FACULTY OF BIOSCIENCE ENGINEERING

HOW DOES MCCOPE HELP PLANTS TO COPE WITH NEMATODES?

Jasper Matthys
Student number: 01608601

Promotor: prof. dr. ir. Tina Kyndt
Tutor: ir. Jonas De Kesel

Master's Dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Science in Bioscience Engineering: Cell and Gene Biotechnology

Academic year: 2020 - 2021

GHENT UNIVERSITY

Word of thanks

First of all, I would like to express my gratitude to prof. dr. ir. Tina Kyndt, for the great opportunity to join her team and to investigate this interesting research project. I'm grateful for her valuable feedback, the possibility to execute a great variety in assays and the immense support.

Many thanks go to ir. Jonas De Kesel, for the great assistance and guidance that proved very valuable in conducting this research. He was always available for answering questions, to provide (loads of 😉) feedback and support, sharing tips and tricks, and much more. He allowed me to work with a healthy dose of independence in a pleasant and loose atmosphere, and was always open-minded to suggestions and discussions from my side. His motivation to obtain a great thesis with a comprehensive story worked infectious.

Further, I would like to thank the EPIC and NEMA research groups, for providing support when needed, for teaching me specific techniques, for the valuable feedback on the presentation of my results and for the occasional (online) activities organised by 'The party Committee'. I would like to specifically express my gratitude to ir. Eva Degroote, for the support during the mCCOPE boiling experiment and DHA measurements in the extract. I would also like to thank Satish Namdeo Chavan, for the assistance during the mRNA-seq experiment and for allowing me to use the data of 5 and 20 mM DHA treated plants from his experiment. I would also like to thank Lien De Smet, for showing me how to count and classify nematodes. Next, I would also like to thank ir. Anikó Meijer for the carpooling on our bi-weekly excursions in the first semester to the UGhent Greenhouse facilities.

Finally, a very special thank you goes to my family and friends for their unconditional support and encouragement. Completing this master thesis would not have been possible without them.

Abstract

Rice is an important monocot model crop that sustains approximately half the world's population. Recent advances in agricultural practices, with the aim to reduce the environmental impact of rice production, have resulted in increased rice yield losses due to *Meloidogyne graminicola* infections, while nematicides have become increasingly banned. As an alternative pathogen control strategy, 'induced resistance' (IR) is of interest. In IR, a stimulus is applied to the plant, potentiating the plant immune response. Here, a new IR-stimulus, the plant extract 'melon *Cucurbitaceae* COld Peeling Extract' (mCCOPE), will be investigated. Plant protection via this extract is based on the use of melon peels, a waste stream from food industry and agriculture, thus contributing to a circular economy.

In this study, the activation of the rice root immune system upon foliar mCCOPE application was analysed using mRNA-seq and subsequent biochemical validation assays. Further, it was investigated if the IR-stimuli ascorbate oxidase and dehydroascorbic acid are the active compounds present in mCCOPE. The additional activity of mCCOPE as a nematicide to *M. graminicola* was assessed as well. Finally, potential fitness costs related to augmented defence activation were assessed upon repetitive and life-long mCCOPE application.

The results showed that mCCOPE application results in systemic redox signalling in the rice root, transient root lignification, early auxin and ethylene signalling, and secondary metabolism activation, which clearly illustrates a successful establishment of the IR-phenotype. It was shown that neither ascorbate oxidase, nor dehydroascorbic acid were responsible for the observed IR. Moreover, mCCOPE was shown to negatively affect *M. graminicola* viability in a dehydroascorbic acid independent way. Finally, mCCOPE does not reduce rice growth or yield upon repeated application, even though it directly affects thousands of genes.

In conclusion, mCCOPE could contribute to a sustainable solution to deal with the growing problems of *M. graminicola* rice infections.

Table of contents

Abbreviation list

AA	Ascorbic acid (vitamin C)
ABA	Abscisic acid
ACC	AMINOCYCLOPROPANECARBOXYLATE
AO	Ascorbate oxidase
AOS	ALLENE OXIDE SYNTHASE
BABA	β-aminobutyric acid
bp	Base pair
BTH	Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester
CCOPE	*Cucurbitaceae* cold peeling extract
DAMP	Damage-associated molecular pattern
DHA	Dehydroascorbic acid
DHAR	Dehydroascorbic acid reductase
DKG	2,3-diketo-L-gulonic acid
DNA	Deoxyribonucleic acid
dpi	Days post infection/inoculation
dpt	Days post treatment
ET	Ethylene
FOX	Ferrous oxidation
GO	Gene ontology
hpi	Hours post infection/infestation
HPLC	High-performance liquid chromatography
hpt	Hours post treatment
HR	Hypersensitive response
IAA	Auxin
IPM	Integrated pest management
IQR	Interquartile range
IR	Induced resistance
IRGSP	International Rice Genome Sequencing Project
ISR	Induced systemic resistance
J2	Second stage juvenile
J3	Third stage juvenile
J4	Fourth stage juvenile
JA	Jasmonic acid
LC-MS	Liquid chromatography-mass spectrometry
log2FC	Log2 fold change
LRR	Leucine rich repeat
MAPK	Mitogen-activated protein kinase
mCCOPE	Melon *Cucurbitaceae* cold peeling extract
MDHA	Monodehydroascorbate
MDHAR	Monodehydroascorbate reductase
MeJA	Methyl jasmonate
Mg	*Meloidogyne graminicola*
mRNA-seq	Messenger RNA sequencing
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
Pep	Plant elicitor peptide
PIP	Plasma membrane intrinsic proteins
ppm	Parts per million
PR	Pathogenesis-related

PRR	Pattern recognition receptor
RBOH	Respiratory burst oxidase homologue
RdDM	RNA-directed DNA methylation
RKN	Root-knot nematode
RLK	Receptor-like kinase
RLP	Receptor-like protein
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT-qPCR	Reverse transcription quantitative PCR
SA	Salicylic acid
SAP	Sand/absorbent polymer
SAR	Systemic acquired resistance
SEM	Standard error of the mean
SOD	Superoxide dismutase
T	Temperature
TIR	Transgenerational induced resistance
UDP	Uridine 5'-diphosphate
UHPLC	Ultra-high-performance liquid chromatography
UHT	Ultra-high temperature
UV	Ultraviolet
XO	Xylenol orange

1. Literature

1.1. Introduction

Rice (*Oryza sativa*) is an important monocot model crop that sustains approximately half the world's population (Seck *et al.*, 2012). It is traditionally grown in paddy fields (flooded fields), which consume large amounts of water (844.5 m^3/ton rice) and result in high methane production (107 mg/m^2day) (Sass *et al.*, 1992; Seck *et al.*, 2012; Yoo *et al.*, 2014). Therefore, there is a recent trend towards producing rice under aerobic conditions, to improve the sustainability of rice farming (51% less water usage and 88% lower methane production) (Sass *et al.*, 1992; Prot and Matias, 1995; Soriano and Reversat, 2003; Bouman *et al.*, 2005). However, the root-knot nematode (RKN) *Meloidogyne graminicola* (*Mg*), the major plant-parasitic nematode in the rice field, thrives under these aerobic conditions (Mantelin, Bellafiore and Kyndt, 2017). Thus, to improve the sustainability of rice agriculture, *Mg* must be kept under control. For many years this was done using chemical nematicides, but recently nematicides and their active compounds have become increasingly banned (Wesemael, Viaene and Moens, 2011; UTZ, 2015; Mantelin, Bellafiore and Kyndt, 2017). Together, this results in an increased need for alternative, sustainable and environmentally friendly control strategies. Recently, a lot of focus has been given towards the use of natural plant immune mechanisms to deal with pathogens and pests. An important example is the use of so-called induced resistance (IR)-stimuli, which are stimuli that induce a state of enhanced defence in the plant, called IR. IR can (nonexclusively) rely on direct induction of defences upon IR-stimulus perception and 'defence priming' (De Kesel *et al.*, 2021). Defence priming is the induction of a 'memory' that allows the plant to stimulate earlier, stronger, and/or more sustained defence responses upon a challenge by a pathogen (Balmer *et al.*, 2015; Conrath *et al.*, 2015). Multiple examples of IR-stimuli in the rice-*Mg* pathosystem have already been described, albeit only under lab conditions (Anita and Samiyappan, 2012; Ji *et al.*, 2015; Huang *et al.*, 2016; De Kesel *et al.*, 2020). Recently, the IR-stimulus *Cucurbitaceae* COld Peeling Extract (CCOPE) was patented (patent application: WO2021/009164A1; Kyndt *et al.*, 2020) by the Kyndt-group. This IR-stimulus is a natural resource based on waste streams (cucurbit peels), highlighting its sustainable and circular economy character. It is forecasted that the global biopesticide (substances derived from naturally occurring resources that control diseases or pests) market will reach 6.77 billion US dollar by 2026 and will have a growth rate of 116% during the period 2021-2026 (Market Data Forecast, 2020). This thesis focusses on foliar applications of CCOPE derived from melon (*Cucumis melo* var. *cantalupensis*; mCCOPE) and its effects on rice root immunity towards nematodes. Hereto four major research questions will be answered:

1. Are the enzyme ascorbate oxidase (AO), and/or its end-product, dehydroascorbic acid (DHA), the central IR-stimulating compound(s) present in mCCOPE?
2. What is the mode-of-action of mCCOPE-IR?
3. Does mCCOPE affect *Mg* viability?
4. Is mCCOPE applicable in the long-term in agriculture, without negative effects on plant growth and yield?

The role of AO and DHA as central IR-stimulating couple is investigated for two main reasons. First, it is well known that the mature fruits of the *Cucurbitaceae,* and especially melon peels, are abundant in the AO enzyme and its substrate, vitamin C (ascorbic acid, AA) (Mosery and Kanellis, 1994; Diallinas *et al.*, 1997; Stevens *et al.*, 2018). As AO catalyses the reaction of vitamin C to DHA (Pignocchi and Foyer,

2003), large levels of DHA are present as well. Second, these compounds have already been associated with the induction of IR against nematodes in both rice and sugar beet (*Beta vulgaris* subsp. *vulgaris*) (Singh, Nobleza, *et al.*, 2020; Singh, Verstraeten, *et al.*, 2020). Further, the mode-of-action of mCCOPE on rice root immunity will be elucidated, together with its potential to act as a nematicide. Previously, different cucurbit extracts were shown to possess such a nematicidal activity, but this was not yet shown against *Mg* (Elbadri *et al.*, 2008; Rizvi and Fayyaz, 2014; Gad *et al.*, 2018; Eman and El-Nuby, 2019). The effects on the rice root transcriptome will be analysed by mRNA sequencing (mRNA-seq) and independent biochemical validation assays. Finally, potential rice growth and yield inhibition upon long-term application will be analysed.

1.2. The *Meloidogyne graminicola*-rice pathosystem

Rice (*Oryza sativa*) is the most-consumed cereal food in the world with human consumption exceeding 100 kg per capita annually in many Asian and some African countries (Seck *et al.*, 2012). It sustains approximately half of the world's population (Seck *et al.*, 2012; Muthayya *et al.*, 2014). Asia is the main rice-growing region of the world, accounting for 90% of the global production (Papademetriou, Dent and Herath, 1999), which is estimated at 782 tonnes of paddy rice (unprocessed rice) annually (FAOSTAT, 2021). Rice is cultivated in a variety of climatic conditions, ranging from mountainous regions to river deltas, and its cultivation is classified based on the hydrological conditions: irrigated, deepwater (50 cm water for at least one month), rain fed-lowland and rain fed-upland systems (Kende, Van Knaap and Cho, 1998; Kyndt, Fernandez and Gheysen, 2014). The most common system in Asia is the irrigated system (Figure 1.1A), while rain-fed systems are more prevalent in Africa (Seck *et al.*, 2012). Aerobic rice production (Figure 1.1B) is a water-efficient cropping system - it uses 51% less water than paddy fields - that is gaining popularity in India and Southeast Asia. Not only is this strategy more relevant to deal with water shortages, it also limits methane production by rice fields (88% lower methane production) (Sass *et al.*, 1992; Prot and Matias, 1995; Soriano and Reversat, 2003; Bouman *et al.*, 2005; Mantelin, Bellafiore and Kyndt, 2017). Next to being an important crop, rice is also the model monocot plant for molecular studies, due to its fully sequenced genome, the availability of functional genomics tools and the relatively easy production of transgenic plants (Shimamoto and Kyozuka, 2002; Han *et al.*, 2007).

Plant-parasitic nematodes cause more than 80 billion US dollar losses in worldwide agriculture annually (Mitkowski and Abawi George, 2003; Nicol *et al.*, 2011; Jones *et al.*, 2013). The economically most devastating species are the sedentary endoparasitic nematodes, including the RKNs of the genus *Meloidogyne*, and the cyst nematodes of the genera *Heterodera* and *Globodera* (Jones *et al.*, 2013). Annual rice yield losses caused by plant-parasitic nematodes have been estimated between 10% to

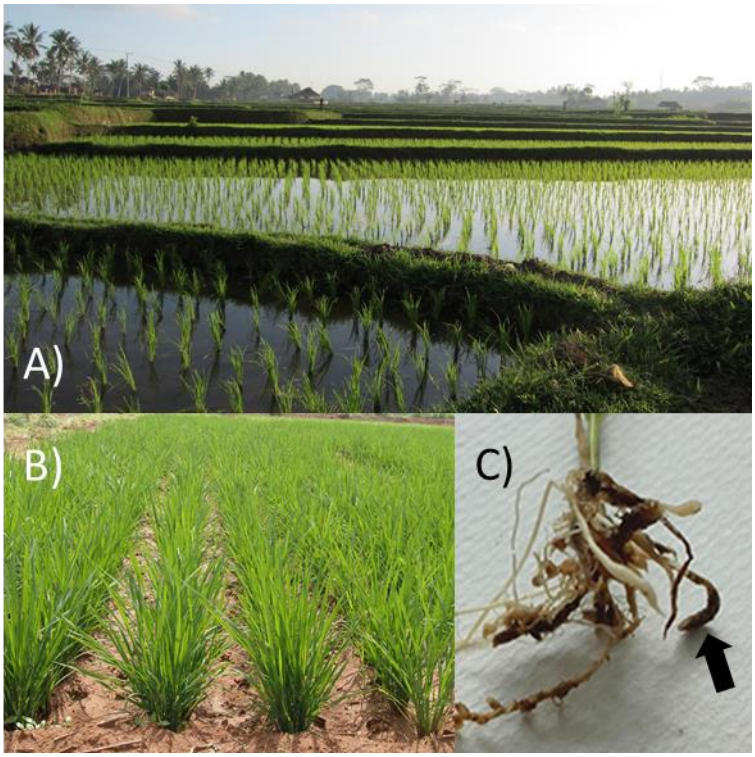

Figure 1.1: (A) Paddy fields for rice cultivation (Wen, 2011). (B) Aerobic rice cultivation (TNAU, 2016). (C) *Mg* infested rice roots, with a black arrow pointing at an *Mg* gall (European and Mediterranean Plant Protection Organization, 2020).

25% worldwide (Bridge, Plowright and Peng, 2005). One of the most damaging types of nematodes attacking rice are the RKNs. *Mg* and *Meloidogyne incognita* can cause up to 70% rice yield losses on the field (Bridge, Plowright and Peng, 2005). *Mg*, or commonly named the rice RKN, is the most abundant nematode in the various rice-growing systems (Mantelin, Bellafiore and Kyndt, 2017). It is considered as a major threat to rice agriculture, particularly in Asia, as changes in agricultural practices in light of environmental and socioeconomic conditions have caused a dramatic increase in *Mg* populations (De Waele and Elsen, 2007; Mantelin, Bellafiore and Kyndt, 2017). However, not only *Mg* strongly infects rice, *Hirschmanniella oryzae* is strongly flooding-adapted, causing it to dominate the paddy fields and mainly deepwater rice (Kyndt, Fernandez and Gheysen, 2014). Typical symptoms of an *Mg*-infection are hook-shaped galls mainly formed at the root tips (Figure 1.1C), stunting, chlorosis, loss of vigour, patches of stunted growth in the field, reduced tillering, poor reproduction and substantial yield losses (Kyndt, Fernandez and Gheysen, 2014; Mantelin, Bellafiore and Kyndt, 2017). Since the above-grounds symptoms are not specific, it is likely that *Mg* is an underestimated pathogen, as many farmers likely attribute this damage to nutrient and water deficiency, or secondary pathogens (Mantelin, Bellafiore and Kyndt, 2017).

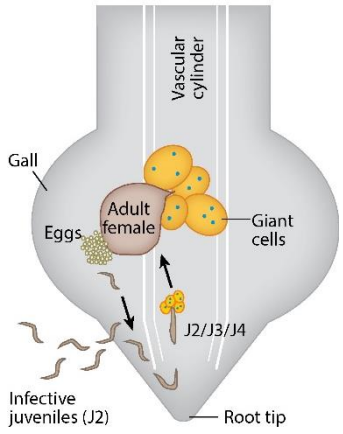

Figure 1.2: The *M. graminicola* life cycle. The second stage juveniles (J2) invade the rice root at the elongation zone and migrate intercellularly to the root tip. Here they invade the vascular cylinder and establish a feeding site with giant cells, and they become sedentary. Proliferation of the cells surrounding the feeding site results in gall formation. Egg-laying females lay their eggs inside the cortex. J2s hatching from these eggs can infect new roots or infect the same root as the parent. (Kyndt, Fernandez and Gheysen, 2014)

The life cycle of *Mg* (Figure 1.2) takes about 19-27 days on rice, depending on the temperature and flooding conditions (Bridge and Page, 1982; Kyndt, Fernandez and Gheysen, 2014; Mantelin, Bellafiore and Kyndt, 2017). Second stage juveniles (J2s, 350-510 µm long) invade the root at the elongation zone and migrate intercellularly in the rice cortex towards the root tip, where they invade the central stele (Mantelin, Bellafiore and Kyndt, 2017). Since the J2s move intercellularly, contrarily to most migratory and cyst nematodes, they cause less extensive damage to the cells (Holbein, Grundler and Siddique, 2016). Nevertheless, the movement can be sensed by the plant due to the recognition of minor plant damage and pathogen-associated molecular patterns (PAMPs). This allows the plant to respond to the infection (Holbein, Grundler and Siddique, 2016; Mantelin, Bellafiore and Kyndt, 2017). In the stele, the biotrophic *Mg* will create a feeding site, which is a nematode nursing tissue induced in the plant. *Mg* establishes its feeding site in the vascular cylinder close to the root meristem, as opposed to the other RKNs, which typically migrate further away through the stele (Mantelin, Bellafiore and Kyndt, 2017). Therefore, *Mg*-galls will typically be found at the root tips. Within the stele, the J2s select vascular parenchyma tissue that contains xylem and phloem in which they will induce their feeding site and become sedentary (Kyndt, Fernandez and Gheysen, 2014). The *Mg* feeding site typically consists out of five to eight so-called 'giant cells' (Kumar *et al.*, 2014; Kyndt, Fernandez and Gheysen, 2014; Nguyễn *et al.*, 2014; Mantelin, Bellafiore and Kyndt, 2017). These giant cells are multinucleate, hypertrophied cells with thickened cell walls and a dense cytoplasm, formed by repeated rounds of nuclear division and cell growth in the absence of cytokinesis (Kyndt *et al.*, 2013; Kyndt, Fernandez and Gheysen, 2014). *Mg* may already begin to induce giant cells two days post infection (dpi) and together with surrounding cell proliferation this will result in so-called root gall formation (Kyndt, Fernandez and Gheysen, 2014; Nguyễn *et al.*,

2014). After the feeding site formation, the nematode will moult three times to reach the adult stage (Mantelin, Bellafiore and Kyndt, 2017). Thus, infecting nematodes can be categorised as J2s, third stage juveniles (J3s), fourth stage juveniles (J4s) and adults.

Mg reproduces via facultative meiotic parthenogenesis in which amphimixis (mating) can occur at low frequency, about 0.5% (Triantaphyllou, 1969). Adult females produce eggs, which hatch and release J2s. Egg-laying females will develop within 14 dpi and remain inside the root, where they are protected from the external environment (Fernandez, Cabasan and De Waele, 2014; Kumar *et al.*, 2014; Kyndt, Fernandez and Gheysen, 2014; Mantelin, Bellafiore and Kyndt, 2017). The eggs are laid inside the cortex, which is rather unusual compared to other RKN species, but is an advantage when the host plant grows in flooded conditions, since the nematode cannot penetrate new rice roots in flooded conditions (Kyndt, Fernandez and Gheysen, 2014; Mantelin, Bellafiore and Kyndt, 2017). The next generation developing in these eggs are therefore more likely to make new feeding sites in the same root (Mantelin, Bellafiore and Kyndt, 2017). Additionally, *Mg* can survive in flooded soil, allowing it to cause problems in all types of rice agrosystems (Kyndt, Fernandez and Gheysen, 2014; Mantelin, Bellafiore and Kyndt, 2017). However, the increasingly popular aerobic system, as well as summer-irrigated lowland and upland rice systems, are extremely vulnerable to *Mg*, causing the nematode to increase in importance due to increased damage (Prot and Matias, 1995; Soriano and Reversat, 2003; Win *et al.*, 2013; Boubakri *et al.*, 2016; Martinez-Medina *et al.*, 2016). Therefore, it is important to also evaluate the negative impact by *Mg* on the sustainability of aerobic rice production in an attempt to resolve water shortages.

For *Mg*-control, crop rotation has only limited effects. Although rice is the main host of *Mg*, the nematode has been reported to infect over 100 plant species, frequently other cereals, as well as grasses and dicotyledons (Mantelin, Bellafiore and Kyndt, 2017; Shukla *et al.*, 2017). Among its hosts are many weeds typically found in rice fields that may form a major reservoir for the nematode (Rich *et al.*, 2009; Mantelin, Bellafiore and Kyndt, 2017). Therefore, using crop rotation, in which non-host plants are cultivated by farmers, such as mustard, sesame, miller or mung bean, only has limited effect in tropical fields (Ventura *et al.*, 1981; Rahman, 1990; Mantelin, Bellafiore and Kyndt, 2017). The use of nematicides has been the most efficient way to control plant-parasitic nematodes in the field for many years (Mantelin, Bellafiore and Kyndt, 2017; Oka, 2020). However, most of these chemicals are extremely toxic and therefore many have been banned from most countries, including methyl bromide (Wesemael, Viaene and Moens, 2011; Vos *et al.*, 2012; Oka, 2020). Additionally, the active ingredients are becoming increasingly restricted, thus causing the need for alternative control strategies (UTZ, 2015; Oka, 2020). In this thesis we will be looking into a potential 'IR-stimulus' (see Section 1.3.3.) that could form an alternative, more sustainable nematode control strategy.

1.3. The plant immune system

1.3.1. Constitutive defence

Plant defence consists of multiple layers which each must be dealt with for the pathogen to successfully infect. The first obstacle for a pathogen is the constitutive defence, which is always present and/or active in the plant. This defence includes preformed anatomical structures, the cell walls, phytoanticipins (preformed antipathogenic chemicals), *etc*. (Underwood, 2012; Holbein, Grundler and Siddique, 2016; Anupama, 2020). In defence to nematodes, the cell wall rigidity is of major importance, as all plant-parasitic nematodes penetrate cells to feed on (Holbein, Grundler and Siddique, 2016). Particularly lignin, a recalcitrant aromatic polymer, forms an important barrier for pathogen

penetration (see Section 1.3.2.4.) (Chaouch, Queval and Noctor, 2012). Further, the preformed phytoanticipins can be nematicidal and help stop infection and/or nematode development. Phytoanticipins include molecules like alkaloids, terpenoids, phenolics, cyanogenic glucosides, … (Bryan *et al.*, 2006; Anupama, 2020).

1.3.2. The inducible immune system

1.3.2.1. General

Upon detection of a threat, the plant will activate defence responses additional to its constitutive defence, referred to as the inducible plant immune system (not to be confused with 'induced resistance'; see Section 1.3.3.). The latter can be divided in two parts, cell surface mechanisms and intracellular immunity (Deng *et al.*, 2020). Cell surface immune receptors (pattern recognition receptors, PRRs) detect molecular patterns originating from pathogens (pathogen-associated molecular patterns, PAMPs) or damaged plant cell molecules (damage-associated molecular patterns, DAMPs) (Bentham *et al.*, 2020). This layer of immunity is also known as pattern-triggered immunity (PTI) (Jones and Dangl, 2006). The PRRs consist of membrane-localised receptor-like kinases (RLKs) and associated receptor-like proteins (RLPs) (Deng *et al.*, 2020). When detection occurs and a ligand binds to the receptor, these receptor-kinase complexes get activated. RLKs obtain kinase activity typically by dimerization and subsequent phosphorylation of the intracellular kinase domains, allowing transduction of immune signals, initiating a broad range of downstream events, such as reactive oxygen species (ROS)-bursts, calcium influx, activation of mitogen-associated and calcium-dependant protein kinases (MAPKs and CDPKs, respectively), cell wall reinforcement and changes in defence-related expression patterns (Bentham *et al.*, 2020). This immune response occurs in both susceptible and resistant plants, and is typically fast, short-lived and relatively low in intensity.

To overcome this immune response and thus induce disease, successful pathogens secrete effectors into the host cell, leading to the so-called effector triggered susceptibility (ETS) (Jones and Dangl, 2006; Deng *et al.*, 2020). In turn, intracellular immune receptors, called nucleotide-binding leucine-rich repeat receptor proteins (NLRs), can recognise these effectors and trigger a strong and long-lasting immune response, known as the effector-triggered immunity (ETI) (Jones and Dangl, 2006). Genes coding for NLRs are often called resistance (*R*)-genes, as they are typically responsible for resistant phenotypes, by triggering a hypersensitive response and necrosis (Bentham *et al.*, 2020; Deng *et al.*, 2020). When a pathogen can successfully repress the plant defence and infect, we speak of a compatible interaction. An incompatible interaction occurs when the plant defence was successful in supressing infection (Ponzio *et al.*, 2016). A schematic overview of the most important components in the nematode defence response can be found on Figure 1.3.

