1. Introduction

In their natural environment, plants are continuously exposed to high levels of different stresses. Non-ideal growth conditions such as inconsistent water supply, alternating temperatures and light irradiation, herbivores, diseases and competition between neighboring plants lead to a more or less threatening loss of biomass and fitness that plants have to cope with and adapt to. If the effects of stress cannot be compensated, these stresses lead to growth retardation, crop penalty, and permanent damage that can even lead to death of the plant. Abiotic stresses originate from the surrounding environment of the plant, whereas biotic stress arises from the presence of living organisms that can cause disease or damage. Adaptation of plants towards stresses are often achieved by modulating their physiology in a fine-tuned network leading to macrobiological changes in appearance. Examples for developmental adaptations are the formation of a thicker cuticle, trichomes and a very selective growth enhancement or retardation. On the complex biochemical level, adaptations such as the biosynthesis of stress-related proteins, signaling molecules and defensive chemicals on the level of secondary metabolites are known. A better understanding of the regulators leading to these drastic changes constantly happening in the plant help to improve the breeding and cultivation of crop variations that can cope better with increasing challenging environmental situations that are a direct result from limited natural resources and climate change.

1.1. Jasmonic acid and its role in growth and defense

One major component in this fine-tuned network facilitating the plant's ability to drastically modulate its appearance and secondary metabolome are jasmonates including the plant hormone jasmonic acid (Vick and Zimmerman, 1984; Schaller and Stintzi, 2009; Wasternack and Hause, 2013; Verma et al., 2016; Ku et al., 2018; Ruan et al., 2019). The biosynthesis of jasmonates is known to be induced not only as a response to wounding, pathogen attack or drought resistance (Farmer and Ryan, 1992; Farmer et al., 2003; Wasternack and Hause, 2013; Savchenko et al., 2014), but also in the course of senescence, pollen and anther development, and the opening of stomates or flowers (Stintzi and

Browse, 2000; Ishiguro et al., 2001; Wasternack et al., 2013; Kim et al., 2015; Yan et al., 2015).

As a response to wounding, plants are able to "switch on" the production of JA very quickly (Koo and Howe, 2009; Mielke et al., 2011; Mayer, 2013). Wounding - very commonly derived from insect feeding - leads to a quick adjustment in plant metabolism. Secondary plant metabolites such as alkaloids (e.g., nicotine, scopolamine, colchicine, morphine), terpenes (e.g., menthol, azadirachtin, pyrethrins) or glycosides (e.g., amygdaline, glucosinolates) are induced, directly preventing insect feeding (Farmer and Ryan, 1990; Dudai et al., 1999; Metcalf and Horowitz, 2010; Bosch et al., 2014a; Benelli et al., 2017; Dhinakaran et al., 2019; Mohammadi et al., 2019). The induction of plant defense proteins (e.g., proteinase inhibitors) impairing starch and protein digestion (Farmer and Ryan, 1990; Jongsma and Bolter, 1997) is also triggered, limiting the herbivore's ability to digest plants and reducing the plants nutritional value.

The sudden JA-burst in response to wounding not only induces defense mechanisms, but also results in an immediate growth retardation due to the fact that jasmonic acid directly inhibits mitosis (Yan et al., 2007; Zhang and Turner, 2008; Havko, 2016; Major et al., 2017). Thus, it is necessary to regulate the production of jasmonates very strictly in order to obtain the growth-defense balance (Huot et al., 2014; Huang et al., 2017).

1.2. Biosynthesis and perception of jasmonates

Jasmonates are a group of lipid-derived phytohormones produced through the oxylipin-pathway, which is localized in the chloroplast and the peroxisome. The production of Jasmonates starts in the chloroplast, where polyunsaturated fatty acids, most importantly α-linolenic acid, are released from the chloroplast membrane by lipases and oxidized by lipoxygenase (LOX) to unsaturated fatty acid hydroperoxides (Bell and Mullet, 1993; Porta and Rocha-Sosa, 2002). Allene oxide synthase (AOS) (Howe and Schilmiller, 2002) converts the hydroperoxides to epoxides. AOC (Hamberg and Fahlstadius, 1990; Stenzel et al., 2003a; Stenzel et al., 2003b) forms the cyclopentenone ring from these epoxides to produce

