Research article

Grape phylloxera (*D. vitifoliae*) manipulates SA/JA concentrations and signalling pathways in root galls of *Vitis* spp.

Markus W. Eitlea,*, Michaela Griesserb, Radomira Vankovab, Petre Dobrevb, Simone Aberera, Astrid Forneckc

a Institute of Viticulture and Pomology, Department of Crop Sciences, University of Natural Resources and Life Sciences (BOKU), Vienna, Konrad Lorenz Straße 24, 3430, Tulln an der Donau, Austria
b Institute of Experimental Botany, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 16502, Praha 6, Prague, Czech Republic
c Institute of Experimental Botany, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 16502, Praha 6, Prague, Czech Republic

1. Introduction

Plants possess a large number of genetically conserved and complex defence mechanisms. Among them the two phytohormones jasmonic (JA) and salicylic acid (SA) mediate biotic stress signals and activate downstream host defence responses in plant tissues under attack (Erb et al., 2012). Phytophagous insects, with different feeding behaviours, were shown to interfere with plant host defences mediated by JA and SA signalling pathways in order to establish compatible host-parasite interactions (De Vos et al., 2005).

The introduction of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in Europe is one of the most famous examples for an introduced insect pest species causing severe economic damages to the viticulture in the 19th century. Today, reports of *D. vitifoliae* root infestation increase worldwide due to the evolution of aggressive *D. vitifoliae* biotypes (Forneck et al., 2016) and in dependence of environmental factors (Powell et al., 2013). Root-galling *D. vitifoliae* feed on the parenchymal cellular content of previously formed organoid root galls on susceptible and tolerant *Vitis* spp. hosts (Forneck et al., 2002). Similar to other piercing-sucking insects, mobile *D. vitifoliae* L1 larvae are observed to probe *Vitis* spp. by puncturing the root tissue with their stylets; thereby selecting a suitable location for feeding site induction (Kingston, 2011). Sedentary L2-L4 larvae establish the root tip feeding site and form hook-shaped, yellowish root galls. Adult insect stages (A) feed on the...
galls and complete the life cycle within 30 days marked by the start of oviposition (Forneck and Huber, 2009). Although morphological and physiological properties of D. vitifoliae root galls have been investigated previously (Forneck et al., 2002; Lawo et al., 2011; Griesser et al., 2015; Eité et al., 2019), little is known about whether and how D. vitifoliae interferes with host plant defences mediated by the JA and SA signaling pathways. Previous experiments show that foliar application of JA systemically decrease root-feeding D. vitifoliae populations on the same host plant by > 50% (Omer et al., 2000). Transcriptionic profiling of D. vitifoliae leaf galls shows that JA defence related transcripts are induced; whereas SA associated transcripts seem to be reduced in affected leaf gall tissue of V. riparia (Nabity et al., 2013).

The JA signaling pathway mediates biotic stress signals and coordinates host plant defense responses against chewing herbivores, necrotophic pathogenes and mechanical wound stress. Once an attacking parasite is perceived JA is readily biosynthesized from its precursor α-linolenic acid by the JA biosynthetic key enzymes lipoygenase 2 (LOX2), allene oxide synthase (AOS), allene oxide cyclase (AOC) and 12-oxophytodienoate reductase 3 (OPR3). JA is transformed by the enzyme jasmonic acid-amido synthetase (JAR1) to its bioactive derivate jasmonate isoleucine (JA-Ile) or by jasmonic acid methyltransferase (JMT) to methyl jasmonate (MeJA). The presence of JA-Ile leads to the degradation of repressive JAZ protein resulting in the transcription of JA responsive genes e.g. VviVSP2, VviPDF1 locally within the attacked host tissue (Wasternack and Hause, 2013).

