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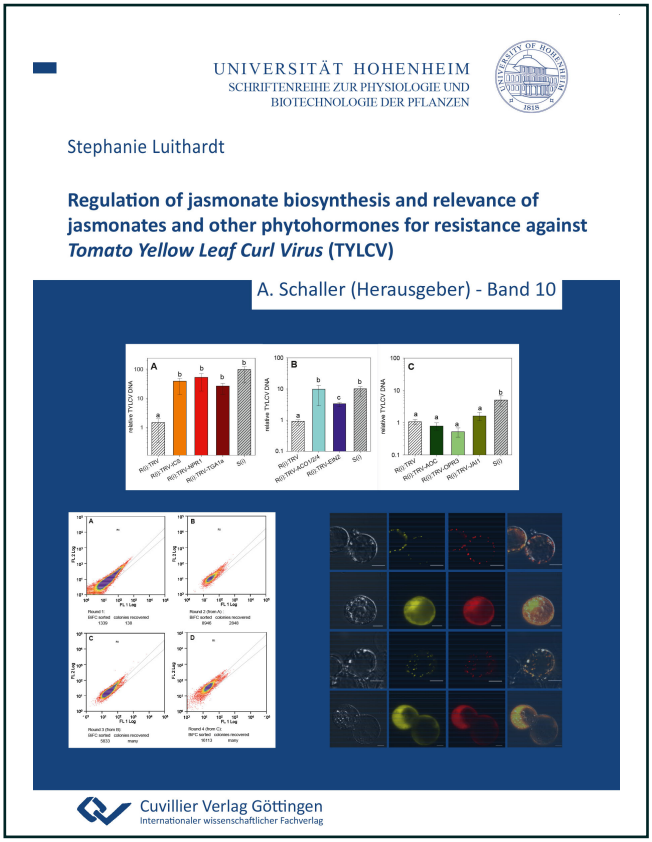
Regulation of jasmonate biosynthesis and relevance of jasmonates and other phytohormones for resistance against Tomato Yellow Leaf Curl Virus (TYLCV)



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1 Introduction

During their whole life cycle plants are exposed to various environmental influences. They are depending on light, water and nutrients for optimal growth. Abiotic stress results when these requirements are not adequately met, either in the right amount or in time. There are also other organisms sharing the same environment and therefore competing for limited resources. To some extent, plants are the “resources” for the other organisms, which creates biotic stress for them. However, plants are not without defensive weapons since they are able to deal with suboptimal conditions as well as pests and pathogens by modulating their own physiology. To gain knowledge about how plants cope with stress is of essential importance in many areas of human life, as plants have significance for human nutrition, in ecology as part of different biotopes, and for global climate homeostasis as carbon sink.

1.1 Defense mechanisms of plants against pathogens and herbivores

Plants have evolved several lines of protection, which include constitutive and induced defense mechanisms, against all possible invaders. There are structural barriers like cell walls and cuticles, but also specialized deterrents like spines, thorns, or prickles (Hanley et al., 2007). Another type of pre-existing defense is the continuous release of toxic allelochemicals which may act either directly, like furanocoumarins, saponins and cardenolides (Wittstock and Gershenzon, 2002), or indirectly through the attraction of beneficial organisms, like the parasitoid wasp attractant linalool (Paiva, 2000).

The prevailing theory is that induced defenses are less costly than constitutive defenses because they are only applied if needed, and because an organism with an already high constitutive response cannot profit from additional induced defenses (Gatehouse, 2002; Heil and Baldwin, 2002). This theory was only recently confirmed by a comprehensive study of 58 different species showing that there is indeed a trade-off between induced and constitutive defense, evident for wild species but not for cultivated species (Kempel et al., 2011). The authors claim that this trade-off is responsible for the high diversity found in resistance traits.

