERK3),	and Li, 2002
		CS00-C3-705-007-D04-CT	46.6	2e-76	interacts with BRI1 *in vitro* and	
		CS00-C3-702-076-D04-CT	37.5	1e-92	*in vivo* to form a heterodimer	
		CR05-C3-702-017-C08-CT	37.1	1e-102		
		CS00-C1-100-123-E06-CT	36.4	6e-98		
		CG32-C1-003-071-D10-CT	29.1	2e-83		
		CS00-C1-100-104-H02-UV	35.8	9e-73		
BAS1	AT2G26710	C1-CS (4)	34.6	2e-99	cytochrome p450, control point	Neff *et al.*,
		C8-PT (2)	30.1	1e-47	between multiple photoreceptor	1999
		CR05-C1-103-084-D10-CT	33.6	1e-54	systems and BR signal	
		CS00-C3-701-100-A11-UV	32.2	1e-72	transduction	

		CR05-C3-701-103-D05-CT	26.2	7e-69	pathways	
		CS00-C1-650-030-H10-CT	22.8	4e-52		
BIN2	AT4G18710	C8-CS (5)	75.5	1e-102	SHAGGY-related PK eta /	Li *et al.*,
		C1-CS/PT (2)	28.4	1e-61	ASK7, cross-talk between	2001b
		CR05-C1-100-026-F08-CT	58.1	1e-108	auxin and BR pathways	
BRS1	AT4G30610	C4-CS (4)	56.6		serine carboxypeptidase II,	Li *et al.*,
		C1-CS (6)	49.2		involved in BRI1-mediated	2001a
					signaling	
BSU1	AT1G03445	CS00-C3-701-035-G06-CT	39.8	1e-74	Kelch repeat, serine/threonine	Mora-Garcia
		CS00-C1-100-119-F05-CT	34.3	2e-80	phosphoesterase,	*et al.*, 2004
		PT11-C9-005-041-C05-CT	30.0	1e-112	metallo-phosphoesterase, BR	
		PT11-C1-900-087-G08-CT	27.6	3e-84	signaling intermediate	
		CS13-C1-001-008-C12-CT	23.2	9e-86		
		transcription factors				
BES1	AT1G19350	C3-CS (2)	40.9	1e-95	plant DUF822, phosphorylated	Yin *et al.*,

		CR05-C1-103-054-F02-CT	58.4	8e-89	by BIN2 GSK3 kinase, binds E- box sequences (CANNTG)	2002; Yin *et al.*, 2005
BIM1	AT5G08130	C1-CS (3)	58.9	1e-112	bHLH, binds E -box sequences (CANNTG)	Yin *et al.*, 2005
		C2-CR/LT (2)	42.4	7e-97		
		CR05-C1-102-035-G07-CT	23.0	9e-60		
BZR2	AT1G78700	CR05-C1-103-054-F02-CT	52.3	1e-148	plant DUF822, positive regulator BR signaling	Wang *et al.*, 2002, He *et al.*, 2005
BZR3	AT3G50750	C3-CS/CR (3)	40.2	e-130	*BZR* family	He *et al.*, 2005

[a]Gene name abbreviations: *BAK*: BRI1-associated receptor kinase, *BAS*: PHYB-activation tagged supressor, *BES*: BRI1-EMS suppressor, *BIM*: BES1-interacting MYC-like protein , *BIN*: brassinosteroid insensitive, *BRI*: brassinosteroid insensitive, *BRL*: BRI1-like protein, *BRS*: BRI1 suppressor, *BSU*: BRI1 suppressor protein, *BZR*: brassinazole-resistant.

[b]C: contig, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Functional domain abbreviations: ASK:, *Arabidopsis* SHAGGY-related protein kinase, ATP: adenosine triphosphate, BR: brassinosteroid, DUF: domain of function unknown, LRR: leucine rich repeat, PK: protein kinase.

Table S6 – Citrus ESTs with homology to genes involved in cytokinin metabolism and transport in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs and**	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**References**
			biosynthesis			
APT3	AT1G80050	CL06-C4-501-001-G02-CT	75.0	1e-81	purine/pyrimidine phosphoribosyl transferase, purine salvage pathway	Allen *et al.*, 2002
AtIPT1	AT1G68460	CS00-C3-704-028-G04-CT	39.2	6e-71	tRNA isopentenyltransferase activity, cytokinin biosynthesis	Kakimoto, 2001; Takei *et al.*, 2001
AtIPT3	AT3G63110	CR05-C1-102-068-C11-CT	36.2	4e-60	*IPT* family	Kakimoto, 2001
AtIPT4	AT4G24650	CR05-C1-102-068-C11-CT	28.8	2e-32	*IPT* family	Kakimoto, 2001
AtIPT5	AT5G19040	CS00-C3-704-018-B02-CT	41.2	9e-55	*IPT* family	Kakimoto, 2001

AtIPT7	AT3G23630	CR05-C1-102-068-C11-CT	33.7	7e-55	*IPT* family	Takei *et al.*, 2001
AtIPT8	AT3G19160	CR05-C1-102-068-C11-CT	20.7	9e-38	*IPT* family	Takei *et al.*, 2001
UGT76C2	AT5G05860	C7-PT (3)	81.6	5e-105	N-glucosylation of adenine moiety (positions N7 and N9) acitivity,	Hou *et al.*, 2004
AtZOX1	F11B9.23	PT11-C1-901-005-B11-CT	78.2	1e-84	zeatin O-xylosyltransferase activity,	Hou *et al.*, 2004
translocation						
AtPUP1	AT1G28230	C2-CS (2)	50.9	7e-66	purine nucleoside transporter activity, cytokinin transport	Burkle *et al.*, 2003
AtPUP2	AT2G33750	C2-CS (2)	43.7	9e-45	*PUP* family	Burkle *et al.*, 2003
Catabolism						

CKX2	AT2G19500	CS00-C1-102-073-H07-CT	27.7	1e-55	amine oxidase activity, cytokinin catabolism, root cap, stomatal complex, stipule, shoot apex	Schmüllig *et al.*, 2003
CKX5	AT1G75450	C1-CS (2)	51.7	2e-86	*CKX* family	Schmüllig *et al.*, 2003

[a]Gene name abbreviations: *APT*: adenine phosphoribosyltransferase, *AtIP*: *Arabidopsis thaliana* isopentenyl transferase, *AtPUP*: *Arabidopsis thaliana* purine permease, *AtZOX*: *Arabidopsis thaliana* zeatin O-xylosyltransferase , *CKX*: cytokinin oxidase/dehydrogenase, *UGT76C2*: UDP-glucose pyrophosphorylase.

[b]C: contig, CL: *Citrus limoni*a, CR: *Citrus reticulata*, CS: *Citrus sinensis,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

Table S7 – Citrus ESTs with homology to genes involved in cytokinin signal transduction in *Arabidopsis thaliana*.