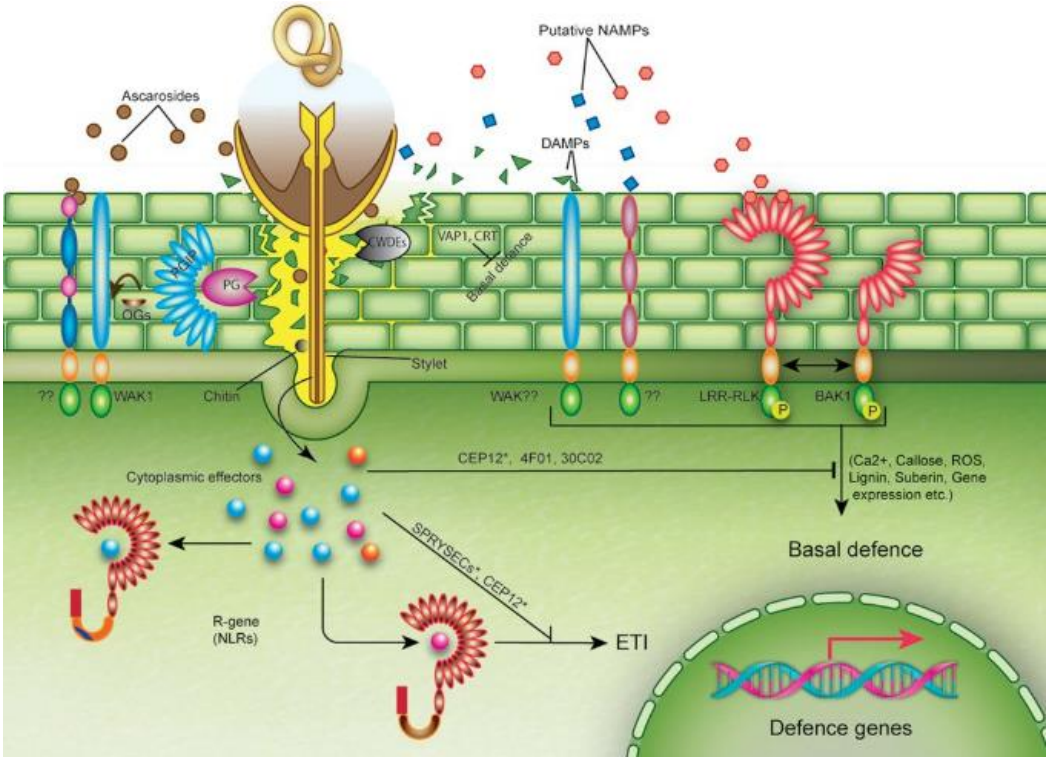

Figure 1.3: Schematic overview of the plant defence response to a nematode infection. CWDEs: cell wall degrading enzymes; LRR-RLK: leucine-rich repeat receptor-like kinases; ETI: effector triggered immunity; NLR: nucleotide-binding leucine-rich repeat receptor proteins; PG: polygalacturonase; PGIP: PG-inhibiting protein; OG: oligogalacturonides. (Holbein, Grundler and Siddique, 2016)

1.3.2.2. Hormone signalling

Plant hormones are essential in plant growth, development and defence regulation. The downstream signalling responses in PTI and ETI are regulated by a complex interaction of defence-related phytohormones, such as ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) (Pieterse *et al.*, 2012). Through synergistic and antagonistic interactions, other phytohormones, that are typically more important in developmental processes and abiotic stress response, wire into the immunity backbone of the classical defence hormones (Pieterse *et al.*, 2012). SA is generally believed to regulate defence against biotrophic pathogens, while ET and JA synergistically operate to manage necrotrophic pathogen and herbivorous insect resistance (Choi and Klessig, 2016; Holbein, Grundler and Siddique, 2016). This generalisation is typically based on dicot-shoot focussed research, but it is known that rice – and monocots in general - deviates from this model and that no simple extrapolations can be made from shoot towards root immunity (De Vleesschauwer, Gheysen and Höfte, 2013). Exogenous application of ET or JA on rice shoots was shown to cause systemic defence to RKNs in the roots (Nahar *et al.*, 2011). Moreover, it is proposed that the JA-pathway is key in the defence to RKNs and that it is modulated and activated by the ET-pathway (Nahar *et al.*, 2011).

Auxin (IAA) is a typical growth and development hormone, and is widely regarded as a negative regulator of plant defence. This regulation is often described as tipping the scale of energy allocation between growth and defence towards growth (Naseem, Kaltdorf and Dandekar, 2015). However, again this generalisation appears not to be adequate. In *Arabidopsis thaliana* (hereafter *Arabidopsis*) it was shown that IAA synergistically interacts with JA to defend against the necrotrophic pathogen *Alternaria brassicicola* (Qi *et al.*, 2012). In turn, JA can stimulate IAA biosynthesis in *Arabidopsis* (Sun *et al.*, 2009; Hentrich *et al.*, 2013). Nevertheless, IAA was also shown to induce expression of a negative regulator

of JA-signalling, *JAZ1* (Grunewald *et al.*, 2009). In rice it was shown that IAA activation upon rice black streaked dwarf virus infection contributes to the JA and ROS mediated viral defence (Zhang *et al.*, 2019). IAA signal transduction consists of three major components, IAA receptors (TIR1 and AFB), transcriptional repressors (Aux/IAA) and auxin response factors (ARF). Upon IAA accumulation, the F-box IAA receptors trigger E3 mediated ubiquitin ligation of the Aux/IAA repressors, freeing the ARF from their repression and activating downstream signalling (Weijers and Wagner, 2016).

Abscisic acid (ABA) is the plant hormone that is generally associated with abiotic stress response, such as stomatal closure, cuticle wax formation, osmotic regulation, *etc.* (Chen *et al.*, 2020). However, it also plays roles in plant development and defence (De Vleesschauwer, Gheysen and Höfte, 2013; Yang, Yang and He, 2013; Chen *et al.*, 2020). In both *Arabidopsis* and rice, ABA is believed to predominantly act as a negative regulator of the defence response and acts antagonistic towards ET, JA and SA (Bailey *et al.*, 2009; Nahar *et al.*, 2012; De Vleesschauwer, Gheysen and Höfte, 2013; Yang, Yang and He, 2013). To illustrate, ABA counters the defence response in rice to the migratory root rot nematode *H. oryzae* (Nahar *et al.*, 2012). However, it should be noted that ABA can also affect plant defence positively, indicating a complex dependence of the effect on the pathogen, plant, tissue, *etc*. (Ton, Flors and Mauch-Mani, 2009; de Vleesschauwer *et al.*, 2010; De Vleesschauwer, Gheysen and Höfte, 2013; Yang, Yang and He, 2013).

1.3.2.3. Reactive oxygen species

Early accumulation of ROS is one of the first biochemical responses of the plant upon pathogen attack. The term ROS includes molecules such as singlet oxygen ($^1O_2$), superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^\bullet OH$) (Noctor, Reichheld and Foyer, 2018). In particular H_2O_2 is often regarded as an important ROS signalling molecule, as it is moderately long lived *in vivo* (half-life of milliseconds to seconds) (Marinho *et al.*, 2014; Smirnoff and Arnaud, 2019). ROS are by-products of the normal aerobic metabolism in mitochondria, chloroplasts and peroxisomes, as well as an autonomously generated product (Camejo, Guzmán-Cedeño and Moreno, 2016; Marcec *et al.*, 2019). ROS play multiple roles in plant cells, namely as important signalling molecules, both locally and systemically, as inducers of programmed cell death, as antipathogenic molecules and as cross-linkers of the plant cell wall (Camejo, Guzmán-Cedeño and Moreno, 2016). The early ROS accumulation upon plant stress exposure is often referred to as the 'oxidative burst', which encompasses a localised and transient ROS production. The oxidative burst is part of PTI and can activate the hypersensitive response (HR), depending on its concentration. The HR is a specific defence response that triggers programmed cell death to limit pathogen spreading (Levine *et al.*, 1996; Apel and Hirt, 2004). During the incompatible interaction, HR results from a transient accumulation of excessive ROS levels (Torres, Jones and Dangl, 2006; Chaouch, Queval and Noctor, 2012; Camejo, Guzmán-Cedeño and Moreno, 2016). During the compatible interaction, ROS is produced as well, but later and at lower levels (Jones and Dangl, 2006). The spatiotemporal pattern of the dynamic ROS concentration change is believed to be stimulus dependent (Marcec *et al.*, 2019). It is widely assumed that the primary ROS production upon pathogen recognition occurs in the apoplast, and is mediated by NADPH oxidases (or respiratory burst oxidase homologues, RBOHs), cell wall peroxidases or polyamine oxidases (Bolwell, 2002; Yoda, Hiroi and Sano, 2006). Intracellular ROS accumulation depends on a balance between ROS generation and antioxidative processes (Fichman and Mittler, 2020). Figure 1.4 provides an overview of pathogen induced ROS signalling.

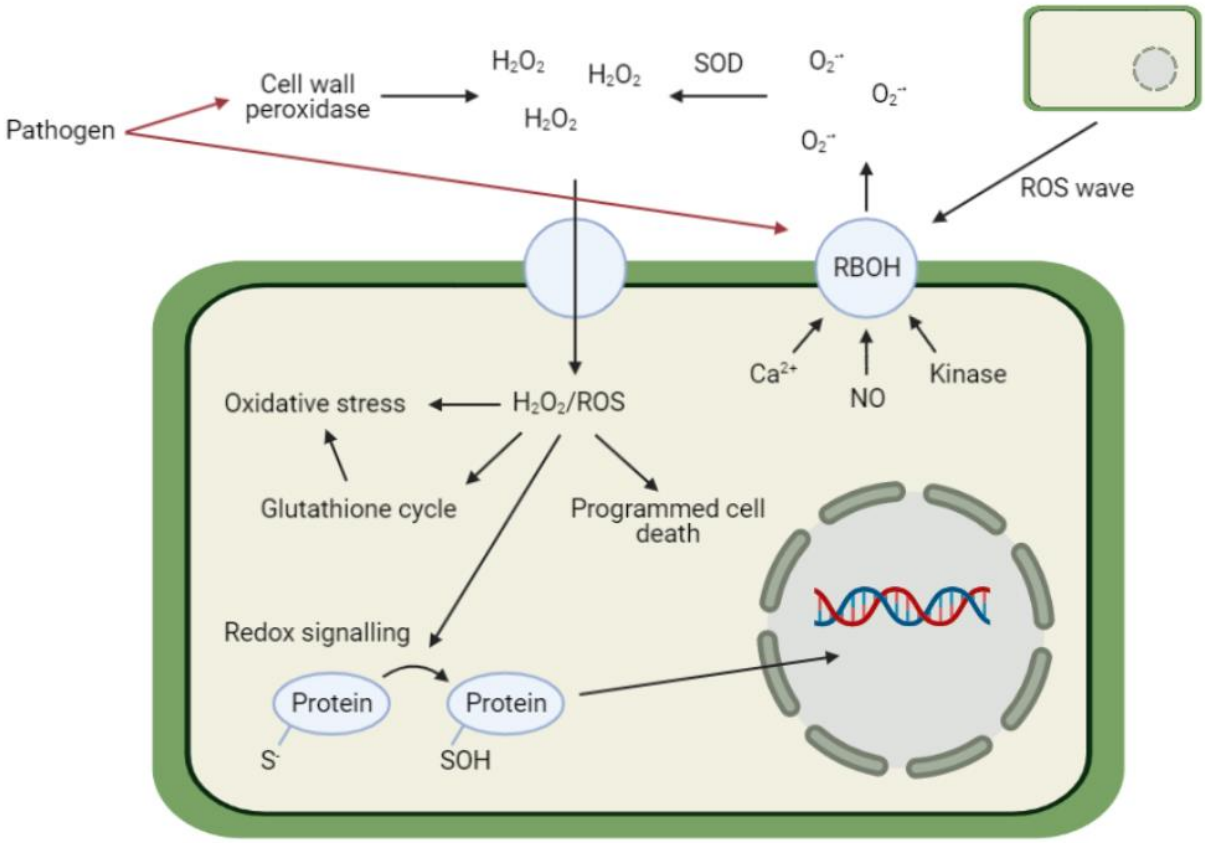

Figure 1.4: ROS mediated signalling upon pathogen detection. Upon pathogen detection, RBOH and cell wall peroxidase mediated ROS accumulation is triggered in the apoplast. The ROS signal is internalised, inducing oxidative stress and redox signalling, altering the glutathione homeostasis, and in high concentrations inducing programmed cell death. RBOH can also be activated by NO, phosphorylation, calcium signalling and the ROS wave (which comes from neighbouring cells). RBOH: respiratory burst oxidase homologue, SOD: superoxide dismutase. Created with BioRender.com.

Redox signalling is typically regarded as a balance between ROS on one hand and antioxidants on the other hand (Noctor, Reichheld and Foyer, 2018). Oxidative signalling shifts the balance, so that ROS accumulate, either via an increase in their production or a decrease in antioxidant capacity. The resulting oxidation causes programmed cell death (HR) and/or acclimation, according to the intensity. Additionally, signalling pathways may require ROS metabolism, with altered flux and/or changes in the antioxidant status being the signal that is perceived (Noctor, Reichheld and Foyer, 2018). ROS are reactive compounds that can directly modify target proteins, in particular the amino acid residues cysteine and methionine (Marcec *et al.*, 2019). Thus, a potential apoplastic ROS sensor are the plasma membrane localised cysteine-rich receptor-like kinases, which have redox-sensitive cysteine residues in the extracellular domain (Feige and Hendershot, 2011; Nagahara, 2011; Lu *et al.*, 2016; Kimura *et al.*, 2017). Alternatively, apoplastic ROS are thought to be able to enter the cytoplasm through aquaporins, allowing them to subsequently trigger intracellular signalling cascades (Tian *et al.*, 2016; Marcec *et al.*, 2019). Intracellular ROS is sensed using redox-based protein modifications (Marcec *et al.*, 2019). For example, H_2O_2 reacts with cysteine thiolate anions oxidising them to their sulfenic form, causing structural changes and potentially altering the protein function (Roos and Messens, 2011). ROS can also influence transcription factors by inducing redox-related translocation to the nucleus or by direct redox-related regulation of DNA binding activity (Marcec *et al.*, 2019). An example of redox-mediated translocation to the nucleus is the well known SA-signalling protein, NPR1 (Tada *et al.*, 2008).

To ensure that ROS accumulation is kept in check, the plant has to protect itself via the presence of antioxidants and enzymes that degrade ROS. Antioxidants can be sacrificial, such as many basic housekeeping compounds like amino acids, sugars, pigments, flavonoids, … or can be dedicated molecules like ascorbic acid (AA, more details in Section 1.4.) and glutathione (Noctor, Reichheld and Foyer, 2018). AA and glutathione can rapidly react with ROS after which they can be regenerated by reductases, allowing this cycling to regulate the cellular redox state (Foyer and Noctor, 2011). ROS degrading enzymes include peroxidases, superoxide dismutase and catalase (Azarabadi *et al.*, 2017). In plants, AA is an important co-factor for peroxidase mediated ROS degradation (Noctor, Reichheld and Foyer, 2018). Glutathione can enzymatically or chemically regenerate AA from its oxidised form, dehydroascorbic acid (DHA) (Noctor, Reichheld and Foyer, 2018). Through its role as an antioxidant that regulates the cellular redox state, glutathione can act as a ROS-related signal. As the most abundant low-molecular thiol of the cell, glutathione acts as a key redox buffer. Its homeostasis of the redox state is highly affected by excess ROS generated under stress, giving it the potential to function as a stress signal (Noctor, Reichheld and Foyer, 2018). Further, glutathione may act as an interfacing molecule between ROS, NO and protein cysteine groups (Noctor, Reichheld and Foyer, 2018). In most subcellular compartments, glutathione is found in a large proportion in its reduced form (glutathione). Excess ROS shifts this balance towards the oxidised form, glutathione disulfide (Queval *et al.*, 2011). This change in redox state may cause glutathione to conjugate to various thiol residues, including cysteine in proteins, resulting in *S*-glutathionylation and potentially affecting protein activity (Dixon *et al.*, 2005; Zaffagnini *et al.*, 2007). Glutathione is known to influence plant stress hormone pathways, including ET, JA and SA, and developmental hormone pathways, such as ABA and IAA (Koprivova, Mugford and Kopriva, 2010; Okuma *et al.*, 2011; Han, Chaouch, *et al.*, 2013; Han, Mhaldi, *et al.*, 2013; X. Yu *et al.*, 2013; Datta *et al.*, 2015). For example, alterations in the glutathione redox state contribute to the increased presence of NPR1 in the nucleus (Kiddle *et al.*, 2003; Pavet *et al.*, 2005).

Next to altered gene expression, ROS can locally cause the crosslinking of glycoproteins and lignin subunits, and trigger the synthesis of callose to strengthen the cell wall (Marcec *et al.*, 2019). In these local responses, the plasma membrane-localised RBOHs are one of the primary enzymes for ROS production (Marcec *et al.*, 2019). Another important mechanism to generate ROS in the apoplast upon challenge is related to changes in the apoplast conditions, affecting the ROS homeostasis. An important example is the alkalinization of the apoplast upon PAMP detection, likely due to proton ATPase inhibition (Ryan and Pearce, 2003; Daudi *et al.*, 2012; Moroz, Huffaker and Tanaka, 2017). This increased pH is favourable for H_2O_2 generation by type III peroxidases in the apoplast (Daudi *et al.*, 2012; Marcec *et al.*, 2019; Smirnoff and Arnaud, 2019). The type III peroxidases are numerous in the apoplast and have an important function in lignin crosslinking (Smirnoff and Arnaud, 2019). Smirnoff and Arnaud (2019) propose that not only inhibition of the proton ATPase could be the cause of the alkalization, but also the activation of the RBOHs could contribute, as they require apoplastic protons to form H_2O_2.

ROS are not only extremely important for their direct toxic effects and local signalling, but also for their systemic cell-to-cell signal transduction. Rapidly communicating danger to distant tissues is an essential plant strategy to deal with potential threats (Fichman and Mittler, 2020). Systemic signalling by ROS molecules is often referred to as the 'ROS wave', which is the cell-to-cell auto-propagating process of ROS production by RBOHD, the RBOH responsible for the ROS wave in *Arabidopsis* (rice ortholog: RBOHB) (Wong *et al.*, 2007; Fichman and Mittler, 2020). When triggered in a single cell, it results in an enhanced production of ROS by the cell, leading to ROS accumulation in the apoplast. This

ROS is then sensed by the neighbouring cells, triggering enhanced ROS production by RBOHD in those cells, thus resulting in an auto-propagating ROS wave that can spread across the entire plant (Miller *et al.*, 2009; Suzuki *et al.*, 2013; Devireddy *et al.*, 2018; Fichman, Miller and Mittler, 2019; Fichman and Mittler, 2020). The ROS wave is thought not to convey specificity to the systemic signal, it is seen as a signal that alerts the cells for incoming stress. It is believed that other accompanying signals contribute to the specificity of the signalling (Suzuki *et al.*, 2013; Gilroy *et al.*, 2014; Kollist *et al.*, 2019; Fichman and Mittler, 2020). Nonetheless it remains a very important signal as research in *Arabidopsis* has shown that systemic signalling is impaired when the ROS wave is absent (Suzuki *et al.*, 2013; Devireddy *et al.*, 2018; Zandalinas *et al.*, 2019). The activation of RBOHs depends on kinase phosphorylation cascades (Kadota *et al.*, 2014; Zhang *et al.*, 2018) or on the binding of calmodulin, thus allowing activation upon calcium fluctuations (Ogasawara *et al.*, 2008). Multiple RLK have been identified that regulate the ROS burst by phosphorylating distinct sites of RBOHD upon pathogen recognition. RBOHD is also assumed to influence developmental processes, as IAA responsive Rac-GTPases can interact with the EF-hand motifs of RBOHD in a calcium-dependent manner and as RBOHD might regulate the transport of oxidised IAA (Wong *et al.*, 2007; Peer, Cheng and Murphy, 2013; Zandalinas and Mittler, 2018).

1.3.2.4. Lignification

Strengthening the cell wall via lignification is a common response to pathogen attack, physically blocking pathogens from further infecting the plant (Chaouch, Queval and Noctor, 2012). This is possible as lignin is an extremely recalcitrant amorphous heteropolymer. It results from the oxidative coupling of the three *p*-hydroxycinnamyl alcohols, *p*-coumaryl, coniferyl and sinapyl, forming the subunits H (hydroxyphenyl), G (guaiacyl) and S (syringyl), respectively (Figure 1.5) (Veronico *et al.*, 2018). These lignin precursors are the end-products of the monolignol branch of the phenylpropanoid pathway (Veronico *et al.*, 2018; Smirnoff and Arnaud, 2019). Once formed, monolignols are transported to the secondary cell wall, where they are oxidised to form radicals and then polymerised to lignin (Veronico *et al.*, 2018; Smirnoff and Arnaud, 2019). These oxidisations are catalysed by oxygen-dependent laccases or H_2O_2-dependent type III peroxidases (Marjamaa, Kukkola and Fagerstedt, 2009; Berthet *et al.*, 2012).

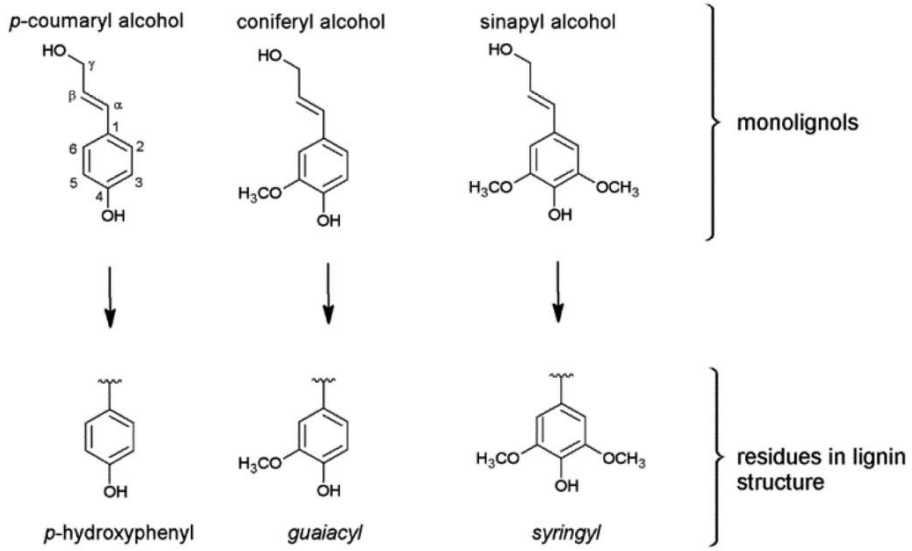

Figure 1.5: Lignin monomers. Structure of the monolignols and their respective lignin subunits when crosslinked in the polymer. Subunit H: *p*-hydroxyphenyl, subunit G: guaiacyl, subunit S: syringyl. (Bergs *et al.*, 2020)

This reinforcement of the cell wall appears to be an effective response to nematode infections. This is for example shown by the fact that most of the nematodes are unable to cross the reinforced endodermis due to the presence of the lignified casparian strips (a lignified band in the radial and transverse cell walls surrounding the vascular cylinder) (Holbein, Grundler and Siddique, 2016). They use alternative methods to reach the vascular cylinder instead, for example by entering the stele at the root tip (see U-turn in Figure 1.2). This way they get inside the stele before the differentiation and reinforcement of the endodermis (Holbein, Grundler and Siddique, 2016). In resistant banana plants (*Musa* sp.) resistance to the nematode *Radopholus similis* was shown to be correlated with increased lignin content in the cell walls (Dhakshinamoorthy *et al.*, 2014). In the tomato (*Solanum lycopersicum*) defence response to *M. incognita*, lignification occurs early upon infection, namely at 1 dpi (Zacheo *et al.*, 1995). In *Arabidopsis* and tobacco (*Nicotiana tabacum*), it was even shown that the lignin monomer composition can affect the plant susceptibility (Wuyts, 2006). The RKN *Meloidogyne javanica* appears to counter lignin-mediated basal defences through repression of genes involved in lignin biosynthesis, already at 1 dpi (Portillo *et al.*, 2013), indicating the importance of lignin in plant defence. It was also found that the IR-stimulus benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) induces enhanced expression of lignin synthesis genes in tomato roots two days post treatment (dpt), conform with the observed increased lignification (Veronico *et al.*, 2018). Similarly, treatment of rice with the plant stress metabolite and IR-stimulus β-aminobutyric acid (BABA, a nonprotein amino acid) increases the lignin content in the rice root, already at 1 dpt (Ji *et al.*, 2015; Schwarzenbacher *et al.*, 2020).

1.3.3. Induced resistance

1.3.3.1. General

In addition to innate immune responses, plants can acquire long-lasting resistance upon appropriate stimulation, which is referred to as induced resistance (IR). IR typically develops in response to transient expression of innate immunity (PTI or ETI) upon exposure to certain pathogens, pests, beneficial micro-organisms, chemicals, physical wounding or herbivory, and allows plants to mount a more efficient immune response against future challenges (Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017; De Kesel *et al.*, 2021). IR functions as an umbrella term for these enhanced resistance phenotypes, but two specific IR-phenomena have been described in detail, systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is a specific type of systemic IR, that is typically induced by necrotizing pathogens, is mediated predominantly by SA and pipecolic acid, and is often associated with the accumulation of pathogenesis-related (PR) proteins (Conrath *et al.*, 2015; De Kesel *et al.*, 2021). ISR, on the other hand, is a more general term that encompasses any form of IR that results in systemic signalling by endogenous signals. If the IR-stimulus itself is systemically transported, the phenomenon is classified as 'IR' (De Kesel *et al.*, 2021). This is for example the case for BABA (Cohen and Gisi, 1994; Balmer *et al.*, 2018). However, it should be noted that this is the most recent definition of the term ISR (De Kesel *et al.*, 2021). In the past this term was frequently (wrongly and very narrowly) defined as a form of IR that is stimulated by non-pathogenic plant growth-promoting rhizobacteria and fungi that depends on JA and ET (Conrath *et al.*, 2015).

IR is generally based on two nonexclusive mechanisms: direct upregulation of inducible defences and 'defence priming' (Figure 1.6, Wilkinson *et al.*, 2019; De Kesel *et al.*, 2021). Following exposure to an IR-stimulus, inducible defences can directly become and remain upregulated, providing IR against subsequent attack (Figure 1.6a, Wilkinson *et al.*, 2019). Examples of this IR mechanism are the accumulation of defence metabolites, induction of anatomical structures, … (Wilkinson *et al.*, 2019).

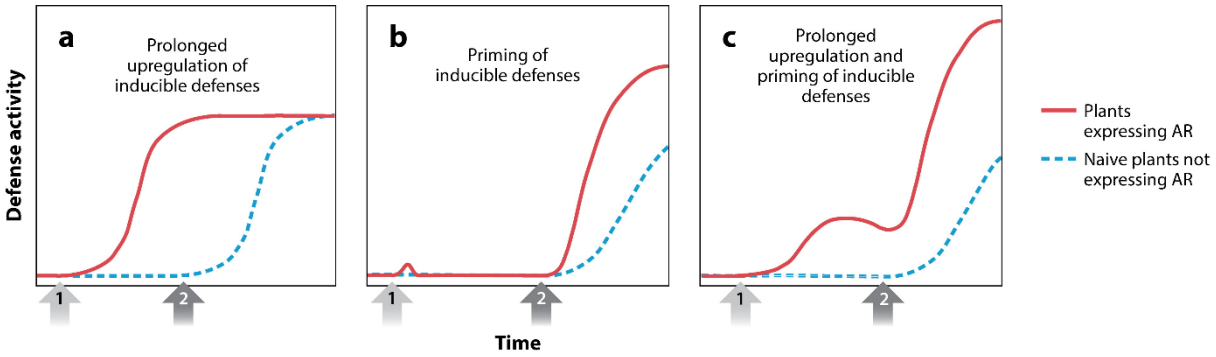

Figure 1.6: The two nonexclusive general mechanisms of IR. Arrow 1: Stimulation by the IR-stimulus. Arrow 2: Challenge by a pathogen. AR in the legend refers to 'acquired resistance', which is another less frequently used term for IR. 'Naive plants' refers to plants that were not stimulated by an IR-stimulus. (a) IR based on direct and prolonged activation of the defence responses upon stimulation by the IR-stimulus. Alternatively, transient activation of the defence response is possible as well. (b) Defence priming, namely no real activation of inducible defences upon IR-stimulus application, but a faster and stronger defence response upon challenge. However, changes do occur upon IR-stimulus application, but are not situated on the level of 'inducible defences'. (c) The nonexclusive mechanism, showing both direct defence responses (transient here, but could be prolonged as well) and defence priming. (Wilkinson *et al.*, 2019)

These direct defence responses can be transient in nature or long lasting. The primed defence, however, is a faster, stronger, and/or more sustained defence response, provoked by a challenging biotic stressor subsequent to IR establishment (Figure 1.6b) (Balmer *et al.*, 2015; Conrath *et al.*, 2015; Martinez-Medina *et al.*, 2016; Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). The primed defence causes an enhanced sensitivity and responsiveness to stress and allows plants to respond to very low levels of stress (Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017). This primed defence response relies on minor direct changes, other than inducible defences (for example epigenetic changes), that establish a primed state that can cause the enhanced responsiveness towards a later challenge (see Section 1.3.3.2.). IR-stimuli can induce a combination of both, or focus on one of both mechanisms of IR establishment. The relative importance of the direct defence response compared to the primed defence response heavily depends on the studied IR-stimulus, as well as many other factors such as the lifestyle of the pathogen, the studied plant tissue, the age of the host plant, the applied concentration of the IR-stimulus, *etc*. (Martinez-Medina *et al.*, 2016).