Induction of any type of defense responses requires detection of invaders. Bacteria and fungi can be recognized by plants as their normal constituents like flagellin, chitin, glycoproteins and lipopolysaccharides, referred to as pathogen/microbe associated molecular patterns (PAMPs/MAMPs), are perceived by plant pattern-recognition receptors (PRRs) (Boller and He, 2009). These interactions then lead to a basal resistance of plants called non-host resistance or PAMP-triggered immunity (PTI) (Monaghan and Zipfel, 2012). Since plants are able to recognize PAMPs, pathogens have evolved effector proteins to suppress PTI or to prevent detection (Bardoel et al., 2011). As a countermeasure plants respond to these secreted effectors by the activity of plant resistance (R)-proteins. The interaction of pathogen effectors and plant R-proteins induces effector-triggered immunity (ETI), a second and more effective line of defense (Jones and Dangl, 2006). PRRs are usually plasma membrane-located receptor proteins, while effector proteins are typically recognized by intracellular nucleotide-binding (NB)-leucine-rich repeat (LRR) receptors (Dodds and Rathjen, 2010). Classically, the recognition of avirulence (Avr) proteins, which include PAMPs and effector proteins, by the products of plants *R* genes is described by the gene-for-gene hypothesis (Flor, 1971). Since the initially predicted direct interaction of R and Avr proteins in a receptor-ligand mode of action could only be confirmed in a limited number of cases (van der Hoorn and Kamoun, 2008) the hypothesis was expanded to include indirect interactions defined by the guard model (van der Biezen and Jones, 1998). In this model R-proteins monitor effector-target-complexes and induce defense mechanisms if the target is modified by the effector (van der Biezen and Jones, 1998; Dangl and Jones, 2001).

In principle, ETI responses are the same as in PTI, but generally more robust and prolonged (Tsuda and Katagiri, 2010). Very early events include increased intracellular Ca^{2+} concentrations, oxidative burst, mitogen-activated protein kinases (MAPKs) activation, protein phosphorylation, receptor endocytosis and diverse protein-protein interactions (PPI) (Nürnberger et al., 2004; Schwessinger and Zipfel, 2008).

Transcriptional changes caused by PTI significantly overlap with those induced by ETI (Navarro et al., 2004) and can also result in a hypersensitive response (HR). HR is a programmed cell-death mechanism restricting pathogen growth. Although both PTI and ETI can induce the HR, the underlying mechanisms are not necessarily the same (Tsuda and Katagiri, 2010). Like in PTI, reactive oxygen species (ROS) are induced by ETI but in a two phase fashion, a weak- and transient burst followed by a prolonged and increased production (Torres et al., 2006).

Plants are not only able to respond to pathogens at the site of attack, but also produce systemic signals which facilitate resistance to the pathogen in distal tissues. Systemic acquired resistance (SAR) is accompanied by systemic expression of *pathogenesis-related (PR)* genes in uninfected distant tissues protecting these sites from secondary infections (Durrant and Dong, 2004). Induction of *PR1*, *PR2* and *PR5* is caused by salicylic acid (SA) treatment and serves as a marker for SAR (Uknes et al., 1992). Although ETI is mainly associated with SAR, it has been shown that PTI can also result in systemic resistance (Mishina and Zeier, 2007). The clear distinction between PTI and ETI is therefore questionable (Thomma et al., 2011).

1.2 Phytohormones regulating biotic defenses in plants

While plant innate immunity is well described by the zigzag model of Dangl and Jones (2006), the downstream effects of PTI and ETI are mainly regulated by the major defense phytohormones SA, jasmonic acid (JA) and ethylene (ET) (Pieterse et al., 2012).

SA is synthesized from chorismate by two distinct routes: either by phenylalanine ammonia lyase (PAL) or by isochorismate synthase (ICS)(Figure 1.1). With the exception of ICS and PAL, all other enzymes involved in SA biosynthesis have not been identified so far. Downstream events triggered by PTI or ETI are mainly controlled by NONEXPRESSOR OF *PR* GENES 1 (also NONINDUCIBLE IMMUNITY 1) (NPR1/NIM1) (Li et al., 1999). Increases in SA concentration induce cytosolic redox changes (Mou et al., 2003) and expression of thioredoxins (Laloi et al., 2004). Thioredoxins reduce the disulfide bonds of the NPR1 oligomer leading to the release of monomers (Tada et al., 2008). As monomer NPR1 is translocated to the nucleus where it interacts with several TGA transcriptional activators that bind to SA responsive promoters e.g. *AtPR-1* (Després et al., 2000; Fan and Dong, 2002). Along with this process, nuclear NPR1 is phosphorylated and ubiquitinated resulting in its degradation by the 26S proteasome (Spoel et al., 2009). There are also negative regulators of the SA pathway like NIM1 interacting 1 (NIMIN1) and SUPPRESSOR OF NPR1-1 INDUCIBLE 1 (SNI1) (Li et al., 1999; Weigel et al., 2005; Pape et al., 2010).

