

Plant-to-plant defence signalling in barley

Alessandro Brambilla

Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München zur Erlangung eines
Doktors der Naturwissenschaften (Dr. rer. nat.)
genehmigten Dissertation.

Vorsitz: Prof. Dr. Ralph Hückelhoven

Prüfer*innen der Dissertation:

1. Prof. Dr. Jörg Durner
2. Prof. Dr. Wilfried Schwab
3. Prof. Dr. Anna Corina Vlot-Schuster

Die Dissertation wurde am 21.07.2023 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 11.12.2023 angenommen.

Summary

A wide range of microbial pathogens can infect plants and alter their health status. To protect themselves from such a threat, plants have evolved complex biochemical responses. Systemic acquired resistance (SAR), for instance, is a priming-based strategy that helps plants to fend off (hemi-) biotrophic microorganisms. In such scenario, as a pathogen attacks parts of plant tissues, a signal is transmitted to uninfected parts of the plant, making them ready to face subsequent attacks.

SAR is a well-studied phenomenon in dicotyledonous plants such as *Arabidopsis thaliana*. Nevertheless, research on SAR in monocotyledonous plants is scarce. In agriculture, monocots such as barley, wheat, rice, maize are considered to be staple crops for humans and livestock. It is thus fundamental, to understand the defence response of these plant species, to be able to protect these crops with more sustainable plant protection strategies.

Barley (*Hordeum vulgare* L.) has been used in this work as a model plant. Its ability to activate SAR-like resistance against the biotrophic fungus *Blumeria graminis* f. sp. *hordei* has been assessed. Specifically, we investigated the role of pipecolic acid (Pip), a well-known molecule that takes part in SAR in dicots, in barley. Pip levels are regulated by the aminotransferase ALD1, thus, we generated *ald1* CRISPR-Cas9 mutants. Interestingly, *ald1* plants displayed reduced Pip levels and a disrupted SAR network. Moreover, at genomic level, the regulation of genes that are involved in plant defence responses such as *HISTONE DEACETYLASE 2* (*HvHDA2*) and *TETRATRICOPEPTIDE REPEAT-LIKE superfamily protein* (*HvTPL*) was altered.

In addition, we characterised the volatile organic compound (VOC) emission from infected barley plants. Within the spectrum of VOCs that are emitted by barley after infection, nonanal and β -ionone were two of the most abundant compounds. Exposure of naïve barley plants to these VOCs bolstered defence responses against a subsequent infection with *Bgh*. At gene level, the aforementioned genes, *HvHDA2* and *HvTPL* were up-regulated after the exposure. Nonetheless, *ald1* plants showed also a jeopardised VOC emission, with nonanal being absent from the list of VOCs that are emitted after infection in wild type barley.

With these results, we achieved a clarification of the SAR network in monocots such as barley. In detail, thanks to the generation of our *ald1* CRISPR-Cas9 mutants, we highlighted the importance of Pip in monocots and how deficiencies in the production of Pip lead to altered SAR pathways, reduced VOC emission and a different gene expression. Furthermore, we

enlightened the ability of barley to communicate with neighbouring plants via volatile cues which takes our results close to be a useful tool in eco-friendly crop protection techniques.

Zusammenfassung

Ein breites Spektrum an mikrobiellen Krankheitserregern kann Pflanzen infizieren und ihren Gesundheitszustand verändern. Um sich vor einer solchen Bedrohung zu schützen, haben Pflanzen komplexe biochemische Reaktionen entwickelt. Die Systemic acquired resistance (SAR) ist beispielsweise eine auf dem Priming basierende Strategie, die Pflanzen hilft, (hemi-) biotrophe Mikroorganismen abzuwehren. Wenn ein Krankheitserreger Teile des Pflanzengewebes angreift, wird ein Signal an die nicht infizierten Teile der Pflanze übertragen, so dass diese für weitere Angriffe gewappnet sind.

SAR ist ein gut untersuchtes Phänomen bei zweikeimblättrigen Pflanzen wie *Arabidopsis thaliana*. Dennoch ist die Forschung zu SAR bei einkeimblättrigen Pflanzen bislang noch sehr begrenzt. In der Landwirtschaft gelten einkeimblättrige Pflanzen wie Gerste, Weizen, Reis und Mais als Grundnahrungsmittel für Mensch und Vieh. Es ist daher von grundlegender Bedeutung, die Abwehrreaktion dieser Pflanzenarten zu verstehen, um diese Kulturen mit nachhaltigeren Pflanzenschutzstrategien schützen zu können.

Gerste (*Hordeum vulgare* L.) wurde in dieser Arbeit als Modellpflanze verwendet. Ihre Fähigkeit, eine SAR-ähnliche Resistenz gegen den biotrophen Pilz *Blumeria graminis* f. sp. *hordei* (Bgh) zu aktivieren, wurde bewertet. Insbesondere haben wir die Rolle der Pipecolinsäure (Pip), eines bekannten Moleküls, das in zweikeimblättrigen Pflanzen an SAR beteiligt ist, in Gerste untersucht. Der Pip-Spiegel wird durch die Aminotransferase ALD1 reguliert, weshalb wir *ald1* CRISPR-Cas9-Mutanten erzeugten. Interessanterweise wiesen *ald1*-Pflanzen reduzierte Pip-Werte und ein gestörtes SAR-Netzwerk auf. Darüber hinaus war auf genomischer Ebene die Regulierung von Genen, die an pflanzlichen Abwehrreaktionen beteiligt sind, wie *HISTONE DEACETYLASE 2* (*HvHDA2*) und *TETRATRICOPEPTIDE REPEAT-LIKE superfamily protein* (*HvTPL*), verändert.

Darüber hinaus haben wir die Emission flüchtiger organischer Verbindungen (VOC) aus infizierten Gerstenpflanzen charakterisiert. Innerhalb des Spektrums der flüchtigen organischen Verbindungen, die von der Gerste nach der Infektion emittiert werden, waren Nonanal und β -Ionon zwei der am häufigsten vorkommenden Verbindungen. Die Exposition naiver Gerstenpflanzen gegenüber diesen VOCs verstärkte die Abwehrreaktionen gegen eine anschließende Infektion mit *Bgh*. Auf genetischer Ebene wurden die oben genannten Gene, *HvHDA2* und *HvTPL*, nach der Exposition hochreguliert. Nichtsdestotrotz zeigten *ald1*-

Pflanzen auch eine beeinträchtigte VOC-Emission, wobei Nonanal in der Liste der VOCs, die nach einer Infektion in Wildtyp-Gerste emittiert werden, nicht vorkommt.

Mit diesen Ergebnissen haben wir eine Klärung des SAR-Netzwerks in Monokotyledonen wie der Gerste erreicht. Dank der Erzeugung unserer *ald1*-CRISPR-Cas9-Mutanten konnten wir die Bedeutung von Pip auch in Monokotyledonen aufzeigen. Wir zeigten zudem, wie Defizite in der Pip-Produktion zu veränderten SAR-Wegen, reduzierter VOC-Emission und einer anderen Genexpression führen. Darüber hinaus haben wir die Fähigkeit der Gerste aufgezeigt, mit benachbarten Pflanzen über flüchtige Signale zu kommunizieren. Unsere Ergebnisse könnten daher einen wichtigen Beitrag zu umweltfreundlichen Pflanzenschutztechniken leisten.

List of publications

Research paper I

Alessandro Brambilla, Anna Sommer, Andrea Ghirardo, Marion Wenig, Claudia Knappe, Baris Weber, Melissa Amesmaier, Miriam Lenk, Jörg-Peter Schnitzler, and A. Corina Vlot. (2021). Immunity-associated volatile emissions of β -ionone and nonanal propagate defence responses in neighbouring barley plants. *Journal of Experimental Botany*, Vol. 73, No. 2 pp. 615–630.

Research paper II

Alessandro Brambilla, Miriam Lenk, Andrea Ghirardo, Laura Eccleston, Claudia Knappe, Baris Weber, Birgit Lange, Jafargholi Imani, Anton R. Schäffner, Jörg-Peter Schnitzler, A. Corina Vlot. (2023). Pipecolic acid drives systemic acquired resistance and impacts nonanal emission in plant-to-plant interactions of barley. *Journal of Experimental Botany*.

Review I

A. Corina Vlot, Jennifer H. Sales, Miriam Lenk, Kornelia Bauer, Alessandro Brambilla, Anna Sommer, Yuanyuan Chen, Marion Wenig, Shahrar Nayem. (2021). Systemic propagation of immunity in plants. *New Phytologist*, Vol. 223, No. 3 pp. 1234-1250.

Review II

Laura Eccleston, Alessandro Brambilla, A. Corina Vlot. (2022). New molecules in plant defence against pathogens. *Essays in biochemistry*, Vol. 66, No. 5 pp. 683–693.

All the listed publications were peer-reviewed

Contents

1. Introduction	9
1.1 Plant early defence responses	9
1.2 The role of ALD1 and pipecolic acid	12
1.3 Volatile organic compounds as airborne cues	13
1.3.1 Monoterpenes	15
1.3.2 Apocarotenoids and β -ionone	15
1.3.3 Aldehydes and nonanal	16
1.4 Sustainable crop protection techniques	17
1.6 Research aims and methods	19
1.6.1 VOC measurements in <i>Psj</i> -infected compared to mock-treated barley plants	19
1.6.2. Resistance induction by VOC exposure	20
1.6.3 Plant-to-plant defence experiments, including relevant barley CRISPR-Cas mutants	20
1.6.4 Transcript profiling to characterise downstream resistance mechanisms	21
2. Results and embedded publications	22
2.1 Publication I: Immunity-associated volatile emissions of β -ionone and nonanal propagate defence responses in neighbouring barley plants	22
2.2 Publication II: Pipecolic acid synthesis is required for systemic acquired resistance and plant-to-plant-induced immunity in barley	23
2.3 Review I: Systemic propagation of immunity in plants	24
2.4 Review II: New molecules in plant defence against pathogens	25
3. Discussion	26
3.1 SAR induction enhances resistance against <i>Blumeria graminis</i> f. sp. <i>hordei</i> in neighbouring plants	26
3.2 Gene regulation in receiver plants	29
3.3 Exposure of barley plants to nonanal and β -ionone enhances defence responses	32
3.4 <i>Hvald1</i> barley plants show reduced Pip levels	34
3.5 <i>Hvald1</i> barley plants are SAR-deficient	35
3.6 Role of <i>HVALD1</i> in plant-to-plant (PTP) defence propagation	37
3.7 Nonanal is a key VOC in PTP barley emissions	41
4. Conclusion	42
5. References	43
6. Acknowledgements	51

1. Introduction

1.1 Plant early defence responses

Plants react to changes in the surrounding environment with specific biochemical responses. Perturbations of their health status activate a cascade of reactions that leads to the elicitation of different phytohormone pathways that help the plant to face the upcoming challenge and optimise their fitness (Erb, 2019; Pieterse et al., 2012). When a plant is threatened with biotic stress caused by pathogens, phytohormones such as salicylic acid (SA), jasmonic acid (JA), or ethylene (ET) are produced (Glazebrook, 2005; Pieterse et al., 2012; Vlot et al., 2009). SA-dependent defence mechanisms are essential in the fight against hemi- and biotrophic pathogens, organisms that thrive on living host tissues. JA- and ET-based responses are, on the contrary, activated when the plant is challenged by a necrotrophic pathogen, that prosper on dead or dying tissues. As the pathogen attacks the host locally, systemic signals generate from the infected area and travel throughout the plant promoting a state of alert in the systemic, uninfected tissues, preparing the plant to a subsequent secondary infection. This phenomenon is also known as induced or systemic resistance, which similarly to ‘priming’, translates into a sort of heightened defence status ready to face upcoming challenges (Conrath et al., 2015).

As briefly mentioned before, hemi- and biotrophic microorganisms activate SA-dependent pathways (Vlot et al., 2009). Firstly, pattern recognition receptors, present on the plant cell surface, allow plants to distinguish pathogen-/microbe-associated molecular patterns (P/MAMPs) present on the surface of pathogens. This enhances the so-called PAMP-triggered immunity (PTI), a primordial resistance that limits the growth of the pathogen (Jones & Dangl, 2006). Nevertheless, some pathogens might make it through this first obstacle and enter the cytoplasm of host cells. By doing so pathogen effectors inhibit PTI, and pathogen growth is reactivated. Yet, instances in which pathogen effectors activate host RESISTANCE (R) protein-dependent responses, lead to what is known as this induces a relatively strong defence response, effector-triggered immunity (ETI), a stronger response by the plant that ends up with the programmed death of the infected and neighbouring tissues with, as a result, a restricted diffusion of the pathogen (Jones & Dangl, 2006). Both PTI and ETI depend on SA and elicit a systemic, SA-dependent defence reaction called systemic acquired resistance (SAR) (Conrath et al., 2015; Spoel & Dong, 2012; Vlot et al., 2009). SAR works as a long-lasting resistance against a wide variety of (hemi-)biotrophic microorganisms. Laboratory experiments showed that the SAR state can stay active on average ~3–10 days but, potentially, it can also be transmitted to the next generation (trans-generational SAR) (Luna et al., 2012).

SAR is a well-known biochemical defence response that is mostly studied in dicots. As mentioned before, when a hemi- or biotrophic pathogen infects local plant tissues, a signal is sent to distal healthy tissues to prepare them to the possible coming threat. This signal depends mainly on two biochemical pathways that can act independently or in together: the SA- and the Pip-dependent response (Vlot et al., 2020). Few studies published similar results in monocots such as wheat, maize, barley and banana (Balmer et al., 2013; Dey et al., 2014; Wu et al., 2013; Y. Yang et al., 2013). Nevertheless, the whole defence mechanism that is activated in monocots after an infection is yet to be clarified.

In *Arabidopsis* and other dicots including tobacco (*Nicotiana benthamiana*) and potato (*Solanum tuberosum*), the production and accumulation of SA increases in local and systemic tissues after a pathogen attack (Manosalva et al., 2010; Vlot et al., 2008). However, multiple studies have demonstrated that the apoplastic movement of SA alone, would not be enough to enhance SAR in plant tissues that are far from the infection site. Far more mobile than SA itself, is its methylated form methyl salicylate (MeSA) (Park et al., 2007). SA is converted in its mobile form MeSA by the *SA methyltransferase*. Once MeSA has reached the uninfected systemic tissues, MeSA is converted again into SA by the methyl esterase SA-binding protein 2. The homeostasis between SA and MeSA levels is then maintained in cells by these two enzymes (Park et al., 2007; Vlot et al., 2021) (Fig. 1).

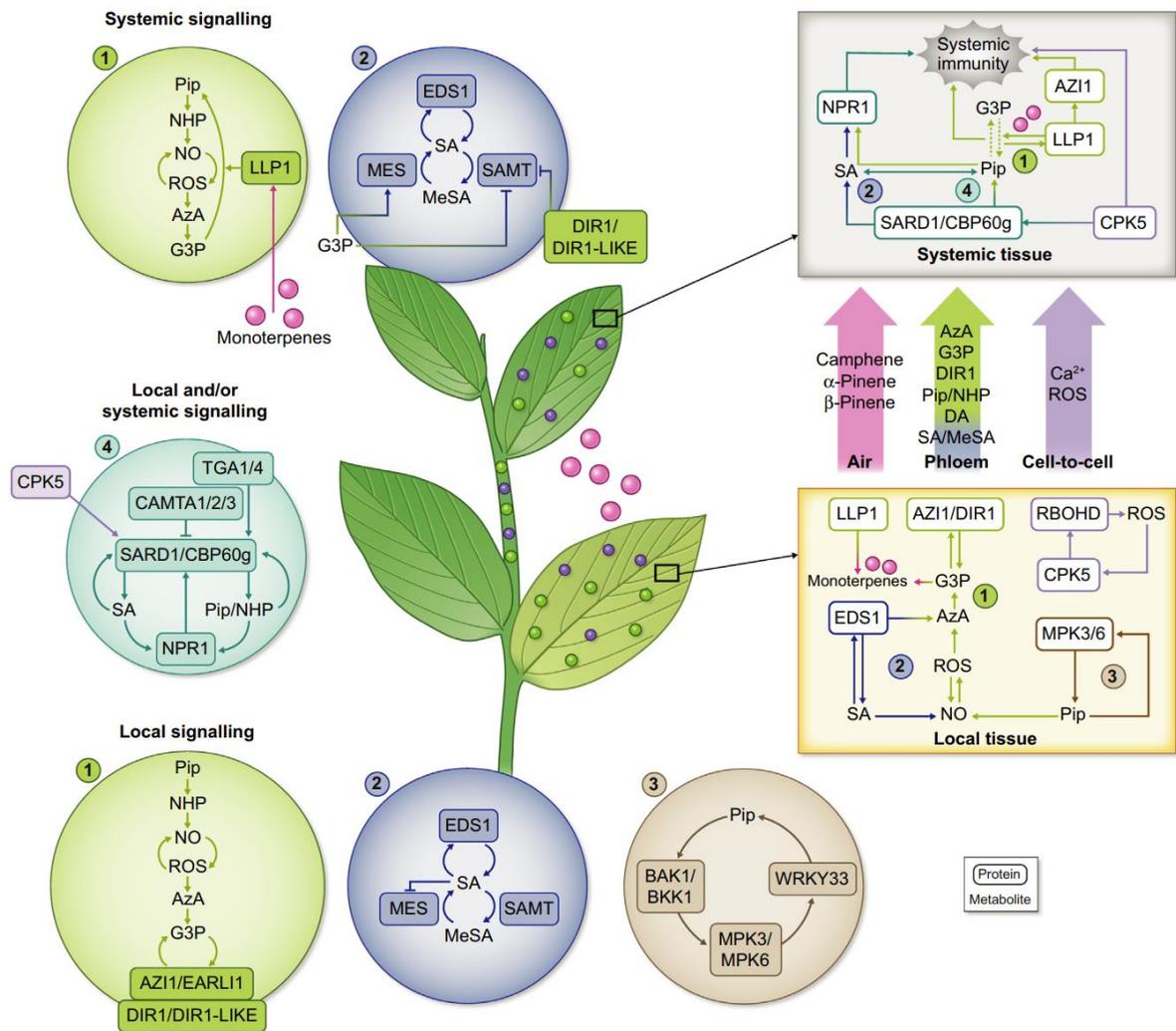


Figure 1 Schematic overview of molecular mechanisms of systemic acquired resistance (SAR). Signaling cascades in the local infected leaf are summarised in the lower yellow box (on right), signaling cascades in the systemic leaf are shown in the upper grey box (on right). Color-coded bubbles with numbers correspond to the larger bubbles on the left with the same color and number, showing the signaling pathways in more detail. Compounds transported between local and systemic tissue are depicted in arrows and color-coded with the respective pathways. Abbreviations: AzA, azelaic acid; AZI1, AZELAIC ACIDINDUCED 1; BAK1, BRI1-ASSOCIATED RECEPTOR KINASE; BKK1, BAK1-LIKE 1; CAMTA, CALMODULIN BINDING TRANSCRIPTION FACTOR; CBP60g, CALMODULIN BINDING PROTEIN 60g; CPK5, CALCIUM-DEPENDENT PROTEIN KINASE 5; DA, dehydroabietinal; DIR1, DEFECTIVE IN INDUCED RESISTANCE 1; EARL1, EARLY ARABIDOPSIS ALUMINUM INDUCED 1; EDS1, ENHANCED DISEASE SUSCEPTIBILITY 1; G3P, glycerol-3-phosphate; LLP1, LEGUME LECTIN-LIKE PROTEIN 1; MES, methyl esterase; MeSA, methyl salicylate; MPK, MITOGEN-ACTIVATED PROTEIN KINASE; NHP, N-hydroxy-pipecolic acid; NO, nitric oxide; NPR1, NON-EXPRESSION OF PR GENES 1; Pip, pipecolic acid; RBOHD, RESPIRATORY BURST OXIDASE HOMOLOG D; ROS, reactive oxygen species; SA, salicylic acid; SAMT, SA methyltransferase; SARD1, SAR-DEFICIENT 1; TGA, TGACG SEQUENCE-SPECIFIC BINDING PROTEIN (Vlot et al., 2021).