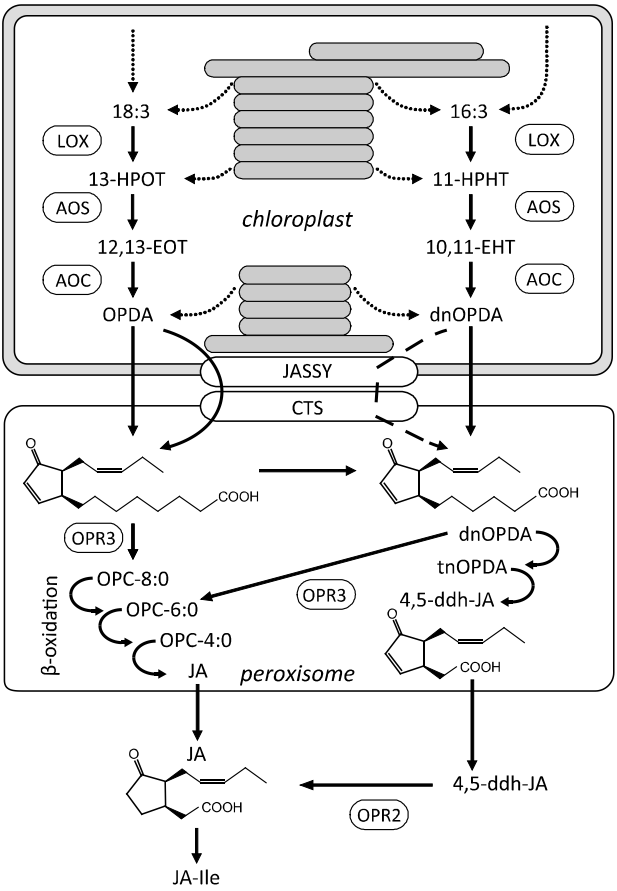

Figure 1.1: Biosynthetic pathway of Jasmonic acid. JA is produced through the octadecanoid-pathway in both chloroplasts and peroxisomes. Poly unsaturated fatty acids (PUFAs) (16:3 and 18:3) are released from the plasma membrane, cyclized and converted to OPDA. OPDA is transported into the peroxisome, where it is reduced through OPR3. β-oxidation shortens the side chain and JA is released into the cytosol, where it is conjugated with isoleucine forming the active plant hormone JA-Ile. An alternative pathway uses OPDA/dnOPDA. The side chain is also shortened by β-oxidation. In the cytosol 4,5-ddh-JA is reduced through OPR2. (modified from Schaller and Stintzi, 2009. Supplemented with Chini et al., 2018 and Guan et al., 2019)

12-oxo-10,15(Z)-phytodienoic acid (OPDA) from linolenic acid (18:3), and (Wasternack, 2007; Schaller and Stintzi, 2009) dinor-OPDA (dnOPDA) from the corresponding 16:3 fatty acid (Weber et al., 1997). OPDA is – at least partially – exported from the chloroplast through the recently discovered chloroplast outer membrane transporter JASSY (Guan et al., 2019) (Figure 1.1, upper part). The ABC-transporter COMATOSE (CTS) (Theodoulou et al., 2005; Footitt et al., 2007; Dietrich et al., 2009; Bussell et al., 2014) imports a large part of the OPDA into the peroxisome. The import of dnOPDA is not clarified yet, but is potentially based on the anion trapping principle (Theodoulou et al., 2005). In the peroxisome, the 9S,13S-stereoisomer of OPDA is reduced through OPR3, forming 3-oxo-2-(2'(Z)-pentenyl)-cyclopentane-1-octanoic acid (OPC-8:0) (Schaller and Weiler, 1997), or OPC-6:0 in the case of dnOPDA. After being activated to its CoA-ester by OPC-8:0 CoA ligase (OPCL) (Koo et al., 2006; Kienow et al., 2008), the carboxylic acid side chain is shortened by three rounds (two for OPC-6:0) of β-oxidation involving the enzymes acyl-CoA oxidase (ACX) (Li et al., 2005), L-3-ketoacyl CoA thiolase (KAT) (Cruz Castillo et al., 2004) and multifunctional protein (MFP) (Richmond and Bleecker, 1999). The result of this reaction is jasmonic acid, which is subsequently released into the cytosol (see Figure 1.1, middle part).