Arabidopsis thaliana		***CitEST***			**Protein motifs and**	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**References**
		receptors and putative receptors				
AHK1/ CKI1	AT2G47430	C2-CS (3)	30.3	5e-67	protein histidine kinase, cytokinin receptor	Inoue *et al.*, 2001
AHK2	AT5G35750	C7-CS (2)	62.2	4e-75	*AHK* family	Higuchi *et al.*, 2004
AHK3	AT1G27320	C1-CR (2)	68.8	5e-92	*AHK* family	Nishimura *et al.*, 2004
		C3-PT (3)	22.9	6e-75		
AHK4/ CRE1/WOL	AT2G01830	C2-CS (16)	51.6	4e-81	*AHK* family	Higuchi *et al.*, 2004
		histidine-phosphotransfer proteins				
AHP1	AT3G21510	C2-CS (4)	23.3	2e-47	histidine kinase activity	Suzuki *et al.*, 2000
AHP2	AT3G29350	C2-CS (4)	25.3	2e-48	*AHP* family	Suzuki *et al.*,

						2000
AHP3	AT5G39340	C2-CS (4)	24.2	3e-49	*AHP* family	Suzuki *et al.*, 2000
AHP4	AT3G16360	CS00-C1-102-072-B03-CT	21.7	3e-40	*AHP* family	Suzuki *et al.*, 2000
AHP5	AT1G03430	CS00-C3-704-018-B02-CT	34.6	9e-55	*AHP* family	Suzuki *et al.*, 2000
		response regulators				
ARR1	AT3G16857	CR05-C3-701-021-A12-CT	49.3	1e-57	two-component response regulator	Mason *et al.*, 2005
ARR2	AT4G16110	CS00-C1-102-045-A08-CT	23.6	5e-54	*ARR* family	Hwang *et al.*, 2002
ARR3	AT1G59940	PT11-C1-901-071-A09-CT	20.9	1e-59	*ARR* family	To *et al.*, 2004
ARR4	AT1G10470	PT11-C1-901-071-A09-CT	21.9	6e-59	*ARR* family	Sweere *et al.*, 2001

ARR5	AT3G48100	PT11-C1-901-071-A09-CT	16.9	3e-50	*ARR* family	Hwang *et al.*, 2002
ARR6	AT5G62920	PT11-C1-901-071-A09-CT	12.9	6e-49	*ARR* family	Hwang *et al.*, 2002
ARR7	AT1G19050	PT11-C1-901-071-A09-CT	11.7	5e-48	*ARR* family	Hwang and Sheen, 2001
ARR9	AT3G57040	CS00-C3-700-065-A09-CT	58.2	3e-60	*ARR* family	Hwang *et al.*, 2002
ARR10	AT4G31920	CS00-C1-102-006-D06-CT	26.3	4e-48	*ARR* family	Hwang and Sheen, 2001
ARR11	AT1G67710	CS00-C1-102-045-A08-CT	53.2	7e-47	*ARR* family	Imamura *et al.*, 2003
		CR05-C1-100-016-F10-CT	14.0	7e-68		
ARR14	AT2G01760	CR05-C3-701-021-A12-CT	40.0	2e-49	*ARR* family	Tajima *et al.*, 2004
ARR15	AT1G74890	PT11-C1-901-071-A09-CT	20.4	3e-44	*ARR* family	Hwang *et al.*, 2002

ARR16	AT2G40670	PT11-C1-900-041-F09-CT	18.2	5e-39	*ARR* family	Hwang *et al.*,
		CS00-C3-700-065-A09-CT	16.7	1e-38		2002
ARR17	AT3G56380	C5-PT (5)	19.5	1e-41	*ARR* family	Tajima *et al.*,
						2004
ARR18	AT5G58080	CR05-C3-701-021-A12-CT	34.1	4e-32	*ARR* family	Hwang and
		CS00-C1-102-045-A08-CT	23.6	4e-27		Sheen, 2001
ARR19	AT1G49190	CR05-C3-701-021-A12-CT	24.8	5e-23	*ARR* family	Hwang and
						Sheen 2001
ARR20	AT3G62670	CR05-C3-701-021-A12-CT	27.4	2e-21	*ARR* family	Hwang *et al.*,
						2002
ARR21	AT5G07210	CR05-C3-701-021-A12-CT	25.2	5e-18	*ARR* family	Hwang *et al.*,
						2002
other signaling components and transcription factors						
KNAT2	AT1G70510	C3-CR (2)	32.4	1e-31	homeodomain protein,	Hamant *et*
		PT11-C2-301-095-B03-CT	40.1	5e-29	meristem development	*al.*, 2002;
						Jasinski *et*

						al., 2005
SPY	AT3G11540	C1-CS/CR (7)	27.4	2e-65	N-acetyl glucosamine	Greenboim-
		CR05-C1-103-015-E09-CT	25.0	4e-43	transferase, signaling positive	Wainberg *et*
		CS00-C1-102-059-G10-CT	15.5	3e-28	regulator	*al.*, 2005
STM	AT1G62360	C4-CR (3)	20.2	3e-43	homeodomain protein,	Rupp *et al.*,
		C1-CS (4)	13.7	7e-32	meristem development	1999
		PT11-C2-301-019-G09-CT	13.8	3e-44		

[a]Gene name abbreviations: *AHK*: *Arabidopsis thaliana* histidine kinase protein, *AHP*: *Arabidopsis thaliana* histidine phosphotransfer protein, *ARR*: *Arabidopsis thaliana* response regulator, *CKI*: cytokinin insensitive , *CRE*: cytokinin receptor, *KNAT*: class I KNOTTED1-like TALE homeodomain, *SPY*: spindly, *STM*: shoot meristemless, *WOL*: wooden leg.

[c]Identity percentage at the amino acid level.

[b]C: contig, CR: *Citrus reticulata*, CS: *Citrus sinensis,* PT: *Poncirus trifoliata*, (number of reads).

Table S8 – Citrus ESTs with homology to genes involved in ethylene biosynthesis in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs[d] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
ACO	AT1G62380	C21-CA/CS/CR/PT (7)	74.3	1e-142	1-aminocyclopropane-1-	Tang *et al.*,
family[u]		C18-PT (15)	63.8	1e-126	carboxylate oxidase, synthesis	1993
		C20-PT (2)	48.0	1e-121	of ethylene from ACC	
		C17-PT (2)	30.7	1e-111		
		C4-CS (2)	30.0	1e-92		
ACS family[v]	AT3G61510	C1-CS (3) ACS3	53.7	3e-97	1-aminocyclopropane-1-	Woeste *et*
		C2-CS/CR (5) ACS12	47.6	1e-93	carboxylate (ACC) synthase,	*al.*, 1999;
		C3-PT (3) ACS12	46.9	5e-90	conversion of AdoMet to ACC	Babula *et al.*,
		C4-CS/CG (9) ACS10	36.6	2e-82		2006
EFE	AT1G05010	C22-CA/CG/CS/CR (34)	75.5	1e-158	*ACO* family	Gomez-Lim
						et al., 1993
ETO1	AT3G51770	C3-CS/CR (4)	71.1	0.0	TRP repeat, BTB/POZ domain,	Guzman and
		C2-CS/CR/PT (11)	57.9	0.0	ACS degradation via 26S	Ecker, 1990,

		C1-CS (3)	47.8	8e-66	proteasome	Yoshida *et*
		CS00-C3-703-024-E09-CT	50.9	6e-73		*al.*, 2006
SAM1	AT1G02500	C1-CS/CR (89)	93.6	0.0	S-adenosylmethionine	Peleman *et*
		C4-LT/PT (4)	81.2	0.0	(AdoMet) synthetase, AdoMet	*al.*, 1989
		C2-CS/CR (11)	80.4	0.0	synthesis	
		C3-CS/CR (3)	80.4	1e-111		

[a]Gene name abbreviations:*ACO*: aminocyclopropane carboxylate oxidase, *ACS*: aminocyclopropane carboxylate synthase, *EFE*: ethylene forming enzyme, *ETO*: ethylene overproduction *SAM*: S-adenosylmethionine synthetase.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Protein motif abbreviations: ACC: aminocyclopropane-1-carboxylate, BTB: broad complex-tramtrack-bric-à-brac, TRP: tetratrico peptide repeat.