Defence priming is typically assumed to be an adaptive, low-cost defensive measure, because the defence responses are not, or only slightly and transiently, activated by the IR-stimulus. Only upon subsequent challenges the defence responses are strongly activated (Conrath *et al.*, 2015). On the contrary, in the past, direct defence responses induced by IR-stimuli were thought to almost certainly be associated with fitness costs, due to an increased investment in the plant defence, the so-called allocation costs (Conrath *et al.*, 2006). However, recent insights in the IR-field have caused the abandonment of this view. Namely, it is nowadays assumed that both primed and direct defence responses may or may not come with an additional cost, requiring the need for long term studies, in a non-hostile set-up, to investigate these potential fitness costs (Martinez-Medina *et al.*, 2016; De Kesel *et al.*, 2021). For example, the IR-stimulus diproline was shown to induce direct defence responses in rice, without leading to fitness costs upon repetitive lifelong treatments (De Kesel *et al.*, 2020).

Stimulation of IR can lead to increased resistance against both biotic and abiotic stress, and this both locally and/or systemically (Conrath *et al.*, 2015; Martinez-Medina *et al.*, 2016; Mauch-Mani *et al.*, 2017; De Kesel *et al.*, 2021). The underlying mechanism of IR can depend on the used IR-stimulus, the encountered challenge, the studied plant tissue, the age of the host plant, the applied concentration,

etc., which makes it rather difficult to pinpoint an exact mechanism (Balmer *et al.*, 2015). The enhanced resistance might be the result of unrelated underlying events, even for a single IR-stimulus.

Upon IR-stimulus perception, changes may occur in the plant at the physiological, transcriptional, metabolic and epigenetic levels (Mauch-Mani *et al.*, 2017). The phase between the IR-stimulus perception and the subsequent challenge is the priming phase (Figure 1.7, phase 1). Upon challenge, the post-challenge primed phase starts (Figure 1.7, phase 2), which results in enhanced defence responses (Balmer *et al.*, 2015). IR can be durable and maintained throughout the plant its life and can even be passed onto the progeny, the so-called transgenerational induced resistance (TIR; Figure 1.7, phase 3) (Pieterse, 2012; López Sánchez *et al.*, 2021).

1.3.3.2. Priming phase

During the priming phase (Figure 1.7, phase 1), direct changes will occur in the plant that will prepare it for enhanced responsiveness. Contrarily to what the name of this phase suggests, these direct changes include direct stimulation of the plant defence and changes that can mediate the primed defence response in the subsequent post-challenge primed phase. Different IR-stimuli may cause similar changes as well as specific ones, and this specificity might arise by activation of only some of all possible responses associated with the priming phase (Mauch-Mani *et al.*, 2017).

A first possible change is the direct induction of a subset of the various plant defence genes. This is mainly associated with direct defence activation, but in some cases contributes to defence priming when inactive products are accumulated. This induction can range from activated defence metabolism, such as lignification, production of antipathogenic compounds such as phytoanticipins or phytoalexins, PR-protein accumulation, *etc.*, to induction of anatomical structures (Wilkinson *et al.*, 2019). The

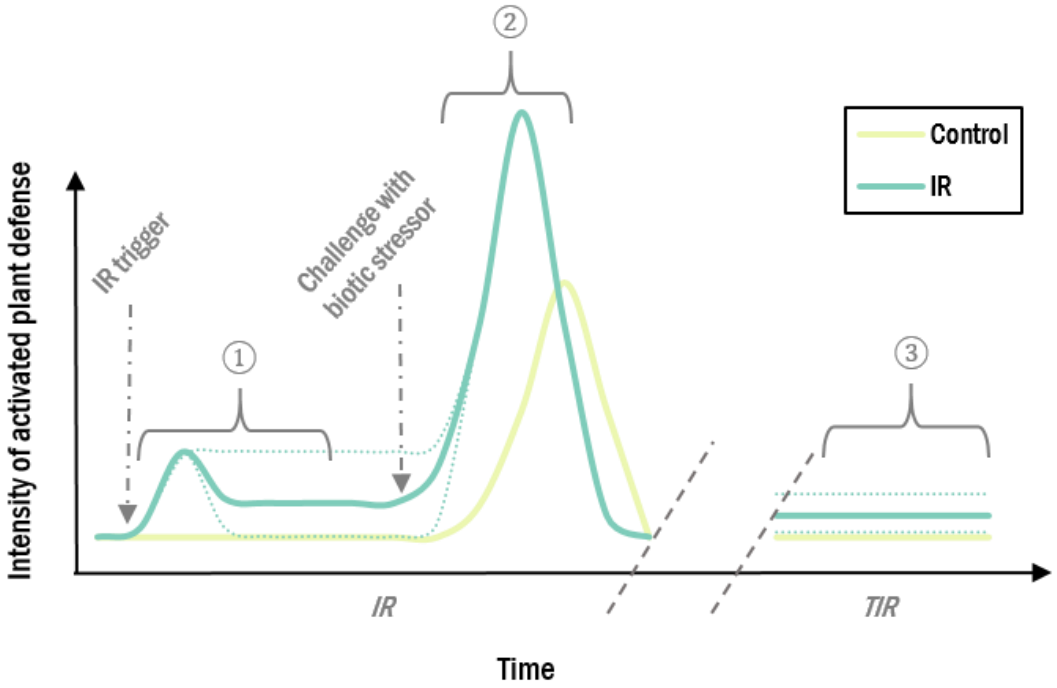

Figure 1.7: The three phases of IR. Phase 1 is the priming phase, phase 2 is the post-challenge primed phase and phase 3 is the transgenerational induced resistance (TIR) phase (Balmer et al., 2015). In the priming phase direct defence responses, defence priming (the lower dotted line) or both can occur. These direct effects can remain as such (upper dotted line) or be partially transient (full line). In phase 2 defence priming is shown, namely a faster and stronger defence response. Phase 3, TIR, can again be characterised by direct defence responses, defence priming (the lower dotted line) or both. In the figure the IR-stimulus is referred to as 'IR trigger'. (De Kesel, unpublished)

metabolism can be affected in two major ways, namely alterations in the primary metabolism and induction of the secondary metabolism (Balmer *et al.*, 2015; Mauch-Mani *et al.*, 2017). The changes in the primary metabolism can constitute increased levels of sugars, amino acids, tricarboxylic acid derivatives, glycerol-3-phosphate, myo-inositol, xylitol, methyl salicylate, … (Balmer *et al.*, 2015; Mauch-Mani *et al.*, 2017). The induction of the secondary metabolism typically causes an accumulation of plant defence metabolites, defence-related phytohormones and phytoanticipins. These compounds can also be produced in their inactive forms, typically their amino acid precursors or conjugated forms (Brotman *et al.*, 2012; Vogel-Adghough *et al.*, 2013; Gamir, Sánchez-Bel and Flors, 2014; Pastor *et al.*, 2014; Balmer *et al.*, 2015; Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). These accumulations allow the plant to react directly or more rapidly to a subsequent attack, the plant is switched to a standby mode (Balmer *et al.*, 2015). Typical changes in roots in this phase include H_2O_2 and lignin accumulation as a direct defence response (Ji *et al.*, 2015; Huang *et al.*, 2016; Veronico *et al.*, 2018). Additionally, specific transcriptional alterations occur during the priming phase, both locally and systemically (Mauch-Mani *et al.*, 2017). For example, systemically induced expression of defence related transcription factors could be observed in *Arabidopsis* upon BABA treatment (Van Der Ent *et al.*, 2009). It is important to notice that the transcriptional changes induced by different IR-stimuli are partially specific (Mauch-Mani *et al.*, 2017). To illustrate, 30% of the induced genes were different between grapevine (*Vitis vinifera*) leaves treated with laminarin and its sulphated derivative (Gauthier *et al.*, 2014), and only 10% of the induced genes overlapped after treatment with BABA or inoculation with the biotic IR-stimulus *Pseudomonas fluorescens* in *Arabidopsis* (Van Der Ent *et al.*, 2009).

Next, defence priming can be mediated by accumulation of dormant, inactive MAPKs. This has been reported in *Arabidopsis* and cucumber (*Cucumis sativus*) (Shoresh *et al.*, 2006; Beckers *et al.*, 2009). This accumulation can, upon subsequent challenge, facilitate an augmented induction of the defence responses due to their importance in cellular signal amplification and downstream defence signalling (Meng and Zhang, 2013; Conrath *et al.*, 2015; Wilkinson *et al.*, 2019). Upon challenge, the accumulated dormant MAPKs will become activated, causing higher levels of active MAPKs and thus resulting in increased defence responses (Beckers *et al.*, 2009; Conrath *et al.*, 2015). Another change mediating priming involves the augmented biosynthesis and secretion of diverse PRRs and their coreceptors (Tateda *et al.*, 2014; Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017). This indicates that plants can enhance their sensitivity against a broad-spectrum of potential new challenges upon IR-stimulus perception (Mauch-Mani *et al.*, 2017). Additionally, it should be noted that the PRR receptors trigger MAPK-cascades upon recognition of attackers, therefore, in combination with elevated levels of MAPKs, this can result in augmented defence responses upon future challenge.

Finally, increasing evidence is supporting that alterations in the chromatin structure appear to be an important change in establishing IR (Wilkinson *et al.*, 2019). Gene activating and chromatin density reducing histone modifications in defence gene promotors have been shown to be associated with IR (Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). Examples of such modifications are trimethylation of lysine 4 in histone H3 (H3K4me3), H3K4me2, and acetylation of H3K9 (H3K9ac), H4K5ac, H4K8ac and H4K12ac (Pokholok *et al.*, 2005; Alvarez-Venegas *et al.*, 2007; Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). Such histone modifications are induced during the priming phase, with or without activating gene expression directly (Jaskiewicz, Conrath and Peterhänsel, 2011). They can facilitate a faster and stronger future defence gene expression (Jaskiewicz, Conrath and Peterhänsel, 2011; Conrath *et al.*, 2015). Next to histone modifications, there is increasing evidence that the DNA methylation status regulates IR as well (Conrath *et al.*, 2015; Espinas, Saze and Saijo, 2016; Mauch-

Mani *et al.*, 2017; Wilkinson *et al.*, 2019). It has frequently been suggested that DNA hypomethylation is associated with priming of SA-dependent defence genes and transgenerational SAR against biotrophic pathogens (Akimoto *et al.*, 2007; Luna *et al.*, 2012; A. Yu *et al.*, 2013; Espinas, Saze and Saijo, 2016; López Sánchez *et al.*, 2016; Wilkinson *et al.*, 2019). However, hypomethylation has also been associated with increased sensitivity to necrotrophs, likely due to the antagonistic cross-talk between SA- and JA-mediated defence responses in *Arabidopsis* (Luna *et al.*, 2012; López Sánchez *et al.*, 2016). Kuźnicki *et al.* (2019) stated however, that TIR is likely related to hypomethylation of defence genes, while within-generation IR is a complex interplay of DNA methylation and other epigenetic factors, as often the correlation between DNA (de)methylation and defence gene expression is not very clear at this stage. Namely, they observed, upon BABA treatment in potato (*Solanum tuberosum*), an initial hypermethylation, that later became a hypomethylation. Interestingly, this pattern was poorly associated with the observed expression patterns (Kuźnicki *et al.*, 2019). Further, Wilkinson *et al.* (2019) proposed that hypomethylation of transposable elements by biotic stress can prime defence genes via cis- and trans-regulatory mechanisms (Pavet *et al.*, 2006; A. Yu *et al.*, 2013). Finally, it has also been suggested that RNA-directed DNA methylation (RdDM) is involved in establishing IR (Agorio and Vera, 2007; Jin, 2008; Luna and Ton, 2012; Mauch-Mani *et al.*, 2017). RdDM is one of the major mechanisms to control DNA methylation and chromatin remodelling in plants (Zhang *et al.*, 2006). In this pathway, small interfering RNAs guide *de novo* methylation of homologous DNA sequences requiring long non-coding RNAs as scaffold to define target sequences (Matzke and Mosher, 2014). DNA methylation is likely to play a central role in establishing TIR, as it can be faithfully transferred over meiosis onto the next generation (Conrath *et al.*, 2015; Wilkinson *et al.*, 2019). Together, the histone modifications and altered DNA methylation can influence the chromatin structure at the promotor regions, and eventually destabilize the chromatin structure of neighbouring regions, facilitating later access for transcriptional activators and thus play a pivotal role in defence priming (Mauch-Mani *et al.*, 2017).

In conclusion, during the priming phase, the plant is prepared on a molecular level to respond to future challenges, via enhanced sensitivity and response by accumulation of PRR receptors and dormant MAPKs, and by going into standby mode by preparing/activating the metabolism and altering the chromatin density.

1.3.3.3. Post-challenge primed phase

During the post-challenge primed phase (Figure 1.7, phase 2), the preparations of the priming phase are used against the infecting pathogen. This either via the direct defence response, in which the pathogen faces a host at higher defence capacity than under unstimulated conditions, or via the primed defence, due to increased responsiveness and downstream defence activation compared to unstimulated plants. It is important to notice that the reaction of the IR-stimulated plant heavily depends on the plant-challenger combination and likely is influenced by other factors, such as the environment and developmental stage of the plant (Balmer *et al.*, 2015). To illustrate, tomato plants colonized by the arbuscular mycorrhizal fungus (a fungus that penetrates the plant root cortical cells and forms arbuscules) *Funneliformis mosseae*, that functions as an IR-stimulus, showed different gene expression activation upon challenge by the caterpillar *Helicoverpa armigera* compared to challenge by the fungal pathogen *Alternaria solani* (Song *et al.*, 2013, 2015). Also, the augmented perception during this phase is an important aspect of defence priming (Mauch-Mani *et al.*, 2017). To illustrate, in *Arabidopsis*, increased responsiveness to flagellin and chitin is observed, due to elevated levels of PRR receptors and coreceptors upon BTH-mediated IR (Tateda *et al.*, 2014). Boosted perception is not

necessarily mediated by cell receptors, also physical structures can help the plant to monitor the environment, such as trichomes (Boughton, Hoover and Felton, 2005; Peiffer *et al.*, 2009; Mauch-Mani *et al.*, 2017). Next to the perception, also the signal transduction could be enhanced. Namely, ROS generation can be boosted upon challenge, which is not only important in the signalling, but also in an enhanced HR (Dubreuil-Maurizi *et al.*, 2010; Pastor *et al.*, 2013; Baccelli and Mauch-Mani, 2016). Also, the MAPKs could show enhanced activation, under the form of longer and more intense phosphorylation (Beckers *et al.*, 2009; Mauch-Mani *et al.*, 2017). Additionally, earlier and/or increased gene expression is a common response in the post-challenge primed phase, with as a result protein accumulation, higher enzyme activity, and increased metabolite, amino acid and phytoalexin synthesis (Mauch-Mani *et al.*, 2017). Finally, IR can sometimes influence tritrophic interactions, by displaying an increased attractiveness of the host plant to pest predators (Hoffmann, Vierheilig and Schausberger, 2011; Mauch-Mani *et al.*, 2017).

1.3.3.4. Transgenerational induced resistance

During the TIR phase (Figure 1.7, phase 3), IR becomes established in the progeny, likely through inheritance of epigenetic marks (Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). An important candidate for mediating TIR is DNA methylation, as it can remain stable over meiosis, and therefore can be faithfully inherited (Niederhuth and Schmitz, 2014; Wilkinson *et al.*, 2019). In plants, three major sequence sites can be cytosine methylated, CG (CpG), CHG and CHH (with H representing A, C or T) (Matzke and Mosher, 2014). More specifically, it has been suggested that non-CpG DNA methylation plays a pivotal role in TIR (Luna and Ton, 2012; Luna *et al.*, 2012). It has been proposed that DNA demethylation is essential for the elicitation, transmission and expression of TIR (Luna and Ton, 2012; Luna *et al.*, 2012; Mauch-Mani *et al.*, 2017; Kuźnicki *et al.*, 2019; Wilkinson *et al.*, 2019). However, the exact mechanism still requires further study, although there is little doubt about its epigenetic character (Wilkinson *et al.*, 2019). As a first argument, TIR can still be observed in isogenic progeny after multiple stress-free generations, indicating that TIR is transmittable through the germline and not merely through physiological maternal effects (Luna *et al.*, 2012; Rasmann *et al.*, 2012; Stassen *et al.*, 2018; Wilkinson *et al.*, 2019). Secondly, TIR requires active DNA demethylation pathways (López Sánchez *et al.*, 2016; Wilkinson *et al.*, 2019). Thirdly, TIR is reversible, and its durability over stress-free generations seems to depend on the level of stress applied on the parent (Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). As plants are sessile and cannot really communicate with their offspring, they need endogenous ways to "communicate". If the stress persists every generation, the plant can genetically adapt to it, but if it only occurs within a few generations TIR can provide this "communication" (Pieterse, 2012). Since TIR is based on epigenetic marks, it is a reversible phenotype. This transient nature is beneficial with respect to the ecological costs associated with (T)IR. These ecological costs are the enhanced susceptibility towards another attacker due to hormone cross-talk (Luna *et al.*, 2012; Mauch-Mani *et al.*, 2017; Wilkinson *et al.*, 2019). Therefore, forgetting a previous stress might be of advantage in the long run. Also, TIR is more likely to be sustained when the initial stress is applied repeatedly, thus warning progeny of a persistent stress (Singh and Roberts, 2015; Mauch-Mani *et al.*, 2017; Stassen *et al.*, 2018). The importance of TIR to a plant might also depend on the plant its lifestyle (Wilkinson *et al.*, 2019). Namely, when comparing a tree with an annual angiosperm, it is likely that the challenges a tree faces will often vary during its lifetime, while the offspring of the annual angiosperm is likely to be faced with the same challenges as its parent(s), indicating a potential greater benefit of TIR for the annual angiosperm (Wilkinson *et al.*, 2019).

TIR has mainly been studied in dicot model plants such as *Arabidopsis*, tobacco, tomato, … In *Arabidopsis*, TIR has been detected upon treatment with chemical IR-stimuli, upon bacterial infection and upon wounding (Luna *et al.*, 2012; Rasmann *et al.*, 2012; Slaughter *et al.*, 2012). Namely, offspring of plants infected with *P. syringae* pv. *tomato* DC3000 showed increased resistance against *P. syringae* and *Hyaloperonospora arabidopsidis*, even across one stress-free generation (Luna *et al.*, 2012). In tomato and *Arabidopsis*, TIR has been observed in mechanically damaged plants, methyl jasmonate (MeJA) treated plants and caterpillar challenged plants. For these IR-stimuli TIR even persisted for two generations in *Arabidopsis* (Rasmann *et al.*, 2012). TIR against *P. syringae* pv. *tomato* and *H. arabidopsidis* was also detected in *Arabidopsis* plants treated with BABA or an avirulent *P. syringae* pv. *tomato* isolate (Slaughter *et al.*, 2012). Slaughter *et al.* (2012) even found an enhanced capacity for establishing IR in offspring of initially IR-stimulated *Arabidopsis* plants. In tobacco, repeated inoculation of the parental plants with tobacco mosaic virus also induced TIR in the next generation (Roberts, 1983). For monocots, Walters and Paterson (2012) observed TIR in barley (*Hordeum vulgare*) treated with acibenzolar-S-methyl or saccharin against *Rhynchosporium commune*. However, to the best of our knowledge, no reports on TIR in rice exist. Nonetheless, it was shown that rice can obtain a transgenerational memory to deal with future heavy metal stress, which could indicate that TIR will be detected in the near future in rice (Cong *et al.*, 2019). On the other hand, TIR against nematodes was observed in tomato by De Medeiros *et al.* (2017). They showed enhanced resistance in the next generation to *M. javanica*, when treating the parent tomato plants with the biocontrol fungus (and IR-stimulus) *Trichoderma atroviride*.

1.3.3.5. IR against nematodes

Multiple examples of IR against nematodes have been described in literature. In tomato, enhanced resistance to *M. incognita* was observed upon BTH treatment. This IR-phenotype was associated with mainly primed lignin accumulation and H_2O_2 accumulation (Melillo, Leonetti and Veronico, 2014; Veronico *et al.*, 2018). In rice, Anita and Samiyappan (2012) showed that inoculation with *Pseudomonas fluorescens* induces ISR to *Mg*. Treatment with the plant stress metabolite, BABA, is known to protect plants against various pathogens (Jakab *et al.*, 2001; Schwarzenbacher *et al.*, 2020). BABA treatment of rice enhances resistance to *Mg*, without apparent involvement of the ET, JA and SA pathways. However, the treatment directly resulted in a strong H_2O_2 accumulation, increased lignin contents and callose accumulation in the roots (Ji *et al.*, 2015). Next, thiamine (vitamin B1) was also shown to enhance resistance to *Mg* in rice by causing primed H_2O_2 and lignin accumulation (Huang *et al.*, 2016). Both AO and DHA were previously shown to stimulate IR in rice to *Mg*. It was shown that foliar treatment of rice with AO results in direct ET and JA accumulation and primed H_2O_2 accumulation in the roots (Singh, Verstraeten, *et al.*, 2020). Recently, new IR-stimuli against *Mg* in rice were identified, namely diproline (De Kesel *et al.*, 2020) and CCOPE (based on butternut pumpkin (*Cucurbita moschata* 'Butternut'), cucumber, melon, muscat pumpkin (*Cucurbita moschata* 'Muscat') or zucchini (*Cucurbita pepo* var. *cylindrica*)) (Kyndt *et al.*, 2020).

1.3.3.6. IR in agriculture

A major advantage of IR is that it is based on the general activation of the plant immune system, rather than being toxic to the challenger. Therefore, contrarily to pesticides, IR-stimuli typically do not affect off-target bacteria, insects, *etc*. This allows to combine the IR-stimulus with biocontrol strategies and avoids toxicity to the environment and human consumers. Additionally, this reduces the rate of resistant pathogen development (Alexandersson et al., 2016). Furthermore, IR has been associated with broad spectrum activity, against biotic and even abiotic stresses (Martinez-Medina et al., 2016).

However, the adoption of IR-stimuli in agriculture also faces some problems. First, it is important to notice that an IR-stimulus can function well in the lab, but fail to perform or be consistent in the field, requiring field tests to ensure its applicability (Walters, Havis, Paterson, *et al.*, 2011; Alexandersson *et al.*, 2016; Sandroni *et al.*, 2020). Additionally, IR-stimuli sometimes are insufficiently tolerated by some crops, limiting their practical application (Conrath *et al.*, 2015). This is for example the case for the SA mimic 2,6-dichloroisonicotinic acid and its methyl ester (Ryals *et al.*, 1996). Finally, IR-stimuli seldomly lead to full pathogen/pest control, while farmers are familiar with the strong curative performance of pesticides (Walters and Fountaine, 2009; Conrath *et al.*, 2015; Alexandersson *et al.*, 2016). This is for example the reason of the limited economic success of BTH (Conrath *et al.*, 2015).

To overcome these possible drawbacks of IR, several solutions can be thought of. To deal with its limited control, IR-stimuli can be used in a so-called integrated pest management (IPM) strategy. This is a strategy that uses a combination of different pest control measures to keep the pathogens under the so-called economic injury level. An IPM strategy can for example also be used to delay pathogen resistance development to chemicals or resistance genes used in conjunction with the IR-stimulus (Alexandersson *et al.*, 2016), to reduce the applied pesticide doses, or to improve problems associated with IR (Yassin *et al.*, 2021). Moreover, IR-stimuli can show synergistic interactions with regular pesticides, supporting the development of mixtures of these compounds to obtain a suitable defence strategy, or to combine them in an IPM strategy (Cohen, 2002; Conrath *et al.*, 2015). Mixing with pesticides reduces the applied doses, thus reducing the potential negative effects of high dose applications, such as phytotoxicity, environmental damage, ... For example, mixing BABA with the fungicide mancozeb synergistically increased the treatment its effect, reducing the to be applied doses of both (Baider and Cohen, 2003). Further, some pesticides have already been shown to function as both an IR-stimulus and pesticide, such as probenazole, imidacloprid and strobilurins, indicating potential for compounds that can exert this dual action (Yoshioka *et al.*, 2008; Conrath *et al.*, 2015; Yassin *et al.*, 2021).

Alternatively, IR-stimuli can be combined to obtain a synergistic effect, boosting the level of pathogen control (Yassin *et al.*, 2021). In barley, a combination of acibenzolar-S-methyl, BABA and JA resulted in increased IR compared to their individual treatments (Walters, Havis, Sablou, *et al.*, 2011). In wheat (*Triticum aestivum*), the combination of MeJA and the biological IR-stimulus *Trichoderma harzianum* significantly decreased spot blotch (*Bipolaris sorokiniana*) symptoms compared to their single treatments (Singh *et al.*, 2019). In tomato, the combination of MeJA, SA and *T. harzianum* synergistically stimulated plant defence (Zehra *et al.*, 2017). In most cases, the additional protection by mixing IR-stimuli is not complete, but in some cases it induces a high level of protection (Yassin *et al.*, 2021). For example, combining *T. harzianum* with acibenzolar-S-methyl resulted in complete protection of faba bean plants (*Vicia faba*) against *Botrytis cinerea* (Abd El-Rahman and Mohamed, 2014).

Furthermore, IR establishment appears to be dependent on the genotype or cultivar under study, allowing to use breeding to obtain cultivars with stronger IR-responsiveness (Walters, Havis, Paterson, *et al.*, 2011; Sandroni *et al.*, 2020). However, this requires a suitable selection strategy (Sandroni *et al.*, 2020). This highlights the importance of studying the IR mechanism, to define the elements responsible for the IR response and thus allow breeding (Balmer *et al.*, 2015). In addition, efforts have recently been made to computationally screen or rationally develop IR-stimuli (Yassin *et al.*, 2021). Kukawka *et*

al. (2018) found that IR-stimulus ion pairing with the cholinium cation can influence the IR-stimulating capacity and the phytotoxicity of IR-stimuli.

In this work, a plant extract that functions as an IR-stimulus is being studied. Such extracts provide some additional advantages. They typically have low toxicity, are biodegradable and environmentally safe, and are allowed to be used in organic farming (Šernaitė, 2017). This also makes them attractive for consumers and food industry. Some plant extracts can stimulate IR, but they are often antipathogenic, providing an additional protective potential (Šernaitė, 2017; Krzyzaniak *et al.*, 2018). Specifically, it has been shown that many plant extracts can function as nematicides (Elbadri *et al.*, 2008; Eman and El-Nuby, 2019). For example, *Cucurbita pepo* seed extracts were shown to be nematicidal to the free living nematode *Caenorhabditis elegans* and the mice pathogen *Heligmosoides bakeri* (Grzybek *et al.*, 2016). *Cucumis melo* var. *agrestis* fruit extracts were shown to be nematicidal to the RKN *M. incognita* (Elbadri *et al.*, 2008). A hot peel extract from Cantaloupe melons was shown to be nematicidal to *M. incognita* as well (Eman and El-Nuby, 2019). Similar to pesticides, some plant antimicrobials have been shown to act as both an IR-stimulus and antipathogenic compound, such as 1-isothiocyanato-4-methylsulfinylbutane and some saponins (for example aescin) (Schillheim *et al.*, 2018; Trdá *et al.*, 2019). A disadvantage of plant extracts, however, is their variability and complexity in composition. To avoid this variability, the active compound could be identified, but this can be hard due to the complex nature of plant extracts (Šernaitė, 2017; Krzyzaniak *et al.*, 2018).