The activation of the SA signaling pathway coordinates host defence responses against biotrophic pathogens (Chong et al., 2008) and piercing-sucking insect species (Thaler et al., 2012). Although SA biosynthesis in plants is not yet fully understood, studies indicate that the enzyme isochorismate synthase 1 (ICS1) plays a key role in SA biosynthesis triggered by attacking parasites (Chen et al., 2009). SA induces local defence responses (LAR) at the site of the attack, whereas systemic acquired resistance (SAR) is mediated by mobile compounds among them volatile methyl salicylate (MeSA) (Shah and Zeier, 2013); ladder one synthesised from SA by S-adenosyl-L-methioninesalicylic acid carboxyl methyltransferases (SAMTs). Presence of SA leads to the monomerisation of the oligomeric NPR1 complex in plants, resulting in the expression of SA host defensive genes such as VviPR2 and VviPR5 encoding for pathogenesis-related proteins known for their antimicrobial properties (Renault et al., 2015; Le Henanff et al., 2009). Recent studies reveal the importance of insect-induced modifications of the SA signaling pathway for the establishment of compatible host-parasite interactions via SA/JA antagonistic crosstalk (Thaler et al., 2012). In particular biotrophic phloem-feeding insects are reported to benefit from the SA mediated suppression of JA host responses as shown for mealybugs Phenacoccus solenopsis (Zhang et al., 2015) and the silver white fly Bemisia tabaci (Zarate et al., 2007).

In the presented descriptive study we analyse phytohormonal changes of the host defensive SA and JA signaling pathways following the compatible root interaction between D. vitifoliae on the grapevine rootstock TSC. We determine concentration levels of free SA, JA, the JA precursor cis-12-oxophytodienoic acid (cisOPDA) and bioactive jasmonic acid isoleucine (JA-Ile) by HPLC-MS as well as relative expression of SA/JA marker genes (listed in Appendix A.1). Analytical root tip and gall samples are taken during larval probing (14 hai; hours after inoculation), early gall formation (24–48 hai) and gall formation (5–14 dai; days after inoculation). Additionally we include “put infested root tips” from infested plants (NIRTs) in order to study potential interactions of systemic SA/JA stress signals translocated towards non-infested parts of the root system; as well as a mechanical wound stress treatment to correlate transcriptional changes of JA marker genes caused by wounding with those during insect probing. Control root tips of same ages are obtained from not infested plants. We hypothesize that (1) the JA signalling pathway is transcriptionally induced during larval probing (14 hai). Compatible root gall formation (24 hai – 14 dai) involves (2) the transcriptional reduction of the JA, but (3) the induction of the SA signaling pathway.

2. Material and methods

2.1. Plant and insect material

All plants used in the presented study derive from single bud cuttings obtained from dormant cuttings of the rootstock Teleki SC (TSC; V. berlandieri x V. riparia) taken from field-grown mother vines at the research vineyard at BOKU university in Tulln (Austria) in early spring and stored at 4°C until propagation. Cuttings are propagated vegetatively by dipping them into a solution of 0.1% IBA and 0.07% NAA to promote rooting in April to May. Potted cuttings develop and grow for two months under greenhouse conditions before being transferred into controlled climate chambers for the experiments.

Grape phylloxera (D. vitifoliae) eggs and larvae employed for the experiments derive from a D. vitifoliae single founder lineage field-sampled on roots of grafted vines in Burgenland Austria in 2007 and since then maintained on excised roots. The employed D. vitifoliae lineage has been selected based on its previous classification as biotype C (Forneck et al., 2016).

2.2. Experiment 1: Probing and early gall formation (14–48 hai)

Fifty rooted TSC cuttings are cultivated in plastic pots (5.5 cm height; 7.0 cm diameter) containing a mixed substrate of clay pebbles:perlite (1:1) in isolated climate chambers (25 ± 3°C, 45% rH, 16 h photoperiod at 80–100 μmol m⁻² s⁻¹). Two weeks after acclimatisation each plant is inoculated with 30 D. vitifoliae L1 larvae hatched at 25°C in darkness the previous day. Twenty vines remain not infested, of which ten are used as untreated controls and ten receive a mechanical wound stress treatment simulated by manually squeezing of the plastic pots to injure root surfaces. Root tip samples are collected from D. vitifoliae infested plants 14, 24 and 48 h after infection (hai), from wound stress treated plants after 14 h and from not infested control plants. Each sample consists of 30 pooled root tips of approximately 1.5 cm length (50–100 mg root FW). Attached insects and substrate particles are quickly removed with a soft brush under a binocular. Root samples are immediately snap frozen in liquid N₂ and stored at ~80°C. Three biological replicates, each corresponding to pooled root tips taken from three individual vines, are collected per treatment.