1.2 The role of ALD1 and pipecolic acid

Although SA and MeSA might be the most known molecules that take part in the SAR network, they surely are not the only ones. The complexity of SAR-like defence responses in plants, are as fascinating as sophisticated to study. Other molecules that are considered to be involved in SAR are Pip and its bio-active derivative *N*-hydroxy-Pip (NHP), together with glycerol-3-phosphate (G3P), azelaic acid (AzA), reactive oxygen species (ROS), calcium (Ca²⁺), nitric oxide (NO) and the proteins AZELAIC ACID INDUCED 1 (AZI1), LEGUME LECTINE LIKE PROTEIN 1 (LLP1), DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1) and volatile organic compounds (Vlot et al., 2021; Wenig et al., 2019). All these molecules work together and support the SA-based defence pathway to confer the plant immunity against the infection (Fig. 2).

Following an infection caused by a pathogen, Pip, a non-proteinogenic amino acid is synthesised from L-lysine by the aminotransferase AGD2-like Defense Response Protein 1 (ALD1). Afterwards, FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1) converts Pip into its bioactive derivative *N*-hydroxy-pipecolic acid (NHP) (Chen et al., 2018; Yildiz et al., 2021). Studies carried out by Gao and co-worker (2015) demonstrated that SA and Pip can work as two independent pathways in the defence response of a plant. Nonetheless, recently has been demonstrated that SA and Pip/NHP reinforce each other's levels via a positive feedback loop favoured by a set of overlapping transcription factors (Vlot et al., 2021). In this cascade of reactions, the production of Pip is followed by a feedback loop between nitric oxide (NO) and reactive oxygen species (ROS). This drives the production of azelaic acid (AzA) and finally glycerol-3-phosphate (G3P), which again, stimulates increased levels of Pip (Wang et al., 2018). Previous studies on *Arabidopsis* showed that mutations of the gene *ALD1* causes a reduced accumulation of Pip, which is takes to dysfunctional SAR (Návarová et al., 2013; Wenig et al., 2019). Nevertheless, in the same plant model, irrigation with exogenous Pip resume immunity in *ald1* mutant plants (Návarová et al., 2013). In monocots like barley, Pip-drench also induced defence mechanisms against an infection with *Blumeria graminis* f. sp. *hordei* (*Bgh*), causal pathogen of barley powdery mildew (Lenk et al., 2019).

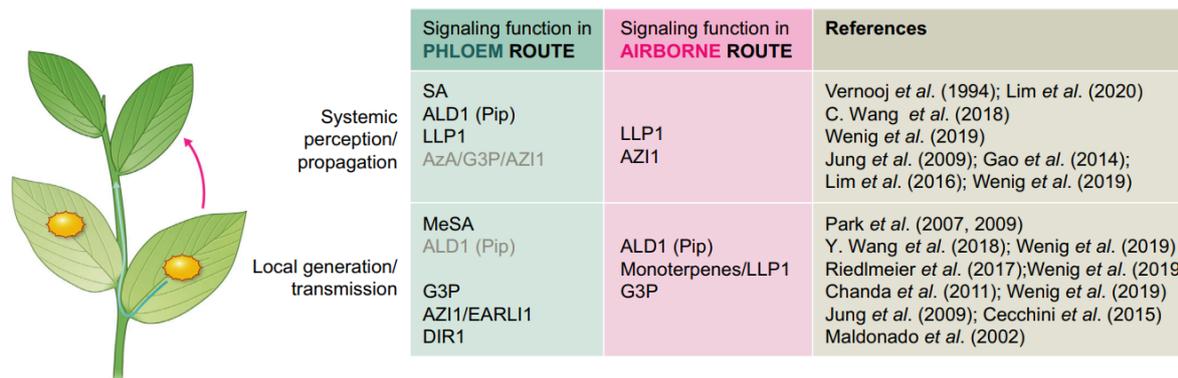


Figure 2 Signaling functions of systemic acquired resistance (SAR) signaling components in local SAR signal generation/transmission and systemic SAR signal perception/propagation. Signaling components are listed in groups of potentially co-operating signals and assigned to phloem-mediated (green arrow) and airborne (pink arrow) routes. The assignment of spatially distinct roles of signaling components in SAR is based on data from grafting, petiole exudate, and plant-to-plant communication experiments from literature sources cited in the column on the right. Gray letters indicate indirect evidence. Abbreviations: ALD1, AGD2-like DEFENSE RESPONSE PROTEIN1; AzA, azelaic acid; AZI1, AZELAIC ACID INDUCED 1; DIR1, DEFECTIVE IN INDUCED RESISTANCE 1; EARLI1, EARLY ARABIDOPSIS ALUMINUM INDUCED 1; G3P, glycerol-3-phosphate; LLP1, LEGUME LECTIN-LIKE PROTEIN 1; MeSA, methyl salicylate; Pip, pipecolic acid; SA, salicylic acid (Vlot *et al.*, 2021).

1.3 Volatile organic compounds as airborne cues

Volatile organic compounds (VOCs) are low molecular weight compounds that easily evaporate at room temperature (Mofikoya *et al.*, 2019). Plants generate these compounds constitutively or after facing a biotic or abiotic stress (Brilli *et al.*, 2019; Loreto & Schnitzler, 2010). Several works (Baldwin & Schultz, 1983; Liu & Brettell, 2019; Markovic *et al.*, 2019b; Piesik *et al.*, 2013), show that when an insect is feeding on parts of a plant or as a pathogen infects a tissue, plants that are in the proximity of the affected neighbours respond with enhanced/primed defences to future insect/pathogen attacks. Riedlmeier *et al.* (2017) demonstrated that VOCs emitted by SAR-induced plants are eavesdropped as defence cues by neighbouring plants. In details, experiments on SAR-induced *Arabidopsis* inoculated with *Pseudomonas syringae* pv. *tomato* (*Pst*) expressing the effector *AvrRpm1* explained that monoterpenes, such as α -pinene, β -pinene, and camphene, work as infochemicals.

Plant-to-plant (PTP) interaction happens when plants share information with each other (Baldwin & Schultz, 1983). Studies with different plant species confirmed that PTP occurs between plants of the same or different species (Frank *et al.*, 2021a; Markovic *et al.*, 2019a; Moreno *et al.*, 2020; Ninkovic *et al.*, 2019; Riedlmeier *et al.*, 2017). As a plant is challenged by either biotic or abiotic stress, emits a blend of VOCs in the environment. These molecules can

be intercepted by neighbouring plants, but also by distal parts of the same plant, which gives them time to promptly activate a response to the possible upcoming change (Bouwmeester et al., 2019; Brillì et al., 2019; Erb, 2018). The mechanisms through which these airborne cues are perceived and interpreted is still unclear (Bouwmeester et al., 2019). However, new findings elucidated the role of LEGUME LECTIN-LIKE PROTEIN 1 (LLP1) in signalling events that follow the production of volatiles, and its involvement in establishing SAR (Wenig et al., 2019). In plants that eavesdrop VOCs coming from infected plants, LLP1 drives a positive feedback loop with Pip and G3P to enhance new VOC biosynthesis and emission, inducing the propagation of a PTP interaction moving between neighbouring plants (Fig. 3) (Wenig et al., 2019).

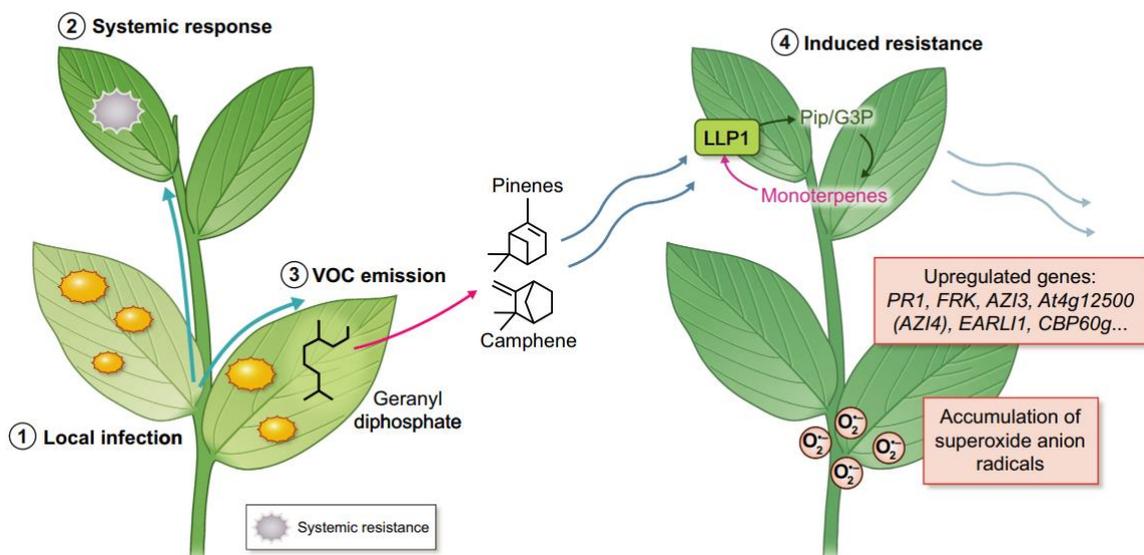


Figure 3 Plant-to-plant propagation of systemic acquired resistance (SAR). Following a pathogen inoculation on a local leaf (1), plants activate a cascade of reactions that trigger a systemic response (2) in distal tissues. Riedlmeier et al. (2017) showed that infected plants emit volatile organic compounds (VOCs) such as pinenes and camphene (3) that are synthesised from a geranyl diphosphate precursor. These VOCs are recognised as defence cues through LEGUME LECTIN-LIKE PROTEIN1 (LLP1; 4) which activates a positive feedback loop, including pipecolic acid (Pip) and glycerol-3-phosphate (G3P), subsequently leading to the production and emission of further volatile defence cues. Headspace exposure of Arabidopsis to pinenes induces reactive oxygen species (ROS) accumulation and upregulates a series of SAR-associated genes, including *PATHOGENESIS-RELATED 1 (PR1)*, *FLG22-INDUCED RECEPTOR-LIKE KINASE1 (FRK1)* and the *AZII* paralogs *AZI3* and *At4g12500 (AZI4)*, *EARLY ABIDOPSIS ALUMINUM INDUCED 1 (EARLI1)*, *CALMODULIN BINDING PROTEIN 60g (CBP60g)*, and more (Vlot et al., 2021).

1.3.1 Monoterpenes

The group of terpenes is vast and several of these VOCs participate in various biological processes in plants (Brilli et al., 2019; Brosset & Blande, 2022; Erb, 2018; Rosenkranz et al., 2021). The initial part of the biosynthesis of terpenes takes place in the plastids. The MEP (methylerythritol phosphate) pathway, that is responsible for the production of these volatiles (Dudareva et al., 2013; Tholl, 2006) begins with the production of 1-deoxy-D-xylulose 5-phosphate that comes from the condensation of pyruvate and glyceraldehyde 3-phosphate. Afterwards, a series of enzymatic steps leads to the biosynthesis of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). IPP and DMAPP either condense and generate geranyl pyrophosphate (GPP) (Fig. 3), precursor of monoterpenes (C₁₀) or induce the production of geranylgeranyl pyrophosphate (GGPP), precursor of diterpenes (C₂₀) and carotenoids (C₄₀) (Barja et al., 2021; Dudareva et al., 2013; Sandmann, 2021). In the specific case of SAR-induction, monoterpenes such as camphene and pinenes are emitted in *Arabidopsis* (Riedlmeier et al., 2017). This group of VOCs seems to be fundamental in dicots for SAR and PTP establishment (Riedlmeier et al., 2017; Wenig et al., 2019). However, we elucidate in this work that other groups of volatile compounds such as apocarotenoids and aldehydes are more important when the plant is a monocot like barley.

1.3.2 Apocarotenoids and β -ionone

Apocarotenoids are a class of metabolites that are involved a wide spectrum of functions in plants (Felemban et al., 2019a; Moreno et al., 2020; Murata et al., 2019; Paparella et al., 2021). They are produced from the cleavage of carotenoids, molecules that are mostly known to be give the typical yellow, orange, and red colour to various flowers, fruits and vegetables (Pu et al., 2020) and, concomitantly, to allow absorption of specific wavelengths during the photosynthesis (Ruban, 2015; Sandmann, 2021). However, this group of molecules is also known for participating in plant defence against a considerable number of pests and pathogens (Brambilla et al., 2022; Murata et al., 2019). The early beginning of the biosynthetic pathway is shared with other signalling-related VOCs, including terpenes. Carotenoids start to differentiate from terpenes after the biosynthesis of GGPP and the subsequent production of 15-cis-phytoene, synthesised by PHYTOENE SYNTHASE (Moreno et al., 2020). 15-cis-phytoene goes through a series of desaturation and isomerisation reactions catalysed in plants by enzymes such as PHYTOENE DESATURASE, ζ -CAROTENE ISOMERASE, ζ -CAROTENE DESATURASE, and CAROTENOID ISOMERASE that lead to the formation of lycopene (Moreno et al., 2020). Lycopene, an acyclic carotenoid, can experience a cyclisation reaction by LYCOPENE β -CYCLASES (Moreno et al., 2020). When this event happens, β -

ionone rings are formed, and β -carotene is biosynthesised (Moreno et al., 2020). CAROTENOID CLEAVAGE DIOXYGENASEs (CCDs) then catalyse the conversion of β -carotene into apocarotenoids (Barja et al., 2021; Dudareva et al., 2013; Sandmann, 2021)(Fig. 4). In addition, the production of apocarotenoids such as β -cyclocitral and β -ionone can take place through nonenzymatic attack of β -carotene by ROS (D'Alessandro et al., 2019; Havaux, 2020; Moreno et al., 2020; Ramel, Birtic, Ginies, et al., 2012). Nine enzymes are known to be part of the CCD family in Arabidopsis (Felemban et al., 2019a). CCD1 and CCD4 are two enzymes that are responsible for the production of several volatile apocarotenoids in citrus (Citrus spp.) and Arabidopsis (Felemban et al., 2019a; Moreno et al., 2020; Murata et al., 2019). Apocarotenoids, such as β -ionone and β -cyclocitral, have been characterised in barley, grapevine, and Arabidopsis emissions following an infection with pathogenic microorganisms (Brambilla et al., 2022; Lazazzara et al., 2018; Sharifi et al., 2018).

1.3.3 Aldehydes and nonanal

A further group of plant defence-related VOCs includes fatty acid derivatives(Brambilla et al., 2022; Dudareva et al., 2013; Yi et al., 2009a). These molecules are biosynthesised through the lipoxygenase (LOX) pathway, that begins with the oxygenation of linoleic acid, an unsaturated fatty acid. This process is performed by two enzymes, 13-LOX and 9-LOX (Dudareva et al., 2013), respectively, oxidising linoleic acid into 13(S)-hydroperoxy linoleic acid or 9(S)-hydroperoxy linoleic acid. Later, 13(S)-hydroperoxy linoleic acid can be subject to a further oxidation by allene oxides (AO) to produce jasmonates and, following, methyl jasmonate (Chauvin et al., 2013). Alternatively, 13(S)-hydroperoxy linoleic acid is converted by 13-hydroperoxide lyase (13-HPL) into unsaturated aldehydes and alcohols that compose the group of the so-called Green Leaf Volatiles (GLVs) (Ameje et al., 2018). GLVs are known to be emitted directly after a mechanical damage and can function as airborne, plant-to-plant interaction cues (Engelberth, 2019; Scala et al., 2013). The unsaturated aldehyde trans-2-pentenal has been detected in grapevine VOC emissions and shown to inhibit symptoms of downy mildew (*Plasmopara viticola*) on exposed grapevine plants (Lazazzara et al., 2018). In the other branch of the LOX pathway, 9(S)-hydroperoxy linoleic acid is converted into aldehydes such as nonanal (Dudareva et al., 2013) (Fig. 4). The exposure of plants to nonanal results in priming or up-regulation of defence-related genes in lima bean (Yi et al., 2009b) and barley (Brambilla et al., 2022).

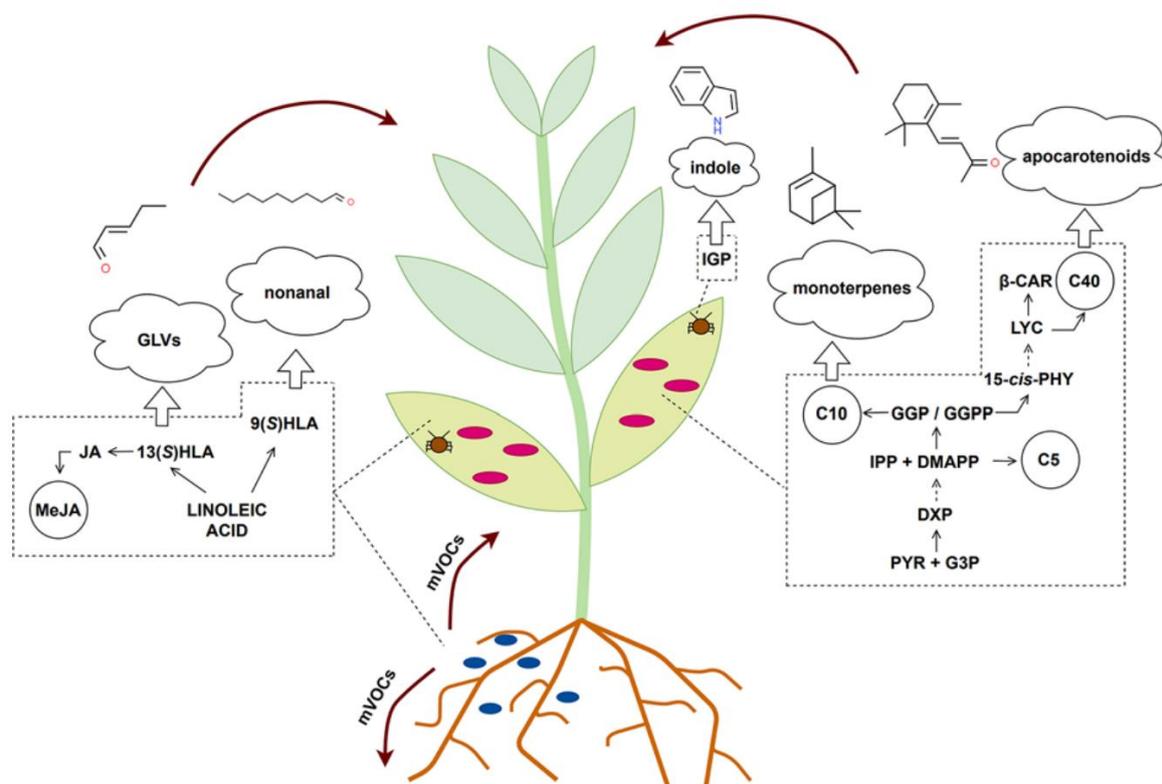


Figure 4 Biosynthesis of defence-related VOCs. Red spots on leaves represent infections by pathogenic microorganisms. On the right: biosynthetic pathway that leads to the production of monoterpenes and apocarotenoids. Abbreviations: β -CAR, β -carotene; 15-cis-PHY, 15-cis-phytoene; C5, hemiterpenes; C10, monoterpenes; C40, tetraterpenes (carotenoids); DMAPP, dimethylallyl pyrophosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; G3P, glyceraldehyde 3-phosphate; GGPP, geranylgeranyl pyrophosphate; GPP, geranyl pyrophosphate; IPP, isopentenyl pyrophosphate; LYC, lycopene; PYR, pyruvate. Biosynthetic pathway of indole: IGP, indole-3-glycerol phosphate. On the left: biosynthetic pathway that leads to the production of green leaf volatiles (GLVs) and other fatty acid derivatives. Abbreviations: 9(S)HLA, 9(S)-hydroperoxy linoleic acid; 13(S)HLA, 13(S)-hydroperoxy linoleic acid; JA, jasmonic acid; MeJA, methyl jasmonate; mVOCs, microbial volatile organic compounds. Red spots on leaves represent infections by pathogenic microorganisms; blue spots near roots represent beneficial microorganisms. Brown arrows indicate the effects of the VOCs on systemic defence (Eccleston et al., 2022).