Upon wounding or herbivory, JA and other jasmonates are synthesized from free tri-unsaturated fatty acids like linolenic acid (18:3) and hexadecatrienoic acid (16:3) or from galactolipids containing esterified oxylipins (Buseman et al., 2006; Schaller and Stintzi, 2008). The subsequent steps take place in plastids and are catalyzed by a lipoxygenase (LOX) (Bell and Mullet, 1993), allene oxide synthase (AOS) (Laudert et al., 1996; Howe et al., 2000) and

allene oxide cyclase (AOC) (Ziegler et al., 1997; Stenzel et al., 2003b; Stenzel et al., 2003a) resulting in formation of cis-(+)-12-oxo phytodienoic acid (OPDA). OPDA is then transported into peroxisomes either through the peroxisomal ABC transporter COMATOSE (CTS) or by an alternative mechanism like ion trapping (Theodoulou et al., 2005). There, OPDA is reduced by OPDA reductase 3 (OPR3) in an NAPDH-dependent manner (Schaller et al., 2000). The resulting 3-oxo-2-(2'(Z)-pentenyl)-cyclopentane-1-octanoic acid (OPC8:0) is activated to its CoA-ester by an OPC-8:0 CoA ligase (OPCL) and shortened by acetyl-CoA-oxidase (ACX), multifunctional protein (MFP) and 3-keto-acyl-CoA-thiolase (KAT) (Cruz Castillo et al., 2004; Koo et al., 2006). After three rounds of ß-oxidation, JA is released (Vick and Zimmerman, 1984; Schaller and Stintzi, 2009) (Figure 1.1).