[e]ACO family: AT2G19590, AT3G47190.

[f]ACS family: AT1G01480, AT1G62960, AT2G22810, AT3G49700 (ETO3), AT4G08040, AT4G11280, AT4G26200, AT4G37770, AT5G51690, AT5G65800, AT3G47190.

Table S9 – Citrus ESTs with homology to genes involved in ethylene signal transduction in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs[d] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
			receptors			
ETR1	AT1G66340	C2-CS/CR (18)	80.8	0.0	GAF domain, histidine kinase	Chang *et al.*,
		C12-CS/CR/PT (6)	59.5	1e-158	A, response regulator receiver,	1993;
		C7-CS/CR/PT (15)	58.8	0.0	ethylene perception	Chiwocha *et*
		C15-PT (2)	45.9	1e-142		*al.*, 2005
ETR2	AT3G23150	CR05-C1-102-100-D04-CT	45.1	9e-47	*ETR* family	Sakai *et al.*,
						1998
ERS1	AT2G40940	C3-CS/CR (4)	72.5	0.0	GAF domain, histidine kinase	Hua *et al.*,
		C4-CR/PT (3)	44.9	1e-151	A, response regulator receiver,	1998, Sakai
		PT11-C1-901-090-C03-CT	51.8	1e-96	ethylene perception	*et al.*, 1998;
		CS00-C1-101-103-E11-EU	50.5	1e-103		Qu and
						Schaeller,
						2004

ERS2	AT1G04310	CR05-C1-102-100-D04-CT	38.5	2e-41	*ERS* family	Hua *et al.*, 1998, Sakai *et al.*, 1998
EIN4	AT3G04580	C2-CS/CR (4)	43.3	2e-80	GAF domain, histidine kinase A, response regulator receiver, ethylene perception	Hua *et al.*, 1998
		C1-CS/CR/PT (20)	35.2	0.0		
		C4-CS (10)	34.9	0.0		
		C3-CS/CR (5)	34.6	1e-167		
		CR05-C1-102-100-D04-CT	51.0	7e-62		
		PT11-C9-005-026-F06-CT	41.7	1e-59		
		early events				
CTR1	AT5G03730	C3-CS (4)	81.4	1e-137	serine/threonine protein kinase, MAP kinase, signaling partner, forms a complex with ethylene receptor	Kieber *et al.*, 1993
		C30-CG (2)	50.4	3e-75		
		C2-CS (6)	40.6	1e-141		
		CR05-C3-700-099-B10-CT	60.5	6e-61		
		CS00-C3-702-066-C07-CT	45.6	1e-142		
EIN2	AT5G03280	C3-PT (3)	60.1	1e-100	NRAMP, metal transporter	Guzman and

		C1-CA/CS (9)	43.7	1e-137	family, signaling partner	Ecker, 1990; Alonso *et al.*, 1999
RAN1	AT5G44790	CR05-C1-100-099-E06-CT	57.3	9e-85	E1, E2 copper-exporting ATPase, signaling partner	Hirayama *et al.*, 1999
primary transcription factors						
EIN3	AT3G20770	C1-CS/PT (6)	63.7	1e-65	EIN3 domain, transcriptional activator	Guo and Ecker, 2003
		C2-CS/PT (2)	35.9	1e-127		
		C4-CS (4)	33.3	4e-47		
EIL family[e]	AT2G27050	C6-CS/PT (13) EIL1	57.4	0.0	similar to EIN3, transcriptional activator	Alonso *et al.*, 2003
		C5-CS/CR (14) EIL1	53.1	1e-178		
		C3-CS (2) EIL1	45.4	3e-67		
		CS00-C3-700-066-A07-CT	33.3	3e-34		
secondary transcription factors						
EBF1	AT2G25490	C2-CR/CS (32)	64.0	4e-95	LRR cysteine-containing	Guo and

		C3-CR/PT (5)	41.8	3e-51	subtype, cyclin-like F box, EREBP, transcriptional regulator	Ecker, 2003
HLS1	AT4G37580	C2-CR/PT (2) C1-CS (3)	51.3 45.9	7e-69 3e-59	N-acetyltransferase, putative, histone deacetylation	Lehman *et al.*, 1996; Li *et al.*, 2004

[a]Gene name abbreviations: *CTR*: constitutive triple response; *EBF*: ethylene binding factor; *EIL*: EIN3-like; *EIN*: ethylene insensitive; ERS: ethylene response sensor; ETR:ethylene receptor; *HLS*: hookless; *RAN*: responsive to antagonist.

[b]C: contig, CG: *Citrus aurantifolia*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Protein motif abbreviations: ATP: adenosine triphosphate; GAF: cGMP-specific and –stimulated phosphodiesterases, *Anabaena* adenyMte cydases and *Escherichia coli* FhlA; GMP: EREBP: ethylene response element binding protein, LRR: leucine-rich repeat; MAP: mitogen-activated protein kinase; NRAMP: natural resistance-associated macrophage protein.

[e]EIN3/EIL family: AT5G21120, AT1G73730.

Table S10 – Citrus ESTs with homology to genes involved in gibberellic acid biosynthesis, metabolism and signal transduction in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs and**	
Name[a]	**Gene**	**EST**[b]	**%**[c]	**e value**	**biological process**	**References**
biosynthesis and metabolism						
KAO-1	AT5G25900	C1-CS (6)	35.3	2e-65	*ent*-kaurene acid oxidase,	Hedden and
GA3					oxides *ent* -kaurene to *ent*-	Phillips,
					kaurenoic acid	2000
GA3	AF537321.1	C2-CS (16)	28.2	3e-55	*ent* -kaurene acid oxidase	Hedden and
KAO-1					family	Phillips,
						2000
GA3	AT5G25900	C3 – CS (2)	31.2	3e-62	*ent* -kaurene acid oxidase	Hedden and
KAO-1					family	Phillips,
						2000
GA3	AT5G25900	C4-CS (3)	51.6	7e-81	*ent* -kaurene acid oxidase	Hedden and
KAO-1					family	Phillips,

						2000
KAO 2	AF537321.1	C5-CS (3)	56.1	1e-76	*ent* -kaurene acid oxidase	Hedden and
		CS00-C3-700-003-C06-CT	42.0	2e-47	family	Phillips,
						2000
KAO2	AT2G32440	C6-CS/CR (6)	17.5	6e-24	*ent* -kaurene acid oxidase	Hedden and
		CS00-C2-003-029-C06-CT	10.6	1e-15	family	Phillips,
						2000
KAO2	AT2G32440	C7-PT (2)	21.1	6e-45	*ent* -kaurene acid oxidase	Hedden and
					family	Phillips,
						2000
GA4	AT5G24530	C8-CS/CR (2)	15.8	2e-21	GA3b hydroxilase, converts	Yamaguchi
		CS00-C1-102-018-C03-CT	56.8	5e-40	GA_9 to GA_4 e GA_{20} to GA_1	and Kamiya,
						2000
GA5	AT4G10490	C9-CS (3)	18.2	0.0	GA20 oxidase1 biosynthesis,	Yamaguchi
GA20OXI					converts GA_{12} to GA_9 and GA_{53}	and Kamiya,
					to GA_{20} Converts GA_{12} to GA_{25}	2000