1.4. Defence functions of ascorbic acid, ascorbate oxidase and dehydroascorbic acid

It is well established that mature fruits of the members of the *Cucurbitaceae* family are abundant in the ascorbate oxidase (AO) enzyme and its substrate ascorbic acid (AA) (Mosery and Kanellis, 1994; Diallinas *et al.*, 1997; Stevens *et al.*, 2018). High levels of the enzyme can be detected in the fruit epidermis and in actively growing tissues (Diallinas *et al.*, 1997). It has been shown that melon (*Cucumis melo* var. *reticulatus*) contains the highest levels of AO in its fruit outer mesocarp compared to its other tissues (Mosery and Kanellis, 1994). Additionally, AO, its substrate and end-product, AA and dehydroascorbic acid (DHA), respectively (Figure 1.8), were shown to play roles in the plant immune system (see below). Therefore, it is of interest to elaborate on the defence functions of these three compounds.

Vitamin C (L-ascorbic acid, ascorbate, AA) is the most abundant water-soluble plant antioxidant (Davey *et al.*, 2000; Smirnoff and Wheeler, 2000) and is known to be at the heart of the peroxide processing and redox signalling in plants, although the exact mechanisms remain elusive (Foyer, Kyndt and Hancock, 2020). AA participates in the reactive nitrogen species (RNS) and ROS signalling pathways

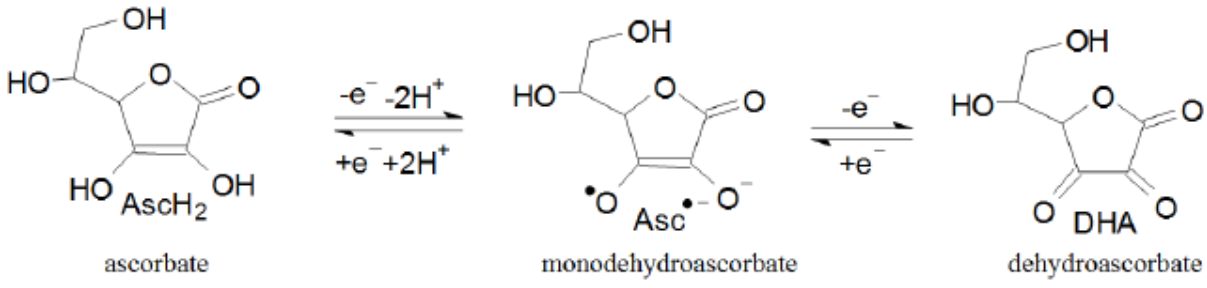

Figure 1.8: Ascorbate oxidase mediated ascorbate degradation. Ascorbate oxidase catalyses the reaction from ascorbate to dehydroascorbate (from left to right) in the apoplast. (Truffault *et al.*, 2014)

(Foyer, Kyndt and Hancock, 2020). Firstly, it has been shown that the production of NO - the most important type of RNS - in flowers is proportional to the AA accumulation and that AA can act as a scavenger of RNS (Rockel *et al.*, 2002; Groß, Durner and Gaupels, 2013). Secondly, the ascorbate-glutathione pathway has been recognised as a key player in the H_2O_2 metabolism, in which reduced glutathione regenerates AA by reducing DHA, either chemically or via DHA reductase (DHAR) (Ding et al., 2020). Most importantly, the apoplastic AA/DHA ratio, which is regulated by AO, designs the apoplastic ROS signal that controls polarised cell growth, biotic and abiotic defence, cell-cell signalling and photosynthesis (Foyer, Kyndt and Hancock, 2020). The homeostasis of the apoplastic ROS signal depends on the local AA concentration, as it is the only abundant antioxidant in the apoplast (Pignocchi and Foyer, 2003; Foyer, Kyndt and Hancock, 2020). Compared to the cytoplasm, the apoplastic AA pool is relatively oxidised, due to the action of AO, which is exclusively localised in the apoplast (Pignocchi and Foyer, 2003). The apoplast is the first line of defence against both biotic and abiotic threats and contains between 5% and 10% of the total leaf AA pool (Foyer, Kyndt and Hancock, 2020). Additionally, the apoplast contains many peroxidases that can metabolise ROS and use ROS in the cell wall metabolism. Together, this indicates the importance of the apoplastic AA pool as a central player in apoplastic ROS processing, and thus in local and systemic signalling. AA also contributes to redox buffering in the apoplast, protecting the cells from oxidative damage (Pignocchi and Foyer, 2003). In addition, AA serves as a cofactor for many reactions in plants, such as the stabilisation of 2-oxoglutarate-dependent dioxygenases, which is important in the synthesis of phytohormones and secondary metabolites (Smirnoff, 2000; Gallie, 2013). AA has a role in the production of anthocyanins, flavonoids and glucosinolates (Turnbull *et al.*, 2004), zeaxanthin (Müller-Moulé, Golan and Niyogi, 2004; Giacomelli, Rudella and Van Wijk, 2006), and ABA, gibberellic acid and ET (Arrigoni and De Tullio, 2002; Mirica and Klinman, 2008).

Arabidopsis low AA mutants, *vtc1* and *vtc2*, were shown to exhibit microlesions, express PR-proteins and contain higher glutathione levels. These higher glutathione levels in turn caused increased presence of NPR1, a SA-signalling protein, in the nucleus (Kiddle *et al.*, 2003; Pavet *et al.*, 2005). Together, this resulted in enhanced resistance against *P. syringae* (Pavet *et al.*, 2005). AA deficiency was shown to also increase the resistance against the oomycete *Hyaloperonospora parasitica* and the aphid *Myzus persicae* in *Arabidopsis* (Barth *et al.*, 2004; Kerchev *et al.*, 2013). These observations are likely mediated by enhanced oxidative signalling in the *vtc* mutants due to a shift in the redox homeostasis (Foyer, Kyndt and Hancock, 2020). However, in *Brassica rapa* it was shown that cultivars with a naturally high level of AA show enhanced resistance to the turnip mosaic virus (Fujiwara *et al.*, 2016). Additionally, in rice, it was shown that low AA mutants are more susceptible to RKN, while containing higher levels of ROS. This indicates that ROS signalling in the rice root might be AA-dependent (Singh, Verstraeten, *et al.*, 2020). AA biosynthesis is enhanced by JA in tobacco and *Arabidopsis* (Wolucka, Goossens and Inzé, 2005; Fujiwara *et al.*, 2016).

AO is responsible for the apoplastic degradation of AA to DHA via the unstable radical monodehydroascorbate (MDHA) (Figure 1.8). The enzyme catalyses the four-electron reduction of oxygen with concomitant one-electron oxidation of AA, producing DHA using oxygen (2 L-ascorbate + $O_2 \rightarrow$ 2 DHA + 2 H_2O) (Stevens *et al.*, 2018). On the other hand, ROS-dependent oxidation of AA occurs in most cellular compartments, for example by ascorbate peroxidase (Foyer, Kyndt and Hancock, 2020). AA can however be recycled from its oxidation products via the MDHA reductase (MDHAR) and DHAR enzyme families, via the glutathione cycle (Foyer, Kyndt and Hancock, 2020). This recycling is believed to only take place in the symplast, requiring DHA to be transported from the apoplast to the

cytosol (Pignocchi and Foyer, 2003). AO is known to participate in the control of cell wall metabolism and the redox status of the apoplast, thus playing an important role in plant defence (Pignocchi *et al.*, 2003, 2006; Foyer, Kyndt and Hancock, 2020). The activity and expression of AO are induced by IAA and AO can catalyse the oxidative decarboxylation of IAA (Kerk, Jiang and Feldman, 2000; Pignocchi *et al.*, 2003). AO expression is also enhanced by JA root drench, but repressed by ABA and SA (Pignocchi *et al.*, 2003; Sanmartin *et al.*, 2007). Transgenic tobacco plants with increased AO expression showed much more DHA than AA in the apoplast, while the AA/DHA ratio in the whole leaf was similar to that of the wild type (Pignocchi *et al.*, 2003, 2006). This led to an increased susceptibility to *P. syringae* and *B. cinerea*, and higher MAPK activity (Kerk, Jiang and Feldman, 2000; Pignocchi *et al.*, 2006). *Arabidopsis ao* mutants showed increased resistance to the turnip mosaic virus and the *vtc1* mutants showed increased susceptibility (Fujiwara *et al.*, 2016). However, Singh, Nobleza, *et al.* (2020) showed that in sugar beet systemic application of AO resulted in IR in the root to the cyst nematode *Heterodera schachtii*. In rice an infection with the rice stripe virus induces AO, boosting the antiviral defences (Sanmartin *et al.*, 2007). AA deficient rice lines are more susceptible to RKN and contain lower levels of JA in the roots. Treating rice leaves with exogenous AO increases tolerance to RKN in the roots, increases the root ET level and activates systemic JA production in the roots, suggesting a role for the apoplastic AA oxidation status as a systemic defence signal. This also indicates that foliar AO treatment can affect the root redox status. Finally, AO treatment primes the roots for increased H_2O_2 generation upon subsequent infection (Singh, Verstraeten, *et al.*, 2020). In *Mg* galls on rice, differential expression of AA recycling protein (e.g. DHAR and ascorbate peroxidase) genes was detected (Kyndt *et al.*, 2012; Ji *et al.*, 2013). The results of these studies indicate the importance for a tight surveillance of the apoplastic AA pool and the apoplastic ROS signals. AO allows to trigger short-term increases in the apoplastic DHA/AA ratio, which can simulate an oxidative burst leading to defence responses. Therefore, constitutive AO overexpression in transgenic lines might cause hypersusceptibility, due to a loss of responsiveness. AO may therefore play a role in the plant defence control by mediating redox signalling (Foyer, Kyndt and Hancock, 2020).

Upon pathogen infection in shoots, accumulation of ROS directs the AA pool towards a more oxidative state, namely increased levels of DHA are found (Foyer and Noctor, 2005). DHA was also shown to accumulate in legume nodules, in *Arabidopsis* roots upon cyst nematode infection and in rice galls upon RKN-infection (Matamoros *et al.*, 2006; Siddique *et al.*, 2014; Singh, Verstraeten, *et al.*, 2020). DHA accumulation/AA oxidation thus seems to be related to oxidative stress. Singh, Verstraeten, *et al.* (2020) suggest that AA oxidation might be important in the early interaction between rice and *Mg*. Additionally, they found that spraying AO or DHA (but not AA) foliarly causes IR to *Mg*, thus indicating a systemic effect. When applying DHA or AA to the root, both cause decreased susceptibility to *Mg*. AA is known to preferentially cross cellular membranes in its oxidised form (DHA), potentially explaining why DHA and not AA causes systemic defence (Horemans, Asard and Caubergs, 1997).

1.5. Aim and research objectives

As nematicides, and pesticides as a whole, become increasingly banned, new strategies are required to assure global food security (Wesemael, Viaene and Moens, 2011; Vos *et al.*, 2012; Mantelin, Bellafiore and Kyndt, 2017; Mauch-Mani *et al.*, 2017). Therefore, the exploitation of the plant immune system in conjunction with other strategies may hold the potential to achieve better and sustainable crop protection (Mauch-Mani *et al.*, 2017). Specifically, IR is of interest under the condition that we select for IR-stimuli without fitness costs. Balmer *et al.* (2015) even suggest that studying the IR

mechanisms could provide us with insights to produce plants with an enhanced capacity for IR, thus creating plants that can better cope with stress in the field and require less protective and curative chemicals. This thesis focusses on a new IR-stimulus, mCCOPE, to sustainably protect rice from parasitic nematode infections. mCCOPE application in agriculture could help to validate an eight to twenty million tonnes waste stream of non-edible melon parts (peels and seeds) produced by food industry per year (Rolim, Seabra and de Macedo, 2020), while contributing to food security.

This thesis has four research objectives:

1. It will be examined whether AO or DHA are the active compound(s) present in mCCOPE.
2. The mode-of-action of mCCOPE as an IR-stimulus to protect rice from *Mg* infection will be identified.
3. It will be investigated whether mCCOPE can also function as a nematicide.
4. The long-term applicability and fitness costs of repeated mCCOPE treatment of rice plants will be assessed.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of wild-type rice *Oryza sativa* cv. 'Nipponbare' were obtained from the United States Department of Agriculture (GSOR-100). The seeds were germinated on wet filter paper for four days at 32°C and the seedlings were transferred to a sand/absorbent polymer (SAP)-substrate. SAP-substrate is a mixture of 25 kg sand (Sibelco) and 28 g Absorbent Polymer AquaPerla® (DCM) solubilised in 3 L water. The seedlings were further grown at 28°C, under a light regime of 12h/12h (light/dark). Hoagland solution (10 mL/plant, composition denoted in Supplementary table S.1) was given as a source of nutrients and water three times a week.

2.2. Plant treatments

Plants were treated when they were two-weeks old, unless stated otherwise. Plants were treated with a fine mist till run-off. Via several application rounds, with minimal interval time, a total of 6.25 mL solution was applied to each plant. To each solution the surfactant Tween20 (Duchefa Biochemie) was added (0.02 v/v%) to promote adherence and uptake of the compounds. The control group was mock treated by spraying the plants with the buffer used for mCCOPE preparation (see next paragraph) containing 0.02% Tween20.

mCCOPE was made by extracting Cantaloupe or Gallia melon (*Cucumis melo* var. *cantalupensis*) peels in a 0.1 M sodium phosphate buffer (pH = 6.5; Acros Organics, Vel n.v.). Extraction and blending were performed at 4°C to preserve potentially crucial enzyme activity. After blending 100 g of peels in a small amount of buffer, the homogenous solution was filtered through a miracloth filter (VWR). The filtrate was collected, and buffer was added till 200 mL was reached. mCCOPE was made fresh, shortly prior to use and always kept cool.

Boiled mCCOPE was prepared by boiling the extract for fifteen minutes in an oil bath with reflux cooling. Room temperature mCCOPE represents an extract that was left for fifteen minutes in the fume hood at room temperature.

Endogenous DHA levels were determined in mCCOPE (see below). Using this endogenous concentration of 0.15 mM, the individual effect of DHA (Sigma Aldrich; stored at -20°C) could be compared to the effects stimulated by crude mCCOPE. Other used DHA concentrations were 5 mM and 20 mM. DHA was solubilised in the sodium phosphate buffer used for mCCOPE preparation and kept on ice before treatments.

2.3. Nematode extraction, inoculation and measurements

M. graminicola was cultured on rice plants and grasses (*Echinocloa crus-galli*). Second-stage juveniles (J2s) were extracted using a sieve/tray extraction method and 250 J2s were inoculated per plant. The extraction was done using the modified Baermann method (Luc, Bridge and Sikora, 2005). Here, cleaned roots were cut in fine pieces, allowing nematodes to leave the galls. These root fragments were incubated for three days on a sieve with large mesh (200 µm or larger) covered with filter paper and just immersed in water. This allowed the nematodes to migrate through the sieve and accumulate in the water. The water was then brought repeatedly over a fine sieve (25 µm), to retain the nematodes. Nematodes were ultimately obtained by washing the 25 µm sieve. To inoculate plants during the infection assay, 250 J2s were applied to the root of each two-week-old rice plant. This was

done via addition of 0.75 mL nematode extract to both sides of the root system, via two holes in the soil. Inoculation was done one day after treatment. Fourteen days post inoculation (dpi), the roots were collected and stained using 12.5% raspberry red (Alcoferm). The roots were destained in acid glycerol (1% (v/v) HCl/glycerol (Acros Organics)), so only nematodes and galls remained red. Next, nematodes and galls were counted and nematodes were categorised according to their developmental phase (J2s, J3/J4s, adult females and egg-laying females, see Figure 2.1). Per treatment, eight biological replicates were used. Significant differences upon various treatments and/or control treatment were assessed by conducting heteroscedastic two-sided t-tests in Excel (version 16.0.13127.21210).

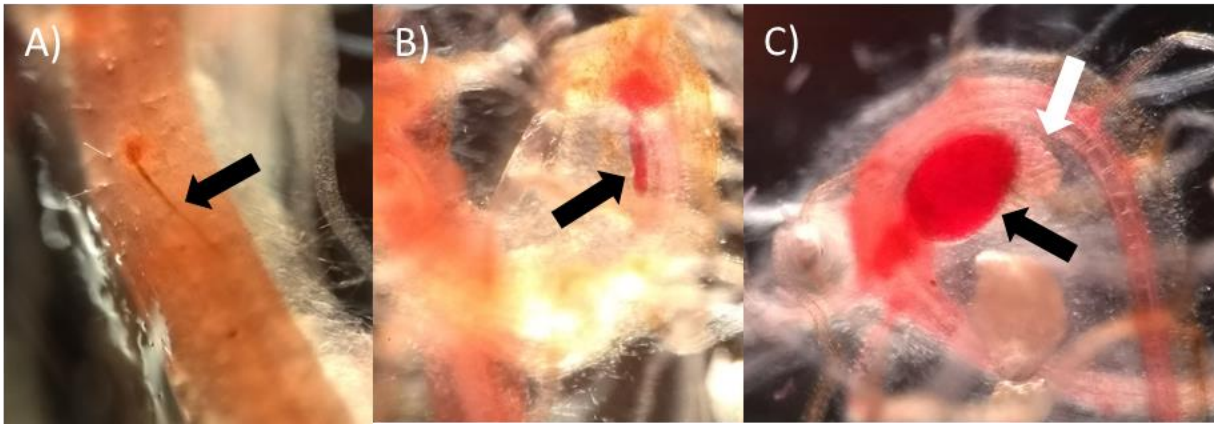

Figure 2.1: Categories of nematode developmental phases counted in this thesis. From left to right, the nematodes (pointed at with a black arrow) were characterised as J2 (A), J3/J4 (B) and egg-laying female (C). In (C) the white arrow points at the produced sack of eggs. Nematodes classified as an adult female look as in (C), but without the eggs.

2.4. DHA measurement

Liquid chromatography-mass spectrometry (LC-MS) analyses to determine the DHA concentration in mCCOPE were performed with an Agilent 1200 Series liquid chromatograph equipped with a Kinetex LC column (150 x 4.6 mm, 2.6u), a UV-detector, an Agilent 1100 series MS mass spectrometer with an electron spray-ionisation geometry (ESI, 4000 V, 70 eV) and a quadrupole detector. 1 mL of extract was filtered (0.22 µm; Whatmann), put in an high-performance liquid chromatography (HPLC)-vial and analysed using the LC-MS system (injection volume: 10 µL; pump flow rate: 0.5 mL/minute; T: 25°C; mobile phase composition: [0-20] = 100% H_2O (HPLC-grade; Fisher Chemical) with 0.1% (v/v) formic acid (Chem-lab)). Per sample, three technical replicates were analysed. A calibration curve of dissolved DHA (0.125, 0.0625, 0.03125 and 0.015625 g/L DHA; analysed in the same way) was constructed and used to convert the peak area to the DHA concentration. Significant differences between different extracts were assessed by conducting heteroscedastic two-sided t-tests in Excel.

2.5. mRNA sequencing

For mRNA-seq, three biological replicates were analysed per time point per treatment. Each biological replicate was the pooled material of four different root systems. Upon collection of the roots, the samples were snap-frozen in liquid nitrogen. Next, they were ground in a mortar and RNA was extracted using the QIAGEN RNeasy Plant Mini kit, according to the manufacturer's instructions. In the RNA extraction protocol, one additional step was performed: a sonication step (three times for ten seconds; Bransonic CPX1800-E) was executed after addition of the RLT extraction buffer. RNA quantity and purity were checked using the Nanodrop 2000 (Thermo Fisher Scientific). Extracted RNA was stored at -80°C till it was sent for further library preparation and sequencing at NXT-GNT. Illumina sequencing was conducted by NXT-GNT (single end, read length = 76 bp).

The quality of the generated reads was first checked, using FastQC (version 0.11.5; Andrews, 2010). Then, all reads were trimmed, using Trimmomatic (version 0.36; Bolger, Lohse and Usadel, 2014). A sliding window of five nucleotides was used and a phred score cut-off of 20. Trimmed reads that had become shorter than twenty nucleotides were removed. Next, quality control was repeated to ensure correct execution of the trimming step. The trimmed reads were then mapped to the *O. sativa* subsp. *japonica* reference genome (build 'International Rice Genome Sequencing Project' (IRGSP)-1.0.39) using STAR (version 2.5.2a; Dobin *et al.*, 2013). Only uniquely mapped genes were kept for further analysis. The BAM-files of the multiplexed samples were merged using SAMtools (version 1.3; Li *et al.*, 2009). Count tables were generated by the 'summerizeOverlaps' function of the 'GenomicAlignments' package in R (version 1.10.1, Lawrence *et al.*, 2013). Differential gene expression was analysed using the 'DESeq2' package (version 1.14.1; Love, Huber and Anders, 2014), with an adjusted p-value of 0.05 for differential expression.

The differentially expressed genes were annotated using the UniProt and RAP-DB-database (Sakai *et al.*, 2013; Bateman *et al.*, 2021). Pathway analysis was conducted on the differentially expressed genes using Gene Ontology (GO) enrichment analysis using the online tool g:Profiler, with an ordered query (allowing to conduct an incremental enrichment analysis) (Raudvere *et al.*, 2019). Finally, a pathway analysis with all genes (i.e. both significantly differentially expressed genes as unaffected genes) was performed using MapMan (version 3.6.0RC1; Usadel *et al.*, 2005). Here, a Wilcoxon rank sum test was executed with Benjamini-Hochberg correction of the p-values to indicate a significant alteration of the pathways under study (Usadel *et al.*, 2005). To compare the effect of multiple treatments (relative to the control), heatmaps and Venn diagrams were constructed of the differentially expressed genes. Venn diagrams were made using a tool provided by the University of Ghent and VIB (http://bioinformatics.psb.ugent.be/webtools/Venn/). Heatmaps were constructed using the differentially expressed genes of the different treatments. This resulted in a gene set containing each gene that was differentially expressed in at least one treatment. Genes that were differentially expressed in only some of the treatments had to be assigned the value 0 for treatments in which they were not differentially expressed to obtain a complete matrix. The heatmap was constructed using the 'aheatmap' function of the 'NMF' R-package (version 0.23.0; Gaujoux and Seoighe, 2010).

2.6. Lignin measurement

Lignin levels in rice roots were quantified using the acetyl bromide assay (Barnes and Anderson, 2017). For each treatment and time point, five biological replicates were used, each containing four pooled root systems. Samples were first ground in liquid nitrogen and then subjected to four subsequent extraction steps. Each extraction step involved a 30 minutes incubation of the sample in 1 mL solvent at a matching temperature, followed by centrifugation at full speed (20800 g) and discarding the supernatant. The used solvent-temperature-centrifugation time combinations subsequently were: water-98°C-three minutes, 96% ethanol (Fisher Scientific)-76°C-three minutes, chloroform (Chem-lab)-59°C-five minutes and acetone (Chem-lab)-54°C-five minutes. After discarding the supernatant, the pellets were left to dry overnight in the fume hood. The dried samples were weighed to assure a mass between 2-7 mg and the pellet was redissolved by adding 200 µL 25% acetyl bromide (Sigma Aldrich) in glacial acetic acid (Sigma Aldrich) to be incubated for two hours at 50°C. As acetyl bromide degrades plastic, a blank was included in the incubation step to normalise the data. Then 1 mL of glacial acetic acid was added to the sample, followed by centrifugation at full speed for ten minutes. 300 µL of the sample was then transferred to a new Eppendorf tube, and 300 µL 2.0 M NaOH (Sigma Aldrich) and

300 µL 0.5 M hydroxylamine (Sigma Aldrich) was added. Upon through mixing the sample was centrifuged at full speed until it was no longer cloudy. Finally, the absorbance was measured at 280 nm in a Tecan (Infinite F200 Pro), using three technical replicates per sample.

The mass of the dried samples was used to normalise the obtained absorbance values. Obvious outliers were removed from the technical replicates as they diverged strongly from the other values. Outliers concerning biological replicates were removed based on the interquartile range (IQR)-method (Seo, 2002). The data were analysed using a heteroscedastic one-sided t-test in excel. This choice for a one-sided t-test was based on prior mRNA-seq results.

2.7. H_2O_2 measurement

The root H_2O_2 content was analysed using a xylenol orange (or ferrous oxidation, FOX) assay. Ten biological replicates were used per treatment per time point, each consisting of four pooled root systems. The material was ground in liquid nitrogen and 70-100 mg sample was transferred to an Eppendorf tube. Per mg sample, 10 µL of 5% (w/v) trichloroacetic acid (Sigma Aldrich, stored at 4°C) solution was added to the samples. Next, the samples were sonicated (three times for twenty seconds; Bransonic CPX1800-E) and subsequently centrifuged for ten minutes at 20800 g at 4°C. To each well of a UV-transparent 96-well plate (Thermo Electron Corporation) 100 µL FOX-buffer, with or without xylenol orange (XO; Sigma Aldrich, stored at 4°C), was added. The buffer without XO was used as a blank, while the buffer with XO was used to measure the H_2O_2 content. 20 µL trichloroacetic acid extract was added to both the buffer with and without XO, and this in technical triplicates. Also, a H_2O_2 (Sigma Aldrich, stored at 4°C) standard curve was included (0, 1.5625, 3.125, 6.25, 12.5, 25, 50 and 100 mM), in duplicates. The plate was then incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 560 nm in a Tecan machine (Infinite F200 Pro). Significance was assessed by conducting heteroscedastic two-sided t-tests in Excel.

2.8. Hormone measurements

To determine the concentration of the gaseous hormone ET in roots, respectively five and eight biological replicates were analysed over two biologically independent repeats. Each biological replicate consisted of at least three fresh root systems. The roots were collected and dried with tissue paper, and subsequently cut in very fine pieces to be collected in a glass vial sealed with a screw cap having a septum (Sigma Aldrich). The tissue was left to incubate for four hours at room temperature, allowing ET accumulation in the headspace. The headspaces were then analysed using a gas chromatograph (Thermo Finnigan TRACE GC Ultra; CP-PoraBOND CP7354, 25 m, 0.53 mm, 10 µm, 35°C) with a flame ionisation detector. Standards of 0.4 ppm and 1 ppm ET were used for calibration. The leftover tissue was then dried for three days at 50°C and weighed to normalise the obtained ET levels for the respective root masses. Significant differences were assessed by heteroscedastic two-sided t-tests in Excel.