1.4 Sustainable crop protection techniques

In the last decades, crop protection in agriculture has been managed predominantly with the application of synthetic agrochemicals. The use of these plant protection products often comes with a fast development of resistance mechanisms in pests and pathogens. In addition, the raising of a public awareness against the potential negative impact to ecosystems and human health, brought the demand for eco-friendly crop protection strategies to growth (Pickett, 2013; Pickett & Khan, 2016). The so called “Push-and-pull” technique is up-to-day one of the most successful management strategies that integrates the use VOCs agriculture (Brilli et al., 2019; Cook et al., 2007). Originally ideated by Pyke et al. (1987), the ‘push-and-pull’ strategy points

at making the main crop plants hard to locate, unattractive, or unsuitable to the pest. The aim of this technique is to use companion plants that emit either repellent (push) or attractive (pull) VOCs, and that can then redirect the pest away from the main, economically valuable host. This method has demonstrated successful contributes to pest reduction in the field (Pickett & Khan, 2016). SAR is known to be correlated with specific VOC emissions, that can induce a SAR-like state in neighbouring plants (Riedlmeier et al., 2017). Furthermore, plants that eavesdrop these cues can further propagate it, and potentially create a wave of information that can spread throughout natural or agricultural ecosystems (Wenig et al., 2019). Interestingly, this signal can be transmitted both within and between plant species: *Arabidopsis* can react to signals from spruce needles, and barley exposed to volatile cues from co-cultivated weed plants under both laboratory and field conditions showed enhanced resistance (Ninkovic et al., 2019; Riedlmeier et al., 2017). For these reasons, alternating high VOC emitters with crops, could represent an innovative system to protect plants from disease and pests (Fig. 5). As pest insects are easier to repel/attract than microbial pathogens, a feasible fight against bacteria, fungi and viruses in agriculture could happen through priming of SAR, a biochemical response that puts plants in a state of alertness and makes them ready to enhance defence upon a subsequent pathogen attack (Conrath et al., 2015; Martínez-Medina et al., 2017). Studies demonstrated that priming does not, or only in a minor part, negatively influence plant growth or yield (Van Hulten et al., 2006). Mechanistically, this might depend on epigenetic reprogramming, including histone modification of defence genes (Jaskiewicz et al., 2011). SAR signalling molecules such as Pip and Aza are well known priming agents and induce defence gene expression upon pathogen attack (Bernsdorff et al., 2016; Jung et al., 2009; Návárová et al., 2013). In *Arabidopsis*, Pip production is up-stream to VOC emissions that are recognised as defence cues by neighbouring plants (Wenig et al., 2019). This said, these SAR inducers can be applied in different forms, e.g. via Pip irrigation, VOC fumigation, or intercropping, in order to prime plant defences without negatively affecting the productivity. If incorporated together, SAR inducers and intercropping could represent a highly effective, complementary, and self-fortifying technique to prime the immune status of crop plants. Notably, the effectiveness of SAR and ISR in the field remains largely unclear. Also, the effectiveness of PGPR/F inoculants under field conditions appears to differ substantially from that in glasshouse experiments, while the ecological effects of such treatments also remain largely unclear. Nevertheless, as the effectiveness of SAR and the persistency of VOC in the environment in the field are unclear (Bostock, 2005; Kaminsky et al., 2019; Mitter et al., 2019), more research, particularly in

economically important crop plants such as potato, barley, and wheat, is required to provide a full knowledge and understanding of such interactions.

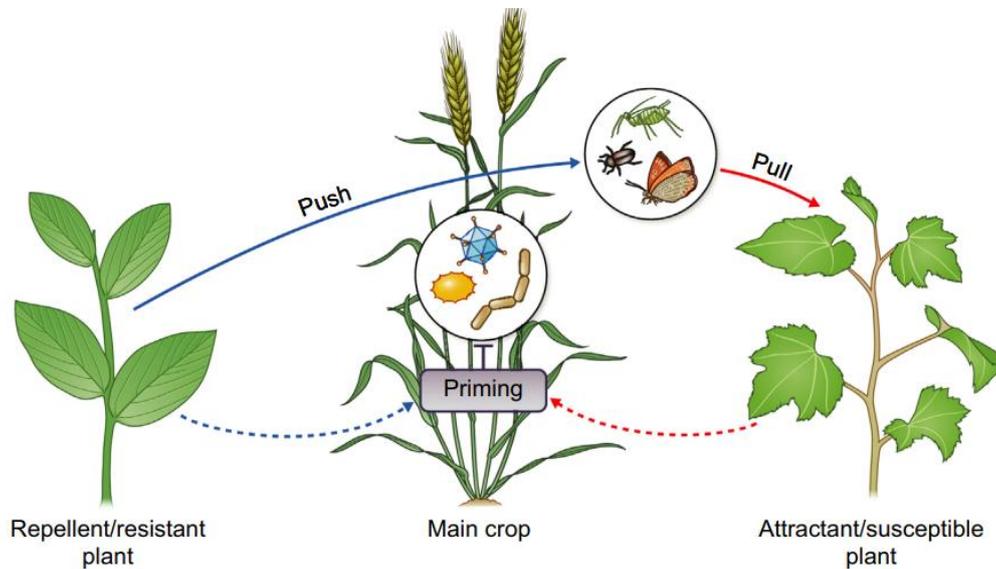


Figure 5 Plant protection strategies based on volatile organic compounds (VOCs). In push-and-pull intercropping strategies VOC-emitting plants are used to divert pests from the main (crop) host. The technique, established by Pyke et al. (1987), combines repellent plants able to ‘push’ (blue arrow) insects away from the crop with attractant plants that ‘pull’ (red arrow) them to the outer sides of a field. Wenig et al. (2019) show that VOCs induce resistance against microorganisms in neighbouring plants. Dashed arrows indicate that airborne defence cues emitted by companion plants might be able to prime a main crop’s defences against microbial pathogens (Vlot et al., 2021).

1.6 Research aims and methods

1.6.1 VOC measurements in *Psj*-infected compared to mock-treated barley plants

Here, we will characterise the emissions of *Psj*-infected compared to mock-treated barley plants using GC-MS (in collaboration with Jörg-Peter Schnitzler, HMGU, Riedlmeier et al., 2017). To this end, *Psj*-infected and mock-treated 3-week-old barley plants will be kept in airtight cuvettes that are flushed with clean (VOC-free) air supplemented with CO₂ and kept at constant environmental conditions. A part of the air exiting the cuvettes will be diverted to PDMS (absorbance) cartridges and collected over 8-hour intervals on clean cartridges every day of the analysis. The collected VOCs will be analysed by GC-MS as described (Riedlmeier et al., 2017), allowing the detection of low abundant VOCs in the barley headspace. Comparative analysis of the data will allow us to identify VOCs that are regulated by *Psj* infection as compared to the mock treatment. Together, this comprehensive analysis of infection-induced VOCs in barley emissions will allow a stringent selection of potentially defence-associated VOCs for further analysis.

1.6.2. Resistance induction by VOC exposure

Defence-associated VOCs from § 1.6.1 will be purchased as pure compounds and tested for their defence-inducing potential. To this end, barley plants will be enclosed in vacuum desiccators and fumigated with individual VOCs as described (Riedlmeier et al., 2017). Each VOC will be applied at different concentrations to ensure the detection of defence induction at the appropriate concentration (Riedlmeier et al., 2017). After the exposure to the selected VOCs, barley plants will be challenged with *Bgh*. Together with the characterisation of *Psj*-induced VOCs, these results will firmly establish which barley VOCs are associated with defence and biotic stress tolerance.

1.6.3 Plant-to-plant defence experiments, including relevant barley CRISPR-Cas mutants

Here, we will study possible barley-to-barley plant-to-plant responses, including correlations between plant-to-plant defence signalling and one or more defence-inducing barley VOCs from described in the previous paragraphs. Plant-to-plant experiments will be performed as follows (Riedlmeier et al., 2017). *Psj*-infected or mock-treated sender plants will be incubated in open-vases with naïve receiver plants. After 3 days, the receiver plants will be inoculated with *Bgh*. A reduction of the resulting *Bgh* infection at 7 days post-inoculation in response to the emissions of *Psj*-infected sender plants will indicate the induction of plant-to-plant resistance.

In order to mutagenize *HvALDI*, we designed two guideRNA (gRNA) sequences, which were spaced ~1,800 bp apart on the *HvALDI* gene. The variable parts of the gRNA sequences were cloned in Golden Gate shuttle vectors by inserting sets of complementary oligonucleotides behind *H. vulgare* U3 promoters driving the expression of a gRNA scaffold as described (Kumar et al., 2018; Ordon et al., 2017). Subsequently, the HvU3:gRNA expression cassettes were transferred to a binary vector, additionally encoding *Streptococcus pyogenes* *CAS9* using a codon-optimised version for monocotyledonous plants (Kumar et al., 2018). After transfer of the CRISPR/Cas vector to *Agrobacterium tumefaciens*, immature embryos of *H. vulgare* cultivar Golden Promise were transformed. Transformants were selected during plant regeneration on media containing hygromycin. The methods which were used for transformation and regeneration of plants are described in (Imani et al., 2011; Kumar et al., 2018). All experiments were performed with T2 plants, which were homozygous for deletion mutations between both gRNA target sites. Two lines from independent transformation events met this criterion. Because both lines were also homozygous for the *CAS9* transgene, all experiments were performed with these two independent lines. Together, the data will provide

insights into the possible identity of causative, defence-inducing airborne signals in the emissions of *Psj*-infected barley plants and the potential application of plant-to-plant signalling for protection of crops, including barley.

1.6.4 Transcript profiling to characterise downstream resistance mechanisms

Before considering VOC-induced resistance or plant-to-plant signalling for field application, it is fundamental to learn more about downstream mechanisms that are triggered in the plant. Here, our goal is to characterise downstream resistance mechanisms in response to defence-inducing VOCs and plant-to-plant signals in barley. Therefore, we will study barley transcriptional responses to exposure to individual VOCs and to plant-to-plant signals. Barley transcriptional responses will be then analysed using Illumina-based RNA-sequencing. The library preparations were sequenced on an Illumina platform and paired-end reads were generated. Reference genome and gene model annotation files were downloaded from the IPK Gatersleben (Monat et al., 2019). We mapped paired-end clean reads to the Morex V2 reference genome using HISAT2 software (Monat et al., 2019). In total 512 313 920 sequences were read with an average of 42 692 826 reads per sample. Within this number, 478 313 528 reads were mapped to the reference genome with an average of 39 859 460 reads per sample. The analysis was performed with R.

2. Results and embedded publications

2.1 Publication I: Immunity-associated volatile emissions of β -ionone and nonanal propagate defence responses in neighbouring barley plants

Plants activate biochemical responses to combat stress. (Hemi-)biotrophic pathogens are fended off by systemic acquired resistance (SAR), a primed state allowing plants to respond faster and more strongly upon subsequent infection. Here, we show that SAR-like defences in barley (*Hordeum vulgare*) are propagated between neighbouring plants, which respond with enhanced resistance to the volatile cues from infected senders. The emissions of the sender plants contained 15 volatile organic compounds (VOCs) associated with infection. Two of these, β -ionone and nonanal, elicited resistance upon plant exposure. Whole-genome transcriptomics analysis confirmed that interplant propagation of defence in barley is established as a form of priming. Although gene expression changes were more pronounced after challenge infection of the receiver plants with *Blumeria graminis* f. sp. *hordei*, differential gene expression in response to the volatile cues of the sender plants included an induction of *HISTONE DEACETYLASE 2* (*HvHDA2*) and priming of *TETRATRICOPEPTIDE REPEAT-LIKE superfamily protein* (*HvTPL*). Because *HvHDA2* and *HvTPL* transcript accumulation was also enhanced by exposure of barley to β -ionone and nonanal, our data identify both genes as possible defence/priming markers in barley. Our results suggest that VOCs and plant–plant interactions are relevant for possible crop protection strategies priming defence responses in barley.

Authors: [Alessandro Brambilla](#), Anna Sommer, Andrea Ghirardo, Marion Wenig, Claudia Knappe, Baris Weber, Melissa Amesmaier, Miriam Lenk, Jörg-Peter Schnitzler, and A. Corina Vlot.

Editor: Journal of Experimental Botany, Vol. 73, No. 2 pp. 615–630, 2022

Contribution: ACV: conceptualization and funding acquisition; AB, AG, MW, ML, JPS, and ACV: conceptualization and planning experiments; AB, AG, MW, CK, BW, and MA: executing experiments; AB, AS, AG, and ACV: data analysis; AB, AS, and ACV: writing first draft, which was critically reviewed by all authors; AG, ML, and JPS: editing.

2.2 Publication II: Pipecolic acid synthesis is required for systemic acquired resistance and plant-to-plant-induced immunity in barley

Defence responses in plants are based on complex biochemical processes. Systemic acquired resistance (SAR), helps to fight infections by (hemi-)biotrophic pathogens. One important signalling molecule in SAR is pipecolic acid (Pip), accumulation of which is dependent on the aminotransferase ALD1 in *Arabidopsis thaliana*. While exogenous Pip primes defence responses in the monocotyledonous cereal crop *Hordeum vulgare* (barley), it is currently unclear if endogenous Pip plays a role in disease resistance in monocots. Here, we generated barley *ald1* mutants using CRISPR/Cas9, and assessed their capacity to mount SAR. Endogenous Pip levels were reduced after infection of *ald1* barley, and this altered systemic defence against the fungus *Blumeria graminis* f. sp. *hordei*. Furthermore, *Hvald1* plants did not emit nonanal, one of the key volatile compounds that are normally emitted by barley plants after the activation of SAR. This resulted in the inability of neighbouring plants to perceive and/or respond to airborne cues and prepare for an upcoming infection, although *HvALD1* was not required in the receiver plants to mediate the response. The present study highlights the crucial role of endogenous *HvALD1* and Pip for SAR and associate Pip, in particular together with nonanal, with plant-to-plant defence propagation in the monocot crop barley.

Authors: Alessandro Brambilla, Miriam Lenk, Andrea Ghirardo, Laura Eccleston, Claudia Knappe, Baris Weber, Birgit Lange, Jafargholi Imani, Anton R. Schäffner, Jörg-Peter Schnitzler, A. Corina Vlot.

Journal: Journal of Experimental Botany, Vol. 74, No. 10 pp. 3033-3046, 2023.

Contribution: JPS and ACV conceived the project, AB, ML, AG, JI, ARS, JPS, and ACV planned experiments, AB, ML, AG, LE, CK, BW, BL, and JI performed experiments and analysed the data, AB, ML, AG, ARS, JPS, and ACV interpreted the data, AB and ACV wrote the manuscript, AG, JI, ARS, and JPS edited the manuscript, all authors agreed to the content of the manuscript.

2.3 Review I: Systemic propagation of immunity in plants

Systemic immunity triggered by local plant–microbe interactions is studied as systemic acquired resistance (SAR) or induced systemic resistance (ISR) depending on the site of induction and the lifestyle of the inducing microorganism. SAR is induced by pathogens interacting with leaves, whereas ISR is induced by beneficial microbes interacting with roots. Although salicylic acid (SA) is a central component of SAR, additional signals exclusively promote systemic and not local immunity. These signals cooperate in SAR- and possibly also ISR-associated signalling networks that regulate systemic immunity. The non-SA SAR pathway is driven by pipecolic acid or its presumed bioactive derivative N-hydroxy-pipecolic acid. This pathway further regulates interplant defence propagation through volatile organic compounds that are emitted by SAR induced plants and recognised as defence cues by neighbouring plants. Both SAR and ISR influence phytohormone crosstalk towards enhanced defence against pathogens, which at the same time affects the composition of the plant microbiome. This potentially leads to further changes in plant defence, plant–microbe, and plant–plant interactions. Therefore, we propose that such inter-organismic interactions could be combined in potentially highly effective plant protection strategies.

Authors: A. Corina Vlot, Jennifer H. Sales, Miriam Lenk, Kornelia Bauer, [Alessandro Brambilla](#), Anna Sommer, Yuanyuan Chen, Marion Wenig and Shahrar Nayem.

Journal: *New Phytologist*, 2021, Volume 229, Issue 3, Pages 1234-1250

2.4 Review II: New molecules in plant defence against pathogens

Plants host a multipart immune signalling network to ward off pathogens. Pathogen attack upon plant tissues can often lead to an amplified state of (induced) defence against subsequent infections in distal tissues; this is known as systemic acquired resistance (SAR). The interaction of plants with beneficial microbes of the rhizosphere microbiome can also lead to an induced resistance in above-ground plant tissues, known as induced systemic resistance. Second messengers such as calcium (Ca^{2+}), reactive oxygen species (ROS), and nitric oxide (NO) are necessary for cell-to-cell signal propagation during SAR and show emergent roles in the mediation of other SAR metabolites. These include the lysine-derived signals pipercolic acid (Pip) and N-hydroxypipercolic acid (NHP), which are key signalling metabolites in SAR. Emerging evidence additionally pinpoints plant volatiles as modulators of defence signalling within and between plants. Plant volatile organic compounds (VOCs) such as monoterpenes can promote SAR by functioning through ROS. Furthermore, plant-derived and additionally also microbial VOCs can target both salicylic acid and jasmonic acid signalling pathways in plants and modulate defence against pathogens. In this review, an overview of recent findings in induced defence signalling, with a particular focus on newer signalling molecules and how they integrate into these networks is discussed.