GA5	AT5G51810	C10-CS (2)	43.0	2e-61	Gibberellin GA20 oxidase,	Yamaguchi
GA20OXI		CS00-C3-704-020-H12-CT	34.0	5e-35	converts GA_{12} to GA_9 and GA_{53}	and Kamiya,
					to GA_{20}	2000
GA 2OX1	AT1G50960	C1-CS/CR (21)	21.9	2e-32	Gibberellin 2-oxidase1 ,	Yamaguchi
		PT11-C1-901-051-F06-CT	17.8	8e-26	converts C19-GA to	and Kamiya,
					biologically inactive GA. GA	2000
					catabolism.	
GA2OX1	AT3G63010	C2-CS (2)	64.4	1e-112	Gibberellin 2-oxidase1 family	Yamaguchi
						and Kamiya,
						2000
CPS/GA1		CS00-C1-100-036-B10-CT	12.2	2e-54	converts geranyl-geranyl	Yamaguchi
					diphosphate to *ent*- Copalyl	*et al.*, 1998
					diphosphate	
		signal transduction				
DELLA-	AT5G27320	C1-CS/CL (3)	18.2	2e-43	cytochrome P450, GAI-RGA	Bolle *et al.*,

GAI/RGA					like gibberellin response modulator	2004
DELLA-GAI/RGA	AT1G50420	C2-CS (5)	33.1	7e-69	GAI-RGA family	Bolle *et al.*, 2004
DELLA-GAI/RGA	AT2G14920	C3-CS/CR (2)	74.0	0.0	GAI-RGA family	Bolle *et al.*, 2004
DELLA-GAI/RGA	AT2G01570	C4-CS/CR/PT (6)	17.4	2e-42	GAI-RGA family	Bolle *et al.*, 2004
DELLA-GAI/RGA	AT1G14920	C5-CG (2)	35.0	4e-71	GAI-RGA family	Bolle *et al.*, 2004
DELLA-GAI/RGA	AT2G01570	C6-CS/PT (2)	32.3	1e-69	GAI-RGA family	Bolle *et al.*, 2004
GAI	AT1G14920	LT33-C1-003-051-C11-CT	6.8	8e-26	GAI-RGA family	Pysh *et al.*, 1999
GAMYB	AT1G77180	CR05-C1-103-051-C01-CT	13.6	6e-23		Gocal *et al.*, 2001

GID1	AT3G63010	C1-CS/CR (8)	50.4	6e-89	putative protein GID1-like. Soluble receptor to GA, hormone sensitive lípase.	Sasaki *et al.*, 2001
GID1-Like	AT3G63010	C2-CS (2)	49.3	2e-84	GID1 family	Sasaki *et al.*, 2001
SLY	At4G24210	C1-CS/CR (11)	53.6	8e-102	F box domain, positive regulator, targets DELLA proteins for proteasomal degradation	Steber *et al.*, 1998
SPY	AT3G11540	C54-CS/CR (3)	50.3	3e-89	O-linked GlcNAc transferase; negative regulator	Jacobsen *et al.*, 1996
		CR05-C3-700-108-H02-CT	19.0	6e-99		
		CS00-C1-102-059-G10-CT	19.0	3e-28		

[a]Gene name abbreviations: *CPS*: *ent*-copalyl diphosphate synthase, *GAI*: gibberellic acid insensitive, *GAMYB*: gibberellic acid-induced MYB transcription factor, *GAOX*: gibberellic acid oxidase; *GID*: gibberellin insensitive dwarf-1, *KAO*: kaurene acid oxidase, *RGA*: repressor of *ga1-3*, *SLY*: sleepy, *SPY*: spindly.

[b]C: contig, CL: *Citrus limoni*a, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

Table S11 – Citrus ESTs with homology to genes involved in jasmonic acid biosynthesis in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs[c] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
AOC/	AT3G25760	C1-CS (5) AOC4	55.1	8e-70	oxide cyclase, formation of	He *et al.*,
ERD12		C3 –CS/CR (3) AOC2	48.4	5e-58	cyclic allene oxide,	2002
family		PT11-C9-005-019-B11-CT - AOC2	58.9	4e-74	13-hydroxyperoxyde	
					metabolism	
AOS1	AT5G42650	C4-CS/PT (2)	44.5	1e-165	cytochrome p450 CYP74,	von Malek *et*
		C3-CR/PT (4)	41.1	1e-101	allene oxide synthesis,	*al.*, 2002
		C1-CS/CR (20)	39.2	1e-122	9-hydroxyperoxyde metabolism	
		C2- CS (8)	36.0	2e-76		
		PT11-C1-900-026-D07-CT	32.3	1e-66		
AtSS family	AT1G74020	C6-CS/PT (2)	34.9	1e-78	strictosidine synthase, alkaloid	Devoto *et*
		C5-CS/PT (4)	34.6	1e-74	and terpenoid biosynthesis	*al.*, 2005
		C4-CS/CR/LT/PT (7)	31.9	6e-78		
		C3-CS (4)	27.5	1e-47		

		CR05-C1-103-066-G10-CT	21.4	5e-56		
		CS00-C3-704-013-C11-CT	20.8	5e-35		
DAD1	AT2G44810	C1-CS/CG (2)	33.7	2e-87	esterase/lipase/thioesterase,	Matsui *et al.*,
		C2-CS (5)	25.7	2e-81	chloroplast phospholipase A1,	2004
		C3-CR (7)	25.0	2e-80	oxylipin biosynthesis	
		CR05-C1-102-055-E10-CT	24.4	1e-79		
		CR05-C3-701-019-C07-CT	23.5	1e-37		
FAS	AT4G05160	C13-CG (2)	54.0	3e-148	AMP-dependent synthetase and	Liechti *et al.*,
		C5-CS/CR/CL (3)	40.8	1e-150	ligase, fatty acid esterification	2006
		C3-CS/CR/CA (12)	33.6	1e-91		
		C7-CS-CG (6)	31.7	1e-115		
		C2-CS/PT (7)	30.7	1e-102		
		C9-CS/CR/PT (9)	23.5	1e-96		
		C4-CR/CS (3)	21.4	5e-72		
		C11-CS/CR/PT (15)	21.0	1e-71		
		CG32-C1-003-036-C11-CT	40.5	1e-136		

		PT11-C1-900-014-E04-CT	40.5	1e-63		
		CR05-C3-701-007-F02-CT	37.4	6e-42		
		PT11-C1-901-019-D03-CT	31.4	1e-46		
		LT33-C1-003-020-D08-CT	26.5	1e-36		
		CS00-C3-704-092-H02-CT	25.8	3e-37		
		CS00-C3-700-089-H08-CT	24.6	1e-93		
		CS00-C3-700-016-E08-CT	23.2	4e-75		
		CR05-C1-102-040-B05-CT	22.2	2e-31		
JMT	AT1G19640	C7-CS/CR (53)	45.5	9e-89	s-adenyl-methione- dependent	Seo *et al.*,
		C5-CS (2)	35.4	2e-46	carboxyl methyltransferase,	2001;
		C4-CS (4)	33.2	8e-30	formation of methyljasmonate	Zubieta *et*
		C6-CR (2)	30.8	1e-32	from jasmonic acid	*al.*, 2003
		C1-CS/CR (2)	30.1	6e-40		
		C2-CS/CR (11)	30.1	2e-33		
		C3-CS (2)	20.7	1e-28		
		CR05-C1-102-008-E06-CT	37.8	1e-47		