2.3. Experiment 2: Root gall formation (5–14 dai)

Forty-eight rooted TSC cuttings are planted in four growth boxes of 38 × 28 × 20 cm filled with the same substrate and cultivated as described in Experiment 1. Each growth box is inoculated with 150 D. vitifoliae eggs by placing the eggs on a moist filter paper adjacent to the roots. D. vitifoliae L1 larva hatch, develop and multiply on TSC roots. After 50 days (two insect reproduction cycles) root galls of different stages and NIRTs are harvested simultaneously by differentiating between: Root tips of not infested plants (control), not infested root tips of infested plants (NIRTs) and root gall stages categorized by the attached D. vitifoliae larval stages: L2 (5–7 dai), L3 (8–14 dai), L4 and the adult stage (A) (> 14 dai) (Forneck and Huber, 2009). Each biological sample consists of 30 pooled root tips or 20 galls (50–150 mg). Three biological replicates, each corresponding to root tissues taken from one growth box, are collected per treatment.

2.4. Gene expression analysis

RNA extraction of root tips and gall tissue is performed according to Reid et al. (2006). RNA concentration, purity and integrity are confirmed spectrophotometrically by Nanodrop2000 (Thermo Scientific, Wilmington, US) and agarose electrophoresis gels (1.5%, 100 V, 40 min). Reverse transcription is performed with 500 ng initial RNA
Fig. 1. Alterations of JA marker genes during D. vitifoliae probing (14 hai) and early gall formation (24–48 hai). Expression levels of marker genes associated to the JA signaling pathway are presented in log2 FC. Treatments consist of root tips sampled 14 h after wound stress treatment (Wounding), root tips sampled 14 hai during D. vitifoliae probing (Probing 14 hai) and early gall formation at 24 and 48 hai (Early gall formation 24 hai/48 hai). Gene expression analyses are conducted by employing the two reference genes actin (VIT_04s0044g00580) and ubiquitin (VIT_16s0098g01190). A primer list is attached in (Appendix A.1). Columns demonstrate means of three independent biological replicates each consisting of 20–30 pooled root tissues. Error bars represent standard deviations. Asterisks mark significant differences obtained by Mann-Whitney U testing (P < 0.05).

2.5. Phytohormone quantification

Phytohormone analyses are conducted based on root tip and gall samples during gall formation (5–14 dai) and the corresponding controls. Ground root material is purified and analysed according to Dobrev and Kaminek (2002); Dobrev and Vankova (2012). In short, 50 mg of root fresh weight (FW) is homogenized and extracted in cold methanol/water/formic acid (15/4/1 v/v/v) extraction buffer (~20°C). To account for sample losses and for quantification by isotope dilution method with multilevel calibration curves (r2 > 0.99). Hormone fraction was eluted with methanol. Fraction is evaporated to dryness in a vacuum concentrator and dissolved into 30μl of 10% methanol. An aliquot (10μl) is analysed using a high-performance liquid chromatograph (HPLC) column Luna C18(2) (100 × 2 mm, 3 μm, Phenomenex, Torrance, USA) at flow rate of 0.25 ml/min. The mass spectrometer is run in electrospray (−)-ionization mode. Quantification of hormones is done using the isotope dilution method with multilevel calibration curves (r2 > 0.99). Data processing is carried out with Analyst 1.5 software (Applied Biosystems Foster City, USA). Final compound determination values are given as absolute concentration in pmol g−1 of root gall FW.

2.6. Statistical data analysis

Statistical analyses are performed using SPSS v21 (IBM, Armonk, USA). Gene expression levels of three independent biological samples are analysed with Whitney U tests (P < 0.05) comparing the root gall stages L2/3/4-A and NIRTs with not infested control tips. Phytohormone (derivate) concentrations, determined by HPLC/MS, are compared between all root tip/gall treatments with one-way ANOVA and Tukey post-hoc tests (P < 0.05).

3. Results

3.1. Larval probing and early gall formation (14–48 hai)

Mobile D. vitifoliae L1 larvae probe Vitis spp. root tissue prior to feeding site establishment marked by repeated stylet insertions. In preliminary trials we observe that L1 larvae probe on Teleki SC root tips at 14 hai, before becoming sedentary at 24 hai for the given experimental setup (data not shown). Accordingly root tips collected at 14 hai are categorized as belonging to the probing period, whereas root tips taken at 24 hai are defined as the earliest sampling time point for the compatible interaction (= early gall formation).