ABA, IAA, JA and SA levels were determined using liquid chromatography. Here, root material was sampled from five biological replicates, each consisting of a pool of at least four individual plants. The roots were snap-frozen, ground and further stored at -80°C. Following a cold solvent (modified Bieleski) extraction, filtration and clean-up, the hormone levels were measured on a ultra-high-performance liquid chromatograph (UHPLC) Q-Exactive™ high-resolution Orbitrap mass spectrometer (Thermo Fisher Scientific), according to the protocol described in Haeck *et al.* (2018). For ABA, IAA and SA, the output was corrected using internal standards, namely their respective deuterated forms (Haeck *et al.*,

2018). JA was not corrected as no such internal standard was available. Data outliers were removed based on the IQR-method (Seo, 2002). IAA and JA data were analysed by a heteroscedastic one-sided t-test in Excel. This choice for a one-sided t-test was based on prior mRNA-seq results. ABA and SA data were analysed using a heteroscedastic two-sided t-test in Excel, as no prior assumptions could be made concerning the levels of these phytohormones based on the mRNA-seq results.

2.9. Nematicidal assay

Nematodes (*M. graminicola*) were extracted as described above (Section 2.3) and surface sterilised. Hereto, nematodes were centrifuged in a falcon at 446 g for three minutes with a slow deceleration. The lower 5-10 mL, containing the nematodes, was kept to sterilise. Gentamycin (Duchefa Biochemie, stored at 4°C), kanamycin (Duchefa Biochemie, stored at 4°C) and spectinomycin (Fluka Biochemika, stored at 4°C) were each added to a final concentration of 200 µg/mL. Nematodes were incubated in this solution for 30 minutes on a shaker. Next, the nematodes were washed twice, by refilling the falcon tubes with autoclaved tap water, pelleting and removing the upper 40-45 mL solution. Then, hospital antiseptic concentrate (3.3 µL/mL nematode solution; Mölnlycke) was added and the nematodes were shortly shaken. The falcon was filled till 50 mL with water, followed by centrifugation to pellet the nematodes down. The upper 40-45 mL was removed, and the nematodes were washed two times (as described above).

Crude plant extracts such as mCCOPE are turbid and thus limit nematode counting with the microscope. To avoid this, mCCOPE was centrifuged twice at full speed for five minutes, discarding the pellet after both centrifugations. All treatment solutions were then filter sterilised to avoid contamination. Three biological replicates were analysed per treatment. A validated positive control, 0.2% (v/v) vertimec (Globachem), leading to full nematode mortality within a few hours, was included as well. 12-well plates were filled with 100 J2 nematodes per well - added via a minimal volume - and 2 mL treatment solution per well. Nematodes were then incubated on a shaker and viability was assessed by counting the nematodes after six and 48 hours of treatment. Nematodes were categorized according their mobility: (1) living nematodes being nematodes that were clearly moving (Figure 2.2A), (2) dead nematodes, being immobile and completely stretched out (Figure 2.2B), and (3) static nematodes, which were not moving but were not stretched out either, but curled (Figure 2.2A).

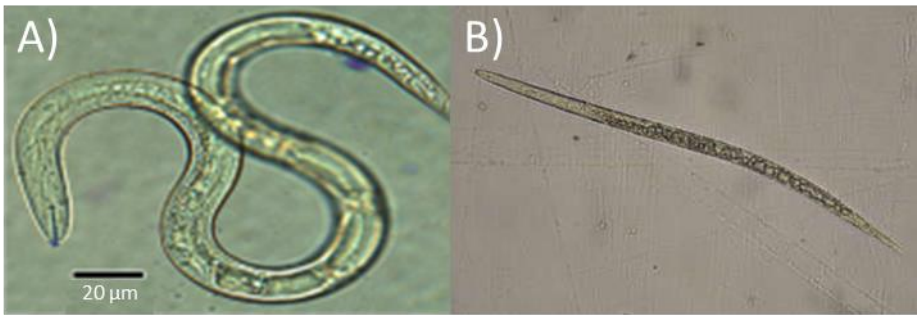

Figure 2.2: Categories of J2 viability. (A) Either a living nematode (when moving) or a static nematode (when immobile). (Wahid, 2019) (B) A dead nematode, recognisable by being completely stretched out and immobile. (Santos, 2013)

To assess nematode viability after 48 hours of treatment, additional purification steps were included prior to counting, as the plant extract had become turbid. To allow comparison, these steps were also performed for the mock treated samples. A 15 mL falcon was filled with 12 mL 100% sucrose (Duchefa Biochemie) solution and 1.9 mL solution under study containing the nematodes. Falcons were thoroughly shaken and a top layer of 1 mL tap water was gently applied. The samples were centrifuged

(446 g for three minutes with slow deceleration) and the upper 3-4 mL – being transparent and containing the nematodes - was gently sucked up without disturbing the various layers using a glass pipet. These 3 to 4 mL solutions were then diluted to 15 mL with tap water and recentrifuged under the same conditions. The upper 13 mL was then removed without disturbing the lower 2 mL, which was a transparent liquid containing the pelleted nematodes. This solution with nematodes was incubated two hours on the shaker to recover, after which the nematodes were categorised as explained above.

Statistical inference was performed based on the theory of Aitchison for compositional data (Aitchison, 2015). To remove the constraint character of the percentual data, a log ratio transformation was conducted using the percentage of static nematodes as a base. The data of vertimec treated nematodes could not be statistically analysed due to the so-called zero-proportions, that do not allow analysis by log ratio transformation (Butler, Bierman and Marion, 2005). The log ratio transformed data were analysed using heteroscedastic two-sided t-tests in excel.

2.10. Long-term effects of mCCOPE on growth and yield

For the evaluation of long-term effects, husked rice seeds were sterilised by subsequently immersing them in 70% ethanol (Fisher scientific) for five minutes and 4% bleach (Haz-tabs) for twenty minutes while continuously rotating. Then the seeds were washed six times with sterile water. Next, they were incubated for five days at 32°C on sterile filter paper in a sterile petri dish containing 10 mL of 0.2 mg/mL thiram solution (Eastman). Three seedlings were planted per pot in potting soil (m. Snebbout, 'Structural'). The plants were grown for four months at the Greenhouse facilities of UGhent (32°C, 12h/12h light/dark) with automatic watering. Starting from week two, every two weeks, the plants were treated with either mCCOPE or mock solution. At these time points the length of the longest leaf of each plant was measured. At the end of the growth period (eighteen weeks after sowing), the number of panicles and tillers was counted, and the produced seeds were weighed. To weigh the seeds, they were first dried for 48 hours at 32°C. For each treatment four pots and thus twelve plants were monitored.

3. Results

3.1. mCCOPE functions as an IR-stimulus, but AO and DHA are not the active compounds hereto

To investigate the importance of AO and DHA in mCCOPE to stimulate IR, the susceptibility of treated rice to *Mg* was analysed. Rice was treated with mCCOPE, boiled mCCOPE (no longer containing AO and DHA, see below) and a solution of pure DHA.

Using LC-MS, the DHA concentration in fresh mCCOPE was measured twice (Figure 3.1 and Degroote, unpublished). In Figure 3.1 the concentration was estimated at 0.20 mM (standard error of the mean (SEM) = 0.067) and a previous measurement estimated the concentration at 0.11 mM (Degroote, unpublished). Therefore, in the rest of this thesis, the endogenous DHA concentration in mCCOPE will be assumed to be 0.15 mM.

To determine the importance of both AO and DHA in mCCOPE to stimulate IR, the extract was boiled for fifteen minutes under reflux. Boiling ensures loss of the AO activity, as the enzyme denatures. Additionally, Figure 3.1 shows that upon boiling DHA could no longer be detected in the extract. Moreover, DHA significantly degraded in mCCOPE that was left for fifteen minutes at room temperature (p = 0.0035).

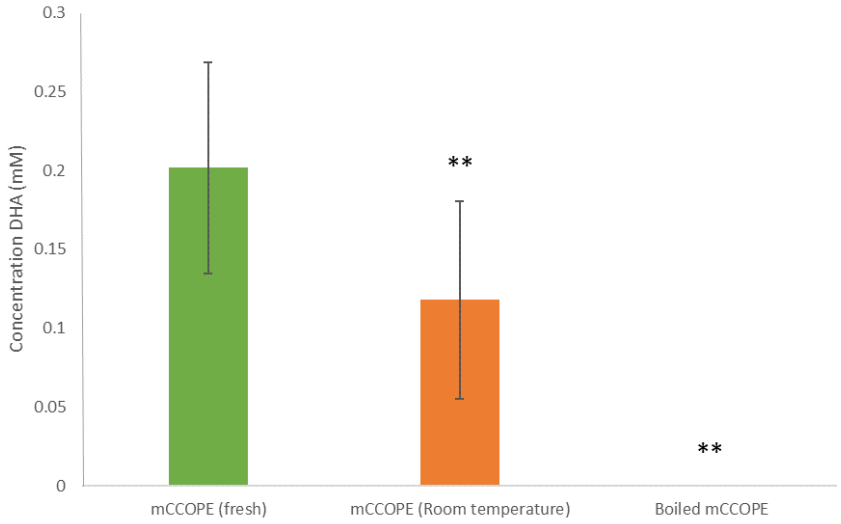

Figure 3.1: DHA degrades in mCCOPE at room temperature and upon boiling. DHA concentrations determined via LC-MS. 'mCCOPE (Room temperature)' corresponds with fresh mCCOPE left for fifteen minutes at room temperature in the fume hood. 'Boiled mCCOPE' represents fresh mCCOPE boiled under reflux for fifteen minutes. Significant differences, calculated via a heteroscedastic two-sided t-test, are illustrated via one or two asterisks (p = 0.1 and p = 0.05, respectively). The error bars represent the standard error of the mean (SEM). Three technical replicates were analysed per sample.

Figure 3.2 shows the results of an infection experiment in which two-week-old rice plants were inoculated with *Mg* at 1 dpt. The studied treatments were fresh mCCOPE, boiled mCCOPE, 0.15 mM DHA and 20 mM DHA. 20 mM DHA - a known IR-stimulating DHA concentration (Singh, Verstraeten, *et al.*, 2020) – was included as a positive control. Infection levels were assessed by counting the number of galls and nematodes in the inoculated root systems at 14 dpi. Figure 3.2 shows that fresh mCCOPE treatment significantly reduced the number of females (p = 0.003) and close to significantly reduced the number of egg-laying females (p = 0.067). Boiled mCCOPE significantly reduced the number of females (p = 0.002) and egg-laying females (p = 0.037). Moreover, boiled mCCOPE also significantly

increased the number of J2s (p = 0.024) and close to significantly increased the number of J3s/J4s (p = 0.073). 0.15 mM DHA treatment did not significantly affect the rice root infection, although 20 mM DHA treatment significantly reduced the number of females (p = 0.005) and egg-laying females (p = 0.037). Further, none of the treatments affected the root or shoot size.

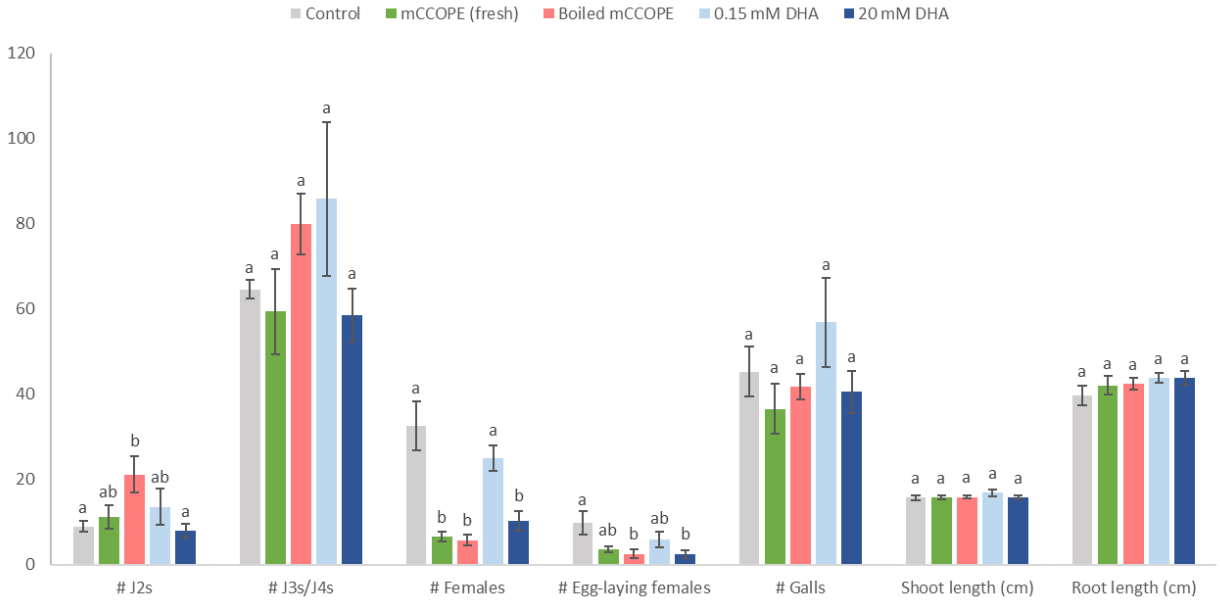

Figure 3.2: Boiling mCCOPE does not reduce the capacity of the extract to establish IR, nor does DHA at the concentration found in mCCOPE stimulate IR. Rice plants were treated with mock solution, fresh mCCOPE, boiled mCCOPE, 0.15 mM DHA and 20 mM DHA. Boiled mCCOPE was prepared by boiling fresh mCCOPE for fifteen minutes under reflux. At 1 dpt, plants were inoculated with 250 J2s of *Mg*. 14 dpi the roots were collected to assess infection levels. Significant differences, calculated via heteroscedastic two-sided t-tests, are illustrated via letters (p = 0.05). Bars that were not significantly different from each other carry the same letter, bars carrying multiple letters were not significantly different from neither of the included groups. Error bars represent the SEM. Eight biological replicates were analysed per treatment.

3.2. mCCOPE but not 0.15 mM DHA drastically affects the rice root transcriptome

To investigate the mode-of-action of mCCOPE on the rice root immunity, mRNA-seq was performed. Rice plants foliarly treated with mCCOPE were compared to mock and 0.15 mM DHA treated plants. The transcriptional response was investigated at 1 and 4 dpt. The mRNA-seq data were analysed by investigating significantly enriched and/or differentially expressed pathways using an online GO-enrichment tool (g:Profiler; Raudvere *et al.*, 2019) and MapMan (Usadel *et al.*, 2005). An overview of all significantly altered pathways upon mCCOPE treatment can be found in Supplementary table S.4. In Table 3.1 the significantly affected pathways upon mCCOPE treatment relevant to plant immunity are shown. Supplementary tables S.2 and S.3 show the annotated differentially expressed genes upon mCCOPE treatment with the 30 most drastically affected log2 fold change (log2FC) values at 1 and 4 dpt, respectively. The latter lists will be referred to as the 'top 30 genes'.

At both 1 and 4 dpt, mCCOPE treatment induced genes related to **cell wall formation** (MapMan: p = <1e-20 and 1.85e-9, respectively) in the rice roots. Specifically, genes involved in **lignin formation** were activated (MapMan: p = 6.55e-6 and 1.70e-3, respectively). Moreover, upregulation of the phenylpropanoid pathway genes (MapMan: p = 6.81e-8 and 2.85e-5, at 1 and 4 dpt, respectively; GO: p = 4.21e-3, at 1 dpt) supports this observation. For both time points, genes coding for enzymes involved in lignin crosslinking, such as laccases were upregulated. Indeed, *LACCASE-10* (Os03g0273200) was among the top 30 genes (Supplementary table S.2). Among the significantly upregulated genes

Table 3.1: Pathway analysis of mCCOPE treated plants at 1 and 4 dpt according to GO enrichment and Mapman (selection of pathways with known roles in plant immunity). A selection of relevant and significantly altered pathways 1 dpt (left) and 4 dpt (right) with mCCOPE is listed. Red asterisks indicate pathways that were also upregulated upon 0.15 mM DHA treatment at the same time point (Supplementary table S.5). p-values can be found between brackets. For MapMan analyses, Benjamini-Hochberg p-value correction was used. The full table can be found in Supplementary table S.4.

1 dpt	4 dpt
Upregulated:	
GO:	
Cellulose metabolism (6.78e-5)	Reactive oxygen species metabolism (3.74e-7)
Cell wall biogenesis and organisation (1.00e-2)	Hydrogen peroxide metabolic process (6.32e-8)
Phenylpropanoid metabolic process (4.21e-3)	Hydrogen peroxide catabolic process* (4.79e-7)
Oxylipin biosynthetic process (9.87e-3)	Response to oxidative stress* (2.38e-4)
	Water transport (3.26e-2)
	Nitrate transport (2.46e-4)
	Nitrate assimilation/reactive nitrogen species metabolism (2.74e-2)
	Cellular oxidant detoxification (3.60e-4)
	Plant-type cell wall organisation (8.19e-4)
Mapman:	
Cell wall (<1e-20)	Cell wall (1.85e-9)
Lignin biosynthesis (6.55e-6)	Lignin biosynthesis (1.70e-3)
Phenylpropanoid pathway (6.81e-8)	Phenylpropanoid pathway (2.85e-5)
Peroxidases (6.51e-6)	Peroxidases (6.63e-10)
Glutathione *S*-transferases (4.38e-3)	Major Intrinsic Proteins (1.45e-3), specifically PIP[1] (1.67e-4)
Ascorbate and glutathione redox (3.13e-2)	Glutathione *S*-transferases (1.75e-7)
Major Intrinsic Proteins PIP[1] (1.44e-3)	Nitrate transport (3.97e-3)
Receptor kinase signalling (4.35e-9)	ET synthesis and degradation (4.04e-2)
Secondary metabolism (1.25e-8)	AP2[2]/EREBP[3], APETALA2/ET-responsive element binding protein family (9.66e-3)
Cytochrome P450 (2.86e-2)	WRKY[4] domain transcription factor family (2.85e-5)
ET synthesis and degradation (1.85e-2)	Cytochrome P450 (5.16e-12)
JA synthesis and degradation (1.69e-2)	Receptor kinases (3.14e-7)
WRKY[4] transcription factor family (4.37e-3)	Calcium signalling (9.46e-5)
Calcium signalling (1.67e-6)	
Downregulated:	
Mapman:	
NA	ARR[5] transcription factor family (8.08e-3)

involved in the phenylpropanoid pathway, the majority was specifically related to lignin biosynthesis, including various laccases and for example Os09g0399800 (log2FC = 1.38 and 1.46, at 1 and 4 dpt, respectively), a gene encoding CINNAMYL ALCOHOL DEHYDROGENASE, which catalyses the final step in the lignin monomer formation (Tobias and Chow, 2005). The early core genes in this pathway were significantly upregulated as well, including for example at 1 dpt Os08g0448000 (*Os4CL5*, Supplementary table S.2) and Os02g0177600 (*Os4CL3*, log2FC = 1.24), which encode two 4-coumarate-coenzyme A ligases, and Os02g0627100 (*OsPAL*, log2FC = 1.43). Further, gene expression of uridine 5'-

[1] Plasma membrane intrinsic protein
[2] APETALA 2
[3] Ethylene-responsive element binding protein
[4] Containing a WRKY amino acid sequence domain
[5] *Arabidopsis thaliana* response regulator

diphospho (UDP)-glucosyl and -glucoronyl transferases was significantly upregulated (Supplementary table S.4, MapMan: p = 1.25e-8 and 8.69e-10, at 1 and 4 dpt, respectively).

At both time points under study, enhanced **redox signalling** was observed as well. This is exemplified by altered expressions of genes encoding glutathione *S*-transferases (MapMan: p = 4.38e-3 and 1.75e-7, at 1 and 4 dpt, respectively), peroxidases (MapMan: p = 6.51e-6 and 6.63e-10, at 1 and 4 dpt, respectively) and genes involved in the ascorbate-glutathione cycle (MapMan: p = 3.13e-2, at 1 dpt), ROS (GO: p = 3.74e-7, at 4 dpt) and RNS metabolism (GO: p = 2.74e-2, at 4 dpt) (Table 3.1). At both 1 and 4 dpt, *NITRATE REDUCTASE* (Os02g0770800), responsible for NO - the most important type of RNS - generation (Astier *et al.*, 2019), was significantly upregulated (Supplementary table S.2 and log2FC = 1.18, at 1 and 4 dpt, respectively). Additionally, expression of nitrate transporters was activated at both time points (Supplementary table S.2 and S.3). At 4 dpt, the nitrate transport pathway was significantly upregulated (MapMan: p = 3.97e-3). At both time points, enhanced expression of aquaporins (GO: 'water transport', p = 3.26e-2, at 4 dpt; MapMan: 'Major Intrinsic Proteins PIP', p = 1.44e-3 and 1.67e-4, at 1 and 4 dpt, respectively) was detected. Examples of significantly upregulated genes were Os04g0559700 (*OsPIP1-2*, log2FC = 0.46 and 1.39, at 1 and 4 dpt, respectively), Os02g0823100 (*OsPIP1-3*, log2FC = 0.49 and 1.00, respectively) and Os02g0629200 (*OsPIP2-2*, log2FC = 0.14 and 1.19, respectively).

Receptor kinase signalling was upregulated by mCCOPE treatment as well (MapMan: p = 4.35e-9 and 3.14e-7, at 1 and 4 dpt, respectively), which could play a role in defence signalling. Correspondingly, at 1 dpt, MapMan analysis showed upregulation of type III leucine rich-repeat (LRR) receptor kinases, which are typically associated with PTI and RLKs (Supplementary table S.4, p = 9.88e-4).

At both time points, **calcium signalling** - a typical and early defence signal in plant immunity (Zhang, Du and Poovaiah, 2014) - was significantly upregulated (MapMan: p = 1.67e-6 and 9.46e-5, respectively).

mCCOPE treatment also affected **hormone signalling** at both time points. First, the oxylipin biosynthesis – amongst others leading to JA biosynthesis (Wasternack and Feussner, 2018) - was activated at 1 dpt (GO: p = 9.87e-3). Additionally, *ALLENE OXIDASE SYNTHASE* (*OsAOS,* Os03g0225900), a key JA biosynthesis gene, was present among the 1 dpt top 30 genes (Supplementary table S.2). Consistently, at 1 dpt, JA metabolism was upregulated as well, as can be seen in Table 3.1 (MapMan: p = 1.69e-2). Secondly, the ET pathway appeared to be affected by mCCOPE treatment at the transcriptional level, as, at 1 dpt, the ET biosynthesis gene *AMINOCYCLOPROPANECARBOXYLATE (ACC) OXIDASE* was differentially upregulated (Supplementary table S.2). At both 1 and 4 dpt, transcription of *ACC SYNTHASE*, synthesising the ET precursor ACC, was significantly upregulated (log2FC = 1.70 and 1.85, respectively). Additionally, MapMan analysis showed upregulation of the ET metabolism (Table 3.1, p = 1.85e-2 and 4.04e-2, at 1 and 4 dpt, respectively). Moreover, MapMan also showed that ET-responsive transcription factors were upregulated at 4 dpt (p = 9.66e-3). Thirdly, at 4 dpt, the cytokinin related ARR transcription factor family was downregulated (MapMan: p = 8.08e-3). Examples of significantly downregulated genes were Os11g0143300 (log2FC = -1.27) and Os12g0139400 (log2FC = -1.44), both related to cytokinin signalling (Ferreira and Kieber, 2005). Fourth, Os12g0617400 (Supplementary table S.2), one of the ABA biosynthesis genes, was downregulated at 1 dpt (Y. Huang *et al.*, 2019). Finally, IAA-signalling was affected as well. At 1 dpt, *INDOLE-3-ACETATE O-METHYLTRANSFERASE* and *SAUR36* (Supplementary table S.2), negative regulators of IAA-signalling,

biosynthesis and transport, were downregulated. At 4 dpt, the top 30 genes included the IAA-AMINO ACID HYDROLASE ILR1-LIKE 3 enzyme, which hydrolyses and thus activates IAA amino acid conjugates (Supplementary table S.3, Sanchez Carranza *et al.*, 2016). In short, ET-, IAA- and JA-signalling were transcriptionally upregulated at both 1 and 4 dpt, cytokinin signalling was transcriptionally downregulated at 4 dpt, and ABA- and SA-signalling were respectively mildly and not significantly affected at either time point.

Finally, **downstream plant defence** genes were transcriptionally activated at both time points, indicating activated rice root immunity. These general plant defence genes included WRKY-transcription factors (MapMan: p = 4.37e-3 and 2.85e-5, at 1 and 4 dpt, respectively), genes of the secondary metabolism (MapMan: p = 1.25e-8, at 1 dpt), cytochrome P450 genes (MapMan: p = 2.86e-2 and 5.16e-12, at 1 and 4 dpt, respectively), phytoalexin biosynthesis genes, germin proteins, harpins, heat shock proteins, PR-proteins, … (Supplementary tables S.2, S.3 and S.4).

To assess the role of DHA in mCCOPE as potential mediator of the above-described transcriptional changes, plants treated with 0.15 mM DHA were analysed via an mRNA-seq experiment. Contrarily to what we observed for mCCOPE, at both time points, no significant upregulation of **cell wall formation, receptor kinase signalling**, **calcium signalling** or **hormone signalling** was observed. At 4 dpt, **redox signalling** was activated, as the glutathione metabolism (GO: p = 8.32e-4) and H_2O_2 catabolism (GO: p = 4.40e-6) were upregulated (Table 3.1, red asterisks, and Supplementary table S.5). At 1 dpt, GO-enrichment (Supplementary table S.5) only showed upregulation of genes related to the heat response (p = 4.00e-2).

Table 3.2 illustrates the intensity of the transcriptomic changes upon treatment with mCCOPE in comparison with 0.15 mM DHA. It can be noted that the effect of 0.15 mM DHA treatment at 1 dpt is very limited compared to mCCOPE treatment. Only five genes were differentially expressed for DHA treated plants, compared to 1306 and 788 differentially up- and downregulated genes, respectively, for mCCOPE treatment. This large difference remains at 4 dpt, with 313 and 68 differentially up- and downregulated genes, respectively, for DHA treatment, compared to 2341 and 1224 for mCCOPE treatment.

Table 3.2: Number of differentially expressed genes for mCCOPE and 0.15 mM DHA treatment at 1 and 4 dpt. This table provides the total number of differentially expressed genes at 1 and 4 dpt. The data are split over the categories of upregulated and downregulated genes.

		mCCOPE	0.15 mM DHA
1 dpt:	*Upregulated:*	1306	5
	Downregulated:	788	0
4 dpt:	*Upregulated:*	2341	313
	Downregulated:	1224	68

Next, the overlap in differentially expressed genes between mCCOPE and 0.15 mM DHA treated plants was represented using Venn diagrams (Figure 3.3). At 1 dpt, all five differentially upregulated genes by 0.15 mM DHA treatment were also present in the differentially upregulated gene set of the mCCOPE treatment (Figure 3.3A). These genes are shown in Supplementary table S.6. The gene *ACC OXIDASE* was among the five significantly upregulated genes upon DHA treatment and is involved in ET biosynthesis. Earlier, this gene was also found to be the 27th most upregulated gene upon mCCOPE treatment (Supplementary table S.2). However, as only a limited number of genes were upregulated

upon treatment with 0.15 mM DHA, the biological relevance of the observed overlap cannot be assessed. At 4 dpt, approximately 50% of the 0.15 mM DHA upregulated genes overlapped with those induced by mCCOPE (Figure 3.3B). As this is a substantial percentage, the overlapping genes were analysed via GO-enrichment. The same was done for the downregulated genes at 4 dpt, for which nineteen overlapping genes were found (Figure 3.3C). GO-enrichment of the overlapping upregulated genes at 4 dpt showed upregulation of H_2O_2 catabolism (p = 6.84e-9). Moreover, this gene list contained several peroxidases and general defence related proteins, such as harpins, chitinases, … (Supplementary table S.7). At 4 dpt, the differentially expressed gene set upon 0.15 mM DHA treatment contained nineteen upregulated peroxidases, while the mCCOPE set contained 45. So, even though overlap in H_2O_2 catabolism could be observed, it only accounted for a minor part of the mCCOPE effect. When analysing the nineteen overlapping downregulated genes at 4 dpt, no clear function was enriched in this gene set.