		CR05-C1-102-065-B11-CT	33.2	1e-41		
		CS00-C5-003-042-D10-CT	29.0	2e-48		
		CS00-C3-705-072-E08-CT	27.2	8e-34		
		CS00-C3-703-095-H07-CT	27.2	6e-32		
		CS00-C1-100-036-G09-CT	25.1	4e-53		
		CL06-C4-500-008-D11-CT	24.3	5e-27		
LOX family	AT1G55020	C3-CS/CR/LT/PT (7) *At*LOX3	71.9	0.0	lipoxygenase, LH2 domain,	Bell and
		C5-CR (2) *At*LOX3			hydroperoxidation of lipids	Mullet,
		C1-CS/CR (12) *At*LOX2	62.8	1e-121		1993;
		C8-CL (2) *At*LOX2	61.5	1e-168		Montillet *et*
		C2-CS (2) *At*LOX1	58.4	2e-90		*al.*, 2004
		C4-CS/CR/PT (89) *At*LOX2	58.2	9e-93		
		C6-CS (5) *At*LOX3	57.0	0.0		
		C7-CS/PT (3) *At*LOX1	53.6	0.0		
		CS00-C3-701-113-F10-CT-	38.8	0.0		
		*At*LOX2	65.2	3e-90		

		CR05-C1-102-100-D10-CT - *At*LOX2	59.4	1e-105		
		CG32-C1-003-091-C03-CT - *At*LOX1	53.1	1e-92		
		PT11-C2-301-049-G03-CT- *At*LOX2	51.4	4e-97		
		CR05-C3-701-084-G10-CT- *At*LOX2	50.5	4e-84		
		CR05-C1-103-013-A07-CT- *At*LOX1	47.7	2e-64		
		CS00-C3-701-073-E12-CT - *At*LOX2	29.6	1e-84		
OPR/DDE family	AT1G76680	C1-CS (2) OPR2	74.6	1e-168	NADH:flavin oxidoreductase/NADH oxidase, oxylipin metabolism	Biesgen and Weiler, 1999; Costa *et al.*, 2000
		C4-CS (7) OPRL2	71.4	1e-141		
		C5-CA/CR (2) OPR1	66.5	8e-88		
		C2-CS/CR (7) OPR3/DDE1	57.4	1e-108		

		C7-PT (2) OPR3/DDE1	48.9	6e-77		
		C3-CS/CR (2) OPR1	30.6	3e-57		
		C6-CS/CR (11) OPR2	28.1	2e-26		
		CR05-C3-702-015-A10-CT- OPR2	66.9	1e-104		
		PT11-C1-901-020-B03-CT- OPR1	60.7	1e-108		
PED1	AT2G33150	C4-CS/CR/PT (14)	88.7	0.0	thiolase, fatty acid β-oxidation	Pinfiel-Wells
		C3-CS/LT (12)	77.7	0.0		*et al.*, 2005;
		C5-CG (4)	38.2	8e-31		Weber *et al.*,
		C1-CS (3)	33.5	1e-69		1997
		C2-CS/CR/PT (8)	31.7	1e-63		
		PT11-C1-900-034-A08-CT	55.7	3e-76		
		CS00-C1-101-061-E07-CT	51.1	6e-71		
SSI2	AT2G43710	C1-CA/CS/CR/PT (9)	83.5	0.0	fatty acid desaturase, type 2,	Kachroo *et*
		C2-CS (4)	47.0	7e-74	fatty acid desaturation	*al.*, 2001;
		PT11-C9-005-040-F11-CT	45.8	2e-71		Kachroo *et*

CS00-C3-701-010-D08-CT	35.9	8e-34	*al.*, 2003
CA26-C1-002-053-D07-CT	33.9	2e-54	
CA26-C1-002-049-D02-CT	30.6	2e-38	

[a]Gene name abbreviations: *AOC*: allene oxide cyclase, *AOS*: allene oxide synthase, *AtSS*: strictosidine synthase, *DAD*: defective in anther dehiscence, *DDE*: deiscence dficient, *ERD*: early-responsive to dehydration stress protein, *FAS:*fatty acid esterification, *JMT*: jasmonic acid carboxyl methyltransferase, *LOX*: lipoxygenase, *OPR*: 12-oxophytodienoate reductase, *PED*: peroxisome defective, *SS*: stearoyl-ACP desaturase.

[b]Identity percentage at the amino acid level.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CL: *Citrus limoni*a, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Functional domain abbreviations: AMP: adenosine monophosphate; CYP: cytochrome protein; LH: leucine and histidine; NADH: reduced nicotinamide adenine dinucleotide.

Table S12 – Citrus ESTs with homology to genes involved in jasmonic acid-mediated signal transduction in *Arabidopsis thaliana*.

Arabidopsis thaliana		**CitEST**			**Protein motifs[d] and**	
Name[a]	**Gene**	**EST[b]**	**%[c]**	**e value**	**biological process**	**References**
AS1	AT2G37630	C25-CS/CR (4)	67.3	1e-138	MYB-type DNA binding factor,	He and Gan,
		C31-PT (2)	20.7	1e-68	transcriptional regulation,	2001
		C19-CS (8)	20.4	2e-67	development	
ATAF2	AT5G08790	C12-PT (2)	54.3	6e-80	NAM family, NAC domain,	Delessert *et*
		C14-CS/CA (8)	50.7	4e-80	putative transcriptional	*al.*, 2005
		C13-PT (2)	41.7	1e-77	activator	
		C7-CS/PT (8)	39.6	2e-87		
		C9-CS/CR/CG (3)	33.1	3e-79		
		C5-CS/CR (6)	32.5	2e-83		
		C1-CS (2)	32.2	6e-81		
		C2-CS (2)	30.4	2e-77		
		C4-CS (7)	30.0	7e-68		
		C10-CS (8)	29.7	1e-49		

		C8-CR (4)	29.0	4e-69		
		C11-CS/PT (2)	27.0	1e-57		
		C3-CS (5)	26.1	6e-56		
		PT11-C1-901-017-B05-CT	25.8	3e-47		
		LT33-C1-003-033-C02-CT	24.7	2e-49		
		CR05-C3-700-031-C10-UV	20.3	1e-28		
AtCHL2	AT5G43860	C1-CS/CR (32)	38.4	4e-67	esterase/lipase/thioesterase,	Tsuchiya *et*
		C2-PT (3)	30.6	5e-34	chlorophyll degradation	*al.*, 1999
		CR05-C3-702-087-F05-CT	37.6	5e-53		
AtHCH1B	AT3G12500	C3-CS/CR/PT (4)	56.2	1e-102	basic endochitinase, signaling	Ellis and
		C4-CR (4)	56.2	1e-105	pathway during systemic	Turner, 2001
		C7-PT (2)	52.6	2e-99	acquired resistance	
		C8-PT (2)	52.5	4e-91		
		C1-CS/CR (10)	51.6	3e-93		
		C6-CS/CR (40)	50.3	2e-91		
		C5-CR (8)	48.1	4e-89		

		C2-CS (4)	46.7	3e-71		
		PT11-C2-301-044-E11-CT	43.1	5e-84		
AtMPK4	AT4G01370	C7-CS (3)	76.9	1e-159	MAP kinase, mediates	Grant *et al.*,
		C8-CS/PT (9)	73.7	1e-163	pathogen-induced responses	2000
		C2-CS (9)	68.6	1e-150		
		C9-CS/PT (6)	65.7	1e-143		
		C1-CS/CR (9)	52.9	1e-117		
		C4-CS/CR (4)	44.4	1e-104		
		C6-CR (7)	43.4	1e-106		
		C5-CR (3)	42.8	2e-99		
		C11-CS/CG (2)	39.0	3e-94		
		C10-CR/PT (2)	35.4	1e-71		
		C3-CS/CR (4)	28.4	3e-72		
		PT11-C2-300-090-C08-CT	54.0	6e-95		
		CR05-C1-100-043-E08-CT	31.9	3e-81		
AtPRB1	AT2G14580	C1-CS/CL (2)	44.1	7e-38	allergen V5/Tpx-1 related	Santamaria