Authors: Laura Eccleston, [Alessandro Brambilla](#), and A. Corina Vlot.

Journal: Essays in Biochemistry, 2022, Volume 66, Issue 5, Pages 683-693

3. Discussion

3.1 SAR induction enhances resistance against *Blumeria graminis* f. sp. *hordei* in neighbouring plants

The interaction between plants is a well-known phenomenon. In order to interact with their surrounding neighbours, plants have evolved strategies that encompass air- or soilborne volatile cues (L. L. Li et al., 2020; Zhao et al., 2018). Plants that happen to perceive such cues, enhance or prime their resistance to a possible change of the environment, namely biotic or abiotic stresses. In this work, we elucidated part of this biochemical scheme in barley responses to infection. Previous studies demonstrated that Arabidopsis plants that eavesdrop airborne cues emitted by infected neighbouring plants, display an enhanced resistance to a subsequent infection (Frank et al., 2021b; Riedlmeier et al., 2017; Wenig et al., 2019). Nevertheless, little is known about plant-to-plant interaction in monocots such as barley. Tolosa et al. (2019) showed that volatile blends released by the monocot *Melinis minutiflora*, also known as molasses grass, successfully repelled the stemborer, *Chilo partellus*, from neighbouring maize plants. In monocots such as maize and rice, the emission of indole is induced as the plants are infested with insects (Erb et al., 2015; Zhuang et al., 2012). Additionally in maize, indole emissions induced by *Spodoptera littoralis* primes resistance not only in the infested plant but also in maize plants that are in the vicinity (Erb et al., 2015). The role of indole was further investigated by Ye et al. (2021) who confirmed its effect in priming defence signalling in tea (*Camellia sinensis*) plants. Furthermore, studies published by Li et al. (2020) assessed the effectiveness of VOCs emitted by monocotyledonous Chinese chive (*Allium tuberosum*) against *Fusarium oxysporum* f. sp. *cubense*, the causal agent of Panama disease, in banana plantations. These works confirm the presence of defence-related PTP interaction in monocots and their role in blocking or slowing down the course of disease caused by insect pests or microorganisms in the field. Experiments on barley carried out by Glinwood et al. (2009) explored the function of VOCs emitted from different barley cultivars in attracting ladybirds as an effective strategy in limiting phloem-feeding aphids' activity. Similarly, Jud et al. (2018) characterised barley VOC emissions after treating the plants with benzothiadiazole (BTH) through proton transfer reaction time-of-flight MS (PTR-ToF-MS). This last work affirms that barley is a low emitting plant species and that VOCs such as methanethiol, monoterpenes, and GLVs, including hexenal isomers, are released after a BTH treatment. Our achievements suggest that a barley-PTP system with *Psj*-infected sender plants drastically reduces a following *Bgh* infection in surrounding plants (Fig. 6).

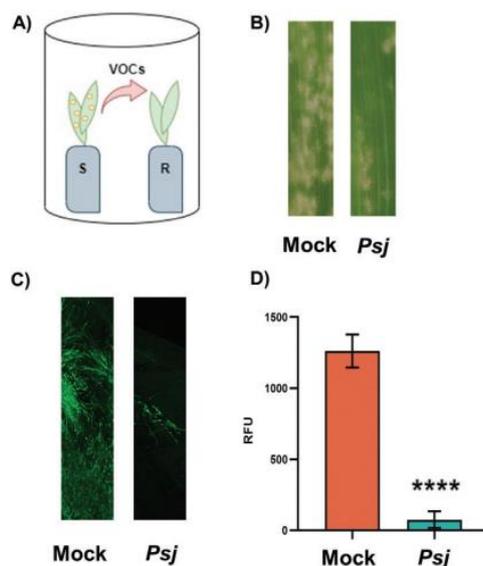


Figure 6 Plant-to-plant (PTP) propagation of defence in barley. (A) Set-up of a PTP experiment. Naïve receiver (R) plants were placed in open-top glass vases together with sender (S) plants, which were either mock-treated (Mock) or inoculated with *Pseudomonas syringae* pv. *japonica* (*Psj*). After 3 d, the receiver plants were inoculated with *Blumeria graminis* f. sp. *hordei* (*Bgh*). (B) *Bgh* on barley leaves. Pictures were taken at 7 dpi. (C) Fluorescence microscopy images of *Bgh* hyphae on leaf discs stained with DAF-FM-DA at 7 dpi. (D) Quantification of *Bgh* propagation in DAF-FM-DA-stained leaf discs. *Bgh*-associated relative fluorescence units (RFU) were calculated by normalizing the measured fluorescence values to those of uninfected controls. Bars represent average RFU values of 12 samples \pm SE. Values are taken from a representative experiment. We repeated the experiment 12 times and obtained comparable results. **** $P < 0.0001$ (unpaired *t*-test) (Brambilla et al., 2022).

After the infection with *Psj*, the relative abundance of 15 VOCs changed in the volatile blend that was emitted by barley (Fig. 7). Within these volatile compounds, we identified alkenes, aldehydes, ketones, aromatic compounds, diterpenes, and apocarotenoids. Specifically, the majority of these compounds are known secondary metabolites that are emitted by several plant species after different types of stresses (Cellini et al., 2021). In short, 1-Undecene is an alkene that is also present in *Farfugium japonicum* essential oils and is a known plant metabolite (Kim et al., 2008). Yi et al. (2009a) illustrated that the exposure of lima bean (*Phaseolus lunatus*) to nonanal induces the transcript accumulation of the defence-associated PR2 gene in the exposed plants. 2-Undecanone is a ketone that is present naturally in banana, guava, and other plant species (Kamal et al., 2019). More interestingly, another structural group of VOCs which we characterised in the emissions of *Psj*-inoculated barley plants are the apocarotenoids, including α -ionene, dehydro- β -ionone, α,β dihydro- β -ionone, β -ionone, β -ionone-epoxide, β -cyclocitral, and dihydroactinidiolide. The production of these VOCs starts from the oxidative cleavage of carotenoids, which are C40 isoprenoids synthesised in plastids (Rodriguez-Concepcion et al., 2018). Murata et al. (2020), but also Paparella et al. (2021), pointed out the

biological roles of these molecules in plant stress responses, growth, and herbivore resistance. Some of these compounds are already known as infochemicals: β -ionone is known to have repellent effects against the crucifer leaf beetle (*Phyllotreta cruciferae*) (Wei et al., 2011) and silverleaf whiteflies (*Bemisia tabaci*) (Cáceres et al., 2016) in *Arabidopsis* plants overexpressing CAROTENOID CLEAVAGE DIOXYGENASE1 (CCD1), which is involved in the biosynthesis of apocarotenoids. Dihydroactinidiolide is present naturally in several plant species (Shumbe et al., 2014) and originates from the degradation of β -ionone. β -Cyclocitral is a VOC common in several plant species (Felemban et al., 2019b) and has been seen to have a role in lateral root development and in inducing resistance against salt (Dickinson et al., 2019) and photo-oxidative stress (Ramel, Birtic, Cuiné, et al., 2012).

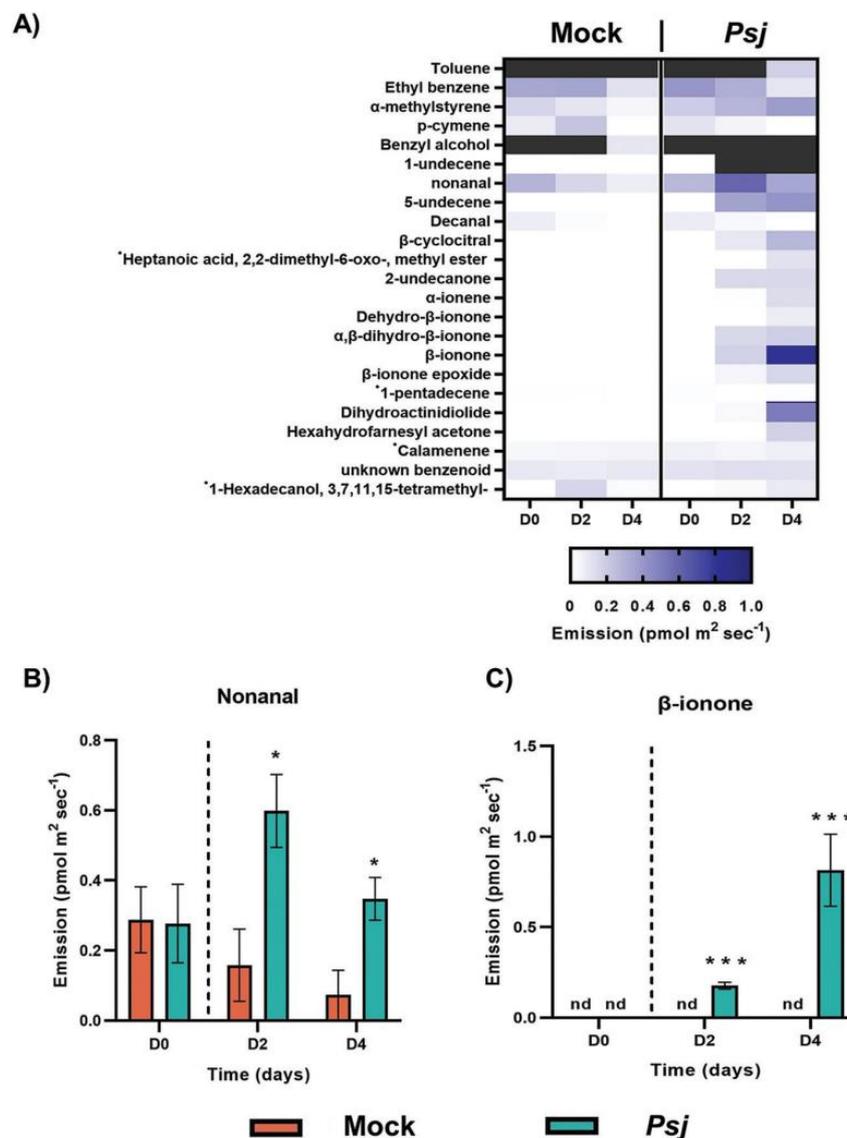


Figure 7 Characterisation of VOC emissions in barley PTP experiments. (A) Heat map of the VOCs detected in the emissions of mock-treated (Mock) and *Psj*-inoculated plants at D0 (before treatment), D2 (24 hpi), and D4 (72 hpi). Darker colours indicate higher emission rates; black-coloured cells indicate out-of-range values (>1). Each cell represents average values from

eight independent replicates. ° indicates tentatively identified compounds. (B and C) VOC emission rates of nonanal (B) and β -ionone (C) in mock-treated (Mock) and *Psj*-inoculated plants. Dashed lines separate time points before and after treatment. Bars represent average values of eight independent replicates \pm SE. * $P < 0.05$; *** $P < 0.0005$ (two-way ANOVA) (Brambilla et al., 2022).

3.2 Gene regulation in receiver plants

In this study, we investigated whether plants that eavesdrop VOC emitted from *Psj*-infected plants display induced or primed defence responses. With this aim, we observed PTP-induced gene expression variation in PTP receiver barley plants before and after a subsequent *Bgh* challenge. Within the changes in the receivers, a marked shift in the number and nature of DEGs that were associated with a the *Bgh* infection (compare group III with group II in Fig. 8) was denoted. Furthermore, we observed a higher number of primed DEGs (group IV) as compared with DEGs that were induced by the exposure of receivers to *Psj*-induced emissions (group I) (Fig. 8). Thus, we propose that the quantitative and, most importantly, the qualitative shift in the *Bgh*-induced transcriptional profile in group III as compared with group II (Fig. 8) was linked to a general primed status of the receiver plant. In Arabidopsis, such events, including transcriptional and metabolic reprogramming, as well as fostered activation of defences after subsequent microbial attacks, can be triggered by SAR-associated molecules, including azelaic acid, Pip, and NHP (Jung et al., 2009; N avarov a et al., 2013; Yildiz et al., 2021). In this context, in previous works we demonstrated that exogenous irrigation with Pip primes ROS accumulation and enhanced defence responses against *Bgh* in barley (Lenk et al., 2019). Further results on Pip-drench and the importance of this molecule will be also discussed in the next paragraphs. As for now, our collective data suggest that PTP-induced disease resistance in barley is established as a form of priming. Remarkably, quite a high number of DEG products are located in the ribosome and plastids (Fig. 8), accentuating the role of these organelles in plant–microbe interaction and in plant defence mechanisms in general (Kretschmer et al., 2020; Lu & Yao, 2018; F. Yang et al., 2021). In detail, photosynthesis-related genes are primed for down-regulation following the challenge of PTP-primed plants with *Bgh* (Fig. 8). This is also confirmed in findings of Molitor et al. (Molitor et al., 2011), who observed an over-representation of photosynthesis-related transcripts, which were down-regulated by *Bgh* at 24 hpi in barley leaves. These collective results that we gathered from barley further support data from Arabidopsis, which demonstrate a decreased photosynthesis and respiration rates in the systemic, primed tissues of SAR-induced plants (Bernsdorff et al., 2016). The decline in net photosynthesis in primed plants, and thus the less production of assimilates, is believed by many researchers as an indirect cost of priming (Douma et al., 2017; Molitor et al., 2011). Our results

further support this hypothesis with reduced expression of genes that are associated with photosynthetic light reactions, particularly within the primed group IV genes (Fig. 8).

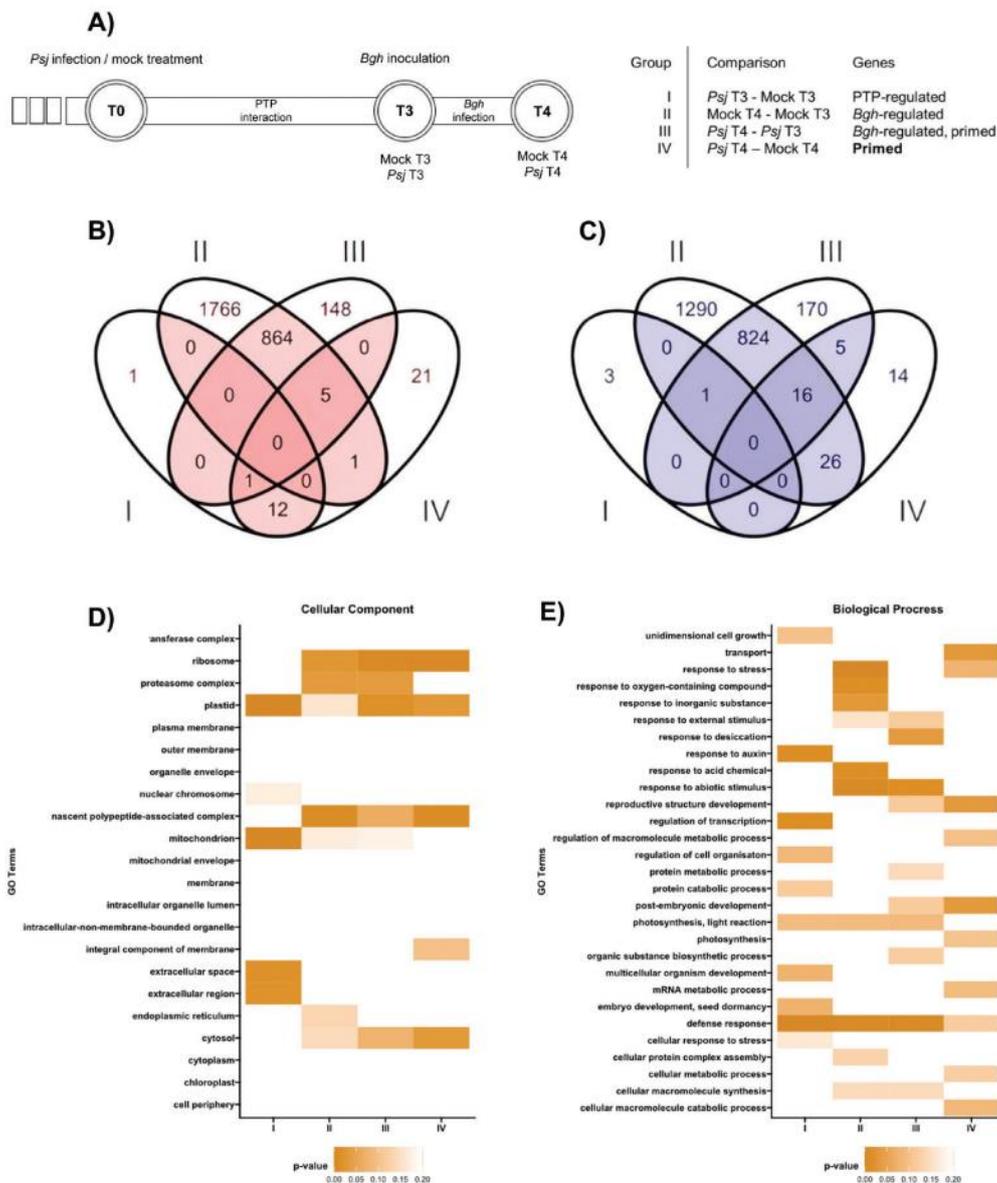


Figure 8 RNA-seq analysis of transcript accumulation in receiver plants in PTP experiments. Plants were either mock treated or inoculated with *Psj*, and subsequently harvested at 3 dpi (T3) or inoculated with *Bgh* and harvested 1 d later (T4). (A) Timeline of the experiment. The RNA-seq data from two biologically independent replicate experiments were used to determine differentially expressed genes (DEGs) in four comparison groups (group definitions to the right of the timeline). (B and C) Venn diagrams of up-regulated (B) and down-regulated DEGs (C) in the different comparison groups. (D and E) GO term enrichment in the categories cellular component (D) and biological process (E) among DEGs in the different comparison groups. Colours indicate the P-value (Brambilla et al., 2022).

In the DEGs, two genes drove particularly our attention, namely *HISTONE DEACETYLASE 2* (*HvHDA2*) and *TETRATRICOPEPTIDE REPEAT-LIKE* superfamily protein (*HvTPL*). Interestingly, our results showed that *HvHDA2* is up-regulated in plants that eavesdrop the emission coming from *Psj*-infected barley (Fig. 9). This might highlight a possible epigenetic-

driven primed status in plants that were neighbours of *Psj*-infected barley. Other works showed that in wheat, *HISTONE DEACETYLASE 2* was characterised as a negative regulator of defence responses against *Blumeria graminis* f. sp. *tritici* (Zhi et al., 2020). Similar results were observed in rice, where the overexpression of HD2-type histone deacetylase *OsHDT701* triggers rice susceptibility to the biotrophic pathogen *Magnaporthe oryzae* and the hemibiotrophic bacterium *Xanthomonas oryzae* pv. *oryzae* (Ding et al., 2012). In our work, after the challenging primed plants with *Bgh*, the expression of *HvHDA2* was down-regulated if compared with the expression of the same gene in the same plants before their inoculation with the fungus (Fig. 9). Given these results, it is believable that *HvHDA2* takes part in the establishment of PTP priming, but not to the execution of the subsequent primed defence response.