Beside the already know pathway, in *opr3-3* mutants there was a second pathway for biosynthesis of JA discovered (Chini et al., 2018). In this pathway, the carboxylic acid side chain of OPDA, or dnOPDA, is shortened by β-oxidation similar to the side chains of OPCs. The product, 4,5-didehydro-jasmonic acid (4,5-ddh-JA) is released into the cytosol analogical to JA. In the cytosol, the mainly uncharacterized oxo-phytodienoic acid reductase 2 (OPR2) reduces the double bond of 4,5-ddh-JA forming JA (see Figure 1.1, bottom right). Further evidence also suggests a reduction of 4,5-ddh-JA by OPR1 (Chini et al., 2018).

Cytosolic JA is further conjugated with isoleucine by the JA-amido synthetase JAR1. JA-Ile in its (+)–7-iso-JA-Ile-form is the active plant hormone and it is in equilibrium with the inactive epimer (−)-JA-Ile (Staswick and Tiryaki, 2004; Fonseca et al., 2009).

Perception of JA-Ile takes place in the nucleus by binding to the receptor complex formed by the SCF_{COI1}-ubiquitin ligase consisting of the Skp1/Cullin and the F-box protein Coronatine Insensitive 1 (COI1) and Jasmonate ZIM domain (JAZ)-proteins. The family of JAZ proteins are negative regulators of JA-induced gene expression (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). The JAZ proteins bind to positive regulators such as MYC2 which are in turn binding to the

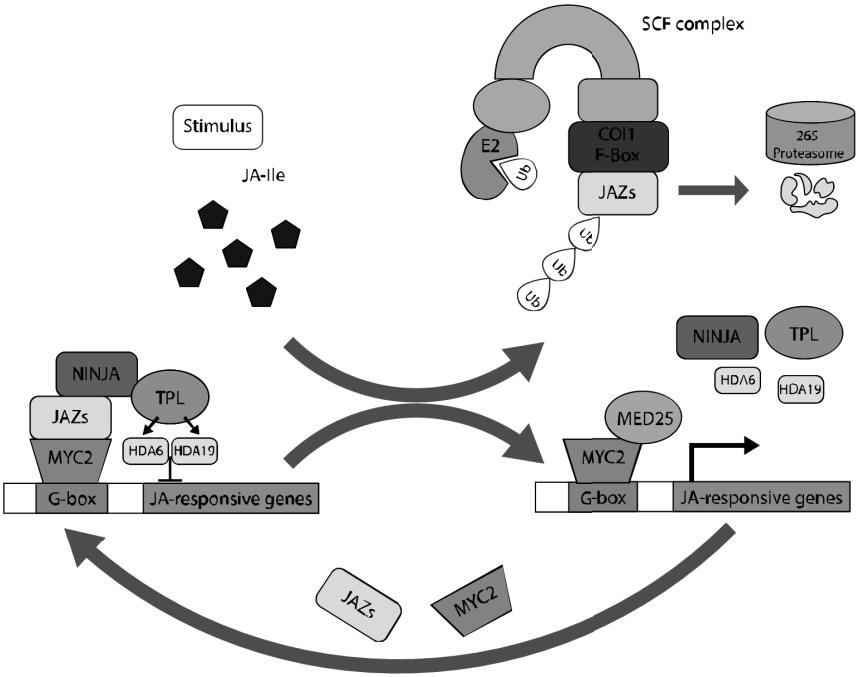

Figure 1.2: JA-Ile perception by COI1-complex. The transcription of JA-responsive genes is repressed on promotor level by JAZ, binding to the G-box binding protein MYC2, and NINJA, binding to TPL. TPL represses transcription via interacting with NINJA and with Histone-acetylating enzymes HDA6 and HDA19. When the JA-Ile level rises upon wounding or developmental signals, JA-Ile binds and activates the SCF_{COI1}-complex which in turn ubiquitinates the JAZ-proteins. JAZ proteins are degraded and the transcription of the JA-responsive genes is no longer repressed. JA-Ile induces the transcription of JAZ and MYC2. (Modified from Wasternack and Hause, 2013)

G-box in the promotor of JA-responsive genes. Novel INteractor of JAZ (NINJA), interacting with both JAZ proteins and the Topless (TPL) repressor, co-represses the transcription of JA-responsive genes. TPL represses the transcription via interaction with histone deacetylases HDA6 and HDA19 (Pauwels et al., 2010).