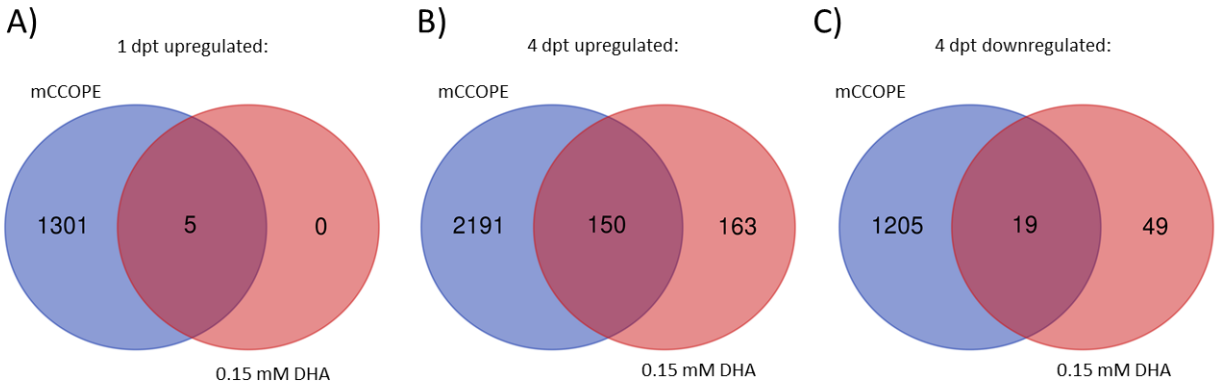

Figure 3.3: The differentially expressed genes of mCCOPE and 0.15 mM DHA treated plants show only minor overlap. Differential expression was assessed compared to the mock treated plants. As no genes were differentially downregulated for 0.15 mM DHA treated plants at 1 dpt, no Venn diagram is shown. Blue: mCCOPE; Red: 0.15 mM DHA.

Arguably, it is possible that mCCOPE acts as a superior formulation for DHA, enhancing the activity of the latter. Therefore, the mCCOPE transcriptome was also compared to transcriptional effects of higher DHA concentrations, namely 5 and 20 mM. Figure 3.4 shows two heatmaps of the differentially expressed genes (compared to the mock treatment) of each treatment at each time point. Figure 3.4 clearly shows that mCCOPE treatment had much more drastic effects on the transcriptome than DHA at the concentration as determined in the extract (i.e. 0.15 mM). Also, 5 mM DHA appeared to only have limited effects. When looking at 1 dpt, we saw that the 20 mM DHA treatment strongly affected the transcriptome. Although there was some overlap between the transcriptional effects evoked by treatment with mCCOPE and 20 mM DHA, the overlap remained very limited compared to their non-overlapping effects at 1 dpt. At 4 dpt, it was clear that mCCOPE alters the transcriptome in a DHA independent way.

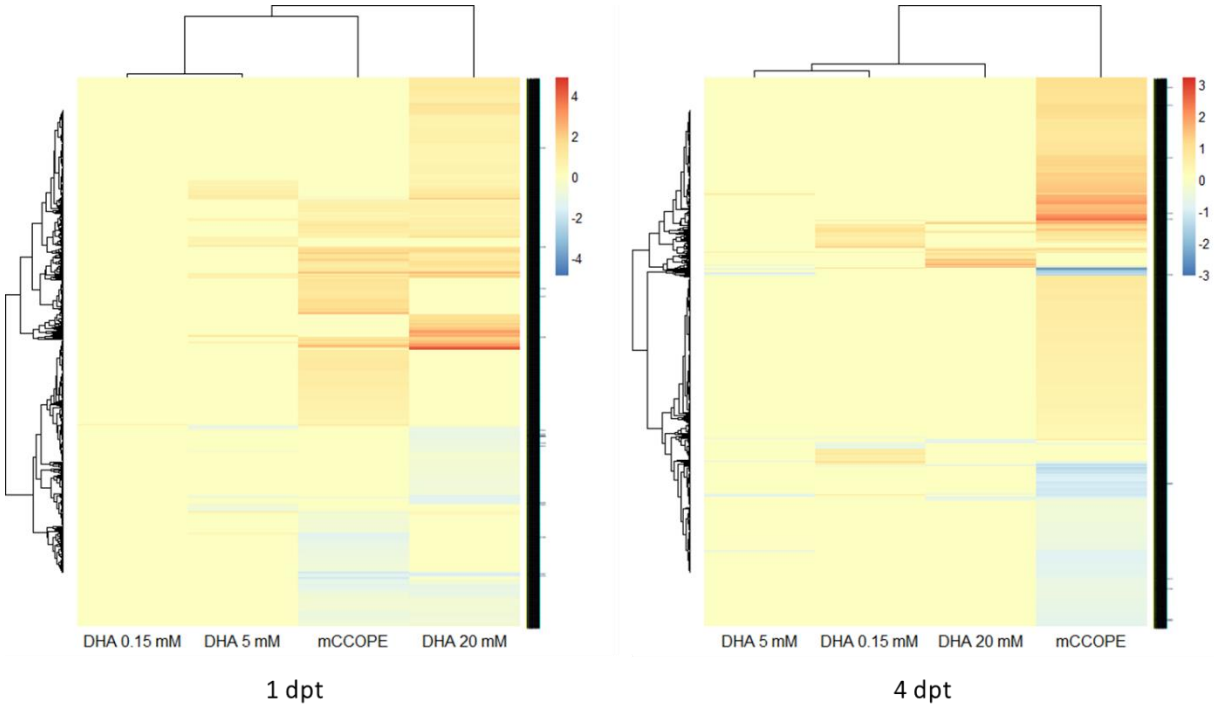

Figure 3.4: mCCOPE treatment shows a largely DHA-independent transcription pattern. The figure shows two heatmaps comparing the differentially expressed genes (relative to the mock treated plants) between mCCOPE, 0.15 mM, 5 mM and 20 mM DHA. Genes that were not differentially expressed in one or more of the treatments, but were in at least one, were assigned the value 0 for the treatments where they were not differentially expressed. At 1 dpt (left image), only mCCOPE and 20 mM DHA show large effects on the transcriptome, although with few overlaps. At 4 dpt (right image), only mCCOPE shows major transcriptional alterations.

3.3. mCCOPE increases the root lignin content at 1 dpt

To validate the increased lignification suggested by mRNA-seq, the root lignin concentration upon mCCOPE treatment was assessed (Figure 3.5). It was found that at 1 dpt, the lignin concentration was significantly increased upon mCCOPE treatment ($p = 0.079$), while the lignin concentration at 4 dpt was the same for both treatments. It should be noted that a p-value of 0.1 was considered significant, as young rice tissues are not strongly lignified, resulting in low effect sizes.

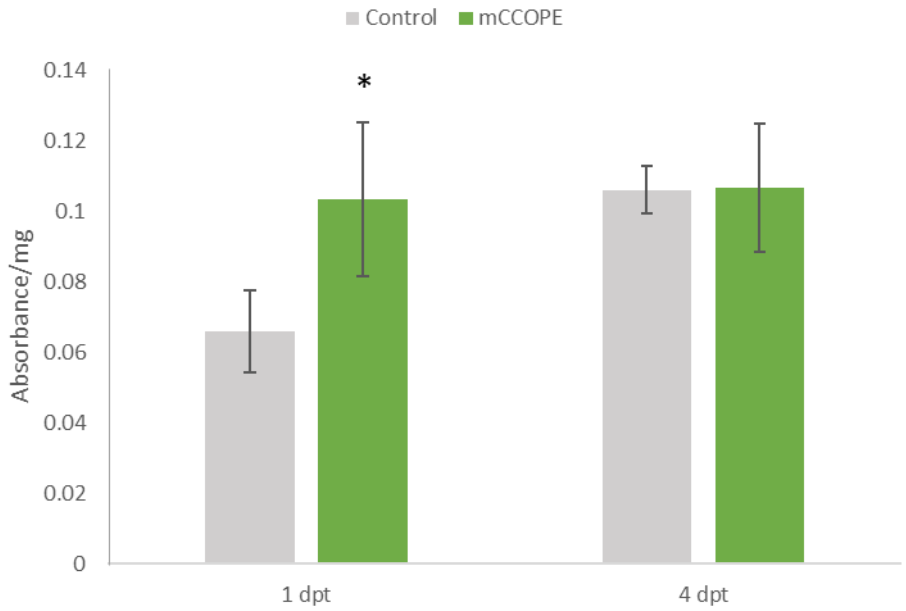

Figure 3.5: mCCOPE treated rice plants show increased root lignification at 1 dpt. Control and mCCOPE treated plant material was analysed using the acetyl bromide assay. Each treatment existed of five biological replicates, each containing four root systems. The lignin levels were compared using the absorbance/mg dried extracted sample. Significant differences, calculated via a heteroscedastic one-sided t-test, are illustrated via one or two asterisks (p = 0.1 and p = 0.05, respectively). The error bars represent the SEM.

3.4. mCCOPE decreases the root H_2O_2 level at 1 dpt

To validate the increased redox signalling suggested by mRNA-seq, the root H_2O_2 levels upon mCCOPE treatment were measured (Figure 3.6). It was observed that at 1 dpt, the root H_2O_2 level was significantly decreased in mCCOPE treated rice roots (p = 0.0001), while no significant difference with the control was observed at 4 dpt.

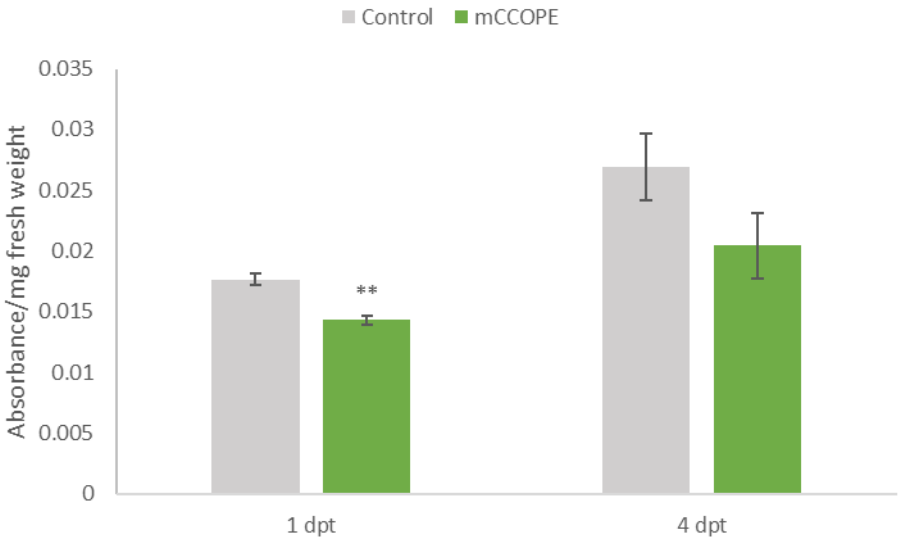

Figure 3.6: mCCOPE treated rice plants show decreased root H_2O_2 concentrations at 1 dpt. Control and mCCOPE treated plant material was analysed using the xylenol orange assay. Each treatment existed of ten biological replicates, each containing four root systems. The H_2O_2 levels were compared using the absorbance/mg fresh weight. Significant differences, calculated via a heteroscedastic two-sided t-test, are illustrated via one or two asterisks (p = 0.1 and p = 0.05, respectively). The error bars represent the SEM.

3.5. mCCOPE increases the IAA concentration at 1 dpt, influences the ET concentration at 1 dpt and decreases the ABA concentration at 4 dpt

To validate the results obtained from mRNA-seq, root hormone concentrations at 1 and 4 dpt were measured in mCCOPE and mock treated rice plants (Figure 3.7 and 3.8).

Figure 3.7 shows the results of two biologically independent repeats of ET measurements. In the first repeat (Figure 3.7A), an increase in ET levels in mCCOPE treated plants was observed at 1 dpt, albeit non-significantly ($p = 0.12$). At 4 dpt, no difference with the control was observed. Contrarily, in the second repeat (Figure 3.7B), ET levels in mCCOPE treated roots were significantly lower than in the control at 1 dpt ($p = 0.020$). Again, no significant differences were observed at 4 dpt. Comparing the peak areas/mg dried root obtained for the controls over the two repeats, no significant differences were observed. This indicates that the results obtained by the gas chromatograph were consistent.

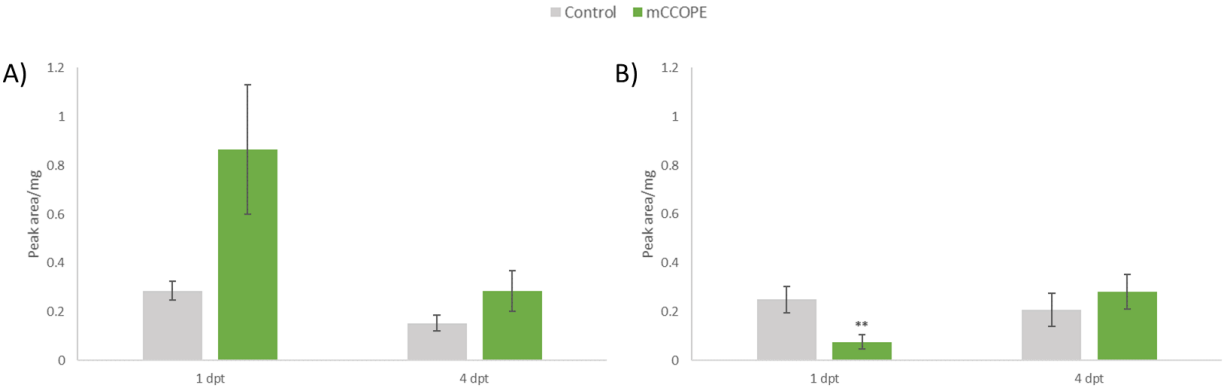

Figure 3.7: mCCOPE treatment influences the rice root ET concentrations at 1 dpt, as assessed in two biologically independent repeats. Control and mCCOPE treated plant material was analysed using gas chromatography. The ET concentration was compared as peak area/mg dried plant material. (A) Each treatment existed of five biological replicates, each containing four root systems. (B) Each treatment existed of eight biological replicates, each containing three to four root systems. Significant differences, calculated via a heteroscedastic two-sided t-test, are illustrated via one or two asterisks ($p = 0.1$ and $p = 0.05$, respectively). The error bars represent the SEM.

In Figure 3.8A and C, it can be seen that the levels of neither JA, nor SA were significantly affected in roots of mCCOPE treated plants at 1 and 4 dpt. Figure 3.8B shows a significant decrease in the ABA levels at 4 dpt ($p = 0.023$), but not at 1 dpt. Figure 3.8D shows a significant increase in IAA concentration at 1 dpt ($p = 0.069$), but not at 4 dpt. It should be noted that a p-value of 0.1 is considered significant for UHPLC-mass spectrometry hormone measurements, due to the inherent high variability of the assay caused by strong stress-responsiveness and variation of the hormones between tissues.

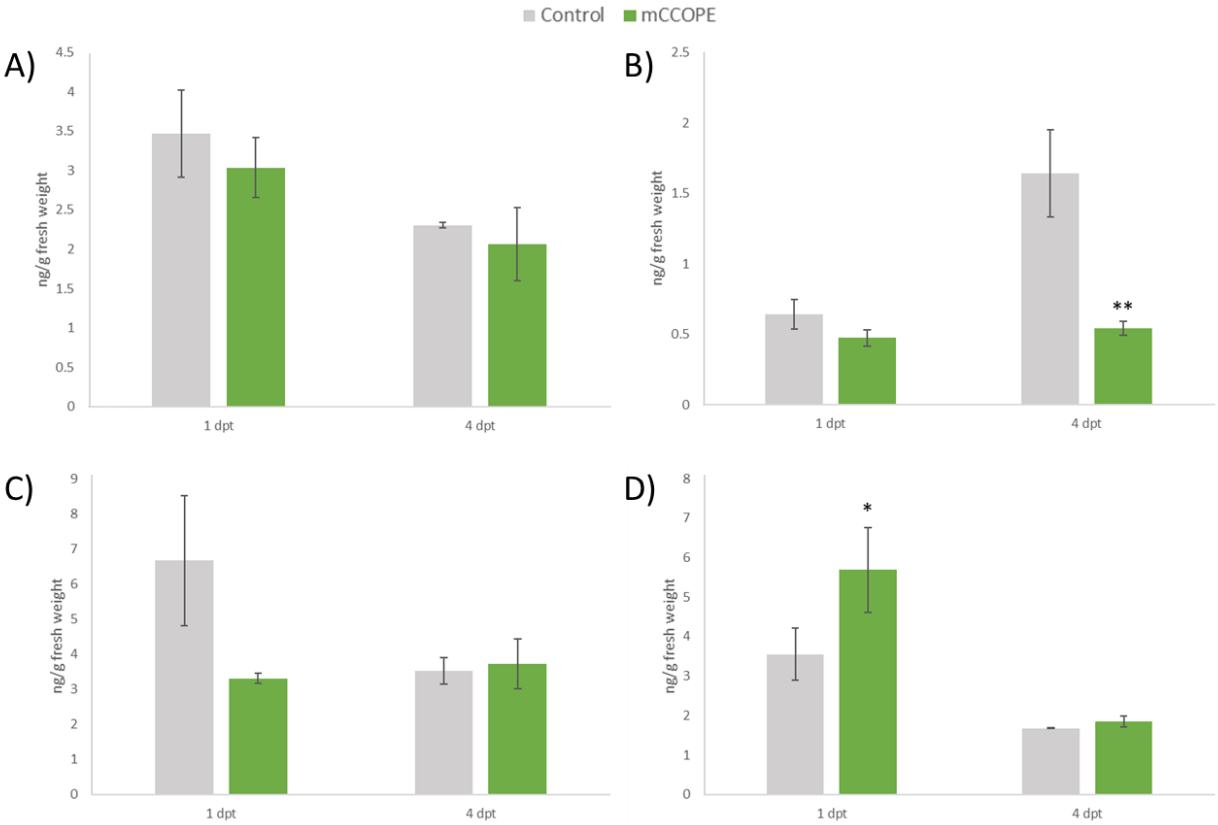

Figure 3.8: mCCOPE treatment does not affect the JA and SA levels in rice roots, but increases the IAA levels at 1 dpt and decreases the ABA levels at 4 dpt. Control and mCCOPE treated plant material was analysed using UHPLC-mass spectrometry. Each treatment existed of five biological replicates, each containing four root systems. (A) SA concentrations in the plant samples, in ng phytohormone/g fresh weight. (B) ABA concentrations in the plant samples, in ng phytohormone/g fresh weight. (C) JA concentrations in the plant samples, in ng phytohormone/g fresh weight. (D) IAA concentrations in the plant samples, in ng phytohormone/g fresh weight. Significant differences, calculated via a heteroscedastic two-sided t-test (for ABA and SA) or a heteroscedastic one-sided t-test (for IAA and JA), are illustrated via one or two asterisks (p = 0.1 and p = 0.05, respectively). The error bars represent the SEM.

3.6. mCCOPE is nematicidal

It is well known that many plant extracts possess a nematicidal activity (Elbadri *et al.*, 2008; Eman and El-Nuby, 2019). To determine if mCCOPE also acts as a nematicide to *Mg*, nematodes were incubated in mCCOPE and their viability was assessed over time. Additionally, 0.15 mM DHA was also assessed for its nematicidal capacity. This was done to determine its importance in the mCCOPE nematicidal activity, rather than contributing to the IR-stimulating capacity. Two treatment durations were analysed, of six and 48 hours. After 48 hours, mCCOPE became too turbid to be analysed under the microscope, which is a recurrent problem for plant extract nematicidal assays. Often this is due to contamination, but in our case a dense layer was formed in the sample, hindering any further counting under a microscope. Therefore, after 48 hours of treatment, the sample was cleared prior to counting (see Section 2.9).

Figure 3.9A shows that mCCOPE decreased the nematode viability already after six hours. The number of living nematodes was significantly lower in the mCCOPE treated sample compared to the control (p = 0.025). This was mainly due to an increased number of dead nematodes, as the number of static nematodes was not affected. After 48 hours, the number of living nematodes upon mCCOPE treatment was still significantly lower than in the control (p = 0.048). However, compared to after six hours the viability did not significantly decrease further after 48 hours of mCCOPE treatment. Figure 3.9B shows

that 0.15 mM DHA did not significantly affect nematode viability after six hours of treatment. After 48 hours, the number of living nematodes was significantly higher in the 0.15 mM DHA treated sample compared to the control ($p = 0.028$), due to a lower number of static nematodes.

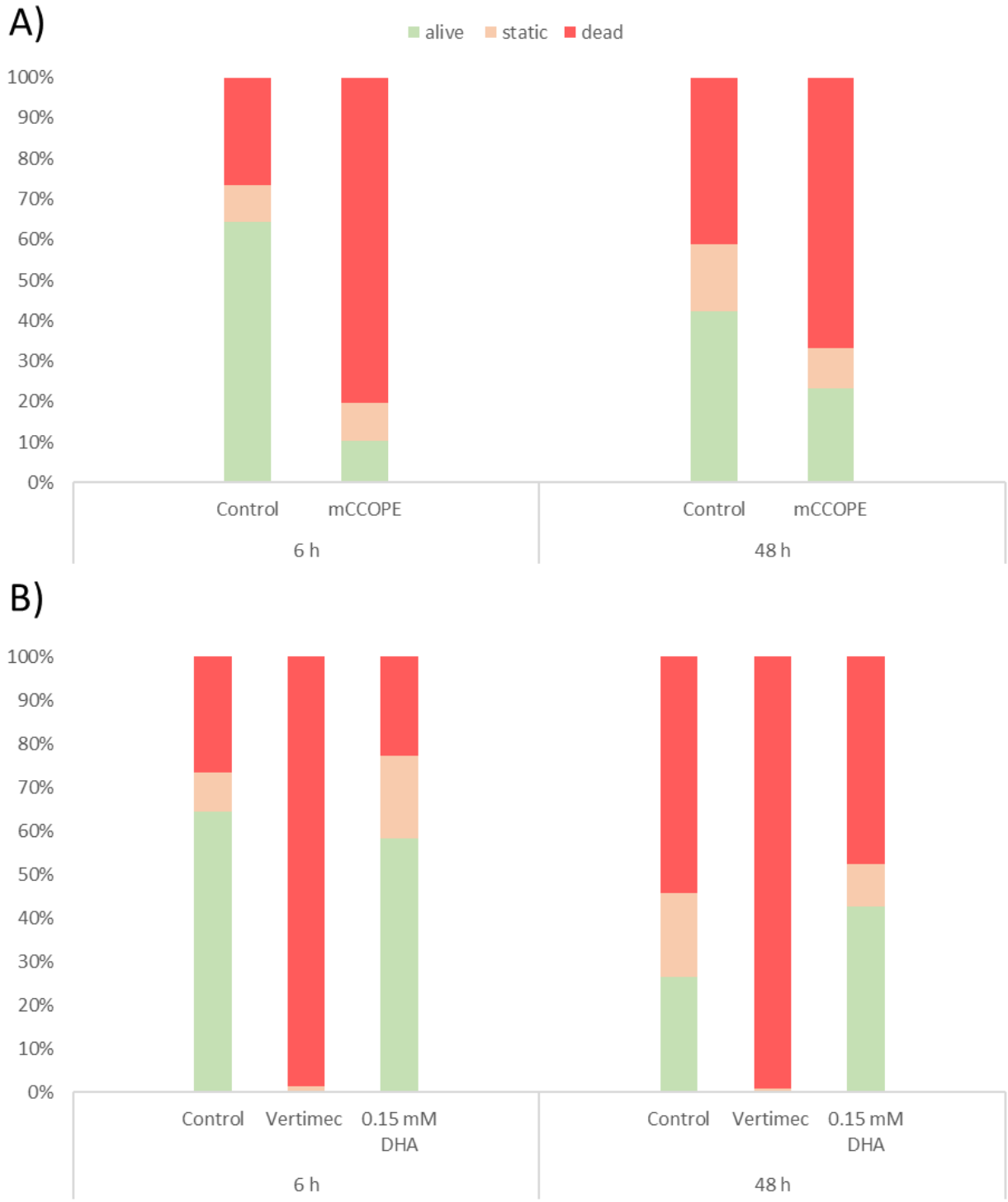

Figure 3.9: mCCOPE, but not 0.15 mM DHA, is nematicidal to *Mg*. 100 *Mg* J2s were incubated in 2 mL treatment solution and nematode viability was assessed after six and 48 hours of treatment. Nematodes were categorized in three groups based on their viability, living nematodes (moving), dead nematodes (stretched and not moving) and static nematodes (curled and not moving). (A) Viability of mock and mCCOPE treated nematodes. After 48 hours of treatment, the nematode solutions were cleared (see Materials and methods). (B) Viability of mock, 0.2% vertimec (nematicide) and 0.15 mM DHA treated nematodes.

3.7. mCCOPE has no negative long-term effect on rice growth and yield

To determine the potential of an IR-stimulus for long-term applicability in agriculture, one needs to look at potential decreases in yield (Martinez-Medina *et al.*, 2016). To investigate this, rice shoots were sprayed every two weeks with mCCOPE, starting from week two. Figure 3.10 demonstrates that neither growth during the whole generation time, nor yield was significantly affected by repeated mCCOPE treatment. Only for ten-weeks-old plants a significant small difference in shoot size was detected ($p = 0.028$), but this trend did not persist. Therefore, the overall trend in growth was not affected by mCCOPE treatment.

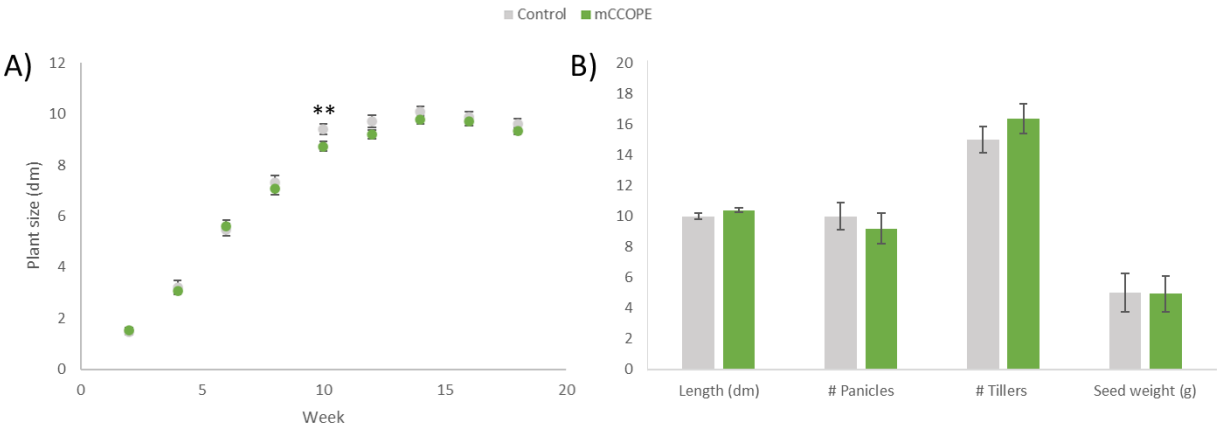

Figure 3.10: mCCOPE does not affect long-term growth and yield. Each treatment involved four pots, each containing three rice plants. The plants were sprayed two-weekly with mock solution or mCCOPE. (A) Plant size throughout the lifecycle, measured by the length of the longest leaf. (B) Measures for size and yield at the end of the growing season, namely length, number of panicles and tillers, and seed weight. Significant differences, calculated via a heteroscedastic two-sided t-test, are illustrated via one or two asterisks ($p = 0.1$ and $p = 0.05$, respectively). The error bars represent the SEM.