The results of the gene expression analysis during D. vitifoliae larval probing show a significant induction of all analysed JA biosynthetic (VviAOS 0.85; VviOPR3 0.93; VviJAR1 1.08; VviJMT 1.15) and responsive (VviPDF1 0.78; VviVSP2 0.56) marker genes 14 hai (Fig. 1). The induced JA gene expression patterns during probing are quantitatively similar to those obtained by the applied mechanical wound stress treatment after 14 h (Fig. 1). All analysed JA biosynthetic (VviAOS 0.84; VviOPR3 0.93; VviJAR1 1.12; VviJMT 1.22) and JA responsive (VviPDF1 1.10, VviVSP2 0.38 log2 FC) are significantly induced at a similar intensity.

In contrast not all of the analysed SA marker genes are affected during D. vitifoliae probing. Our data shows significantly increased expression levels of the SA signalling genes VviSAMT2 (1.04 log2 FC) and VviSAMT2 (1.35 log2 FC), however the two analysed SA responsive marker genes are not induced (VviPR2 -0.39; VviPR3 -0.85) in probed root tips at 14 hai (Appendix S2A).

As soon as D. vitifoliae larvae become sedentary, the analysed genes associated to the JA signaling pathway shift from formerly induced (14 hai) to significantly reduced expression levels by the start of early gall formation (24 hai): VviOPR3 -0.67; VviJAR1 -0.88; VviJMT −1.12;
3.2. Phytohormonal changes detected during root gall formation (5–14 dai)

Among the analysed JA derivates, we observe significantly increased levels of the JA precursor cis-(+)-12-oxo-phytodienoic acid (cisOPDA) through all analysed D. vitifoliae root galls stages with 333.05 ± 9.58 pmol g FW⁻¹ in L2, 273.68 ± 19.52 pmol g FW⁻¹ in L3 and 354.66 ± 13.89 pmol g FW⁻¹ in L4/A root galls, compared to cisOPDA levels in control root tips 103.58 ± 10.05 pmol g FW⁻¹ (Fig. 2A). In contrast levels of free JA are not significantly increased in D. vitifoliae root galls ranging between 71.48 ± 5.61 and 102.16 ± 10.73 pmol g FW⁻¹ as compared to JA levels in control root tips (74.48 ± 10.43 pmol g FW⁻¹, Fig. 2A). Levels of jasmonic acid isolateucine (JA-Ile), the primary bioactive JA derivate triggering JA host responses, are significantly increased in L2 (88.60 ± 9.40 pmol g FW⁻¹) and L4/A (129.93 ± 10.87 pmol g FW⁻¹), but not in L3 (60.33 ± 5.44 pmol g FW⁻¹) root galls, when compared to levels in control root tips (43.41 ± 4.12 pmol g FW⁻¹, Fig. 2A). The observed gap between relatively higher levels of cisOPDA and minor levels of JA and JA-Ile (Fig. 2A) in the analysed D. vitifoliae root galls is consistent with significantly decreased expression levels of the underlying JA biosynthetic genes (VviAOS — 2.84, VviOPR3 — 1.24, VviJAR1 — 0.87 and VviJMT — 4.00 log₂ FC in L4/A galls) transforming the precursor cisOPDA to JA and JA-Ile or volatile MeJA (Fig. 3A). Despite the presence of bioactive JA-Ile, the two analysed JA responsive genes are significantly reduced with VviPDF1 -0.81 and VviVSP2 -6.77 log₂ FC in L4/A galls (Fig. 3A). A similar effect is observed for NIRTs, the precursor cisOPDA is increased, whereas JA and JA-Ile are not significantly different from the concentration levels in control roots (Fig. 2A). Three JA biosynthetic marker genes are significantly downregulated (VviAOS — 0.42, VviOPR3 -0.33 and VviJMT — 1.06 log₂ FC, Fig. 3A). One of the analysed JA responsive marker genes is induced (VviVSP2 0.63 log₂ FC) and the other reduced (VviPDF1 -0.57 log₂ FC in NIRTs (Fig. 3A).