After rising JA-Ile-levels are perceived by the SCF_{COI1}-complex, JAZ-proteins are recruited to and ubiquitinated by the SCF_{COI1}-complex. As JAZ proteins are subsequently degraded at the 26S proteasome, the transcription of JA-responsive genes is triggered by MYC2. Besides, TPL, HDA6 and HDA19 are released from the DNA, also triggering transcription. As a result, JA-responsive genes triggering i.e., responses to wounding or insects feeding, oxidative stress or necrotrophic infection are induced (Koo and Howe, 2009; Bodenhausen and Reymond, 2007; Zhang et al., 2017; Zhang et al., 2018; Browse and Wallis, 2019).In a positive feedback loop, MYC2 and JAZ's are transcribed as positive and negative regulators are transcribed, providing the constant regulation of the JA-Ile response (Chung et al., 2008). (See Figure 1.2).

In addition to the perception of JA-Ile mediated by COI1, other oxylipin-perceiving pathways were identified. The JA-deficient moss *Marchantia polymorpha* lacks OPR3 and JAR1 (Stumpe et al. (2010)), but contains both OPDA and dnOPDA, from which dnOPDA is perceived by the *Mp*COI1-receptor (Monte et al., 2018). *Mp*COI1-receptor was found to be mutated in a single amino acid compared with *At*COI1 in the ligand binding part, leading to a switch in ligand specificity (Monte et al., 2018). The role of OPDA and dnOPDA in *M. polymorpha* is only partially overlapping with the role of jasmonates in vascular plants. Contrasting to the role of JA-Ile in *Arabidopsis*, dnOPDA does not contribute to male fertility in *M. polymorpha*. On the other hand, dnOPDA is induced upon wounding, and growth is inhibited by dnOPDA-treatment in *M. polymorpha*, so some functions of dnOPDA and JA-Ile do overlap. Furthermore, an additional COI1-independent dnOPDA-perceiving pathway in *M. polymorpha* was reported. This pathway triggered plant thermotolerance genes, probably through the electrophilic properties of dnOPDA (Monte et al., 2020). The alternative pathway led to

an enhanced heat resistance of *M. polymorpha*. This finding of an alternative, potentially ancestral (dn)OPDA-perceiving pathway, recently not only reported in *M. polymorpha*, but also in *A. thaliana* and *Klebsormidium nitens*, and its involvement in heat response suggests a vital and conserved pathway in streptophyta evolutionary predating JA (Monte et al., 2020). The dnOPDA-perceiving pathway led to an enhanced survival rate of plants under heat stress.

Consistent with these findings in *M. polymorpha*, similar results regarding distinct OPDA- or JA-functions related to defense were obtained in *Arabidopsis thaliana* and *Solanum lycopersicum*. In Arabidopsis thaliana, *opr3*-knockout plants (deficient in JA/JA-Ile) were much more resistant towards dipteran *Bradysia impatiens* and the fungus *Alternaria brassicicola* compared to plants defective in JA-perception (*coi1*), implying a direct function of OPDA in defense response, potentially through the electrophilic effect of OPDA/dnOPDA (Stintzi et al., 2001). Similarly, insect resistance in *Solanum lycopersicum* is also regulated differently in response to OPDA and JA/JA-Ile (Bosch et al., 2014b). In JA/JA-Ile deficient plants, oviposition of the specialist *Manduca sexta* was not altered in comparison to wild-type *Solanum lycopersicum*, whereas plant resistance against *Manduca sexta* was compromised in plants deprived of JA/JA-Ile signal transduction. It was concluded that JA-signal transduction is directly essential for induced defense, whereas JA/JA-Ile are not (Bosch et al., 2014b).

As this shows a distinct role for OPDA/dnOPDA in plants improving survival and defense, a strict regulation of OPDA-amount through inhibiting the conversion of OPDA through OPR3 seems essential. In consequence, OPR3 is discussed to be a key regulating step in the oxylipin signaling by tightly controlling OPDA/JA-amounts and the fine-tuned response network.