4. Discussion

4.1. mCCOPE functions as an IR-stimulus, but AO and DHA are not the active compounds hereto

Due to the high levels of AO found in melon peels (Mosery and Kanellis, 1994; Diallinas *et al.*, 1997; Stevens *et al.*, 2018) and the previously reported function of AO and DHA as IR-stimuli (Singh, Nobleza, *et al.*, 2020; Singh, Verstraeten, *et al.*, 2020), AO and DHA were hypothesised to be the active compound(s) in mCCOPE. The order of magnitude concerning endogenous DHA levels in mCCOPE was determined at 0.15 mM DHA. At room temperature, DHA in mCCOPE quickly degrades (Figure 3.1), which is in conjunction with the instability of DHA reported in literature (Koshiishi and Imanari, 1997). To investigate if mCCOPE is still active without AO and DHA, the extract was boiled, to ensure degradation of DHA and loss of the AO activity by denaturation. Contrarily to our expectations, boiled mCCOPE still caused IR to *Mg* infection (Figure 3.2), indicating that neither AO nor DHA are essential in establishing this phenotype. Accordingly, 0.15 mM DHA treatment of rice plants did not cause IR to *Mg* (Figure 3.2). However, treating plants with 20 mM DHA resulted in IR, supporting the observations by Singh, Nobleza, *et al.* (2020) and Singh, Verstraeten, *et al.* (2020). Together, these data formed a first indication negating our hypothesis that AO and DHA are the active compound(s) in mCCOPE. In Section 4.2.6. a second indication will be provided, together with suggestions for alternative active compounds.

4.2. Foliar mCCOPE treatment leads to systemic induced resistance

Studying the IR mechanism is interesting from a fundamental point of view, to elucidate the common aspects defining IR and to better understand overall plant immunity. Furthermore, as IR establishment appears to be dependent on the genotype or cultivar under study, breeding could be used to obtain cultivars with stronger IR-responsiveness (Walters, Havis, Paterson, *et al.*, 2011; Sandroni *et al.*, 2020). However, this requires a suitable selection strategy (Sandroni *et al.*, 2020). This highlights the importance of studying the IR mechanism, to define elements responsible for the IR response and thus allow breeding (Balmer *et al.*, 2015). Therefore, in this thesis the effects of foliar mCCOPE treatment on rice root immunity were investigated, by performing mRNA-seq. This thesis specifically focussed on the priming phase (Figure 1.7, phase 1), thus mainly elucidating direct defence responses and hypothesising potential direct alterations contributing to the facilitation of a primed defence response. Contrarily to this work, in literature most attention is payed to the primed defence response and the post-challenge primed phase (Fujikawa *et al.*, 2021; Gomes *et al.*, 2021; Zhu *et al.*, 2021).

4.2.1. mCCOPE stimulates lignification in rice roots

mCCOPE treatment activated genes involved in cell wall formation, specifically in lignin biosynthesis at 1 and 4 dpt in rice roots. Moreover, phenylpropanoid pathway genes were upregulated at 1 and 4 dpt. Important to remark is the double function of the phenylpropanoid pathway. This pathway is an important defence related pathway that can contribute to chemical (including phytoalexins) and structural defence (by lignin formation) (Smirnoff and Arnaud, 2019). Thorough analysis of the upregulated genes showed that the vast majority was related to lignin biosynthesis. Therefore, the phenylpropanoid pathway is likely mainly induced to synthesise lignin. Further, validation of the rice root lignin levels was conducted using the acetyl bromide assay, showing increased lignin levels at 1 dpt. For this validation significance was accepted at p-values lower than 0.1, as the results were also supported by the evidence from the mRNA-seq data. This was done since young rice roots only show

limited lignification, thus resulting in low effect sizes and statistical power. Moreover, it was shown that the acetyl bromide assay shows lower analytical sensitivity for younger compared to mature samples (Fukushima and Dehority, 2000). However, this experiment should be repeated (with a higher number of biological replicates) to confirm lignification at 1 dpt.

Synthesising lignin is a valuable strategy against nematode infections, as it hinders pathogen penetration (Zacheo *et al.*, 1995; Portillo *et al.*, 2013; Dhakshinamoorthy *et al.*, 2014), while all plant-parasitic nematodes have to penetrate cells to feed on (Holbein, Grundler and Siddique, 2016). Validation of the lignin levels in rice roots showed an increase in lignification at 1 dpt, but no longer at 4 dpt (Figure 3.5), indicating a transient effect of mCCOPE treatment on root lignin levels. Stimulation of the lignin biosynthesis has for example also been shown for the IR-stimuli BABA, riboflavin and thiamine in rice, BABA in mango (*Mangifera indica*), vanillyl nonanoate in pepper (*Capsicum* sp.), and BTH in tomato (Taheri and Tarighi, 2010; Ji *et al.*, 2015; Huang *et al.*, 2016; García, Veloso and Díaz, 2018; Veronico *et al.*, 2018; T. Li *et al.*, 2019). Specifically, treatment of rice with BABA and of pepper with vanillyl nonanoate, directly resulted in increased lignification at 1 dpt, confirming the possibility of such an early accumulation (Ji *et al.*, 2015; García, Veloso and Díaz, 2018). Moreover, in tomato, defence against *M. incognita* was associated with lignification at 1 dpi (Zacheo *et al.*, 1995).

In most cases, stimulated lignin biosynthesis upon elicitation by an IR-stimulus is observed to be transient. Lignin biosynthesis genes are transiently expressed resulting in a sped-up lignification compared to the control for a couple of days. Typically, this results in a plateau in the lignin level, similarly to what was observed here upon mCCOPE treatment. However, rarely does the control catch up this higher lignin level, a constant higher lignification is sustained in the treated plants (Sasaki, Yamamoto and Matsumoto, 1996; Smit and Dubery, 1997; Deborah *et al.*, 2001; Mandal *et al.*, 2013). Another infrequent pattern is a transient lignification in which the lignin level returns to the basal level. Such pattern was for example observed for dragon fruit plants (*Selenicereus* sp.) treated with chitosan, in which the lignin levels showed a peak that lasted six to seven days (Ali *et al.*, 2014). An explanation for the mCCOPE mediated transient lignin boost could be that lignin biosynthesis is feedback inhibited to avoid overaccumulation of lignin. As we treated young rice roots, they likely are still lignifying at the time of treatment as part of their development (Ma and Peterson, 2003), thus the control plants can catch up with the mCCOPE treated ones that are experiencing feedback inhibition. Transient lignin biosynthesis stimulation and avoiding lignin overaccumulation make sense, as the strength and defence of the lignin polymer comes at a price, namely a reduction in cell wall permeability and extendibility (Fry, 1979, 1986; Fan *et al.*, 2006; Tamaoki *et al.*, 2006; Růžička *et al.*, 2015; Serk *et al.*, 2015). High lignification results in increased hydrophobicity and seals of the cells, a strategy that is of value in for example xylem vessels but results in an unliveable situation (Boyce *et al.*, 2004; Růžička *et al.*, 2015). Moreover, reduced extendibility of the cell wall counters cell elongation and thus growth (Fan *et al.*, 2006; Tamaoki *et al.*, 2006).

Alternatively, as the acetyl bromide assay measures levels of lignin monomers, named monolignols, it could be that the assay does not validate increased lignification but validates monolignol accumulation. Furthermore, this assay could also detect increased levels of lignans, which are dimers of monolignol moieties (Davin *et al.*, 1997). These lignans are believed to function as phytoalexins and could thus contribute to an alternative way for the lignin biosynthesis pathway to mediate the observed IR-phenotype (Pohjamo *et al.*, 2003; Wang *et al.*, 2013). Further, monolignols themselves can be antipathogenic, but in large concentrations they are also phytotoxic (Väisänen *et al.*, 2015). UDP-

glycosyl transferases can glycosylate monolignols to reduce the phytotoxicity and to store or transport them (Liu, 2012; Speeckaert *et al.*, 2020). Thus, the observed upregulation of both the lignin biosynthesis (via mRNA-seq (Table 3.1) and biochemical assays (Figure 3.5)) and UDP-glycosyl transferases (via mRNA-seq (Supplementary table S.4)) could also be part of a priming mechanism, namely the accumulation of inert monolignol glucosides to react more quickly to a subsequent pathogen attack, either by enhanced lignin biosynthesis or by phytoalexin accumulation/activation. Such a storage and inactivation of secondary metabolites, defence-related phytohormones and phytoanticipins has previously been reported to be part of priming mechanisms (Brotman *et al.*, 2012; Vogel-Adghough *et al.*, 2013; Gamir, Sánchez-Bel and Flors, 2014; Pastor *et al.*, 2014; Wilkinson *et al.*, 2019). Either way, our results indicate that activation of the lignin biosynthesis pathway contributes to mCCOPE-IR, either via structural or chemical defence, or both.

4.2.2. mCCOPE induces redox signalling

A second major response triggered by mCCOPE treatment is redox signalling. Namely, the glutathione cycle and ROS metabolism were transcriptionally activated at both 1 and 4 dpt, and RNS metabolism at 4 dpt. Both ROS and RNS function as second messengers in the defence signalling, whereas glutathione acts as a redox buffer and signals changes in the redox state (Noctor, Reichheld and Foyer, 2018; Marcec *et al.*, 2019; Arnaiz *et al.*, 2021). Redox signalling is frequently associated with defence signalling (Suzuki *et al.*, 2012; González-Bosch, 2018; Mata-Pérez and Spoel, 2019). The observed redox signalling could possess a dual role, namely it could be involved in systemic signal transduction and local activation of defences. Further, H_2O_2 accumulation has previously been shown to be part of the mode-of-action of various IR-stimuli, for example for BABA and thiamine in rice, ulvan in *Arabidopsis*, avirulent *Xanthomonas campestris* in pepper and BTH in tomato (Lee and Hwang, 2005; de Freitas and Stadnik, 2015; Ji *et al.*, 2015; Huang *et al.*, 2016; Veronico *et al.*, 2018). Moreover, treatment of apple plants (*Malus domestica*) with common ivy (*Hedera helix*) extract results in the accumulation of peroxidases (Baysal and Zeller, 2004).

Seemingly contrarily to the observed transcriptionally enhanced redox signalling, H_2O_2 levels in mCCOPE treated roots were significantly lower than in control treated ones at 1 dpt (Figure 3.6). However, accumulation of ROS is known to be one of the first biochemical responses of the plant upon pathogen attack (Noctor, Reichheld and Foyer, 2018). Transcriptomic analysis with two-hour intervals showed that in *B. cinerea* infected *Arabidopsis,* redox signalling is activated at fourteen hours post infection (hpi) (Windram *et al.*, 2012). In *Magnaporthe oryzae* infected rice, oxidative signalling was shown to be transcriptionally activated at least as early as 12 hpi (Wang *et al.*, 2014). In pepper infected with *X. campestris*, H_2O_2 accumulation in local and systemic leaves was observed to already produce a first peak at 2 hpi (Lee and Hwang, 2005). In D-allose treated rice, activated ROS synthesis was observed as early as 12 hpt (Kano *et al.*, 2013). Together, this could indicate that in this thesis we might have just missed the expected H_2O_2 peak, as the early accumulation of ROS – the oxidative burst – is known to be transient (Noctor, Reichheld and Foyer, 2018). Therefore, we might have measured the H_2O_2 levels in a subsequent feedback inhibition phase, explaining the contradictory results. A similar observation of potential feedback inhibition upon mCCOPE treatment was made for the ET levels at 1 dpt (see Section 4.2.4.1.). Such an H_2O_2 feedback inhibition mechanism was previously observed in the mitochondria upon abiotic stress, with the aim to keep the ROS levels under control (Petrov *et al.*, 2015). Moreover, H_2O_2 negative feedback and decreases are also mediated by the ROS-scavenging pathways of the cell (Mittler *et al.*, 2004). Feedback inhibiting H_2O_2 accumulation makes sense, as overaccumulation of ROS would result in programmed cell death (Torres, Jones and Dangl, 2006),

rather than stimulating IR-signalling. Alternatively, the glutathione signalling pathway, which was also transcriptionally activated at 1 and 4 dpt, catabolises H_2O_2, resulting in a new redox signal (Noctor, Reichheld and Foyer, 2018). Additionally, H_2O_2 is also used in the lignin crosslinking by type III peroxidases (Smirnoff and Arnaud, 2019), resulting in an additional H_2O_2 sink at 1 dpt, as we validated lignification at this timepoint (see Section 4.2.1.). Together, the results of mRNA-seq and the validation assay seem to suggest a role for H_2O_2 signalling early upon treatment.

As mCCOPE was applied foliarly and IR was studied in the rice root, an early systemic signal to the root is required. Therefore, it could be hypothesised that both ROS and RNS could act as these systemic signals (Orozco-Cardenas and Ryan, 1999; Valderrama *et al.*, 2007; Miller *et al.*, 2009; Suzuki *et al.*, 2013), as they often tightly co-operate in the plant defence (Groß, Durner and Gaupels, 2013; del Río, 2015; Arnaiz *et al.*, 2021). However, as the RNS metabolism genes were only significantly activated at 4 dpt, it is likely that this pathway did not contribute to this early systemic signalling. Alternatively, calcium signalling could be responsible for the systemic signal, as it was activated at both 1 and 4 dpt. Likely, both ROS and calcium co-operate to form the systemic signal, as they have frequently been associated to contribute to shoot-root signalling (Miller *et al.*, 2009; Choi *et al.*, 2014; Wang *et al.*, 2019) and both waves are well known to interact with each other (Ogasawara *et al.*, 2008; Gilroy *et al.*, 2014; Devireddy, Arbogast and Mittler, 2020). Moreover, it is believed that the specificity of systemic ROS signals is mediated by other accompanying signals, including for example calcium, further supporting their potential interaction (Suzuki *et al.*, 2013; Gilroy *et al.*, 2014; Kollist *et al.*, 2019; Fichman and Mittler, 2020).

Upon arrival in the root system, both ROS and calcium can be internalised and trigger various intracellular defence responses, as both molecules are known to be involved in early signalling responses to pathogen infection (Zhang, Du and Poovaiah, 2014; Noctor, Reichheld and Foyer, 2018; Fichman and Mittler, 2020). Interestingly, Marcec *et al.* (2019) suggested the importance of certain aquaporins to allow H_2O_2 to travel from the apoplast to the cytoplasm and transduce apoplastic ROS signals. This could explain the observed upregulation of aquaporins via mRNA-seq at both 1 and 4 dpt, potentially as a sensitisation towards future ROS signalling. Supporting this theory, the upregulated aquaporins belonged to the Major Intrinsic Protein PIP family. Corroborative, in *Arabidopsis*, *AtPIP1-4* was shown to be upregulated upon PTI response and *Atpip1-4* mutants were more susceptible to bacterial infection and no longer transcribed defence genes (Tian *et al.*, 2016). Moreover, AtPIP2-1 was shown to be involved in PAMP-induced stomatal closure by mediating the H_2O_2 internalisation (Rodrigues *et al.*, 2017). Further, ROS accumulation can trigger local RNS production and alter the glutathione redox state, forming new redox signals that can induce downstream defence responses (Rumer, Gupta and Kaiser, 2009; Queval *et al.*, 2011). Moreover, ROS and peroxidases can contribute to lignin cross-linking (Marcec *et al.*, 2019), which is in conjunction with the observed increase in lignification (see Section 4.2.1.). Together, the data seem to indicate an important role for ROS as one of the central signalling molecules early upon mCCOPE treatment.

4.2.3. mCCOPE sensitises the rice root

Next to enhanced redox signalling, increased receptor kinase expression was detected at both 1 and 4 dpt. This enhancement could be part of a primed defence response, sensitising the plant for future attacks. At 1 dpt, LRR receptor kinase expression was enhanced. These LRR receptor kinases are typically associated with PTI and RLKs (Kwang, 2004; Bentham *et al.*, 2020). RLKs are part of the PRRs, that detect PAMPs and DAMPs. Therefore, this enhancement could also contribute to a primed

defence response by sensitising the plant. Such an upregulation of PRRs has previously been reported as a potential mechanism to establish a primed defence response (Tateda *et al.*, 2014; Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2017).

4.2.4. mCCOPE affects rice root hormone signalling

Fourth, mCCOPE treatment affects the rice root hormone signalling. This was shown both transcriptionally and via measurements of the actual phytohormone levels. Root ABA, IAA, JA and SA levels were determined by UHPLC-mass spectrometry. By assessing significance on the 0.1 p-value level, IAA and ABA were found to be up- and downregulated, respectively. The use of this higher than standard significance level is accepted as UHPLC-mass spectrometry faces inherent high variability caused by strong stress-responsiveness and variation of the hormones between tissues. Variability between tissues complicates the analysis, as the root is considered as one 'tissue' during the measurement (Haeck *et al.*, 2018). However, the root contains various tissues, such as the epidermis, cortex, endodermis, pericycle, vascular cylinder, root meristem, *etc.* Furthermore, each of these tissues can contain various cell types, for example the outer epidermis can be split in root hair and non-root hair forming cells, the meristem contains four sets of initials (stem cells) around the non-dividing quiescent centre, … (Benfey and Scheres, 2000). Each of these cell types can vary in their hormone levels, thus the resulting root samples will show variance due to different proportions of each of these cells in the different samples. A typical example is the uneven distribution of IAA in the root. Namely, IAA maxima can be found in the root meristem stem cell niche and at lateral root primordia (Overvoorde, Fukaki and Beeckman, 2010; Olatunji, Geelen and Verstraeten, 2017). Further, the IAA levels are higher in root hair forming cells than in non-root hair forming cells (Overvoorde, Fukaki and Beeckman, 2010).

4.2.4.1. Classical defence hormones: ET, JA and SA

In mCCOPE-IR, ET biosynthesis was transcriptionally activated at both 1 and 4 dpt, and ET signalling was transcriptionally activated at 4 dpt (Table 3.1). To validate the role of ET, two repeated ET measurements with a gas chromatograph were conducted. These two validations resulted in contradictory results, as at 1 dpt, both ET accumulation (although not significant) and decrease upon mCCOPE treatment was observed. At 4 dpt, in both experiments no effect of mCCOPE on the ET levels was observed. At 1 dpt, the peak area/mg for the control samples over the two repeats was similar, which was also the case for the peak areas/mg for the control and mCCOPE treated samples at 4 dpt. Therefore, it can be argued that the results obtained from the gas chromatograph were consistent, and thus this contradiction likely results from biological processes or variation.

A first explanation could be batch-to-batch variation of mCCOPE, potentially leading to different results. As melons were bought in the supermarket over different seasons to make fresh mCCOPE, lack in standardisation of the origin, season, agricultural and storage practices, … could have resulted in variation in the composition of the extract. Alternatively, in conjunction with the H_2O_2 results, it could be that mCCOPE mediates an early ET peak which was just missed in the first measurement (which explains the non-significant increase in ET that was in conjunction with the mRNA-seq data), while the second measurement could have been taken in a following feedback inhibition phase. Namely it was so, that due to practical limitations, the samples for the first experiment (Figure 3.7A) were taken at 23 hours post treatment (hpt), while for the second experiment (Figure 3.7B) they were taken at 24 hpt. Moreover, feedback inhibition could have initiated earlier in the second experiment due to batch-to-batch differences in the extract, for example a quicker stimulation of the observed responses.

Feedback inhibition of ET mediated defence has been previously reported, on both the biosynthesis and signalling level (Vandenbussche *et al.*, 2012). In tobacco, two ET-responsive inhibitors of the ET signalling have been identified (Cao *et al.*, 2015; Tao *et al.*, 2015). Further, ET biosynthesis upon wounding was found to be subjected to negative feedback in avocado (*Persea americana*), citrus (*Citrus sinensis*), etiolated pea (*Pisum sativum*) and persimmon (*Diospyros kaki*) (Riov and Yang, 1982; Peck and Kende, 1998; Owino *et al.*, 2002; Zheng, Nakatsuka and Itamura, 2006). This feedback inhibition already showed its effects at twelve hours post wounding in persimmon (Zheng, Nakatsuka and Itamura, 2006).

Further, ET accumulation in response to stress has been observed earlier than 1 dpt, supporting the potential of such an early peak. Transcriptomic analysis with two-hour intervals showed that in *B. cinerea* infected *Arabidopsis* ET biosynthesis is activated at 14 hpi and ET signalling at 16 hpi (Windram *et al.*, 2012). In rice, ET mediated defence to *M. oryzae* was shown to be transcriptionally activated at least as early as 12 hpi (Wang *et al.*, 2014). ET accumulation in rice infested with the rice striped stem borer was already observed at 2 hpi (Zhou *et al.*, 2011). In AO treated rice, Singh, Verstraeten, *et al.* (2020) observed a transient ET accumulation at 12 hpt, which already had faded by 24 hpt. Similarly, in 20 mM DHA treated rice, ET accumulated already at 6 hpt to fall back to the basal level at 24 hpt (De Kesel, unpublished).

Together, this could indicate that in this thesis we might have just missed the ET peak, which could explain the here-observed contradictory results as a strong feedback inhibition of the early ET accumulation. Hypothesising such an early ET peak is supported by the defence functions reported in literature for ET, that could mediate mCCOPE-IR. Similarly to our hypothesis, Singh, Verstraeten, *et al.* (2020) report the importance of transient and early (12 hpt) ET accumulation in rice root-AO-IR. Nahar *et al.* (2011) showed that systemically applied ethephon (an ET generator) enhanced the resistance in rice roots to *Mg*, thus ET could act as a systemic signal to the root. Similarly, rice root inoculation with the endophyte *Azospirillum* sp. stimulates ET mediated IR in the shoot (Kusajima *et al.*, 2018). Further, in tomato and *Arabidopsis*, root sclareol treatment caused local ET accumulation and ET dependent root lignification (Fujimoto *et al.*, 2015). Together, this could indicate a potential role for early ET accumulation as an important mediator of mCCOPE-IR. Alternatively, root ET accumulation could be a result of the enhanced redox signalling, rather than a systemic signal. This hypothesis also supports early ET accumulation, as such a pattern was also hypothesised for the H_2O_2 accumulation. In *Arabidopsis* it has been shown that glutathione can induce ET biosynthesis via transcriptional and posttranscriptional regulation of its key enzymes. Further, exogenous glutathione application on an ET signalling mutant no longer enhanced stress tolerance, indicating a form of glutathione ET-dependent signalling in *Arabidopsis* shoots (Datta *et al.*, 2015). Additionally, in *Arabidopsis*, ET biosynthesis could be stimulated by ROS accumulation (Liu and Zhang, 2004). Thus, ET could still be important in the early mCCOPE mediated IR, but as a local root signal transduction pathway.

Even though JA biosynthesis, an important mediator of the rice root defence to nematodes (Nahar *et al.*, 2011), was transcriptionally upregulated at 1 and 4 dpt, JA levels in rice roots were not found to be affected by mCCOPE treatment. However, as root ET signalling was activated as well and ET and JA signalling are well known to cooperate in rice root-nematode defence (Nahar *et al.*, 2011), it is likely that JA is involved in mCCOPE-IR. However, again it could be that JA signalling plays an early signalling role, explaining the unaffected JA levels at 1 and 4 dpt. Further, SA levels were not affected either, indicating that mCCOPE-IR might act independent of this classical defence hormone. Without further

experiments, it cannot be excluded that mCCOPE-IR might also act independent from JA. Similarly, in rice root-BABA-IR, neither ET, JA nor SA, the classical defence hormones, were involved in establishing IR (Ji *et al.*, 2015).

4.2.4.2. IAA

Remarkably, IAA signalling was activated at 1 and 4 dpt (see Section 3.2.) and increased IAA levels were validated at 1 dpt (Figure 3.8). IAA is a typical growth and development hormone, and generally associated with negative regulation of the plant defence (Naseem, Kaltdorf and Dandekar, 2015). However, some reports implicate IAA being involved in defence responses (Sun *et al.*, 2009; Qi *et al.*, 2012; Hentrich *et al.*, 2013; B. L. Huang *et al.*, 2019). The exact mechanism of IAA in defence signalling currently remains unclear, but increasing evidence associates IAA with redox signalling.

In cucumber and maize (*Zea mays*) roots, it has been proposed that excess IAA can result in a redox imbalance, shifting the redox state towards oxidation (Key, 1962; Jiang, Meng and Feldman, 2003; Koprivova, Mugford and Kopriva, 2010). Such a shift could influence the glutathione redox state and activate defence responses. Further, it was shown that mutating the thioredoxin and glutathione redox systems lowers the IAA concentrations in *Arabidopsis* shoots (Bashandy *et al.*, 2010). Moreover, the glutathione redox status appears to affect the auxin signalling in *Arabidopsis* root development (X. Yu *et al.*, 2013). Namely, glutathione decreases the stability of the Aux/IAA repressors of the IAA signalling (Pasternak, Palme and Paponov, 2020). The other way around, IAA was shown to positively affect the glutathione concentration (Marrè and Arrigoni, 1957; Pasternak, Palme and Paponov, 2020). IAA signalling is also associated with ROS signalling, as RBOHs and type III peroxidases were shown to be induced by IAA (Brightman *et al.*, 1988; Mangano *et al.*, 2017; Mhamdi and Van Breusegem, 2018). IAA responsive Rac-GTPases can interact with RBOHD, which is involved in the ROS wave transduction, in a calcium-dependent manner (Wong *et al.*, 2007; Peer, Cheng and Murphy, 2013; Zandalinas and Mittler, 2018). In lateral root formation, this IAA mediated ROS stimulation resulted in subsequent enhancement of the IAA signalling (Biswas *et al.*, 2019). Similarly, IAA stimulates NO accumulation in *Arabidopsis* roots, while NO enhances the degradation of IAA signalling repressors upon IAA accumulation (Hu *et al.*, 2005; Terrile *et al.*, 2012). Additionally, ROS accumulation can trigger the oxidative attenuation of the IAA signalling, mediating its transient nature (Peer, Cheng and Murphy, 2013). Thus, the early IAA accumulation observed upon mCCOPE treatment could be an indication of activated redox signalling and could contribute to mCCOPE-IR as a downstream signal of the redox signalling.

Similar to our results, De Medeiros *et al.* (2017) observed concerted upregulation of IAA response and ROS accumulation in offspring of *T. atroviride* IR-stimulated tomato plants, which resulted in TIR to *M. javanica* infection. De Medeiros *et al.* (2017) propose an alternative model in which IAA triggers the subsequent redox mediated defence. In our case the opposite appears to make more sense, as ROS is more likely to act as the fast, systemic signal coming from the shoot. This is supported by the proposed feedback inhibition of H_2O_2 at 1 dpt, while IAA is still activated. In wheat, the synergistic effect of the combined treatment with the two IR-stimuli MeJA (foliarly applied) and *T. harzianum* was associated with IAA accumulation in the root and activated redox signalling in the shoot (Singh *et al.*, 2019). Alternatively, as IAA is important in root growth and development (Meng *et al.*, 2019), its upregulation could help obtain a balance between defence stimulation and growth, avoiding negative effects on the plant fitness by the induced defence stimulation.