Levels of free SA increase gradually along with D. vitifoliae root gall formation from 970.68 pmol g FW⁻¹ in L2 up to 2303.52 pmol g FW⁻¹ in L4/A galls (Fig. 2B). This remarkable accumulation of SA in D. vitifoliae root galls is not explained by isochorismate synthase 1 (ICS1) known to be the primary SA biosynthetic enzyme in plants, because gene expression levels of VviICS1 formation from 970.68 pmol g FW⁻¹ in L2 up to 2303.52 pmol g FW⁻¹ in L4/A galls (Fig. 2B). However the gene expression data demonstrates means of three independent biological replicates each consisting of 20–30 pooled root tissues. Error bars represent standard deviations. Minor letters indicate significant differences obtained by Tukey post-hoc testing (P < 0.05). Minor numbers in Fig. 2A illustrate that statistical tests are done by comparing each analysed compund between D. vitifoliae root gall stages.

VviPDF1 -0.43 and VviVSP2 -0.83 log₂ FC (Fig. 1); except for VviAOS − 0.46 being not significantly affected (Fig. 1). The reduced expression levels of JA marker genes during 24 hai early gall formation diminish at 48 hai. The expression levels of five out of six analysed JA marker genes are not different from those in control root tips (Fig. 1), merely VviOPR3 remains significantly reduced. Again no clear and consistent gene expression patterns are observed for the SA biosynthesis, signalling and responsive genes during early gall formation (Appendix A.2A).

4. Discussion

Modulations of the host plant defensive SA and JA biosynthesis and signalling pathways could be key factors for the compatible interaction between grape phylloxera (D. vitifoliae) and grapevine rootstocks (Vitis spp.). The presented study describes host physiological alterations regarding phytohormonal SA and JA concentration levels and transcriptional modification of associated SA/JA marker genes following the compatible interaction between a root-galling D. vitifoliae biotype feeding on the rootstock T5C (V. berlandieri x V. riparia). Thereby we differentiate between root tissue under mechanical wound stress (14 h), insect probing (14 hai), early gall formation (24–48 hai), gall formation (5–14 dai) and systemic NIRTs.

4.1. JA signalling during D. vitifoliae probing

Plant tissue woundings caused by phytophagous insect species trigger the induction of the JA signalling pathway depending on the feeding mode and the degree of the related tissue damage (De Vos et al., 2005). Although insects with piercing-sucking mouthparts, such as aphids or phylloxerids, minimize plant tissue wounding, they elicit local JA accumulation and trigger JA related host defence responses
shortly after infestation (Thompson and Goggin, 2006). Due to the experimental set-up, the presented study does not provide information about changes of JA levels in probed root tip tissues. However the results of the transcriptional analysis show the induction of all analysed JA biosynthetic and responsive marker genes during *D. vitifoliae* probing on root tips of Teleki 5C 14 hai. We strongly suggest that the repeated stylet insertions exerted by mobile L1 larvae into the root cortex tissue (Kingston, 2011), might cause a wound stress signal, thereby triggering the induction of the JA marker genes. This is supported by similarly induced JA expression patterns in root tips exposed to the mechanical wound stress treatment. Similar to our results probing of other piercing-sucking insect species is shown to trigger the induction of the JA signalling pathway within the first hours after infestation. Probing of the pea aphid (*Acyrthosiphon pisum*) results in increased levels of JA in leaf tissue of *Pisum sativum* cv. Cysterski 24–48 hai (Drzewiecka et al., 2014). Another experiment shows that transcript levels of JA biosynthetic *AtLOX2* and JA responsive *AtPDF1.2* peak at 72 hai in *Arabidopsis thaliana* leaf tissue probed by the green peach aphid (*Myzus persicae*) (Moran and Thompson, 2001).

The induction of the JA signalling pathway triggers host responses against phytophagous insects (Erb et al., 2012). Here we report the increased induction of two JA responsive genes (*VvVSP2* and *VvPDF1*) associated to JA defence mechanisms during the *D. vitifoliae* probing phase. The question rises, how *D. vitifoliae* establish the compatible interaction despite potentially induced JA defence genes? To mention two possibilities, insect released effectors (Giron et al., 2016) as well as proteases and peptidases within insect guts (Jongsma and Beekwilder, 2011) have been shown to degrade plant defensive proteins allowing compatible host-parasite interactions. In the future targeted (e.g. effector based) research is needed to elucidate how *D. vitifoliae* and other gall-inducing phylloxerids encounter activated host defences in plant tissues in order to establish the compatible host-parasite interaction in particular during larval probing.