		CS00-C2-003-083-H02-CT	43.5	4e-40	domain, pathogenesis related protein	*et al.*, 2001; Mur *et al.*, 2006
CAD1	AT5G44070	C1-CS/CA (2)	80.5	3e-90	phytochelatin synthase domain, cadmium tolerance	Xiang and Oliver, 1998
		CS00-C1-100-076-H06-UV	30.4	1e-36		
COI1	AT2G39940	C4-CS/LT (4)	72.9	1e-115	E3 ubiquitin ligase SCF complex F-box subunit, LRR-containing, transcriptional regulation	Devoto *et al.*, 2002; Ren *et al.*, 2005
		C3-CS (3)	46.3	4e-55		
		C1-CS/LT (4)	29.7	5e-37		
		C7-CR/CL/CA (3)	29.5	1e-27		
CORI3	AT4G23600	C1-CS/CR (2)	32.0	1e-58	aminotransferase domain class I and II, cystine lyase function, responsive to wounding	Jones *et al.*, 2003
		CR05-C1-102-097-D08-CT	38.6	3e-60		
		CS00-C1-102-059-A03-CT	30.8	6e-48		
		CA26-C1-002-066-G04-CT	24.1	1e-45		
CPC	AT2G46410	C3-CS/LT (2)	39.5	3e-46	MYB-type DNA binding factor, transcriptional regulation, root differentiation	Koshino-Kimura *et al.*, 2005;
		C10-CR/LT (3)	39.5	2e-46		
		C9-CR/CG (2)	28.9	5e-33		

		CS00-C2-003-009-A06-CT	40.0	3e-35		Kwak *et al.*, 2005
CPR5 / HYS1	AT5G64930	CS00-C3-700-005-C07-CT LT33-C1-003-078-H06-CT	25.3 21.3	1e-24 5e-18	no functionally characterized domain, senescence	Yoshida *et al.*, 2002
CYT1	AT2G39770	C4-CS/LT (19) C3-CS (2) C1-CS (4) CL06-C4-501-031-D06-CT	89.5 78.9 41.0 51.4	0.0 1e-160 1e-63 1e-86	mannose-1-pyrophosphatase, cell wall carbohydrate biosynthesis and protein glycosylation	Lukowitz *et al.*, 2001
ESP	AT1G54040	C2-CS (7) C1-CS (4) PT11-C2-300-054-C06-CT	42.9 20.5 24.1	e-104 7e-60 1e-85	epithiospecifier protein, Kelch motif (4 repeats)	Zabala *et al.*, 2005
HD1	AT4G38130	C1-CS (2) C2-LT (3) CS00-C2-003-054-C10-CT	85.2 31.1 55.2	0.0 1e-88 1e-107	histone deacetylase, jasmonic acid-induced pathogen resistance	Devoto *et al.*, 2002
JR1	AT3G16470	C2-CS/CR (3) C1-CS (2)	27.3 22.2	5e-29 2e-35	jacalin lectin family, lectin domain; similar to myrosinase-	León *et al.*, 1998; Sun *et*

		CR05-C1-100-093-F10-CT	19.9	1e-35	binding protein, response to	*al.*, 2006
		CG32-C1-003-076-F02-CT	19.3	2e-35	wounding	
LDOX	AT4G22880	C3-CS (9)	40.2	5e-77	2OG-Fe(II) oxygenase	Devoto *et*
		C6-CS/CR (22)	39.9	5e-76	superfamily, anthocyanin	*al.*, 2005
		C4-CS (2)	33.2	1e-45	biosynthesis, vacuole formation	
		C2-CS/CR (2)	32.1	4e-48		
		C1-CS/CR (3)	31.6	2e-51		
		C5-CS/CR (6)	27.0	4e-45		
		PT11-C1-900-048-G06-CT	35.5	6e-65		
		CR05-C1-102-021-H06-CT	34.1	2e-57		
		CS12-G8-000-003-B09-CT	32.2	1e-59		
		CS00-C3-700-004-D07-CT	29.3	6e-54		
		CG32-C1-003-071-C04-CT	28.9	5e-52		
MYC2 /	AT1G32640	C2-CS/CR (2)	39.1	4e-59	basic helix-loop-helix DNA	Boter *et al.*,
JIN1		CR05-C3-701-055-H07-CT	39.6	2e-54	binding domain, transcriptional	2004
					regulator	

OCP3	AT5G11270	C1-CS (2)	52.3	1e-71	homeobox protein, transcriptional regulator, necrotrophic pathogen resistance	Coego *et al.*, 2005
PDF1.2	AT5G44420	CL06-C4-501-009-E08-CT	37.5	7e-24	Gamma thionin family domain, plant defensin	Xu *et al.*, 2001
RCD1	AT1G32230	C1-CS/CR/LT (26)	43.5	9e-75	WWE domain, superoxide radicals-mediated jasmonates signal transduction	Ahlfors *et al.*, 2004
RNS1	AT2G02990	C2-PT (3)	34.8	1e-96	ribonuclease T2 family, inhibitor of anthocyanin production	LeBrasseur *et al.*, 2002
		C1-CS (4)	33.5	1e-85		
		C3-CR/PT (2)	24.1	3e-83		
SEN1	AT4G35770	C1-PT (11)	54.6	2e-45	rhodanese-like, senescence-associated gene	Schenk *et al.*, 2005
TRF1/	AT3G46590	C1-CS/CR/PT (6) TRFL1	35.8	1e-79	MYB DNA-binding domain, telomere binding protein	Yanhui *et al.*, 2006
TRFL		C2-CS/CR/PT (7) TRFL3	33.1	1-73		

family						
VSP family	AT5G24780	C1-CS (2) VSP2	39.3	6e-63	HAD superfamily (subfamily	Berger *et al.*,
		C2-CR (12) VSP2	38.1	1e-62	IIIB) phosphatase, pathogen	1995; Berger
		C3-CS/CR (5) VSP1	37.4	2e-55	and herbivore protection	*et al.*, 2002
		C4-CS (2) VSP2	28.0	5e-56		
		C5-CS/PT (6) VSP1, VSP2	22.6	1e-56		
		CS00-C3-705-032-G02-CT VSP2	20.0	2e-44		
		CA26-C1-002-050-E09-CT VSP2	18.5	1e-48		
VTC2	AT4G26850	C3-CS/CR (82)	72.9	1e-176	novel protein, ascorbate	Pavet *et al.*,
		C2-PT (2)	51.0	1e-72	biosynthesis	2005
		C1-CS/CR (4)	50.0	2e-78		
WRKY70	AT3G56400	C1-CS (2)	35.0	7e-34	WRKY transcription factor;	Li *et al.*,
		C3-CS (2)	29.3	4e-33	Group III	2004
		C2-CS/PT (7)	28.6	3e-34		
		C4-CR (3)	23.5	9e-45		