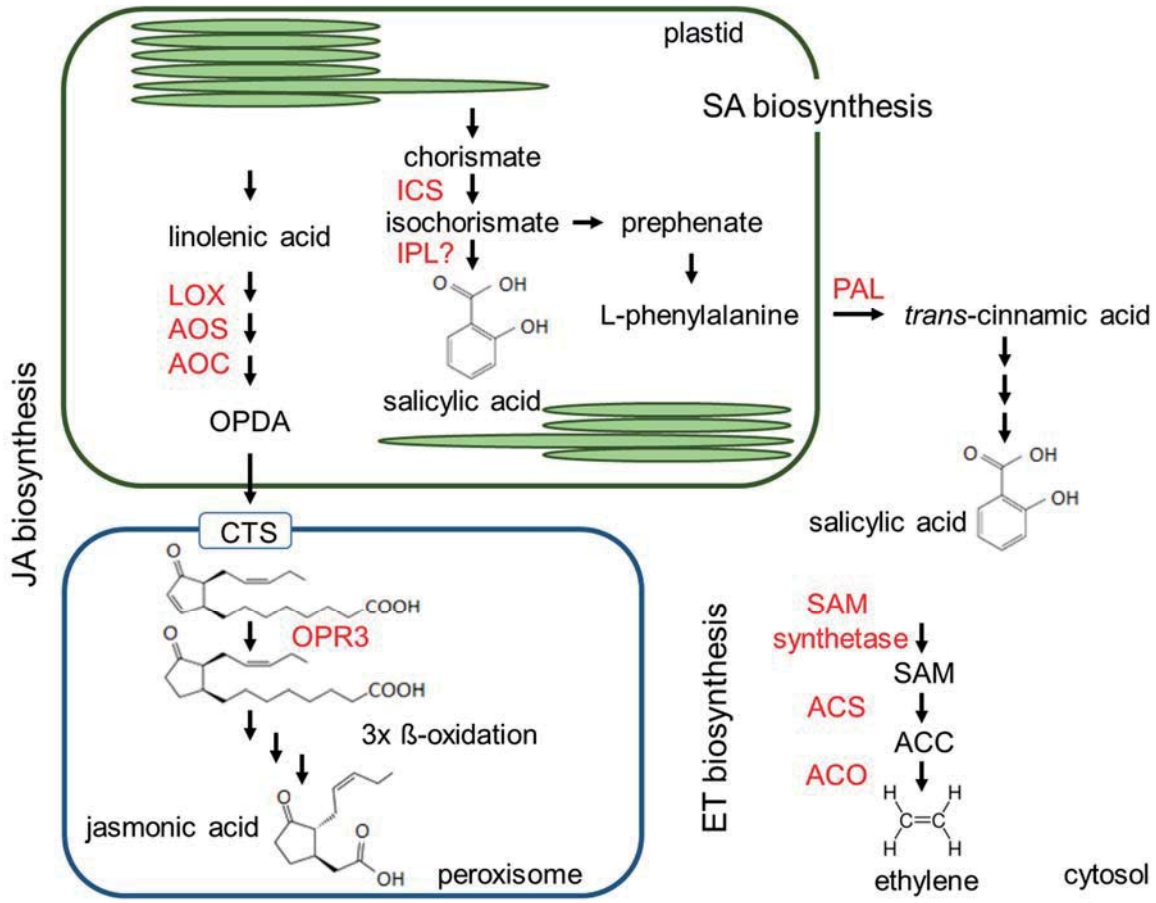

Figure 1.1: JA, SA and ET biosynthesis pathways.

Cellular compartmentalization of the different hormone biosynthetic pathways. JA and SA biosynthesis starts in plastids. JA is synthesized by the octadecanoid pathway from the precursor linolenic acid, through the intermediate OPDA. The alternative route starting from hexadecatrienoic acid (16:3) is not shown. After reduction of OPDA by OPR3 and three cycles of ß-oxidation in peroxisomes, JA is released. SA is synthesized from chorismate via two different mechanisms beginning with either ICS or PAL. The

ICS pathway involves the action of a not yet identified isochorismate pyruvate lyase-like (IPL). Another route of synthesis is i. a. catalyzed by PAL from L-phenylalanine. ET biosynthesis is localized in the cytosol. The ET precursor S-adenosyl-L-methionine (SAM), is generated by SAM synthetase. 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) catalyzes the formation of ACC which is oxidized by ACC oxidase (ACO) to ET.

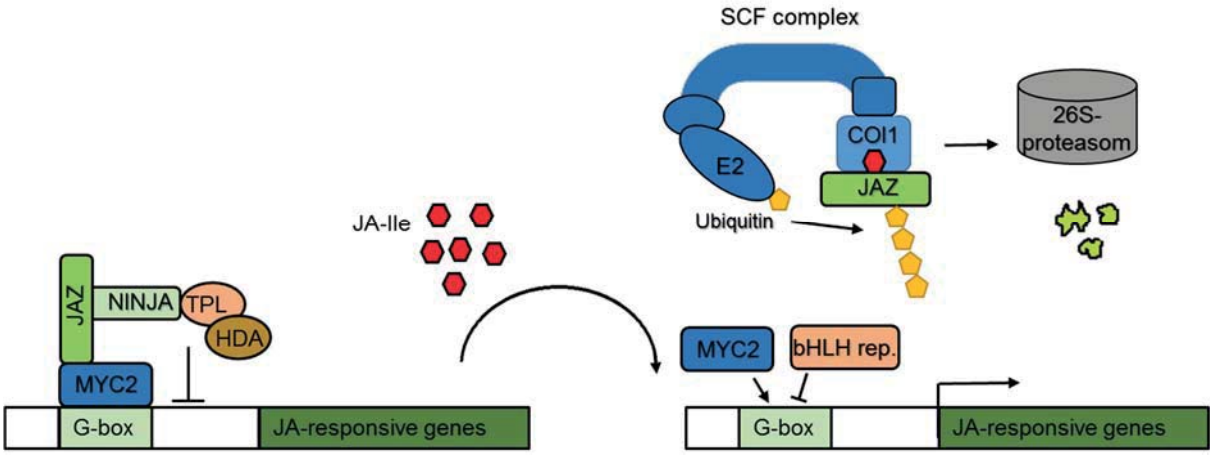

Figure 1.2: Regulation of JA-responsive gene expression.