### 4.2. JA biosynthesis and response during root gall formation

Although the sensitivity of *D. vitifoliae* root populations against activated JA signalling cascades in *Vitis* spp. has been observed previously (Omer et al., 2000), so far no study targets temporal alterations of the JA signalling pathway along *D. vitifoliae* root gall formation. We report
a transcriptional shift from induced JA marker genes during probing (14 hai) to transcriptionally reduced JA marker genes during gall formation (24–48 hai and 5–14 dai) when the larvae becomes sedentary. This observation includes the transcriptional reduction of three JA biosynthetic genes (VviAOS, VviOPR3 and VviJAR1) involved in the stepwise transformation of the JA precursor cisOPDA to JA and later downstream of JA-Ile (Wasternack and Hause, 2013). Consistently we observe higher levels of cisOPDA in parallel with comparably lower levels of JA and JA-Ile at different root gall stages, indicating a disturbed JA biosynthesis in D. vitifoliae root galls at this conversion step. A similar mechanism has been observed for piercing-sucking mealybugs (Phenacoccus solenopsis) feeding on tomato (Lyopersicon esculentum cv. Moneymaker) showing that insect feeding decreased JA concentrations in leaves as part of the aphid’s infestation strategy to prevent JA host responses (Zhang et al., 2015). In contrast to our data genes associated to JA biosynthesis (e.g. VviUMT, VviAOCS) and signaling (VviJAZ28) are induced in D. vitifoliae leaf galls on V. riparia (Nabity et al., 2013), suggesting the involvement of different physiological mechanisms that are depend on the host tissue.

Presence of JA-Ile in physiologically active plant tissue is reported to trigger JA responsive genes. Here we observe that significantly elevated JA-Ile levels detected in L2 and L4-A galls seem to be insufficient to trigger a JA based host response shown by unchanged expression levels of VviVSP2 and VviPDF1 in D. vitifoliae root galls. Similar studies show that increased JA-Ile levels caused by the phytophagous mites Tetranychus urticae and Tetranychus evansi do not induce JA defence responsive genes in leaf tissue of Lyopersicon esculentum cv. Castlemart (Alba et al., 2015). Additionally, physiological JA induction experiments with Arabidopsis thaliana show that JA responses are suppressed downstream of JA-Ile (Van Der Does et al., 2013). On the basis of our data we postulate two scenarios: either detected JA-Ile levels are insufficient to induce the analysed JA responsive genes in D. vitifoliae root galls; and/or JA host responses are transcriptionally suppressed downstream of JA-Ile. However, the presented descriptive experimental approach does not target this specific aspect of JA response regulation, therefore this questions remains open. At the current stage we are not sure about the source of elevated JA-Ile in D. vitifoliae L2 and L4-A root galls, which could have been influenced by the root gall cultivation system, sampling strategy or other physiological mechanisms in the D. vitifoliae - host interaction not targeted by our approach.

4.3. SA accumulates in D. vitifoliae root galls

So far, resistance mechanisms against plant-galling insects have been associated with an increased SA accumulation in plant tissue resulting in the activation of SA based host responses such as PR-gene expression and hypersensitive cell death. Here we report the compatible host-parasite interaction between root-galling D. vitifoliae and Vitis spp. despite elevated levels of SA and the transcriptionally induced SA responsive genes VviPR2/5. Although the SA biosynthetic pathways is not fully understood, recent studies report that more than 90% of the SA in plants is biosynthesized via the isochorismate pathway, with ICS1 being the central isoenzyme for SA production upon biotic stress perception (Chen et al., 2009). Our data fails to correlate remarkably increased SA levels up to 2303.52 pmol gFW⁻¹ with the gene expression levels of the primary SA biosynthesis gene VviICS1. Possible explanations could be the involvement of alternative SA biosynthetic routes e.g. via isochorismate pyruvate lyase (Chen et al., 2009), VviICS2 or the induction of VviICS1 during a time point not specifically tested in our experiment. Alternatively SA might have been imported from other parts of the plant. Free SA and salicylic acid O-β-glucoside (SAG) are shown to be mobile SA triggers within the plant phloem, however so far no convincing evidence provides their functions as efficient systemic SA signals in plant tissues (Bonnemain et al., 2013). Exogenous SA supplied by the insect or mutualistic microorganisms targeting the induction of the SA signalling pathway may be considered to explain the increased SA levels in root galls. Bacterial species have been previously shown to facilitate the establishment and maintenance of compatible host-aphid interactions (Moran and Baumann, 2000). However, currently, no obligate endosymbiont is found impacting the D. vitifoliae - Vitis spp. interaction (Vorwerk et al., 2007). Thus the origin of the elevated SA levels in D. vitifoliae root galls needs further evidence.