In tandem, the expression of *HvTPL* was primed (i.e. up-regulated) only after having inoculated with *Bgh* receivers of *Psj*-induced emissions (Fig. 9). Tetratricopeptide repeats (TPRs) are protein–protein interaction modules present in many proteins. In Arabidopsis, TPR motif-containing proteins are participate in responses to hormones, including ethylene, cytokinins, auxins, and gibberellins (Schapire et al., 2006). In addition, TPR motifs play a further role in substrate recognition and/or in the production of active multiprotein complexes, hence they often play important functions in vital cellular processes (Cervený et al., 2013). In rice, for instance, TPR-containing proteins have been seen to regulate mRNA metabolism (Goebel & Yanagida, 1991). Furthermore, these TPR proteins or the multiprotein complexes they induce are involved in rice defence response against *M. oryzae* and *Xanthomonas oryzae* pv. *oryza* (Goebel & Yanagida, 1991; Zhou et al., 2018). Zhou et al. (2021) showed that TPR-containing proteins in tomato, *Solanum lycopersicum*, participate in responses to the biotic stress caused by necrotrophic fungi. Likewise to *HvTPL* in response to *Bgh* (Fig. 9), *SITPR2* is over expressed following the inoculation of tomato with *Botrytis cinerea*, and this could be linked with its immune response. In conclusion, our collective data suggest that TPR-containing proteins, including *HvTPL*, cover important roles in plant immunity and priming.

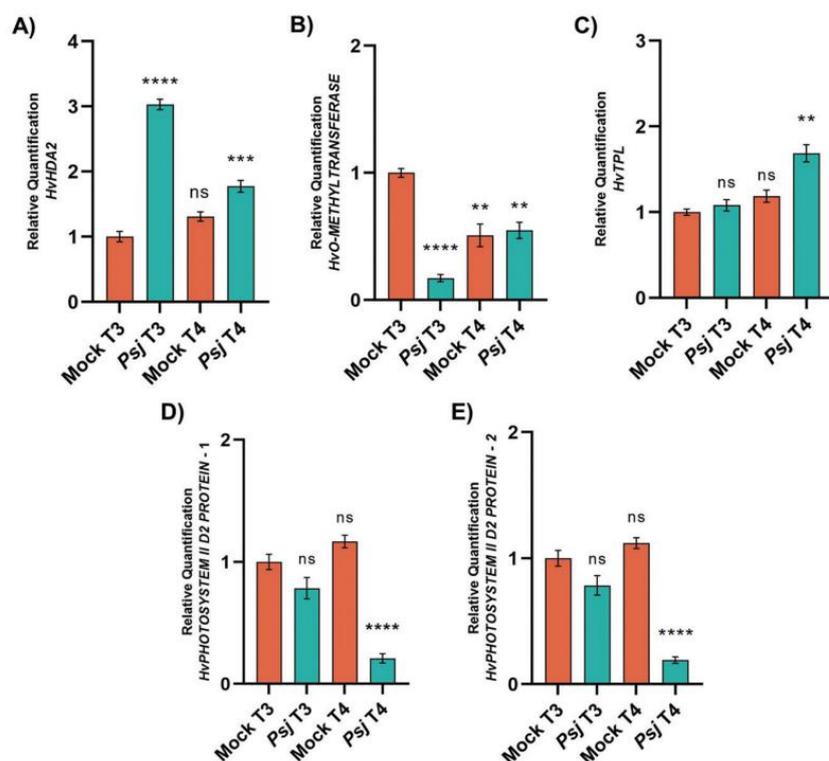


Figure 9 qRT-PCR validation of selected DEGs. Plants were either mock treated or inoculated with *Psj*, and subsequently harvested at 3 dpi (T3) or inoculated with *Bgh* and harvested 1 d later (T4). Transcript accumulation of the indicated genes was analysed by qRT-PCR and normalised to that of *HvEF1a* and *HvUBI*. Accumulation of transcripts is shown relative to that at T3 in mock-treated samples. Bars represent average values from four biologically independent experiments \pm SE. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$; **** $P < 0.0001$ (one-way ANOVA, Tukey's multiple comparison test) (Brambilla et al., 2022).

3.3 Exposure of barley plants to nonanal and β -ionone enhances defence responses

Exposure of barley plants to nonanal or β -ionone, two of the VOCs that are emitted after the infection of barley with *Psj*, activated the defence of the plants against *Bgh* (Fig. 10). After fumigating barley plants with different concentrations of nonanal and β -ionone, we can state that the in experimental set-up were 35 ppbv for nonanal and 75 ppbv for β -ionone, respectively, were the most effective concentrations to trigger defence responses against *Bgh* (Fig. 10). Nevertheless, fair results were also obtained with 55 ppbv nonanal and 50 ppbv β -ionone. The concentrations of nonanal and β -ionone which were used in the exposure experiments were in an estimated >1000 -fold higher range than what was measured in the emissions of *Psj*-inoculated barley, like the observed concentration for terpene-induced defence in *Arabidopsis* (Frank et al., 2021b; Riedlmeier et al., 2017). This endorses other findings that plants in a natural context probably respond to VOC blends rather than to individual compounds, and that such compounds consequently are needed in considerably higher concentrations to elicit a response on their own (Rosenkranz et al., 2021). Nonetheless, the exposure treatments with

either nonanal or β -ionone both induced the transcript accumulation of *HvHDA2* and *HvTPL*. In addition to consolidate the potential role of these genes in plant immunity, these results confirm that nonanal and β -ionone are within the causative VOCs promoting PTP propagation of immunity in barley. Nonanal and β -ionone could cover a central role in PTP interaction by inducing or priming the up-regulation of defence-related genes such as *HvHDA2* and *HvTPL* and by down-regulating *O-METHYLTRANSFERASE* (Fig. 10). Previous studies on PTP interaction displayed the benefits of introducing VOC-emitting plants in agricultural contexts (Brilli et al., 2019; Pickett & Khan, 2016). Moreover, intercropping barley with companion plants that would naturally emit nonanal or β -ionone could help in reducing *Bgh* infections and associated yield losses. As an alternative, recent works demonstrated that the incorporation of VOC-based plant protection products in disease management programmes can reduce the input of chemical pesticides (Brilli et al., 2019; Ricciardi et al., 2021). These crop protection techniques are likely to represent a benefit for human health, as well as to preserve natural ecosystems, and to avoid pesticide resistance in fields.

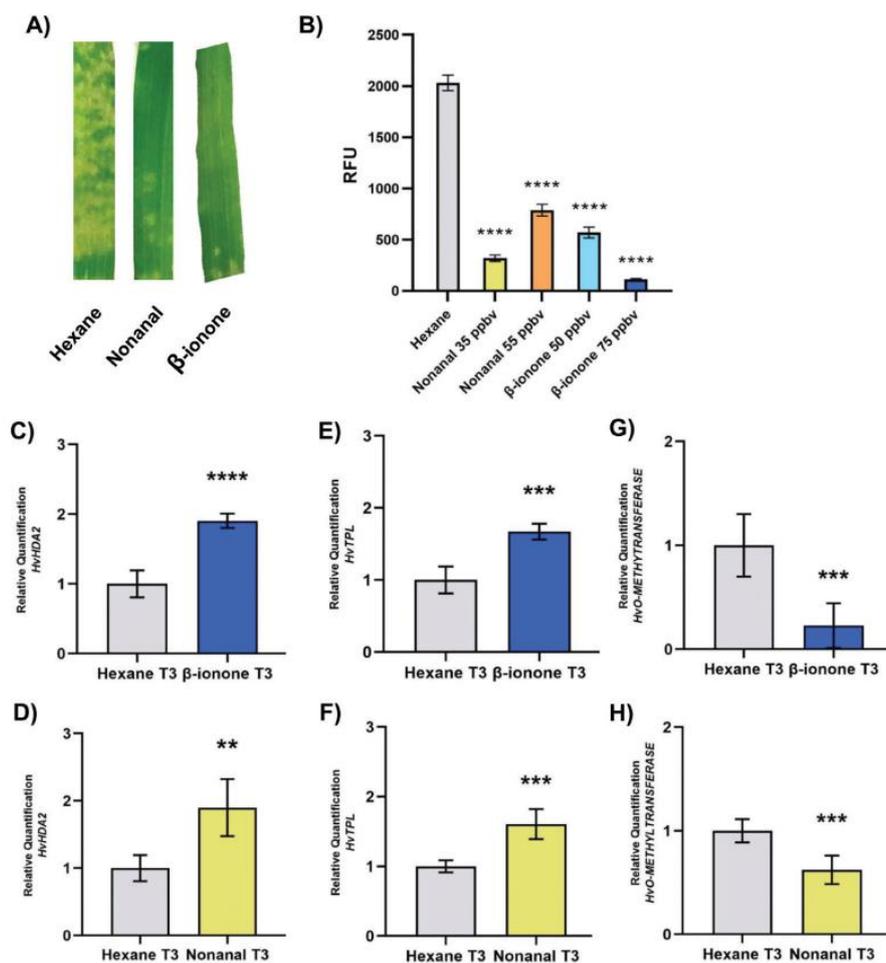


Figure 10 Exposure to nonanal and β -ionone enhances resistance in barley against *Bgh*. Plants were exposed to the indicated concentrations of nonanal or β -ionone (in hexane) or to a comparable amount of hexane as the mock control treatment. Three

days later, leaves were either harvested (T3) or inoculated with *Bgh* and evaluated at 7 dpi. (A) *Bgh* on barley leaves; pictures were taken at 7 dpi. (B) Quantification of *Bgh* propagation in DAF-FM-DA-stained leaf discs. *Bgh*-associated relative fluorescence units (RFU) were calculated by normalizing the measured fluorescence values to those of uninfected controls. Bars represent average values of 12 samples \pm SE. Values are taken from a representative experiment. We repeated the experiment eight times and obtained comparable results. *** $P < 0.0005$ (one-way ANOVA, Tukey's multiple comparison test). (C–H) qRT–PCR analysis of transcript accumulation of the indicated genes after exposure of barley to β -ionone (blue bars) and nonanal (yellow bars). Transcript levels were normalised to that of *HvEF1 α* and *HvUBI* and are shown relative to those in hexane-treated samples (grey bars). Bars represent average values of three biologically independent experiments \pm SE. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$; **** $P < 0.0001$ (unpaired *t*-test) (Brambilla et al., 2022).

3.4 *Hvald1* barley plants show reduced Pip levels

The importance of Pip (as fundamental precursor of its bioactive derivative NHP) in plants' immune responses was previously described (Chen et al., 2018; Návarová et al., 2013; Vlot et al., 2021; Yildiz et al., 2021). Lenk et al. (2019) showed that Pip levels increase in barley leaves following a microbial infection and that, moreover, irrigation of barley plants with exogenous Pip triggers SAR-like responses, defending the plant against an up-coming infection with *Xanthomonas translucens* pv. *cerealis*. In this work, we investigate whether endogenous Pip takes also part in SAR and SAR-associated responses in barley. In this regard, we generated *Hvald1* barley plants using CRISPR/Cas9 mutagenesis (Fig. 11a). Two homozygous *Hvald1* lines, namely L14 and L38, have been selected for the studies mentioned here after (Fig. 11b). First, we measured Pip levels in the transgenic lines comparing them to wild type barley plants. As previously shown in other works, Pip levels increase after the inoculation of a wild type barley plant with *Psj*, compared to a mock treatment (Fig. 11c). Interestingly, in our barley *Hvald1* lines, Pip levels did not increase after such infection. This led us, firstly to a further confirmation of the successful mutagenesis of our CRISPR/Cas9 lines and, secondly, that barley *Hvald1* plants display reduced Pip levels, highlighting the importance of ALD1 and Pip in barley defence response against microbial infections (Fig. 11).

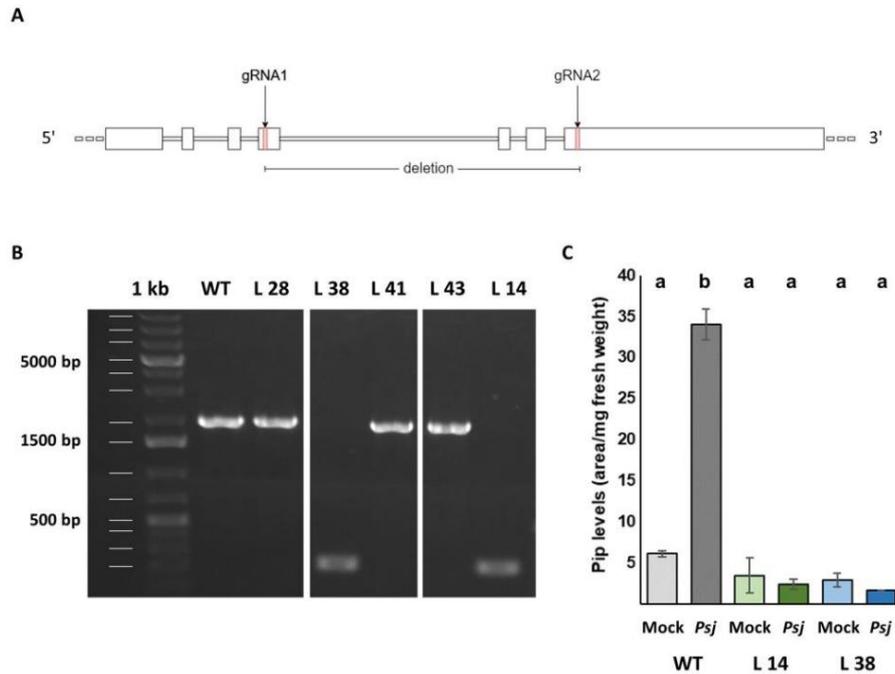


Figure 11 Characterisation of *Hvald1* mutant plants. A) Schematic representation of the *HvALD1* gene. Non-coding sequences are striped, coding sequences are shown with solid lines: bars indicate the positions of the exons. Red marks show the positions of the target sites of gRNAs1 and 2 as indicated. B) PCR analysis of *Hvald1* mutant lines. The gel image shows PCR amplicons covering a 2 kb fragment across both gRNA target sites in *HvALD1*. Lines L38 and L14 were homozygous for deletion mutations between the target sites of gRNA1 and 2. WT, wild type (control). C) Pipecolic acid (Pip) accumulation in WT and *Hvald1* plants. Leaves of the plants were inoculated with *Pseudomonas syringae* pv. *japonica* (*Psj*) or a corresponding control treatment (Mock). Pip accumulation was measured one day post-inoculation (dpi) and is shown as the average LC-MS peak area per mg fresh weight \pm SD from three samples, which were collected from three biologically independent experiments. Different letters above bars indicate significant differences (one-way ANOVA, $P < 0.05$) (Brambilla et al., 2023).

3.5 *Hvald1* barley plants are SAR-deficient

In order to understand whether the limited Pip accumulation in *Hvald1* plants has consequences on barley immunity, we performed a SAR experiment and compared the results obtained with *Hvald1* plants to the ones of WT barley. In WT plants, a decrease of >50-fold was observed in the quantification of *Bgh* infection on the systemic leaves of *Psj*-pre-treated plants as compared to the mock control (Fig. 12A/B). This confirms that the first infection with *Psj* has successfully boosted the defence response in distal parts of the plant and thus had induced SAR. On the other hand, in *Hvald1* plants inoculated with the SAR-inducing bacterium *Psj*, the aggressivity of the following infection with *Bgh* did not change in comparison to that in the mock-treated plants (Fig. 12A/B). Thus, similarly to SAR in *Arabidopsis Atald1* mutant plants (Návarová et al., 2013), SAR was abolished in barley lacking functional *HvALD1*.

SAR in barley shows a different molecular mechanism compared to that of *Arabidopsis*. The role of SA is much less pronounced and so are the associated marker genes (Dey et al., 2014;

Lenk et al., 2018). We previously mentioned the changes in the gene expression of *HvHDA2* and *HvTPL* in barley PTP interactions (Brambilla et al., 2022). Thus, we assessed here, if these genes also respond to SAR. While both *HvHDA2* (Fig. 12C/D) and *HvTPL* (Fig. 12E/F) were up-regulated after *Bgh* infection of *Psj*-treated plants, *HvHDA2* followed a classical priming behaviour in WT plants. In details, its transcript accumulation was not profoundly altered in the systemic tissue three days after the primary *Psj* infection but, on the contrary, reacted to the infection with *Bgh* with a significant increase in *Psj*-pre-treated as compared to mock-pre-treated plants (Fig. 12C/D). This result suggests that a defence-associated induction of *HvHDA2* was primed during SAR, but it became clear and visible only after the challenge infection. Conversely, priming of *HvHDA2* was not present in both *Hvald1* mutant lines L14 and L38 (Fig. 12C/D).

A systemic accumulation of *HvTPL* transcripts was observed three days after the primary *Psj* infection in WT plants but this did not happen in *Hvald1* L38 (Fig. 12F). As *HvTPL* transcript accumulation in *Psj*-pre-treated plants did not increase further after the *Bgh* challenge infection (Fig. 12E/F), we assume that the expression of *HvTPL* was induced and not further primed during SAR in barley. In *Hvald1* L14, the basal *HvTPL* transcript accumulation level in the systemic tissues of mock-treated plants displayed reduced levels as compared to WT (T3 sample, Fig. 12E). Following the infection of the plants with either *Psj* or *Bgh* the transcript level of *HvTPL* in L14 increased comparably to that in mock-treated WT plants, advocating a moderate induction of *HvTPL* in the absence of functional *HvALDI*. After observing that *HvTPL* transcript accumulation in *Hvald1* mutant lines L14 and L38 stayed at or below the mock-induced levels in WT plants, we deduced that SAR-associated gene expression changes were compromised in the absence of functional *HvALDI*. Contrarily to the transcript accumulation of *AtALDI* in systemic tissues during SAR in Arabidopsis (Návarová et al., 2013), *HvALDI* transcript accumulation did not seem to change in the systemic tissue during SAR in barley. Conclusively, our collective results strengthen the hypothesis that the production of Pip is a fundamental event in plant immunity and that this is true in both dicotyledonous and monocotyledonous plants with SAR being fully dependent on functional *ALDI* in both Arabidopsis (Bernsdorff et al., 2016; Návarová et al., 2013) and barley (Fig. 12).