[a]Gene name abbreviations: *AS*: asymmetric leaves, *At*: *Arabidopsis thaliana*, *ATAF*: *Arabidopsis thaliana* activation factor, *CAD*: cadmium sensitive, *CHL*: chlorophyllase, *COI*: coronatine insensitive, *CORI*: coronatine induced *CPC*: caprice , *CPR*: constitutive expressor of pathogenesis-related genes , *CYT*: cytokinesis defective , *ESP*: epithiospecifier modifier , *HCH*: basic endochitinase, *HD*: histone deacetylase, *HYS*: hypersenescence , *JIN*: jasmonate insensitive, *JR*: jasmonic acid responsive , *LDOX*: leucoanthocyanidin dioxygenase , *MPK*: mitogen-activated protein kinase, *OCP*: overexpressor of cationic peroxidase, *PDF*: plant defensin, *PRB*: pathogenesis-related barley protein , *RCD*: radical-induced cell death, *RNS*: ribonuclease , *SEN*: senescence, *TRF*: telomere repeat-binding factor, *TRFL*: TRF-like, *VTC*: vitamin C defective, *VSP*: vegetative storage protein.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CL: *Citrus limoni*a, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

[d]Functional domain abbreviations: CUC: cup-shaped cotyledons, HAD: haloacid dehalogenase, LRR: leucine-rich repeat,, MAP: mitogen-activated protein, MYB: retroviral oncogene v-myb, NAC: *Petunia* NAM and *Arabidopsis* ATAF1, ATAF2, and CUC2 , NAM: no apical meristem, SCF: Skp1, Cdc53, an F-box complex, WWE: domain is named after three of its conserved residues (tryptophan, tryptophan and glutamate), WRKY: a 60 amino acid region that is defined by the conserved amino acid sequence WRKYGQK at its N-terminal end, together with a novel zinc-finger- like motif .

Table S13 – Citrus ESTs homologous to functionally-characterized plant peptide hormones[a].

Arabidopsis thaliana		*CitEST*			Protein motifs and	
Name[b]	**Gene**	**EST**[c]	**%**[d]	**e value**	**biological process**	**References**
PSK1	AT3G44735	CS00-C3-703-089-C10-CT	50.0	2e-11	conserved C-terminal sYIsYTQ, sulfated tyrosine residues	Matsubayashi and Sakagami, 2006
RALFL	AT1G02900	CS12-C1-001-016-E04-CT	55.0	1e-25	conserved C-Terminal domain, funcion unknown, secretory system, less than 200 amino acids in length	Olsen *et al.*, 2002
		CA26-C1-002-074-E06-CT	54.2	7e-26		
		CR05-C3-702-097-F10-CT	54.2	7e-26		
		CS00-C1-101-094-H04-UV	54.2	9e-26		
		LT33-C1-003-017-H11-CT	44.2	4e-24		
		LT33-C1-003-022-B03-CT	38.3	7e-20		
BRI1	AT4G39400	CS00-C3-702-092-G08-CT	80.8	1e-133	leucine-rich repeat receptor kinase, plasma membrane	Scheer *et al.*, 2003
		CS00-C1-650-008-E09-CT	70.9	1e-115		

		PT11-C1-900-089-E02-CT	63.9	1e-99	localization, BR signal	
		CR05-C3-702-027-G11-CT	63.1	5e-90	transduction, systemin receptor	
		CS00-C3-700-068-G06-CT	57.1	3e-88		
		CR05-C1-103-056-H03-CT	42.5	1e-73		
ROT4/DVL	AT2G36985	CS00-C3-703-011-C08-CT	25.6	3e-12	RTF (ROTUNDIFOLIA)	Narita *et al.*,
		LT33-C1-003-050-C02-CT	22.2	2e-05	domain - 29-amino acid	2004; Wen *et*
					domain, whole protein 54	*al*., 2004
					amino acids in length	

[a]Database searches of CitEST have been unable to recover homologs of the following peptide hormones: *Solanum lycopersicum* systemins (TomSys), *S. lycopersicum* small, secreted, cysteine rich proteins (SCR/SP11), *Arabidopsis thaliana* small, secreted, cysteine rich proteins-like (SCRL), *A. thaliana* inflorescence deficient in abscission (IDA, AT1G68765), *A. thaliana* inflorescence deficient in abscission-like IDL (AT3G25655), *A. thaliana* POLARIS (PLS) (AT4G39403), *A. thaliana* CLAVATA3 (CLV3, AT2G27250), *A. thaliana* CLV3-like (CLE, AT1G73165 and approximately other 100 similar sequences).

[b]Gene name abbreviations: *PSK1*: phytosulfokine; *RALFL*: rapid alkalinization factor-like; *BRI1*: brassinosteroid insensitive1; *ROT4/DVL*: rotundifolia4/devil.

[c]CA: *Citrus aurantium*, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata.*

[d]Identity percentage at the amino acid level.

Table S14 – Citrus ESTs with homology to genes involved in salicylic acid metabolism in plants.

Arabidopsis thaliana		**CitEST**[b]			**Protein motifs and**	
Name[a]	**Gene**	**EST**	**%**[c]	**e value**	**biological process**	**References**
			biosynthesis			
ATCM2	AT5G10870	CS00-C1-102-092-A04-CT	60.8	9e-71	chorismate mutase activity, aromatic amino acid family biosynthesis, shikimate pathway, located in cytosol	Eberhard *et al.*, 1996
ICS1	AT1G74710	C1-CR/CS (4)	69.1	5e-72	isochorismate synthase activity, salicylic acid biosynthesis, systemic acquired resistance	Wildermuth *et al.*, 2001
PAL1	AT2G37040	C3-CA (2)	88.4	e-148	phenylpropanoid biosynthesis, phenylalanine ammonia-lyase activity	Rohde *et al.*, 2004
		C1-CG (3)	77.9	4e-75		
		C1-CR (6)	74.9	1e-78		
		C3-CR (4)	71.5	e-100		
		C1-CS (42)	82.5	0		

		C2-CS (5)	82.8	e-130		
		CR05-C3-700-042-D03-CT	66.7	3e-42		
		CS00-C1-100-081-H07-CT	74.1	e-113		
		CL06-C4-500-043-E07-CT	95.9	e-130		
PAL2	AT3G53260	C5-LT/CS (2)	78.5	6e-46	phenylpropanoid biosynthesis,	Rohde *et al.*,
		C4-CA (2)	77.7	3e-61	phenylalanine ammonia-lyase	2004
		C2-CR (2)	79.3	3e-86	activity	
		C1-LT (5)	79.2	e-162		
		C2-LT (2)	77.8	3e-78		
		CS00-C3-702-093-C03-CT	76.6	4e-86		
		CS13-C1-001-005-A02-CT	48.5	5e-42		
prephenate	AT1G11790	C1-CS (3)	58.2	2e-55	amino acid binding, prephenate	
dehydratase		C2-CA/CS (2)	72.3	e-114	dehydratase activity,	
family		C3-CS/CR (4)	65.4	2e-61	metabolism, L-phenylalanine	
protein					biosynthesis, located in	
					mitochondrion	

putative phenylalanine ammonia-lyase	AT3G10340	C3-CA/PT/CS (4) C4-PT (2) CL06-C4-500-043-E07-CT CR05-C3-700-098-F08-CT C1-CA (2)	85.3 92.3 95.9 61.4 87.4	0 e-108 e-130 1e-34 0	biosynthesis, L-phenylalanine catabolism, located in cytoplasm, has ammonia-lyase activity	Appert *et al.*, 1994
		catabolism				
AmSAMT	AAN40745	C1-CR/CS (2) C3-CS (2) C6-CR/CS (3) CS00-C5-003-030-G01-CT	34.9 45.6 38.9 41.4	1e-40 1e-29 5e-29 5e-27	S-adenosyl-L-methionine-dependent carboxyl methyltransferase, formation of methylsalicylate / methylbenzoate respectively from salicylic and benzoic acid	Ross *et al.*, 1999; Negre *et al.*, 2003
NtSAGTase	AAF61647	C1-CS (5) C2-CR/CS (2) CS13-C1-001-006-B05-CT	51.4 57.1 42.3	1e-82 e-131 4e-62	transferase and UDP-glucosyl transferase activity, transferring hexosyl groups	Lee and Raskin, 1999;

[a]Gene name abbreviations: *CM*: chorismate mutase, *ICS*: isochorismate synthase, *PAL*: phenylalanine ammonia-lyase, *AmSAMT*: *Antirrhinum majus* S-adenosyl-L-methionine:salicylic acid methyltransferase; *NtSAGTase*: *Nicotiana tabacum* salicylic acid glucosyltransferase.