4.4. Possible roles of elevated SA levels in root galls

The induction of the SA signalling pathway triggers antimicrobial SA responses, preventing compatible host-parasite interactions in grapevine tissue under phytopathogen attack (e.g. Renault et al., 2015). We show that increased SA levels correspond with induced expression of SA defensive genes (VviPR2/5) in D. vitifoliae root galls 5–14 dai. Both, VviPR2 and VviPR5 encode for pathogenesis-related proteins with known antimicrobial properties against root infesting pathogens (e.g. Molinari et al., 2014). In support of this hypothesis, an earlier study detects the induction of at least three WRKY transcription factors (VviWRKY48/65/75), associated to SA signaling and SA host responses during the compatible interaction (Du et al., 2014). It might be interesting to investigate if D. vitifoliae larvae induce antimicrobial defences in root galls to protect the root gall and in not infested root tips (NIRTs) against secondary microbial infections indicated by induced expression levels of VviPR2 and VviPR5 in this study. A previous study shows that larval secretions of the phytophagous Colorado potato beetle (Leptinotarsa decemlineata) trigger the transcriptional induction of PR-genes in the plant tissue thought to protect the feeding site against microbial infections (Chung et al., 2013). In addition to SA and JA other phytohormones such as ethylene, abscisic acid, auxins, cytokinins and gibberellins co-regulate SA/JA mediated host responses in plant galls (Tooker et al., 2008). So far, evidence for the involvement of other phytohormones in the D. vitifoliae root gall formation is given by induced genes associated to ethylene, gibberellin and auxin signaling (Du et al., 2014). Although our descriptive data cannot provide functional evidence, we suggest that the detected transcriptional reduction of the JA marker genes in D. vitifoliae root galls corresponds to a suppression of the JA signalling pathway; thus representing an important factor for the compatible D. vitifoliae – Vitis spp. interaction. Likewise increased SA levels and subsequent induction of SA signaling and responsive marker genes correspond to the induction of the SA signalling pathway in D. vitifoliae root galls. Recent studies show that phytophagous insect species circumvent the activation of JA host response mechanisms via the induction of the SA signalling pathway (e.g. Zarate et al., 2007; Zhang et al., 2015). Whether the SA/JA antagonistic crosstalk plays a role for the compatible D. vitifoliae gall formation, remains to be further investigated.

5. Conclusion

In conclusion, we present the first report of manipulated SA and JA signalling pathways in root tissue under the control of a galling insect. The study demonstrates the induction of the JA signalling pathway during D. vitifoliae root tip probing. The subsequent suppression of the JA signaling pathway during gall formation represents an important factor for D. vitifoliae feeding. Additionally we report a strong induction of the SA signalling pathway. On the basis of the current knowledge we provide two scenarios as possible explanations for the observed changes of the SA/JA signalling pathways in D. vitifoliae root galls: (1) elevated SA levels and the subsequent activation of the host SA response mechanisms could enhance the defence status of the root system against phytopathogenic microorganisms in the soil thereby protecting D. vitifoliae’s feeding tissue. (2) Simultaneous manipulation of the two signal pathways indicate the possibility of SA/JA antagonistic crosstalk mechanism in root galls resulting in a suppression of JA host defence responses. Future biomolecular research should target both mentioned aspects as well as elucidate the source of SA in D. vitifoliae root galls in
order to further increase our knowledge on this specific compatible host-parasite interaction belowground.

Contributions


Funding

This work was supported by a DOC research fellowship for MW Eitle provided by the Austrian Academy of Sciences (OAW).

Acknowledgements

This work partly contributes to the thesis of S. Aberer M. Sc. and Dr. M. W. Eitle. We greatly acknowledge Prof. Dr. Kevin Powell (CSU, Wagga Wagga, Australia), Prof. Dr. Joseph Strauss (BOKU Vienna, Austria), Dr. Krzysztof Wieczorek (BOKU Vienna, Austria) for discussions and helpful comments to the manuscript interest and Verena Dockner M.Sc. for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.024.

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PMCID: PMC2967469


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