1.3. Regulation of OPR3 activity by dimerization

Since the conversion from OPDA to OPC 8:0 seems to be of crucial importance for the different functionality of the two phytohormones OPDA and JA-Ile, the conversion of OPDA was considered as a potential regulatory step in the pathway. OPR3 as the responsible enzyme was object of closer examination and a

suggestion for a regulatory mechanism was derived from the crystal of *Sl*OPR3 (Breithaupt et al., 2006).

When *Sl*OPR3 was crystalized in the presence of ammonium sulfate, it unexpectedly turned out to as a homodimer (Breithaupt et al., 2006), contrasting to the previously obtained monomeric crystal of *At*OPR3 (Malone et al., 2005; Han et al., 2011). In the *Sl*OPR3-homodimer, the finger-like loop L6 of protomer A was bound to the substrate-binding cavity of protomer B and vice versa by hydrogen bonds, leading to a self-inhibition of both protomers. E291 from protomer B at the tip of loop 6 forms hydrogen bonds to H185 and H188 of Protomer A, thereby stabilizing the dimer (Breithaupt et al., 2006, Figure 1.3). Interestingly, there were also two sulfate ions included in the interaction sites of the crystal, forming hydrogen bonds with both protomers. The sulfate ion in the substrate binding pocket of protomer A hydrogen bonds with R343, Y364 and R366. In addition, it forms hydrogen bonds with R294 of protomer B, potentially stabilizing the dimer. Interestingly, the sulfate ions are in direct proximity of Y364.

It was thus hypothesized that the sulfate — with its similar size and charge — may mimic phosphorylation of Y364. As a consequence, it was hypothesized that phosphorylation of Y364 may lead to the inactivation of OPR3 by promoting formation of the self-inhibiting dimer *in vivo* (Breithaupt et al., 2006). Consistent with the proposed role of E291 und Y364 in dimerization, the E291K and Y364F mutants of tomato OPR3 crystallized as monomers (Breithaupt et al., 2006), similar to *At*OPR3 (Malone et al., 2005; Han et al., 2011). Additionally, dimer of *Sl*OPR3 was confirmed by dynamic light scattering (DLS) and by analytical ultracentrifugation (AUC) in a concentration-dependent manner (Breithaupt et al., 2006). With AUC, a dissociation constant of 30 µM was calculated for wild-type OPR3. Consistent with the crystallization data, dimerization of the E291K-mutant was not observed at any concentration. In addition, OPR3 at high concentrations in a stopped flow experiment was 6 times less active than E291K, which supported the inactivation by dimerization (Breithaupt et al., 2006).

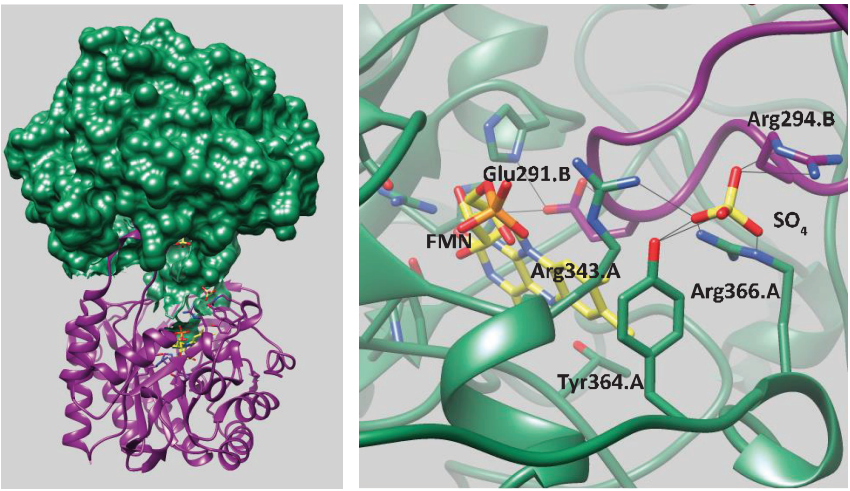

Figure 1.3: Crystal structure of *Sl*OPR3 dimer. The protomers are shown in green and purple respectively with either charged surface (green, protomer A) or ribbon structure (purple, protomer B). In the closeup (right side), the interaction site of both protomers is shown in ribbon structure. The FMN cofactor is depicted in yellow. Amino acids involved in dimerization are labeled. Modified from Breithaupt et al., 2006 (pdb: 2HSA).