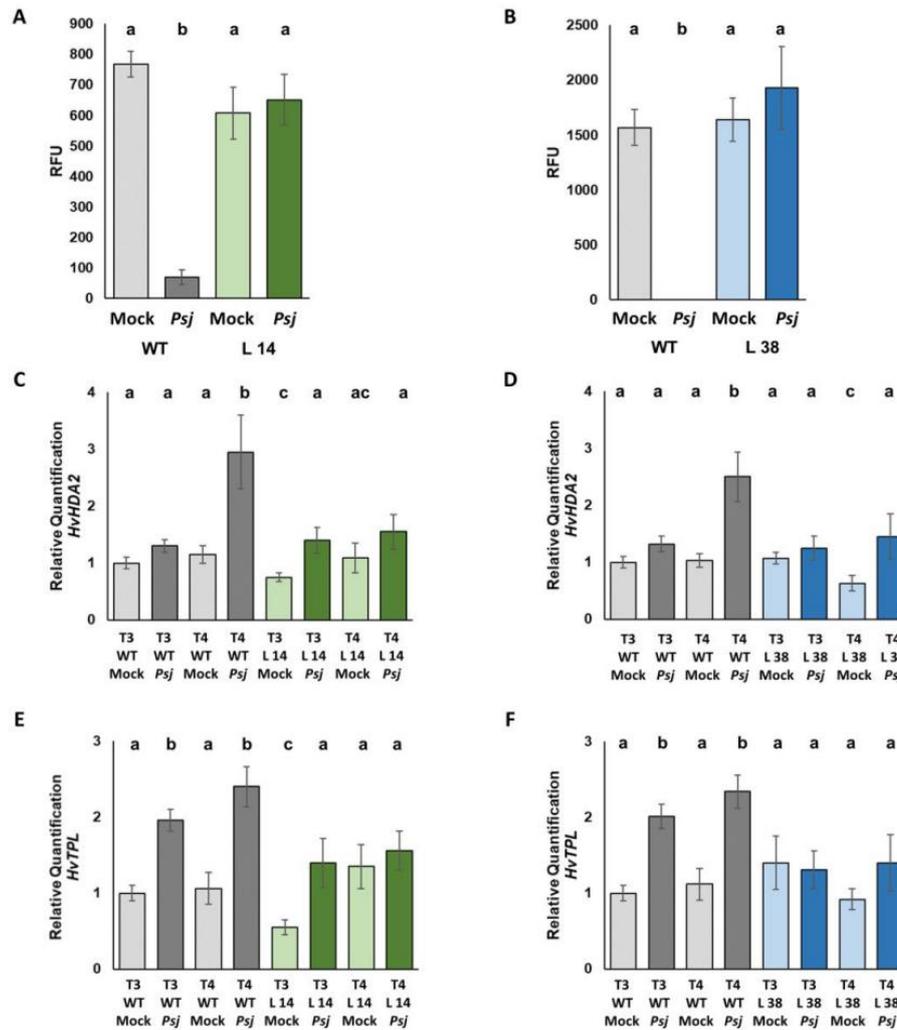


Figure 12 Systemic acquired resistance (SAR) is compromised in *Hvald1* plants. Plants of the genotypes indicated below the panels were treated in their first true leaves with *Psj* or with a corresponding mock control solution. Five days later, systemic leaves were either inoculated with *Blumeria graminis* f. sp. *hordei* (*Bgh*) or harvested for the analysis of transcript accumulation (samples indicated as T3). Further samples were harvested for the analysis of transcript accumulation at one dpi (samples indicated as T4). At seven dpi, *Bgh* hyphae were stained with the fluorescent dye DAF-FM-DA and the *Bgh*-associated relative fluorescence units (RFU) were calculated by normalizing the measured fluorescence values to those of uninfected controls. A-B) RFU indicating the intensity of the *Bgh* infection in mock- and *Psj*-treated WT barley and *Hvald1* line 14 (A) and 38 (B). Bars represent average, normalised RFU values of 12 samples \pm SE. Values were taken from a representative experiment. We repeated the experiment 3 times with comparable results. Different letters above bars indicate significant differences (one-way ANOVA, $P < 0.05$). C-F) Relative quantification of *Hordeum vulgare* HISTONE DEACETYLASE 2 (*HvHDA2*; C-D) and TETRACOPEPTIDE REPEAT-LIKE superfamily protein (*HvTPL*; E-F) transcript accumulation before (T3) and 24h after *Bgh* inoculation (T4) of mock and *Psj*-treated WT plants and *Hvald1* line 14 (C/E) and 38 (D/F). Transcript accumulation was normalised to *HvEF1a* and *HvUBI* and is shown relative to that in mock-treated WT T3. Bars represent average values from three biologically independent experiments \pm SD. Different letters above bars indicate significant differences (one-way ANOVA, $P < 0.05$) (Brambilla et al., 2023).

Interestingly, previous studies in *Arabidopsis* (Návarová et al., 2013) demonstrated that the exogenous application of Pip to *ald1* plants is enough to resume immunity. We thus investigated

if this is also valid in barley. Hence, we drenched *Hvald1* barley with Pip or a corresponding water control, and, after 5 days, challenged the plants with *Bgh*. In comparison to that of water-treated plants, *Bgh* propagation was disrupted on the leaves of Pip-treated WT and *Hvald1* barley (Fig. 13). As *Hvald1* plants responded normally to exogenous Pip with enhanced immunity against *Bgh* we can further supports the functional identification of locus HORVU.MOREX.r3.4HG0399940 as *HvALDI*.

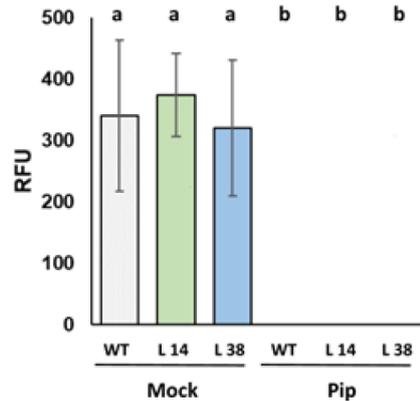


Figure 13 *Hvald1* plants respond to exogenous Pip treatment with enhanced resistance to *Blumeria graminis* f. sp. *hordei* (*Bgh*). Plants of the genotypes indicated below the panel were treated with Pip by drenching the soil near the roots with 40 mL of 30 μ M pipercolic acid (Pip). Five days later, the plants were inoculated with *Bgh*. At seven dpi, *Bgh* hyphae were stained with DAF-FM-DA and the *Bgh*-associated relative fluorescence units (RFU) were calculated by normalizing to uninfected controls. Bars represent average, normalised RFU values of 12 samples \pm SE. Values were taken from a representative experiment. We repeated the experiment three times and obtained comparable results. Different letters above bars indicate significant differences (one-way ANOVA, $P < 0.05$) (Brambilla et al., 2023).

3.6 Role of *HvALDI* in plant-to-plant (PTP) defence propagation

As a next step in this work, we wished to assess the role of *HvALDI* in barley PTP. In detail, we checked whether *Hvald1* plants send volatile cues to neighbouring plants and, concomitantly, if *Hvald1* plants perceive these cues from *Psj*-treated sender plants and, thus, activate defence responses against a following *Bgh* infection. As mentioned before (Brambilla et al., 2022), *Bgh* propagation was limited on barley plants which had been exposed to the emissions of *Psj*-infected WT plants (Fig. 14A/B). Nevertheless, this phenomenon was not observed in a scenario in which sender plants lacked functional *HvALDI* (Fig. 14A/B). In fact, naïve WT receiver plants which had been placed near *Psj*-infected *Hvald1* sender plants were as susceptible to *Bgh* as receivers which had been close to mock-treated senders of any genotype. These results confirm that the emission of airborne defence cues during SAR in barley depends on *HvALDI* and thus most probably on Pip and/or NHP.

Regarding the expression of the two defence related genes that we have taken into consideration in previous experiments in this work, namely *HvHDA2* and *HvTPL*, we also noticed differences in the transcript levels in receiver plants. *HvHDA2* is known, from our previous results, to be induced in WT receiver plants following their exposure to *Psj*-inoculated WT senders (Brambilla et al., 2022). Nevertheless, in the systemic tissues of SAR-induced barley plants *HvHDA2* appears not to be induced, but primed, highlighting the different transcriptional response of this gene between SAR and PTP events. In addition, the existence of PTP interaction entirely depends on VOCs, and, on the contrary, SAR sees other non-volatile phloem-mobile signals that might influence the transcriptional response. This being said, in both SAR and PTP there is a regulation in the expression of *HvHDA2*. We also interestingly noticed that when *Hvald1* plants are used as senders in PTP events, the defence response, thus the expression of *HvHDA2*, in neighbouring plants against a subsequent *Bgh* infection is altered. Similarly, *HvTPL* displayed a different transcriptional response in systemic tissues of SAR-induced plants and in receiver plants. This further takes us to suggest that *HvALD1* and *HvTPL* are fundamental in the establishment of PTP, and thus that the production of Pip and NHP is situated upstream to the one of VOCs.

Moreover, we assessed whether *Hvald1* plants could perceive VOCs coming from WT *Psj*-infected senders. Interestingly, *Hvald1* receivers of the emissions of *Psj*-inoculated WT senders showed significantly less propagation of *Bgh* than *Hvald1* receivers which had been exposed to the emissions of mock-treated senders. Similar results have been described in studies on *Atald1*, where these mutants have been described to perceive volatile cues but not emit them. *HvALD1* is thus located upstream of the emission of volatile defence cues in sender plants, but not as important as for their perception and/or propagation in receivers.

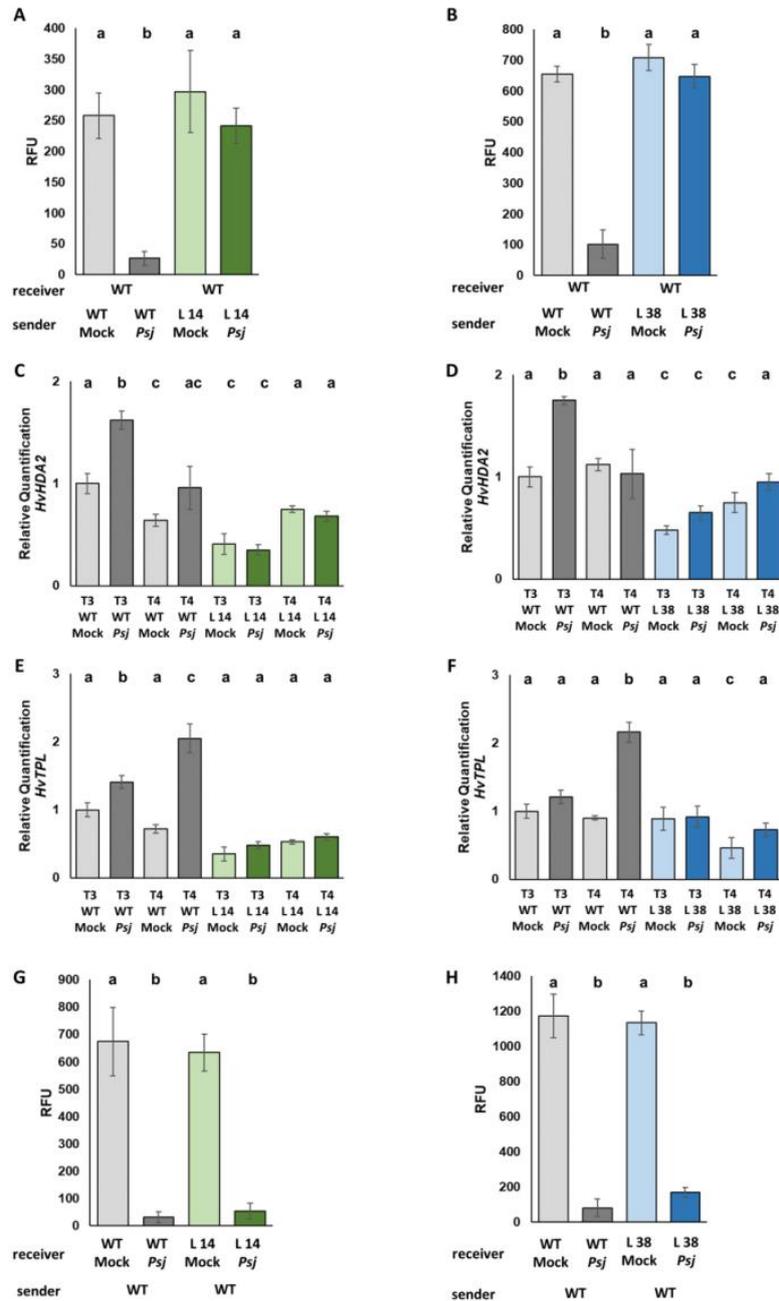


Figure 14 *HvALD1* promotes plant-to-plant interactions in barley. Sender plants of the genotypes indicated below the panels were inoculated with *Psj* or with a corresponding mock control solution. Receiver plants were exposed to the volatile emissions of the senders. After three days, leaves of the receiver plants were harvested for the analysis of transcript accumulation (samples indicated as T3) or the receiver plants were inoculated with *Bgh*. Further samples were harvested for the analysis of transcript accumulation at one dpi (samples indicated as T4). At seven dpi, propagation of *Bgh* was evaluated. A-B) Relative fluorescence units (RFU) indicating the intensity of *Bgh* infection in WT receiver plants which had been exposed to the emissions of mock or *Psj*-inoculated, WT, *Hvald1* line 14 (A) or line 38 (B) sender plants. C-D) Relative quantification of *HvHDA2* transcript accumulation before (T3) and 24h (T4) after *Bgh* inoculation of WT receiver plants and of *Hvald1* line 14 (C) and 38 (D) receivers. E-F) Relative quantification of *HvTPL* before (T3) and 24h (T4) after *Bgh* inoculation of WT receiver plants and of *Hvald1* line 14 (E) and 38 (F) receivers. G-H) RFU indicating the intensity of *Bgh* infection in WT, *Hvald1* line 14 (G) and line 38 (H) receivers which had been exposed to the emissions of mock or *Psj*-inoculated WT sender plants. A-H) Bars in A-B and G-H represent average RFU values of 12 samples \pm SE. Values were taken from representative experiments. Experiments were repeated three times with comparable results. Different letters above bars indicate significant differences (one-way

ANOVA, $P < 0.05$). Transcript accumulation in (C-F) was normalised to *HvEF1 α* and *HvUBI* and is shown relative to that in mock-treated WT T3. Bars represent average values from three biologically independent experiments \pm SD. Different letters above bars indicate significant differences (one-way ANOVA, $P < 0.05$) (Brambilla et al., 2023).

3.7 Nonanal is a key VOC in PTP barley emissions

Our previous data supported the hypothesis that SAR in barley is associated with two specific volatile compounds, nonanal and β -ionone (Brambilla et al., 2022). As naïve WT barley receiver plants did not show enhanced resistance against *Bgh* when placed in the vicinity of *Psj*-infected *Hvald1* plants, we decided to search for these two VOCs in the volatile blend of *Psj*-infected *Hvald1*. Nonanal appeared to be completely absent in the emission of *Hvald1* after the inoculation of *Psj* (Fig. 15). On the contrary, β -ionone emission was comparable in *Hvald1* and WT barley after the infection with *Psj* (Brambilla et al., 2023). These data suggest that nonanal acts as a causative PTP signal in the VOC emissions of barley.

Nonanal is known to have antimicrobial properties (Kubo et al., 1995; Muroi et al., 1993). The growth of *Penicillium cyclopium*, a post-harvest fungal pathogen of tomato, was demonstrated to be inhibited by nonanal treatments (Zhang et al., 2017). Nonanal also displayed antifungal activity on *Aspergillum flavus* where the exposure of this fungus to nonanal provoked severe damage on its cell wall and cell membrane, with the direct effect of reducing *A. flavus* propagation on maize kernels (Q. Li et al., 2021). Moreover, several studies investigated the role of nonanal in plant defence. First, Yi et al. (2009) demonstrated that nonanal is induced in the emissions of SAR-activated lima bean (*Phaseolus lunatus*) plants, and that its emission enhances the resistance of same-species neighbours to pathogenic *P. syringae*. Exposure to nonanal also enhanced the resistance of lima bean against *P. syringae* and this was reported to be associated with priming of the defence-associated *PR-2* gene for enhanced expression after infection of the plants (Yi et al., 2009). Other works demonstrated that the damage caused by caterpillars, as well as a treatment with benzothiadiazole (BTH) on cowpea (*Vigna unguiculata* var. *unguiculata*) plants were observed to emit stress-induced VOCs, including nonanal, resulting in the attraction of parasitoids to protect the plants from infestation (Sobhy et al., 2018). Similarly, potato tubers that were damaged by insects, showed to emit a series of VOCs, nonanal among others, which influences movement of entomopathogenic nematodes (Laznik & Trdan, 2016). Finally, we also reported before that exposure of barley to nonanal enhances immunity against *Bgh* and the expression of *HvHDA2* and *HvTPL* (Brambilla et al., 2022). This suggests that, in addition to its direct antimicrobial activity, nonanal indirectly protects plants from infection by inducing or priming defence responses. Our data show that nonanal is at least in part causative for inducing defence responses in receiver plants of barley PTP emissions.

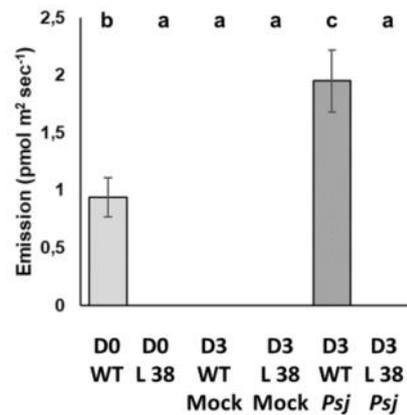


Figure 15 Nonanal emissions are compromised in *Hvald1* barley. Plants of the genotypes indicated below the panels were inoculated with *Psj* or a corresponding mock control treatment. Emissions were collected on the day before (D0) and on the second day (D3) after the treatment and analysed by GC-MS. Bars represent average emission rates of nonanal from five biologically independent samples \pm SE. Different letters above bars indicate significant differences (one-way ANOVA $P < 0.05$) (Brambilla et al., 2023).

4. Conclusion

In conclusion, our findings elucidate firstly the role for VOCs in PTP propagation of immunity in the cereal crop barley. We demonstrated that barley *Psj*-infected plants emit a specific blend of volatile compounds, within them nonanal and β -ionone. These two VOCs are responsible for the regulation of defence-related genes in neighbouring plants that eavesdrop such airborne cues. In sight of a more sustainable agriculture and crop protection techniques, it would be of great use to use plant protection product based on such VOCs or, similarly, to intercrop barley with plants that naturally emit them, for a better defence against barley powdery mildew (*Bgh*). In addition, our work introduces new insights into plant immunity in monocotyledonous plants. We reported the fundamental role of *HvALDI* and thus presumably the one of Pip and/or NHP in SAR in barley and its connection with the emission of airborne defence cues for PTP propagation of SAR (Fig. 16). Importantly, our data revealed the importance of Pip and/or NHP upstream of VOCs, which are recognised in exposed, receiver plants as defence signals in both *Arabidopsis* (Wenig et al., 2019) and barley. This strong conservation of the molecular mechanism leading to PTP propagation of SAR in plants highlights the potential of nonanal as a bio-protectant of cereal crops. If and how this can be integrated into new crop protection strategies for this and other crop species will be subject to further investigation.