[b]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CL: *Citrus limoni*a, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[c]Identity percentage at the amino acid level.

Table S15 – *Citrus* ESTs with homology to genes involved in salicylic acid signal transduction in plants[a].

Arabidopsis thaliana		**CitEST**			**Protein motifs and**	
Name[b]	**Gene**	**EST**[c]	**%**[d]	**e value**	**biological process**	**References**
AtWhy1	AT1G14410	C1-CR/CS/LT/PT (10)	69.7	1e-94	functions in DNA binding, located in chloroplast and in plastid chromosome	Desveaux *et al.*, 2004
CDR1	AT5G33340	C1-CR/CS/PT (4)	52.4	e-118	defense response to pathogenic bacteria; proteolysis; located in apoplast; aspartic-type endopeptidase activity; pepsin A activity	Xia *et al.*, 2004
		C4-CR/CS (19)	55.6	e-119		
		C10-CS/PT (7)	55.8	e-122		
		C11-CG (2)	43.6	6e-47		
		CR05-C1-100-021-A01-CT	49.5	1e-51		
EDS1	AT3G48090	C1-CR/CS (8)	43.6	e-129	lipase activity, signal transducer activity, triacylglycerol	Wiermer *et al.*, 2005
EDS5	AT4G39030	C2-CS/PT (3)	67.3	7e-84	defense response, salicylic acid biosynthesis, multidrug efflux pump activity	Nawrath *et al.*, 2002
		C3-CR (3)	69.3	1e-71		
		C4-CS (7)	66.8	e-146		

		CA26-C1-002-088-C11-CT	51.1	2e-45		
		CG32-C1-003-085-F02-CT	54.7	1e-43		
		LT33-C1-003-064-B03-CT	74.3	1e-96		
ICS1	AT1G74710	C1-CR/CS (4)	69.1	5e-72	isochorismate synthase activity, salicylic acid biosynthesis, systemic acquired resistance	Wildermuth *et al.*, 2001
NDR1	AT3G20600	C1-CS (3)	50.9	8e-52	defense response to pathogenic bacteria and fungi, incompatible interaction, located in membrane	Coppinger *et al.*, 2004
		CR05-C3-701-043-E04-CT	43.2	4e-21		
NPR1	AT1G64280	C9-CG/CS/CA (5)	58.9	2e-82	cell death, response to heat, response to bacteria and insect, systemic acquired resistance, salicylic acid mediated signaling pathway	Cao *et al.*, 1997
		C10-CR (2)	54.1	4e-91		
NPR4	AT4G19660	C11-CS/CR/LT (5)	46.8	5e-95	response to pathogenic bacteria,	Liu *et al.*,

		C12-CS/CA/CR (4)	46.2	1e-95	response to pathogenic fungi;	2005
		C13-CR (3)	46.6	7e-48	located in nucleus; functions in	
		CR05-C1-102-062-E01-CT	43.6	9e-42	protein binding; required for	
		CR05-C3-702-071-B07-CT	49.2	2e-60	basal defense against	
		CS00-C3-704-097-E10-CT	52.5	3e-53	pathogens, and may be	
		PT11-C2-300-016-C11-CT	42.5	5e-39	implicated in the cross-talk	
					between the SA- and JA-	
					dependent signaling pathways	
NtCAT-1	P49319	C1-CA (3)	75.1	e-163	catalase activity; serves to	Chen *et al.*,
		C2-CA (3)	92.4	0.0	protect cells from the toxic	1993
		C1-CG (8)	89.5	0.0	effects of hydrogen peroxide;	
		C1-CR (33)	78.7	e-179	inhibited by salicylic acid	
		C2-CR (57)	89.2	0.0		
		C1-CS (28)	78.9	0.0		
		C2-CS (52)	89.4	0.0		
		C1-LT (4)	73.4	e-173		

		C1-PT (31)	89.4	0.0		
		C2-PT (2)	82.3	4e-84		
NtSABP2	AAR87711	C1-CG/CR/CS (7)	56.0	3e-81	lipase, alpha/beta hydrolase	Kumar and
		C2-CA/CG/CR/CS (2)	42.0	4e-59	fold, may generate a lipid-	Klessig,
		C4-CA/CG/CR/CS (7)	55.0	6e-82	derived signal, responsible for	2003;
		C5-CR/CS/PT (6)	57.0	1e-82	the conversion of MeSA into	Forouhar *et*
					SA	*al.*, 2005
PAD4	AT3G52430	C16-CS (2)	43.6	2e-59	response to insect, systemic	Jirage *et al.*,
					acquired resistance, salicylic	1999
					acid mediated signaling	
					pathway, defense response to	
					pathogenic bacteria,	
					incompatible interaction, leaf	
					senescence; protein binding;	
					has lipase activity	
SAG101	AT5G14930	C17-CS (2)	34.8	2e-29	carboxylic ester hydrolase	Feys *et al.*,

					activity	2005
TGA transcription factors family	AT5G06950 *(TGA2)*	C2-CG/CR/CS (10)	76.0	e-138	transcription factor with a basic region leucin zipper, DNA binding	Johnson *et al.*, 2003
		C3-CR/CS/PT (5)	75.0	e-140		
		CR05-C1-100-077-F10-CT.	59.0	6e-69		
	AT1G22070 (*TGA3*)	C1-CR/CS/PT (3)	58.0	e-114	*TGA* family	Johnson *et al.*, 2003
VAD1	AT1G02120	C1-CR/CS (2)	49.0	1e-18	putative membrane-associated protein containing a GRAM domain, a lipid or protein binding signaling domain	Lorrain *et al.*, 2004
		CR05-C1-103-019-B12-CT	72.0	5e-47		

[a]Database searches of CitEST have been unable to recover homologs of the ACD6, a novel ankyrin repeat and transmembrane-domain containing protein.

[b]Gene name abbreviations: *AtWhy1*: *A. thaliana Whirly 1*, *CDR*: constitutive disease resistance, *EDS*: enhanced disease susceptibility, *ICS*: isochorismate synthase, *NDR*: non-race specific disease resistance, *NPR*: nonexpresser of PR genes, *NtCAT*: *Nicotiana tabacum* catalase, *NtSABP2*: *Nicotiana tabacum* salicylic acid-binding protein, *PAD*: phytoalexin-deficient, *SAG*: senescence-associated gene, *TGA*: transcription factors that interacts with sequence elements containing 'TGACG' motifs, *VAD*: vascular associated death.

[c]C: contig, CA: *Citrus aurantium*, CG: *Citrus aurantifolia*, CL: *Citrus limoni*a, CR: *Citrus reticulata*, CS: *Citrus sinensis,* LT: *Citrus latifolia,* PT: *Poncirus trifoliata*, (number of reads).

[d]Identity percentage at the amino acid level.