Inhibitory JAZ proteins bind to positive regulators like e.g. MYC2 together with NINJA, TOPLESS (TPL), and histone deacetylase (HDA) to prevent the transcription of JA responsive genes. In the presence of JA-Ile, the SCF^{COI} complex interacts with JAZ proteins, ubiquitinating and degrading them by the 26S proteasome. MYC2 competitively antagonizes bHLH repressor proteins and activates JA-responsive genes. (Wasternack and Song, 2016)

JASMONATE RESISTANT 1 (= jasmonic acid-amido synthetase; JAR1) conjugates JA to isoleucine and other amino acids (Staswick and Tiryaki, 2004). The most biologically active JA derivate, (+)-7-iso-JA–Ile (Fonseca et al., 2009), is the substrate of the key regulator of JA responses: the F-BOX protein CORONATINE INSENSITIVE 1 (COI1) (Yan et al., 2009). The JA-Ile receptor is a multiprotein complex consisting of the E3 ubiquitin-ligase Skp/Cullin/F-box (SCF) complex called SCF^{COI1} (Sheard et al., 2010). In cells with a low JA-Ile concentration, JASMONATE ZIM domain (JAZ) proteins bind to transcription factors such as MYC2, 3, 4, (Lorenzo et al., 2004; Fernandez-Calvo et al., 2011; Niu et al., 2011) ETHYLENE RESPONSE FACTOR 1 (ERF1) (McGrath et al., 2005) or OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59 (ORA59) (Pre et al., 2008) and prevent their activity (Figure 1.2). The SCF^{COI1} complex recruits JAZ repressor proteins in the presence of JA-Ile, ubiquitinates and

subsequently targets them for degradation (Pauwels and Goossens, 2011). The release of JAZ repression on key regulatory genes initiates JA-dependent responses.

MYC2 and ERF1 activate two distinct JA signaling pathways (Lorenzo et al., 2004). Defense against necrotrophic pathogens is indicated by the expression of *PLANT DEFENSIN 1.2 (PDF1.2)* (Manners et al., 1998) and induced by ERF1 (Berrocal-Lobo et al., 2002), which requires both JA and ET (Lorenzo et al., 2002). Wounding or herbivorous insects activate the MYC branch (Lorenzo et al., 2004). One marker gene downstream of MYC2 (Kazan and Manners, 2013) and expressed upon wounding is *VEGETATIVE STORAGE PROTEIN 2 (VSP2)* (Berger et al., 2002).

The gaseous plant hormone ET is synthesized from S-adenosyl-L-methionine (SAM) (Adams and Yang, 1977) through the actions of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) (Boller et al., 1979) and ACC oxidase (ACO) (Ecker, 1995) (Figure 1.1). ET is perceived by homodimeric receptor proteins typified by ETR1 (Bleecker et al., 1998), which are located in the endoplasmic reticulum (ER) membrane (Ju and Chang, 2012). They physically interact with the homodimeric kinase CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) (Clark et al., 1998; Gao et al., 2003; Zhong et al., 2008), a negative regulator of ET responses (Kieber et al., 1993). In the absence of ethylene, CTR1 phosphorylates ETHYLENE INSENSITIVE 2 (EIN2) (Ju and Chang, 2012), a transmembrane protein residing in the ER (Bisson et al., 2009), preventing its nuclear localization. In the presence of ethylene, CTR1 is inactivated by an unknown mode of action and no longer phosphorylates EIN2 (Chen et al., 2011) thus allowing the cleavage of its C-terminus (Wen et al., 2012). Nuclear translocation of the EIN2 C-terminus (Wen et al., 2012) activates ET responsive transcription by stabilizing EIN3 and its homologs ETHYLENE INSENSITVE-LIKES (EILs) (Chen et al., 2011; Ju et al., 2012; Wen et al., 2012). EIN3/EILs are positive, master transcriptional regulators and activate downstream ET response genes e.g. *ERF1* (Solano et al., 1998).

Pathogens can be classified into necrotrophic, biotrophic and hemi-biotrophic pathogens based on their lifestyle as either consumers of dead or living tissue. Herbivores are categorized by their tissue-chewing or piercing-sucking feeding behavior (van Loon et al., 2005). Defense mechanisms against these biotic stress categories can be assigned to the different phytohormones. Responses against necrotrophic pathogens are mainly regulated synergistically by JA and ET (Penninckx et al., 1998). In contrast, SA dependent responses are