Based on this hypothetical regulating mechanism, OPR3 was also investigated *in vivo*. As JA plays a distinct role in flower development (Stintzi and Browse, 2000), anthers of *Arabidopsis thaliana* were investigated with a split-YFP-approach (Sperling, 2012). In flower stage 11 and 12, the JA-content in stamen is high (Figure 1.4 B), as JA is needed for the elongation of stamen filaments. Contrasting, the JA-content is much lower in flower stage 13 and 14 (Figure 1.4 B).

In these flowers, the stamen elongation is going on (13) or completed (14). In flower stage 14, the anthers finally have passed the stigma height, promoting self-pollination (Figure 1.4 A, upper lane). In order to link the JA-content to the oligomeric state of OPR3, a split-YFP-assay was performed by Sperling (2012).

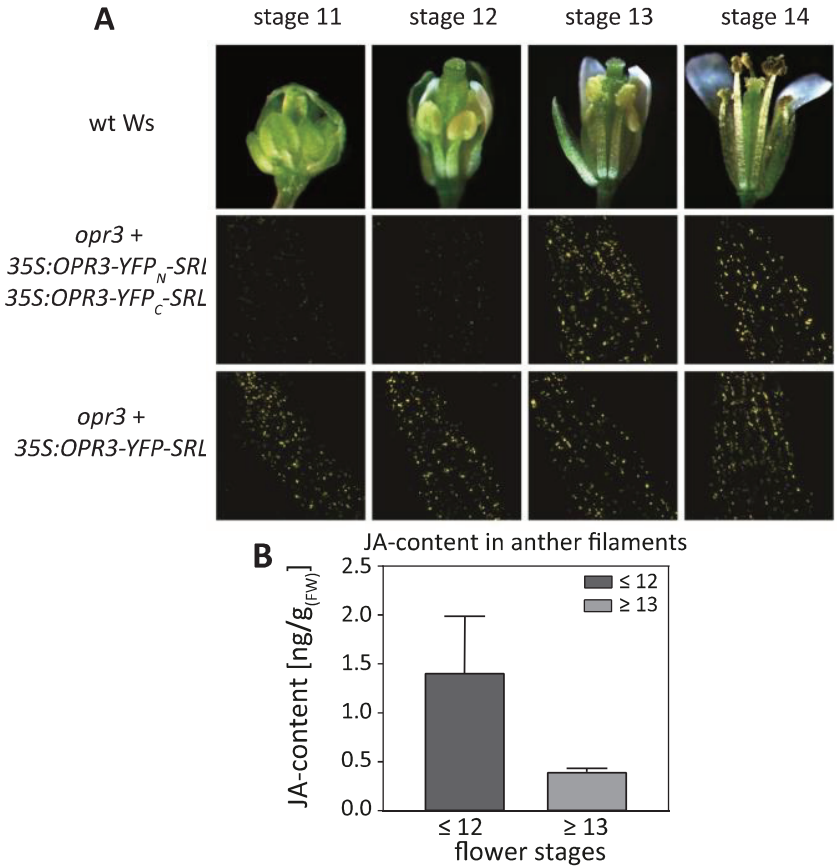

Figure 1.4: OPR3-Dimerization in stamen filaments depending on the JA-requirement. A: Dimerization of OPR3 in stamen filaments was investigated using BiFC. Anther filaments of flower stages 11-14 were investigated in *Ws* wt and *opr3* (upper row pictures). BiFC-experiments (center row) show that OPR3 is present as a monomer in flower stages 11 and 12, whereas the dimer is found in flower stage 13 and 14. OPR3-YFP is expressed at similar levels at all flower stages (lower row). (modified from Sperling, 2012) **B: JA-content in stamen filaments**. Filaments of flower stages 10-12 (≤ 12) and 13-15 (≥ 13) were analyzed for their JA-content (Stintzi and Hause, unpublished).