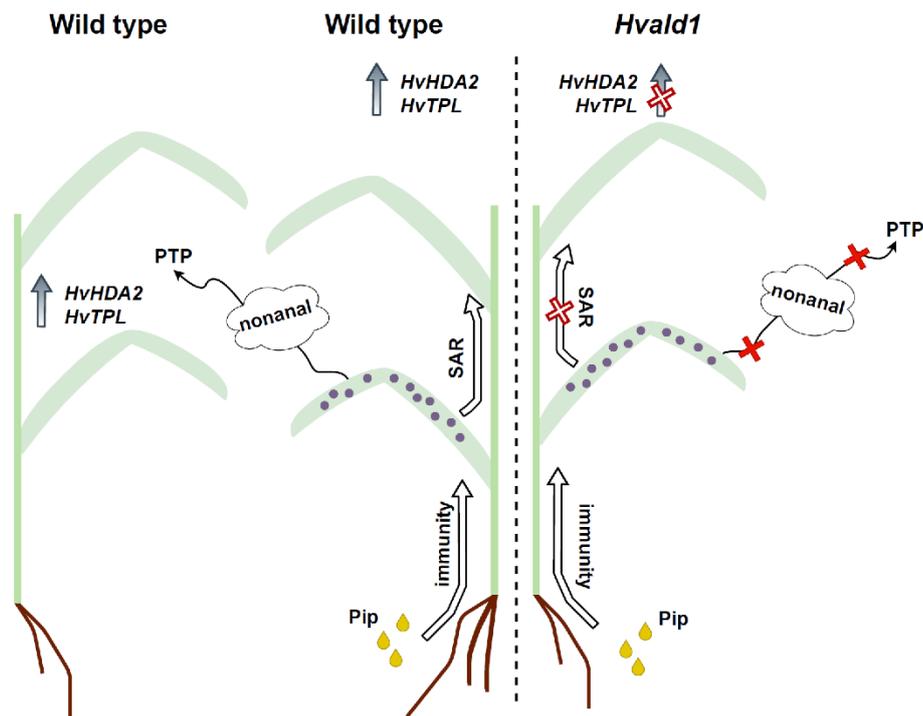


Figure 16 Working model summarizing the role of *HvALD1* and Pip in systemic acquired resistance (SAR) and plant-to-plant (PTP) defence propagation in barley. Abbreviations: Pip, pipelicolic acid; *HvHDA2*, *Hordeum vulgare* HISTONE DEACETYLASE 2; *HvTPL*, *Hordeum vulgare* TETRACOPEPTIDE REPEAT-LIKE superfamily protein (Brambilla et al., 2023).

5. References

- Ameye, M., Allmann, S., Verwaeren, J., Smaghe, G., Haesaert, G., Schuurink, R. C., & Audenaert, K. (2018). Green leaf volatile production by plants: a meta-analysis. *New Phytologist*, *220*(3), 666–683. <https://doi.org/10.1111/NPH.14671>
- Baldwin, I. T., & Schultz, J. C. (1983). Rapid changes in tree leaf chemistry induced by damage: Evidence for communication between plants. *Science*, *221*(4607), 277–279. <https://doi.org/10.1126/SCIENCE.221.4607.277>
- Balmer, D., de Papajewski, D. V., Planchamp, C., Glauser, G., & Mauch-Mani, B. (2013). Induced resistance in maize is based on organ-specific defence responses. *The Plant Journal*, *74*(2), 213–225. <https://doi.org/10.1111/tpj.12114>
- Barja, M. V., Ezquerro, M., Beretta, S., Diretto, G., Florez-Sarasa, I., Feixes, E., Fiore, A., Karlova, R., Fernie, A. R., Beekwilder, J., & Rodríguez-Concepción, M. (2021). Several geranylgeranyl diphosphate synthase isoforms supply metabolic substrates for carotenoid biosynthesis in tomato. *New Phytologist*, *231*(1), 255–272. <https://doi.org/10.1111/NPH.17283>
- Bernsdorff, F., Döring, A. C., Gruner, K., Schuck, S., Bräutigam, A., & Zeier, J. (2016). Pipelicolic acid orchestrates plant systemic acquired resistance and defense priming via salicylic acid-dependent and -independent pathways. *Plant Cell*, *28*(1), 102–129. <https://doi.org/10.1105/tpc.15.00496>
- Bostock, R. M. (2005). Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Annual Review of Phytopathology*, *43*, 545–580. <https://doi.org/10.1146/ANNUREV.PHYTO.41.052002.095505>

- Bouwmeester, H., Schuurink, R. C., Bleeker, P. M., & Schiestl, F. (2019). The role of volatiles in plant communication. *The Plant Journal*, *100*(5), 892–907. <https://doi.org/10.1111/TPJ.14496>
- Brambilla, A., Lenk, M., Ghirardo, A., Eccleston, L., Knappe, C., Lange, B., Imani, J., Schäffner, A. R., Schnitzler, J.-P., & Corina, A. (2023). *Pipecolic acid synthesis is required for systemic acquired resistance and plant-to-plant-induced immunity in barley 2 3*.
- Brambilla, A., Sommer, A., Ghirardo, A., Wenig, M., Knappe, C., Weber, B., Amesmaier, M., Lenk, M., Schnitzler, J. P., & Vlot, A. C. (2022). Immunity-associated volatile emissions of β -ionone and nonanal propagate defence responses in neighbouring barley plants. *Journal of Experimental Botany*, *73*(2), 615–630. <https://doi.org/10.1093/jxb/erab520>
- Brilli, F., Loreto, F., & Baccelli, I. (2019). Exploiting plant volatile organic compounds (VOCS) in agriculture to improve sustainable defense strategies and productivity of crops. *Frontiers in Plant Science*, *10*, 1–8. <https://doi.org/10.3389/FPLS.2019.00264/BIBTEX>
- Brosset, A., & Blande, J. D. (2022). Volatile-mediated plant–plant interactions: volatile organic compounds as modulators of receiver plant defence, growth, and reproduction. *Journal of Experimental Botany*, *73*(2), 511–528. <https://doi.org/10.1093/JXB/ERAB487>
- Cáceres, L. A., Lakshminarayan, S., Yeung, K. K. C., McGarvey, B. D., Hannoufa, A., Sumarah, M. W., Benitez, X., & Scott, I. M. (2016). Repellent and Attractive Effects of α -, β -, and Dihydro- β - Ionone to Generalist and Specialist Herbivores. *Journal of Chemical Ecology*, *42*(2), 107–117. <https://doi.org/10.1007/S10886-016-0669-Z/METRICS>
- Cellini, A., Spinelli, F., Donati, I., Ryu, C. M., & Kloepper, J. W. (2021). Bacterial volatile compound-based tools for crop management and quality. *Trends in Plant Science*, *26*(9), 968–983. <https://doi.org/10.1016/j.tplants.2021.05.006>
- Cervený, L., Strasková, A., Danková, V., Hartlova, A., Cecková, M., Staud, F., & Stulik, J. (2013). Tetratricopeptide repeat motifs in the world of bacterial pathogens: role in virulence mechanisms. *Infection and Immunity*, *81*(3), 629–635. <https://doi.org/10.1128/IAI.01035-12>
- Chauvin, A., Caldelari, D., Wolfender, J. L., & Farmer, E. E. (2013). Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded *Arabidopsis thaliana* leaves: a role for lipoxygenase 6 in responses to long-distance wound signals. *New Phytologist*, *197*(2), 566–575. <https://doi.org/10.1111/NPH.12029>
- Chen, Y. C., Holmes, E. C., Rajniak, J., Kim, J. G., Tang, S., Fischer, C. R., Mudgett, M. B., & Sattely, E. S. (2018). N-hydroxy-pipecolic acid is a mobile metabolite that induces systemic disease resistance in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(21), E4920–E4929. <https://doi.org/10.1073/pnas.1805291115>
- Conrath, U., Beckers, G. J. M., Langenbach, C. J. G., & Jaskiewicz, M. R. (2015). Priming for Enhanced Defense. In *Annual Review of Phytopathology* (Vol. 53, pp. 97–119). Annual Reviews Inc. <https://doi.org/10.1146/annurev-phyto-080614-120132>
- Cook, S. M., Khan, Z. R., & Pickett, J. A. (2007). The Use of Push-Pull Strategies in Integrated Pest Management. *Annual Review of Entomology*, *52*(1), 375–400. <https://doi.org/10.1146/annurev.ento.52.110405.091407>
- D’Alessandro, S., Mizokami, Y., Légeret, B., & Havaux, M. (2019). The Apocarotenoid β -Cyclocitric Acid Elicits Drought Tolerance in Plants. *IScience*, *19*, 461–473. <https://doi.org/10.1016/j.isci.2019.08.003>
- Dey, S., Wenig, M., Langen, G., Sharma, S., Kugler, K. G., Knappe, C., Hause, B., Bichlmeier, M., Babaeizad, V., Imani, J., Janzik, I., Stempf, T., Hüchelhoven, R., Kogel, K. H., Mayer, K. F. X., & Corina Vlot, A. (2014). Bacteria-triggered systemic immunity in barley is associated with WRKY and ETHYLENE RESPONSIVE FACTORS but not with salicylic acid. *Plant Physiology*, *166*(4), 2133–2151. <https://doi.org/10.1104/pp.114.249276>
- Dickinson, A. J., Lehner, K., Mi, J., Jia, K. P., Mijar, M., Dinneny, J., Al-Babili, S., & Benfey, P. N. (2019). β -Cyclocitral is a conserved root growth regulator. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(21), 10563–10567. <https://doi.org/10.1073/PNAS.1821445116>

- Ding, B., Bellizzi, M. del R., Ning, Y., Meyers, B. C., & Wang, G. L. (2012). HDT701, a histone H4 deacetylase, negatively regulates plant innate immunity by modulating histone H4 acetylation of defense-related genes in rice. *Plant Cell*, *24*(9), 3783–3794. <https://doi.org/10.1105/TPC.112.101972/DC1>
- Douma, J. C., Vermeulen, P. J., Poelman, E. H., Dicke, M., & Anten, N. P. R. (2017). When does it pay off to prime for defense? A modeling analysis. *New Phytologist*, *216*, 782–797. <https://doi.org/10.1111/nph.14771>
- Dudareva, N., Klempien, A., Muhlemann, J. K., & Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist*, *198*(1), 16–32. <https://doi.org/10.1111/NPH.12145>
- Engelberth, J. (2019). Primed to grow: a new role for green leaf volatiles in plant stress responses. <https://doi.org/10.1080/15592324.2019.1701240>, *15*(1). <https://doi.org/10.1080/15592324.2019.1701240>
- Erb, M. (2018). Volatiles as inducers and suppressors of plant defense and immunity—origins, specificity, perception and signaling. *Current Opinion in Plant Biology*, *44*, 117–121. <https://doi.org/10.1016/J.PBI.2018.03.008>
- Erb, M. (2019). Plant Biology: Evolution of Volatile-Mediated Plant–Plant Interactions. In *Current Biology* (Vol. 29, Issue 18). <https://doi.org/10.1016/j.cub.2019.07.066>
- Erb, M., Veyrat, N., Robert, C. A. M., Xu, H., Frey, M., Ton, J., & Turlings, T. C. J. (2015). Indole is an essential herbivore-induced volatile priming signal in maize. *Nature Communications*, *6*. <https://doi.org/10.1038/NCOMMS7273>
- Felemban, A., Braguy, J., Zurbriggen, M. D., & Al-Babili, S. (2019a). Apocarotenoids Involved in Plant Development and Stress Response. *Frontiers in Plant Science*, *10*, 1168. <https://doi.org/10.3389/FPLS.2019.01168/BIBTEX>
- Felemban, A., Braguy, J., Zurbriggen, M. D., & Al-Babili, S. (2019b). Apocarotenoids Involved in Plant Development and Stress Response. *Frontiers in Plant Science*, *10*. <https://doi.org/10.3389/FPLS.2019.01168>
- Frank, L., Wenig, M., Ghirardo, A., van der Krol, A., Vlot, A. C., Schnitzler, J. P., & Rosenkranz, M. (2021a). Isoprene and β -caryophyllene confer plant resistance via different plant internal signalling pathways. *Plant Cell and Environment*, *44*(4). <https://doi.org/10.1111/pce.14010>
- Frank, L., Wenig, M., Ghirardo, A., van der Krol, A., Vlot, A. C., Schnitzler, J. P., & Rosenkranz, M. (2021b). Isoprene and β -caryophyllene confer plant resistance via different plant internal signalling pathways. *Plant, Cell & Environment*, *44*(4), 1151–1164. <https://doi.org/10.1111/PCE.14010>
- Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. In *Annual Review of Phytopathology* (Vol. 43). <https://doi.org/10.1146/annurev.phyto.43.040204.135923>
- Glinwood, R., Ahmed, E., Qvarfordt, E., Ninkovic, V., & Pettersson, J. (2009). Airborne interactions between undamaged plants of different cultivars affect insect herbivores and natural enemies. *Arthropod-Plant Interactions*, *3*(4), 215–224. <https://doi.org/10.1007/S11829-009-9072-9>
- Goebel, M., & Yanagida, M. (1991). The TPR snap helix: a novel protein repeat motif from mitosis to transcription. *Trends in Biochemical Sciences*, *16*(C), 173–177. [https://doi.org/10.1016/0968-0004\(91\)90070-C](https://doi.org/10.1016/0968-0004(91)90070-C)
- Havaux, M. (2020). β -Cyclocitral and derivatives: Emerging molecular signals serving multiple biological functions. *Plant Physiology and Biochemistry*, *155*, 35–41. <https://doi.org/10.1016/J.PLAPHY.2020.07.032>
- Imani, J., Li, L., Schäfer, P., & Kogel, K. H. (2011). STARTS—a stable root transformation system for rapid functional analyses of proteins of the monocot model plant barley. *The Plant Journal: For Cell and Molecular Biology*, *67*(4), 726–735. <https://doi.org/10.1111/J.1365-313X.2011.04620.X>
- Jaskiewicz, M., Conrath, U., & Peterhänsel, C. (2011). Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Reports*, *12*(1), 50–55. <https://doi.org/10.1038/EMBOR.2010.186>

- Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323–329. <https://doi.org/10.1038/nature05286>
- Jud, W., Winkler, J. B., Niederbacher, B., Niederbacher, S., & Schnitzler, J. P. (2018). Volatilomics: A non-invasive technique for screening plant phenotypic traits. *Plant Methods*, *14*(1). <https://doi.org/10.1186/S13007-018-0378-4>
- Jung, H. W., Tschaplinski, T. J., Wang, L., Glazebrook, J., & Greenberg, J. T. (2009). Priming in systemic plant immunity. *Science (New York, N.Y.)*, *324*(5923), 89–91. <https://doi.org/10.1126/SCIENCE.1170025>
- Kamal, A. M., El-Tantawy, M. E., Haggag, E. G., Shukr, M. H., El-Garhy, A. M. G., & Lithy, R. M. (2019). Chemical and biological analysis of essential oils and pectins of banana, cantaloupe peels, guava pulp and formulation of banana pectin gel. *Journal of Pharmacognosy and Phytochemistry*, *8*(4), 1808–1816. <https://www.phytojournal.com/archives/2019.v8.i4.9211/chemical-and-biological-analysis-of-essential-oils-and-pectins-of-banana-cantaloupe-peels-guava-pulp-and-formulation-of-banana-pectin-gel>
- Kaminsky, L. M., Trexler, R. V., Malik, R. J., Hockett, K. L., & Bell, T. H. (2019). The Inherent Conflicts in Developing Soil Microbial Inoculants. *Trends in Biotechnology*, *37*(2), 140–151. <https://doi.org/10.1016/J.TIBTECH.2018.11.011>
- Kretschmer, M., Damoo, D., Djamei, A., & Kronstad, J. (2020). Chloroplasts and plant immunity: Where are the fungal effectors? In *Pathogens* (Vol. 9, Issue 1). MDPI AG. <https://doi.org/10.3390/pathogens9010019>
- Kubo, A., Lunde, C. S., & Kubo, I. (1995). Antimicrobial activity of the olive oil flavor compounds. *Journal of Agricultural and Food Chemistry*, *43*(6), 1629–1633. <https://doi.org/10.1021/JF00054A040>
- Kumar, N., Galli, M., Ordon, J., Stuttmann, J., Kogel, K. H., & Imani, J. (2018). Further analysis of barley MORC1 using a highly efficient RNA-guided Cas9 gene-editing system. *Plant Biotechnology Journal*, *16*(11), 1892–1903. <https://doi.org/10.1111/PBI.12924>
- Lazazzara, V., Bueschl, C., Parich, A., Pertot, I., Schuhmacher, R., & Perazzolli, M. (2018). Downy mildew symptoms on grapevines can be reduced by volatile organic compounds of resistant genotypes. *Scientific Reports 2018 8:1*, *8*(1), 1–14. <https://doi.org/10.1038/s41598-018-19776-2>
- Laznik, Ž., & Trdan, S. (2016). Attraction Behaviors of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) to Synthetic Volatiles Emitted by Insect Damaged Potato Tubers. *Journal of Chemical Ecology*, *42*(4), 314–322. <https://doi.org/10.1007/S10886-016-0686-Y>
- Lenk, M., Wenig, M., Bauer, K., Hug, F., Knappe, C., Lange, B., Mengel, F., Dey, S., Schäffner, A., Schäffner, S., & Vlot, A. C. (2019). *Pipecolic Acid Is Induced in Barley upon Infection and Triggers Immune Responses Associated with Elevated Nitric Oxide Accumulation*. *32*(10), 1303–1313. <https://doi.org/10.1094/MPMI-01-19-0013-R>
- Lenk, M., Wenig, M., Mengel, F., Häußler, F., & Vlot, A. (2018). Arabidopsis thaliana Immunity-Related Compounds Modulate Disease Susceptibility in Barley. *Agronomy*, *8*(8), 142. <https://doi.org/10.3390/agronomy8080142>
- Li, L. L., Zhao, H. H., & Kong, C. H. (2020). (-)-Loliolide, the most ubiquitous lactone, is involved in barnyardgrass-induced rice allelopathy. *Journal of Experimental Botany*, *71*(4), 1540–1550. <https://doi.org/10.1093/JXB/ERZ497>
- Li, Q., Zhu, X., Xie, Y., & Liang, J. (2021). Antifungal properties and mechanisms of three volatile aldehydes (octanal, nonanal and decanal) on *Aspergillus flavus*. *Grain & Oil Science and Technology*, *4*(3), 131–140. <https://doi.org/10.1016/J.GAOST.2021.07.002>
- Li, Z., Wang, T., He, C., Cheng, K., Zeng, R., & Song, Y. (2020). Control of Panama disease of banana by intercropping with Chinese chive (*Allium tuberosum* Rottler): Cultivar differences. *BMC Plant Biology*, *20*(1). <https://doi.org/10.1186/S12870-020-02640-9>
- Liu, H., & Brettell, L. E. (2019). Plant Defense by VOC-Induced Microbial Priming. *Trends in Plant Science*, *24*(3), 187–189. <https://doi.org/10.1016/j.tplants.2019.01.008>

- Loreto, F., & Schnitzler, J. P. (2010). Abiotic stresses and induced BVOCs. *Trends in Plant Science*, *15*(3), 154–166. <https://doi.org/10.1016/J.TPLANTS.2009.12.006>
- Lu, Y., & Yao, J. (2018). Chloroplasts at the crossroad of photosynthesis, pathogen infection and plant defense. In *International Journal of Molecular Sciences* (Vol. 19, Issue 12). MDPI AG. <https://doi.org/10.3390/ijms19123900>
- Luna, E., Bruce, T. J. A., Roberts, M. R., Flors, V., & Ton, J. (2012). Next-generation systemic acquired resistance. *Plant Physiology*, *158*(2). <https://doi.org/10.1104/pp.111.187468>
- Manosalva, P. M., Park, S. W., Forouhar, F., Tong, L., Fry, W. E., & Klessig, D. F. (2010). Methyl esterase 1 (StMES1) is required for systemic acquired resistance in potato. *Molecular Plant-Microbe Interactions : MPMI*, *23*(9), 1151–1163. <https://doi.org/10.1094/MPMI-23-9-1151>
- Markovic, D., Colzi, I., Taiti, C., Ray, S., Scalone, R., Ali, J. G., Mancuso, S., & Ninkovic, V. (2019a). Airborne signals synchronize the defenses of neighboring plants in response to touch. *Journal of Experimental Botany*, *70*(2), 691–700. <https://doi.org/10.1093/jxb/ery375>
- Markovic, D., Colzi, I., Taiti, C., Ray, S., Scalone, R., Ali, J. G., Mancuso, S., & Ninkovic, V. (2019b). Airborne signals synchronize the defenses of neighboring plants in response to touch. *Journal of Experimental Botany*, *70*(2), 691–700. <https://doi.org/10.1093/JXB/ERY375>
- Martínez-Medina, A., Van Wees, S. C. M., & Pieterse, C. M. J. (2017). Airborne signals from Trichoderma fungi stimulate iron uptake responses in roots resulting in priming of jasmonic acid-dependent defences in shoots of Arabidopsis thaliana and Solanum lycopersicum. *Plant, Cell & Environment*, *40*(11), 2691–2705. <https://doi.org/10.1111/PCE.13016>
- Mitter, B., Brader, G., Pfaffenbichler, N., & Sessitsch, A. (2019). Next generation microbiome applications for crop production - limitations and the need of knowledge-based solutions. *Current Opinion in Microbiology*, *49*, 59–65. <https://doi.org/10.1016/J.MIB.2019.10.006>
- Mofikoya, A. O., Bui, T. N. T., Kivimäenpää, M., Holopainen, J. K., Himanen, S. J., & Blande, J. D. (2019). Foliar behaviour of biogenic semi-volatiles: potential applications in sustainable pest management. *Arthropod-Plant Interactions 2019 13:2*, *13*(2), 193–212. <https://doi.org/10.1007/S11829-019-09676-1>
- Molitor, A., Zajic, D., Voll, L. M., Pons-Kühnemann, J., Samans, B., Kogel, K. H., & Waller, F. (2011). Barley leaf transcriptome and metabolite analysis reveals new aspects of compatibility and Piriformospora indica-mediated systemic induced resistance to powdery mildew. *Molecular Plant-Microbe Interactions*, *24*(12), 1427–1439. <https://doi.org/10.1094/MPMI-06-11-0177>
- Monat, C., Padmarasu, S., Lux, T., Wicker, T., Gundlach, H., Himmelbach, A., Ens, J., Li, C., Muehlbauer, G. J., Schulman, A. H., Waugh, R., Braumann, I., Pozniak, C., Scholz, U., Mayer, K. F. X., Spannagl, M., Stein, N., & Mascher, M. (2019). TRITEX: Chromosome-scale sequence assembly of Triticeae genomes with open-source tools. *Genome Biology*, *20*(1). <https://doi.org/10.1186/S13059-019-1899-5>
- Moreno, J. C., Mi, J., Alagoz, Y., & Al-Babili, S. (2020). Plant Apocarotenoids: From Retrograde Signaling to Interspecific Communication. *The Plant Journal*, *tpj.15102*. <https://doi.org/10.1111/tpj.15102>
- Murata, M., Kobayashi, T., & Seo, S. (2019). α -Ionone, an Apocarotenoid, Induces Plant Resistance to Western Flower Thrips, Frankliniella occidentalis, Independently of Jasmonic Acid. *Molecules 2020, Vol. 25, Page 17*, *25*(1), 17. <https://doi.org/10.3390/MOLECULES25010017>
- Muroi, H., Kubo, A., & Kubo, I. (1993). Antimicrobial Activity of Cashew Apple Flavor Compounds. *Journal of Agricultural and Food Chemistry*, *41*(7), 1106–1109. https://doi.org/10.1021/JF00031A018/ASSET/JF00031A018.FP.PNG_V03
- Návarová, H., Bernsdorff, F., Döring, A. C., & Zeier, J. (2013). Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant Cell*, *24*(12), 5123–5141. <https://doi.org/10.1105/TPC.112.103564>

- Ninkovic, V., Rensing, M., Dahlin, I., & Markovic, D. (2019). Who is my neighbor? Volatile cues in plant interactions. In *Plant Signaling and Behavior* (Vol. 14, Issue 9). <https://doi.org/10.1080/15592324.2019.1634993>
- Ordon, J., Gantner, J., Kemna, J., Schwalgun, L., Reschke, M., Streubel, J., Boch, J., & Stuttmann, J. (2017). Generation of chromosomal deletions in dicotyledonous plants employing a user-friendly genome editing toolkit. *The Plant Journal: For Cell and Molecular Biology*, 89(1), 155–168. <https://doi.org/10.1111/TPJ.13319>
- Paparella, A., Shaltiel-harpaza, L., & Ibdah, M. (2021). β -Ionone: Its Occurrence and Biological Function and Metabolic Engineering. *Plants (Basel, Switzerland)*, 10(4). <https://doi.org/10.3390/PLANTS10040754>
- Park, S. W., Kaimoyo, E., Kumar, D., Mosher, S., & Klessig, D. F. (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science (New York, N.Y.)*, 318(5847), 113–116. <https://doi.org/10.1126/SCIENCE.1147113>
- Pickett, J. A. (2013). Food security: Intensification of agriculture is essential, for which current tools must be defended and new sustainable technologies invented. *Food and Energy Security*, 2(3), 167–173. <https://doi.org/10.1002/fes3.32>
- Pickett, J. A., & Khan, Z. R. (2016). Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytologist*, 212(4), 856–870. <https://doi.org/10.1111/nph.14274>
- Piesik, D., Pańka, D., Jeske, M., Wenda-Piesik, A., Delaney, K. J., & Weaver, D. K. (2013). Volatile induction of infected and neighbouring uninfected plants potentially influence attraction/repellence of a cereal herbivore. *Journal of Applied Entomology*, 137(4), 296–309. <https://doi.org/10.1111/J.1439-0418.2012.01742.X>
- Pieterse, C. M. J., Van Der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, 28. <https://doi.org/10.1146/annurev-cellbio-092910-154055>
- Pu, X., Li, Z., Tian, Y., Gao, R., Hao, L., Hu, Y., He, C., Sun, W., Xu, M., Peters, R. J., Van de Peer, Y., Xu, Z., & Song, J. (2020). The honeysuckle genome provides insight into the molecular mechanism of carotenoid metabolism underlying dynamic flower coloration. *New Phytologist*, 227(3), 930–943. <https://doi.org/10.1111/NPH.16552>
- Ramel, F., Birtic, S., Cuiñé, S., Triantaphylidès, C., Ravanat, J. L., & Havaux, M. (2012). Chemical Quenching of Singlet Oxygen by Carotenoids in Plants. *Plant Physiology*, 158(3), 1267–1278. <https://doi.org/10.1104/PP.111.182394>
- Ramel, F., Birtic, S., Ginies, C., Soubigou-Taconnat, L., Triantaphylidès, C., & Havaux, M. (2012). Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 109(14), 5535–5540. https://doi.org/10.1073/PNAS.1115982109/SUPPL_FILE/SD01.XLS
- Ricciardi, V., Marciandò, D., Sargolzaei, M., Maddalena, G., Maghradze, D., Tirelli, A., Casati, P., Bianco, P. A., Failla, O., Fracassetti, D., Toffolatti, S. L., & De Lorenzis, G. (2021). From plant resistance response to the discovery of antimicrobial compounds: The role of volatile organic compounds (VOCs) in grapevine downy mildew infection. *Plant Physiology and Biochemistry*, 160(January), 294–305. <https://doi.org/10.1016/j.plaphy.2021.01.035>
- Riedlmeier, M., Ghirardo, A., Wenig, M., Knappe, C., Koch, K., Georgii, E., Dey, S., Parker, J. E., Schnitzler, J.-P., & Vlot, C. (2017). Monoterpenes support systemic acquired resistance within and between plants. *The Plant Cell*, 29(June), tpc.00898.2016. <https://doi.org/10.1105/tpc.16.00898>
- Rodriguez-Concepcion, M., Avalos, J., Bonet, M. L., Boronat, A., Gomez-Gomez, L., Hornero-Mendez, D., Limon, M. C., Meléndez-Martínez, A. J., Olmedilla-Alonso, B., Palou, A., Ribot, J., Rodrigo, M. J., Zacarias, L., & Zhu, C. (2018). A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. *Progress in Lipid Research*, 70, 62–93. <https://doi.org/10.1016/j.plipres.2018.04.004>

- Rosenkranz, M., Chen, Y., Zhu, P., & Vlot, A. C. (2021). Volatile terpenes – mediators of plant-to-plant communication. *The Plant Journal*, 108(3), 617–631. <https://doi.org/10.1111/TPJ.15453>
- Ruban, A. V. (2015). Evolution under the sun: optimizing light harvesting in photosynthesis. *Journal of Experimental Botany*, 66(1), 7–23. <https://doi.org/10.1093/JXB/ERU400>
- Sandmann, G. (2021). Diversity and origin of carotenoid biosynthesis: its history of coevolution towards plant photosynthesis. *New Phytologist*, 232(2), 479–493. <https://doi.org/10.1111/NPH.17655>
- Scala, A., Allmann, S., Mirabella, R., Haring, M. A., & Schuurink, R. C. (2013). Green Leaf Volatiles: A Plant's Multifunctional Weapon against Herbivores and Pathogens. *International Journal of Molecular Sciences* 2013, Vol. 14, Pages 17781-17811, 14(9), 17781–17811. <https://doi.org/10.3390/IJMS140917781>
- Schapiro, A. L., Valpuesta, V., & Botella, M. A. (2006). TPR Proteins in Plant Hormone Signaling. <https://doi.org/10.4161/PSB.1.5.3491>, 1(5), 229–230. <https://doi.org/10.4161/PSB.1.5.3491>
- Sharifi, R., Lee, S. M., & Ryu, C. M. (2018). Microbe-induced plant volatiles. *New Phytologist*, 220(3), 684–691. <https://doi.org/10.1111/NPH.14955>
- Shumbe, L., Bott, R., & Havaux, M. (2014). Dihydroactinidiolide, a high light-induced β -carotene derivative that can regulate gene expression and photoacclimation in Arabidopsis. *Molecular Plant*, 7(7), 1248–1251. <https://doi.org/10.1093/MP/SSU028>
- Sobhy, I. S., Bruce, T. J. A., & Turlings, T. C. J. (2018). Priming of cowpea volatile emissions with defense inducers enhances the plant's attractiveness to parasitoids when attacked by caterpillars. *Pest Management Science*, 74(4), 966–977. <https://doi.org/10.1002/PS.4796>
- Spoel, S. H., & Dong, X. (2012). How do plants achieve immunity? Defence without specialized immune cells. *Nature Reviews Immunology*, 12(2), 89–100. <https://doi.org/10.1038/nri3141>
- Tholl, D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology*, 9(3), 297–304. <https://doi.org/10.1016/J.PBI.2006.03.014>
- Tolosa, T. A., Tamiru, A., Midega, C. A. O., Van Den Berg, J., Birkett, M. A., Woodcock, C. M., Bruce, T. J. A., Kelemu, S., Pickett, J. A., & Khan, Z. R. (2019). Molasses Grass Induces Direct and Indirect Defense Responses in Neighbouring Maize Plants. *Journal of Chemical Ecology*, 45(11–12), 982–992. <https://doi.org/10.1007/s10886-019-01122-z>
- Van Hulst, M., Pelser, M., Van Loon, L. C., Pieterse, C. M. J., & Ton, J. (2006). Costs and benefits of priming for defense in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, 103(14), 5602–5607. <https://doi.org/10.1073/PNAS.0510213103>
- Vlot, A. C., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic Acid, a Multifaceted Hormone to Combat Disease. *Annual Review of Phytopathology*, 47(1), 177–206. <https://doi.org/10.1146/annurev.phyto.050908.135202>
- Vlot, A. C., Liu, P. P., Cameron, R. K., Park, S. W., Yang, Y., Kumar, D., Zhou, F., Padukkavidana, T., Gustafsson, C., Pichersky, E., & Klessig, D. F. (2008). Identification of likely orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic acquired resistance in Arabidopsis thaliana. *The Plant Journal*, 56(3), 445–456. <https://doi.org/10.1111/J.1365-313X.2008.03618.X>
- Vlot, A. C., Sales, J. H., Lenk, M., Bauer, K., Brambilla, A., Sommer, A., Chen, Y., Wenig, M., & Nayem, S. (2020). Systemic propagation of immunity in plants. *New Phytologist*. <https://doi.org/10.1111/nph.16953>
- Vlot, A. C., Sales, J. H., Lenk, M., Bauer, K., Brambilla, A., Sommer, A., Chen, Y., Wenig, M., & Nayem, S. (2021). Systemic propagation of immunity in plants. In *New Phytologist* (Vol. 229, Issue 3, pp. 1234–1250). Blackwell Publishing Ltd. <https://doi.org/10.1111/nph.16953>
- Wang, X. dong, Bi, W. shuai, Gao, J., Yu, X. mei, Wang, H. yan, & Liu, D. qun. (2018). Systemic acquired resistance, NPR1, and pathogenesis-related genes in wheat and barley. *Journal of Integrative Agriculture*, 17(11), 2468–2477. [https://doi.org/10.1016/S2095-3119\(17\)61852-5](https://doi.org/10.1016/S2095-3119(17)61852-5)

- Wei, S., Hannoufa, A., Soroka, J., Xu, N., Li, X., Zebarjadi, A., & Gruber, M. (2011). Enhanced β -ionone emission in *Arabidopsis* over-expressing AtCCD1 reduces feeding damage in vivo by the crucifer flea beetle. *Environmental Entomology*, *40*(6), 1622–1630. <https://doi.org/10.1603/EN11088>
- Wenig, M., Ghirardo, A., Sales, J. H., Pabst, E. S., Breitenbach, H. H., Antritter, F., Weber, B., Lange, B., Lenk, M., Cameron, R. K., Schnitzler, J.-P., & Vlot, A. C. (2019). Systemic acquired resistance networks amplify airborne defense cues. *Nature Communications*, *10*(1). <https://doi.org/10.1038/s41467-019-11798-2>
- Wu, Y., Yi, G., Peng, X., Huang, B., Liu, E., & Zhang, J. (2013). Systemic acquired resistance in Cavendish banana induced by infection with an incompatible strain of *Fusarium oxysporum* f. sp. cubense. *Journal of Plant Physiology*, *170*(11), 1039–1046. <https://doi.org/10.1016/j.jplph.2013.02.011>
- Yang, F., Xiao, K., Pan, H., & Liu, J. (2021). Chloroplast: The Emerging Battlefield in Plant–Microbe Interactions. *Frontiers in Plant Science*, *12*. <https://doi.org/10.3389/fpls.2021.637853>
- Yang, Y., Zhao, J., Liu, P., Xing, H., Li, C., Wei, G., & Kang, Z. (2013). Glycerol-3-Phosphate Metabolism in Wheat Contributes to Systemic Acquired Resistance against *Puccinia striiformis* f. sp. tritici. *PLoS ONE*, *8*(11), e81756. <https://doi.org/10.1371/journal.pone.0081756>
- Ye, M., Liu, M., Erb, M., Glauser, G., Zhang, J., Li, X., & Sun, X. (2021). Indole primes defence signalling and increases herbivore resistance in tea plants. *Plant Cell and Environment*, *44*(4), 1165–1177. <https://doi.org/10.1111/PCE.13897>
- Yi, H. S., Heil, M., Adame-Álvarez, R. M., Ballhorn, D. J., & Ryu, C. M. (2009a). Airborne Induction and Priming of Plant Defenses against a Bacterial Pathogen. *Plant Physiology*, *151*(4), 2152–2161. <https://doi.org/10.1104/PP.109.144782>
- Yi, H. S., Heil, M., Adame-Álvarez, R. M., Ballhorn, D. J., & Ryu, C. M. (2009b). Airborne Induction and Priming of Plant Defenses against a Bacterial Pathogen. *Plant Physiology*, *151*(4), 2152–2161. <https://doi.org/10.1104/PP.109.144782>
- Yildiz, I., Mantz, M., Hartmann, M., Zeier, T., Kessel, J., Thurow, C., Gatz, C., Petzsch, P., Köhrer, K., & Zeier, J. (2021). The mobile SAR signal N-hydroxypipicolinic acid induces NPR1-dependent transcriptional reprogramming and immune priming. *Plant Physiology*. <https://doi.org/10.1093/plphys/kiab166>
- Zhang, J. hong, Sun, H. long, Chen, S. yang, Zeng, L. I., & Wang, T. tao. (2017). Anti-fungal activity, mechanism studies on α -Phellandrene and Nonanal against *Penicillium cyclopium*. *Botanical Studies*, *58*(1), 1–9. <https://doi.org/10.1186/S40529-017-0168-8/FIGURES/6>
- Zhao, M., Cheng, J., Guo, B., Duan, J., & Che, C. T. (2018). Momilactone and Related Diterpenoids as Potential Agricultural Chemicals. *Journal of Agricultural and Food Chemistry*, *66*(30), 7859–7872. <https://doi.org/10.1021/ACS.JAFC.8B02602>
- Zhi, P., Kong, L., Liu, J., Zhang, X., Wang, X., Li, H., Sun, M., Li, Y., & Chang, C. (2020). Histone Deacetylase TaHDT701 Functions in TaHDA6-TaHOS15 Complex to Regulate Wheat Defense Responses to *Blumeria graminis* f.sp. tritici. *International Journal of Molecular Sciences*, *21*(7). <https://doi.org/10.3390/IJMS21072640>
- Zhou, X., Liao, H., Chern, M., Yin, J., Chen, Y., Wang, J., Zhu, X., Chen, Z., Yuan, C., Zhao, W., Wang, J., Li, W., He, M., Ma, B., Wang, J., Qin, P., Chen, W., Wang, Y., Liu, J., ... Chen, X. (2018). Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(12), 3174–3179. <https://doi.org/10.1073/PNAS.1705927115/-/DCSUPPLEMENTAL>
- Zhou, X., Zheng, Y., Cai, Z., Wang, X., Liu, Y., Yu, A., Chen, X., Liu, J., Zhang, Y., & Wang, A. (2021). Identification and Functional Analysis of Tomato TPR Gene Family. *International Journal of Molecular Sciences*, *22*(2), 1–18. <https://doi.org/10.3390/IJMS22020758>
- Zhuang, X., Fiesselmann, A., Zhao, N., Chen, H., Frey, M., & Chen, F. (2012). Biosynthesis and emission of insect herbivory-induced volatile indole in rice. *Phytochemistry*, *73*, 15–22. <https://doi.org/10.1016/j.phytochem.2011.08.029>

6. Acknowledgements

I would like to thank Prof. Dr. Corina Vlot and Prof. Dr. Jörg Durner for supervising this project and giving me the opportunity to work on such an interesting topic.

I would also like to acknowledge the support of Prof. Dr. Wilfried Schwab in giving interesting and useful inputs on this work.

In addition, I heartily thank all the scientists, technicians, colleagues who have given me their scientific and personal help during these years. This includes people at Helmholtz Zentrum München, at the Institute of Biochemical Plant Pathology, at the Institute of Environmental Simulation and at the School of Life Science at TUM.

Finally, a kind thank you to all the friends, relatives and people outside the academic world that have supported me in this journey. Without you, this would have not be possible.