4.2.4.3. ABA

At 4 dpt, the root ABA levels were decreased upon foliar mCCOPE treatment (Figure 3.8). ABA typically mediates the abiotic stress response and is predominantly described as a negative regulator of the rice immunity (Bailey *et al.*, 2009; Nahar *et al.*, 2012; De Vleesschauwer, Gheysen and Höfte, 2013; Yang, Yang and He, 2013). The decrease in ABA concentration might be a result from antagonistic crosstalk with the early IAA or ET accumulations. IAA can interact both synergistically and antagonistically with ABA, depending on the tissue, the conditions, the age of the tissue, … (Tanaka, 2006; Popko *et al.*, 2010; Asghar *et al.*, 2019). Thus, IAA might inhibit the ABA signalling to lift a potential negative regulation of the rice immunity. With a similar goal, ET might antagonise ABA, a response that has been previously observed in rice (De Vleesschauwer, Gheysen and Höfte, 2013; Sharma *et al.*, 2013; Yang, Yang and He, 2013; Yu *et al.*, 2019).

Alternatively, the lower ABA concentration in mCCOPE compared to mock treated plants at 4 dpt could be attributed to an increase in ABA concentration in the control from 1 to 4 dpt, rather than a decrease in concentration in mCCOPE treated plants. Namely, compared to 1 dpt, the ABA levels in the control increased at 4 dpt, while they remained the same for mCCOPE treated plants. As ABA can also influence development, for example lateral root initiation, this increase could be related to the age of the tissue (Chen *et al.*, 2006; De Smet *et al.*, 2006). In this case the decrease in ABA concentration observed in mCCOPE treated plants relative to the control is likely to be predominantly related to antagonistic crosstalk. However, this difference could also be attributed to salt stress induced in the mock treated plants, as they were sprayed with a 0.1 M sodium phosphate buffer, the buffer in which mCCOPE was prepared. Namely, the mock spray consists of only this buffer, while mCCOPE contains a part buffer and a part “peel juice”, potentially resulting in a difference of osmotic stress induced by the treatments. In this case ABA could be part of a systemic signalling mechanism to induce resistance to salt in the mock treated rice root (Sripinyowanich *et al.*, 2013; Suzuki *et al.*, 2013). This could explain the increase in ABA concentration in control treated plants compared to mCCOPE treated ones. Thus, the lower ABA concentration in mCCOPE treated plants could be attributed to antagonistic crosstalk with IAA and/or ET, to salt stress in mock treated plants, or to both, as they do not necessarily exclude each other. Further, it is likely that buffer-induced salt stress cannot fully explain the observation, as no upregulation of osmotic responses in the control compared to mCCOPE treated plants was observed at 4 dpt.

4.2.5. mCCOPE induces the accumulation of stress metabolites and proteins

Next to the signalling pathways described before, mCCOPE treatment also directly enhances the expression of genes encoding for PR-proteins, WRKY-transcription factors, germin-like proteins, heat shock proteins, *etc*., which are common proteins involved in the plant defence response (Dunwell *et al.*, 2008; Jiang *et al.*, 2017; Zhang *et al.*, 2021). Further, genes involved in plant secondary metabolism were activated, probably inducing the synthesis of antipathogenic compounds to limit a subsequent infection. Moreover, the secondary metabolism gene *Os4CL5* was one of the most strongly affected genes at 1 dpt (Os08g0448000, Supplementary table S.2). This gene was previously identified by De Kesel *et al.* (2020) as a PTI-marker gene and can be used to screen for IR-stimulating compounds. Similar to mCCOPE, treatments with other IR-stimulating plant extracts have also shown to induce direct activation of defence responses. Milsana® (Compo Gmgh Company), a commercial IR-stimulus based on giant knotweed (*Reynoutria sachalinensis*) extract, was shown to cause secondary metabolite accumulation in cucumber (Daayf, Schmitt and Bélanger, 1995, 1997; Fofana *et al.*, 2002). In barley

secondary metabolites were shown to accumulate upon treatment with neem (*Azadirachta indica*) extract (Paul and Sharma, 2002). Treatment with sugar beet extract of potato resulted in both secondary metabolite and PR-protein accumulation (Moushib *et al.*, 2013). Treatment of apple plants with common ivy extract also resulted in accumulation of PR-proteins (Baysal and Zeller, 2004).

4.2.6. mCCOPE mediated changes of the rice root transcriptome cannot be attributed to DHA

To assess the role of DHA as a mediator of these above-described defence responses, mRNA-seq was also conducted for plants treated with 0.15 mM, 5 mM and 20 mM DHA. The results showed that foliar application of the endogenous DHA concentration present in mCCOPE (ca. 0.15 mM) only had very minor effects on the rice root immunity (Figure 3.4). It was illustrated that 0.15 mM DHA, to some extent, stimulates IR, as some redox signalling genes and general defence proteins were transcriptionally upregulated. However, a large discrepancy in activity could clearly be observed when comparing to the large mCCOPE effects (Figure 3.4). Further, the only functional overlap between genes induced by 0.15 mM DHA and mCCOPE treatment was the GO category 'H_2O_2 catabolism', which is typical for many IR-stimuli and even defence responses in general (Ji *et al.*, 2015; Huang *et al.*, 2016; Zhou *et al.*, 2018; Bentham *et al.*, 2020; Gao *et al.*, 2020). To ascertain that this discrepancy in activity was not related to a concentration effect, by mCCOPE acting for example as a superior formulation enhancing DHA activity, 5 mM and 20 mM DHA treatments were also analysed. This is supported by previous findings that report enhanced stability or activity of certain pesticides when introduced in a plant extract formulation (Eyheraguibel *et al.*, 2010; Roy *et al.*, 2016; Puthur *et al.*, 2019). Moreover, 20 mM DHA treatment was previously shown to act as an effective IR-stimulus by Singh, Verstraeten, *et al.* (2020). The results showed that 5 mM DHA treatment only had limited effects on the rice root transcriptome, similar to the 0.15 mM DHA treatment (Figure 3.4). The 20 mM DHA treatment strongly affected the rice root immunity at 1 dpt, but not at 4 dpt (Figure 3.4), which is in strong contrast with the mCCOPE mediated effect. Moreover, the overlap between the differentially expressed genes at 1 dpt for the 20 mM DHA and mCCOPE treated plants was only minor, to an extent that indicates that the overlap is related to overlap in typical defence responses, rather than overlap in the specific mCCOPE-IR-stimulating mechanism. Therefore, contrarily to our hypothesis, DHA might contribute to some extent to establishing mCCOPE-IR, however it appears not to be the central compound in mCCOPE. Further, it was confirmed that DHA acts as an IR-stimulus, by both the infection and mRNA-seq experiment, but at higher concentrations than present in mCCOPE.

Thus, this immediately probes the question of which molecule(s) could be responsible for the observed mCCOPE-IR. It should be obvious that this is not a simple question, as plant extracts contain a plethora of components, making it hard to identify single potential active compounds. Further, it could be a question without a simple answer, as each of those compounds could contribute to the total observed phenotype. Supporting this view, Burketova *et al.* (2015) stated:

> IT IS IMPOSSIBLE TO DETERMINE UNAMBIGUOUSLY THE MECHANISM INVOLVED IN TRIGGERING PLANT DEFENSE BY HERBAL EXTRACTS SINCE THESE EXTRACTS REPRESENT A MIXTURE OF DIVERSE COMPOUNDS, BEGINNING WITH DAMPS, THAT ARISE FROM THE HOMOGENIZATION OF PLANT TISSUES, ACROSS INORGANIC COMPOUNDS, SECONDARY METABOLITES AND SIGNALING MOLECULES, TO ESSENTIAL OILS. THESE CONSTITUENTS LIKELY INFLUENCE PLANT METABOLISM SYNERGISTICALLY AND EITHER DIRECTLY ACTIVATE OR PRIME THE TREATED PLANT TO PROMPT A RESPONSE TO PATHOGEN. THIS IS WHY ATTEMPTS TO ISOLATE INDIVIDUAL EFFICIENT COMPOUNDS DO NOT OFTEN BRING THE ANTICIPATED RESULT. (BURKETOVA *ET AL.*, 2015)

To hypothesise potential active compounds in mCCOPE-IR, two major characteristics have to be accounted for. First, the compound should be quite specific to the *Cucurbitaceae* family, as previous screens in the lab and research in the IR-field have shown that not all plant extracts stimulate IR, but multiple CCOPE-types do, for example butternut pumpkin, cucumber, melon, muscat pumpkin and zucchini CCOPE (Kyndt *et al.*, 2020). Second, the compound should be quite heat stable, as mCCOPE still worked upon fifteen minutes of boiling.

Therefore, a first potential direction to investigate could be the IR-stimulating capacity of phytoanticipins or -alexins. Secondary metabolites possess the potential to be thermostable in structure. Moreover, phytoanticipins and -alexins are often quite specific to a certain plant family or a small group of plants, and they are often present in high concentrations in unripe fruits and peels to protect fruits from spoilage (Ribera and Zuñiga, 2012; Slusarenko, Fraser and van Loon, 2012; Tupe *et al.*, 2013; Anupama, 2020). Examples of such phytoanticipins are avenacins in *Avena sativa*, sakuranetin in *Ribes nigrum*, glucosinolates in *Brassicaceae*, …, and examples of such phytoalexins are viniferins in grapevine, tsibulins in *Allium cepa*, lettucenin A in *Lactuca sativa*, … (Slusarenko, Fraser and van Loon, 2012). Some plant antipathogenic compounds have previously been shown to act as IR-stimuli, such as saponins (for example aescin) and 1-isothiocyanato-4-methylsulfinylbutane (Schillheim *et al.*, 2018; Trdá *et al.*, 2019). A typical secondary metabolite class in *Cucurbitaceae* fruits are cucurbitacins. These compounds can be found in the peels and fruits of the various genera of the *Cucurbitaceae* (Jian *et al.*, 2005; Rajasree, Francis and William, 2016). Specifically cucurbitacin A, B, C and D can be found in melon, with cucurbitacin B the most abundant cucurbitacin in melon and presumably unique to the *Cucurbitaceae* family (Jian *et al.*, 2005; Yuan *et al.*, 2019). Cucurbitacin can be extracted from plant material at high temperatures, indicating thermostability (Liu, Ou and Gregersen, 2020). Cucurbitacin has also been extracted at room temperature (Tannin-Spitz *et al.*, 2007; Momma *et al.*, 2008), potentially indicating that this compound can be coldly extracted, however, to the best of our knowledge, no reports have specifically been made on a cold extraction method. Cucurbitacins are highly oxidised tetracyclic triterpenoids that possess antioxidant activity – and thus could alter the redox state and induce signalling - and have been frequently described to affect mammalian health (anticancer, cytotoxic, anti-inflammatory, anti-diabetic, …) (Tannin-Spitz, Bergman and Grossman, 2007; Rajasree, Francis and William, 2016; Yuan *et al.*, 2019). Further, cucurbitacin A and B have been described to be nematicidal (Mashela and Shokoohi, 2021). Together, this makes cucurbitacin B a suitable candidate, as it is a typical cucurbit, stable and biologically active stress metabolite. However, to the best of our knowledge, no previous implications as an IR-stimulus have been reported.

Secondly, DHA degradation products can be hypothesised as active compounds present in mCCOPE. As AO, DHA and DHA degradation products are not specific to cucurbit peels, the first requisite of our potential IR-stimulus is technically not met. However, high levels of AO and DHA are typical for cucurbit peels (Mosery and Kanellis, 1994; Diallinas *et al.*, 1997; Stevens *et al.*, 2018), thus satisfying this requisite under the form of specifically high levels of these compounds. In our results we showed that DHA in the extract degrades quite quickly at room temperature and that it was completely lost upon boiling, while the extract retained its IR-stimulating capacity. Therefore, one may investigate the IR-stimulating potential of DHA degradation products, as those would be present in high levels in the extract as well. Many DHA degradation products have been described, namely 2,3-diketo-L-gulonic acid (DKG) (Slight, Feather and Ortwerth, 1990; Fayle *et al.*, 2000), threose (Lopez and Feather, 1992; Fayle *et al.*, 2000), oxalic acid (Ortwerth *et al.*, 1994; Fayle *et al.*, 2000; Simpson and Ortwerth, 2000),

oxalyl threonate (Simpson and Ortwerth, 2000), L-threonate (Simpson and Ortwerth, 2000), L-xylosone (Sawamura *et al.*, 1994), 3,4-dihydroxy-5-methyl-2(5H)-furanone (Sawamura *et al.*, 1994) and 2-furoic acid (2-furancarboxylic acid) (Sawamura *et al.*, 1994). Still, some new degradation products remain uncharacterised (Dewhirst and Fry, 2018). As a note of criticism, the presence of DHA degradation products in cold, fresh mCCOPE (stored at 4°C) has not been described before. However, based on our results, showing DHA instability at room temperature, it can be argued that upon spraying mCCOPE on rice plants (grown at 28°C), DHA in mCCOPE will start to degrade *in situ* and form these degradation products. Here we will discuss which of these degradation products could be associated with the plant defence based on previous observations in the literature.

First, 2-furoic acid treatment of tomato roots was shown to induce resistance in the shoot to *Fusarium oxysporum* f. sp. *lycopersici* (Miyazawa, Kawabata and Ogasawara, 1998). 3,4-Dihydroxy-5-methyl-2(5H)-furanone and 2-furoic acid are degradation products originating from DKG and are the result of a multiple step degradation starting from DHA (Sawamura *et al.*, 1994). These products are believed to only be formed after a long period of time or under high temperatures (Kärkönen *et al.*, 2017; Dewhirst and Fry, 2018), limiting their significance in the IR-stimulating capacity of mCCOPE, as unboiled mCCOPE serves as an IR-stimulus as well.

Secondly, DKG itself is of interest, as it shows potential to affect the plant redox homeostasis, and thus could initiate redox signalling (Dewhirst and Fry, 2018). In aqueous solution and under low levels of ROS, DHA will predominantly hydrolyse to form DKG (Deutsch, 2000; Dewhirst and Fry, 2018). DKG in turn can react with ROS to form 2-oxo-L-*threo*-pentonate, and thus shows an antioxidant activity (Dewhirst and Fry, 2018). Similar to ascorbic acid, this can contribute to ROS detoxification in the plant. Further, this can disturb the ROS homeostasis, potentially triggering redox signalling. Dewhirst and Fry (2018) hypothesise that DKG could provide specific information on the ROS status, as, depending on the type of ROS that reacts with DKG, specific compounds are formed. Further, they state that high DKG levels in the plant can only occur when the ROS level is low, indicating a further potential signalling role for the molecule. Contrarily, an unidentified degradation product of DKG (presumably 2-oxo-L-*threo*-pentonate), generates apoplastic H_2O_2 (in the presence of trace Cu^{2+}) and might delay/inhibit peroxidase activity (Kärkönen *et al.*, 2017; Hasanuzzaman *et al.*, 2019). Kärkönen *et al.* (2017) propose that this molecule might loosen the cell wall via inhibition of cell wall crosslinking by peroxidases and H_2O_2 mediated loosening of crosslinked molecules. Alternatively, this could result in an oxidative burst, inducing defence responses and potentially stimulating IR. In short, the function of DKG in the plant remains unclear, but it is likely that the molecule could disturb the redox status in the apoplast upon exogenous application, and thus could stimulate IR.

Finally, the degradation product oxalate has previously been associated to plant defence. First of all, oxalate, and more importantly calcium oxalate crystals, were shown to accumulate in some plants as a defence response to herbivores, due to their toxic nature (Khan, 1995). However, more importantly, oxalate in the plant can be converted with O_2 to CO_2 and H_2O_2 by oxalate oxidase. This is an important process in the defence response of plants to oxalate producing pathogens (Zhou *et al.*, 1998; Hu *et al.*, 2003) and it was shown to induce defence responses in sunflower (*Helianthus* sp.) (Hu *et al.*, 2003). In wheat, barley, maize, oat (*A. sativa*), rice, rye (*Secale cereale*) and pine (*Pinus sylvestris*), some germin-like proteins were identified to possess oxalate oxidase activity (Bernier and Berna, 2001; Hu *et al.*, 2003). In our results, expression of genes encoding germin-like proteins was increased, potentially supporting the activity of oxalate on the rice plants. Moreover, in cucumber it was shown that spraying

oxalate on one of the leaves induced resistance to eight unrelated diseases (fungi, bacteria and viruses) on a systemic leaf (Mucharromah and Kuc, 1991). Together, this illustrates the potential effect of oxalate, a DHA degradation product, as an IR-stimulus.

In conclusion, of the above-described molecules, DKG and oxalate appear the most promising as IR-stimuli in mCCOPE, based on the current literature. Both arise from degradation of DHA, DKG from hydrolysis and oxalate from oxidation of DHA. Therefore, in most cases both will be present in a DHA extract, as their formation is a balance between hydrolysis and oxidation based on the extract conditions (Dewhirst and Fry, 2018). Thus, it is likely that both will synergistically contribute to the IR-stimulating capacity of mCCOPE. Synergism between IR-stimuli has been previously reported in literature, namely for MeJA and *T. harzianum* in wheat (Singh *et al.*, 2019), MeJA, SA and *T. harzianum* in tomato (Zehra *et al.*, 2017), acibenzolar-S-methyl and *T. harzianum* in faba bean (Abd El-Rahman and Mohamed, 2014), acibenzolar-S-methyl, BABA and JA in barley (Walters, Havis, Sablou, *et al.*, 2011), and acibenzolar-S-methyl and BABA in grapevine (Reuveni, Zahavi and Cohen, 2001).

Another option is IR-stimulation by a cucurbit specific DAMP. To date, only two types of DAMPs have been identified to be specific to a certain group of plants, namely systemin and Plant elicitor peptides (Peps) (Tanaka and Heil, 2021). Systemin is a peptide specific to the *Solanaceae* (Tanaka and Heil, 2021). Peps are widespread in angiosperms, but each species has its own specific Peps (Bartels and Boller, 2015). Moreover, Peps are not cross recognised between species or genera, eliminating a potential role of Peps in mCCOPE (Lori *et al.*, 2015). Furthermore, as these specific DAMPs are peptides, their role as active compound in mCCOPE can be excluded due to their known lack of stability upon boiling (D'Hondt *et al.*, 2011). Although DAMPs likely, to some extent, do contribute to mCCOPE-IR, they are probably not the cucurbit specific central compound.

Another interesting molecule class are the carbohydrates and sugars. Sugars have been previously described to be involved in plant defence signalling, the so-called high-sugar resistance (Morkunas and Ratajczak, 2014). Sugars can enhance the oxidative burst, induce lignification and secondary metabolite formation, crosstalk with phytohormones, *etc*. (Moghaddam and Van Den Ende, 2012; Morkunas and Ratajczak, 2014). Moreover, some sugars and carbohydrates have been shown to act as IR-stimuli, including D-tagatose, galactinol, palatinose, raffinose, sucrose, trehalose, … (Moghaddam and Van Den Ende, 2012; Morkunas and Ratajczak, 2014; Tayeh *et al.*, 2014; Mochizuki *et al.*, 2020). Furthermore, some IR-stimulating carbohydrates are specific to certain plants or plant groups. For example, the rare sugar (a sugar occurring in limited quantities in nature) D-psicose, found in wheat, *Itea* spp., sugar cane (*Saccharum officinarum*) and sugar beet, stimulates IR in rice (Moghaddam and Van Den Ende, 2012; Patra, Patel and Shah, 2017). The rare sugar D-allose, found in leaves of African shrub (*Protea rubropilosa*), stimulates IR in *Arabidopsis* (Perold, Beylis and Howard, 1973; Moghaddam and Van Den Ende, 2012). Also, the rare sugar turanose induces IR in tomato (Moghaddam and Van Den Ende, 2012; Ratiu *et al.*, 2019). Further, rare sugar-like compounds, such as 2,5-dideoxy-2,5-imino-D-mannitol and arbutin, respectively present in tropical legumes (genera *Lonchocarpus* and *Derri*) and in *Myrothamnus flabellifolia*, can also act as IR-stimuli (Bianchi *et al.*, 1993; Birch *et al.*, 1993; Kuźniak *et al.*, 2015). 2,5-dideoxy-2,5-imino-D-mannitol stimulates systemic IR in tomato against *Meloidogyne* spp., in potato against *Globodera* spp. and in *Petunia* sp. against *Xiphinema diversicaudatum* (Birch *et al.*, 1993). Thus, sugars, carbohydrates or sugar-like compounds (specifically) present in melon peels could contribute to the here-observed IR-phenotype. However, to the best of our knowledge, studies

on the exact composition of trace or rare sugars and carbohydrates present in melon, and cucurbits overall, are still lacking.

Gallic acid is a phenolic acid found in fruits of the members of the *Cucurbitaceae* (Ibrahim, El-Hefnawy and El-Hela, 2010; Kalaskar and Surana, 2014; Busuioc *et al.*, 2020; Omokhua-Uyi and Van Staden, 2020), and in some other plants, including for example tea (*Camellia sinensis*), various berries and grape vine (Tomás-Barberán and Clifford, 2000; Cabrera, Giménez and López, 2003; Pastrana-Bonilla *et al.*, 2003). Gallic acid is present in very high levels in Cantaloupe melon peels (245 mg/100 g dry matter compared to 672.5 and 0.8-6.7 mg/100 g dry matter in tea and black berry (*Rubus fruticosus*), respectively, with tea containing some of the highest levels of gallic acid) (Tomás-Barberán and Clifford, 2000; Gómez-García *et al.*, 2020) and is stable at high temperatures (160°C) (Rawson *et al.*, 2013). Moreover, gallic acid can act as both an antioxidant, in the presence of H_2O_2, and a pro-oxidant, in the presence of iron, when in low concentrations, or in the absence of H_2O_2 (Yen, Duh and Tsai, 2002; Badhani, Sharma and Kakkar, 2015). Further, treatment of rice seeds with gallic acid resulted in higher levels of callose and enhanced defence gene expression in the rice seedlings (Singh, Gupta and Pandey, 2017). Therefore, gallic acid shows potential as an IR-stimulus in mCCOPE. Ellagic acid is another phenolic antioxidant present in the *Cucurbitaceae* (Priyadarsini *et al.*, 2002; Baljeet, Roshanlal and Ritika, 2016; Shodehinde *et al.*, 2016; Sultana, Nahar and Bachar, 2018; Ezzat *et al.*, 2019) and some other plants, such as many berries (of the *Rosaceae*), pomegranate (*Punica granatum*), *etc*. (Gil *et al.*, 2000; Häkkinen *et al.*, 2000). It has also been detected to be present in melon peels (57 mg/100 g dry matter) (Rolim *et al.*, 2018; Ezzat *et al.*, 2019; Gómez-García *et al.*, 2020) and is quite stable upon boiling (Häkkinen *et al.*, 2000). Ellagic acid treatment of chickpea (*Cicer arietinum*) seeds results in enhanced biotic defence gene expression in seedlings grown under osmotic stress (El-Soud *et al.*, 2013), indicating a potential broad spectrum activity for inducing biotic IR as well in the absence of abiotic stress.

Together, cucurbitacin, DKG, ellagic acid, gallic acid and oxalic acid, all meet the criteria of the potential IR-stimulus/stimuli in mCCOPE and have biological activities supporting an IR-stimulating capacity. As stated before and supported by Burketova *et al.* (2015), it is likely that each of these compounds contributed (potentially synergistically) to the observed phenotypic changes upon mCCOPE treatment, rather than a single compound. Further testing of the individual compounds could elucidate to what extent each of the potential IR-stimuli contributes to the here-observed phenotype.

4.3. mCCOPE has a negative effect on *Mg* viability

As many plant extracts possess a nematicidal activity (Elbadri *et al.*, 2008; Eman and El-Nuby, 2019), it was investigated whether mCCOPE can act as a nematicide next to stimulating the plant immune system. For the same reason, rice plants were treated foliarly in this thesis, to avoid confounding effects of potential nematicidal mCCOPE activity during the infection experiments. Further, rice was inoculated at 1 dpt, to minimize direct contact between *Mg* and mCCOPE. Run-off could still be possible, however mRNA-seq confirmed that mCCOPE does stimulate the plant immunity.

Even though DHA did not act as the central compound in stimulating the rice root immunity, it could still play a central role in the mCCOPE nematicidal activity. Therefore, the nematicidal activity of the endogenous DHA concentration was assessed as well. A recurrent problem for nematicidal assays with plant extracts is that these extracts tend to become turbid over time, hampering microscopic analysis. After 48 hours, mCCOPE formed a dense layer blocking further analysis. Therefore, optimisation of the

protocol was needed. To solve this, some additional nematode solution clearing steps were added prior to counting (see Section 2.9.).

Our results showed that mCCOPE affects the *Mg* viability already after six hours of treatment (Figure 3.9). However, after 48 hours, the effect of mCCOPE treatment was limited compared to the control and no further decrease in viability compared to after six hours was observed. Therefore, mCCOPE could either act as a nematicide with large effects when freshly applied, or as a nematistatic extract, non-lethally inhibiting the nematodes shortly after being applied. To elucidate the exact action of mCCOPE on the nematode viability, further experiments will be required. However, it is clear that mCCOPE exerts a negative effect on *Mg*. Contrarily, 0.15 mM DHA treatment did not affect the *Mg* viability, thus DHA does not act as the nematicidal/-static agent in mCCOPE.

Again, many candidates as potential nematicidal agent(s) in mCCOPE can be hypothesised, due to the complex nature of the extract. Obviously, the extract contains many secondary metabolites and phytoanticipins, which each could exert a nematicidal activity. Plant extracts contain various compounds such as coumarins, essential oils, flavones, flavonoids, flavonols, phenols, phenolic acids, quinones, tannins, *etc.*, that all show potential antipathogenic activity (Šernaitė, 2017). Two typical cucurbit compounds with nematicidal activity are the cucurbitacins and gallic acid. As previously discussed, cucurbitacin A and B are both present in melon peels and have been commercialised as active components in nematicides (Yuan *et al.*, 2019; Mashela and Shokoohi, 2021). Further, gallic acid, which is present in high levels in Cantaloupe melon peels, was shown to be highly nematicidal to *M. incognita* (Nguyen *et al.*, 2013; Gómez-García *et al.*, 2020).

4.4. mCCOPE does not affect the rice yield upon life-long application

In light of economical applications, an important aspect of an IR-stimulus is whether or not allocation costs are present. As nowadays it is becoming more and more accepted that direct stimulation of defences by an IR-stimulus is not always associated with these costs, it is required to execute a long-term experiment to evaluate potential loss of fitness upon IR-stimulation (Martinez-Medina *et al.*, 2016; De Kesel *et al.*, 2021). To do so, it is necessary to evaluate plant growth and yield in non-hostile conditions, so the potential benefits of IR cannot mask the potential costs (Martinez-Medina *et al.*, 2016). Therefore, in this thesis, rice plants were treated two-weekly with mCCOPE and the growth was followed throughout the growing season. The results showed that repeated mCCOPE treatment of rice does not affect the growth throughout the rice life cycle nor does it affect the yield at the end of the growing season (Figure 3.10). Therefore, mCCOPE could be successfully applied in an agricultural setting. This result fully supports the new theory on allocation costs of IR-stimuli (De Kesel *et al.*, 2021), as mCCOPE directly induces thousands of defence related genes, without decreased growth capacity. Such an activation of defence responses without subsequent growth-related costs was previously reported for the IR-stimulus diproline on rice (De Kesel *et al.*, 2020). Similarly, treatment of rice seeds with gallic acid or rutin was shown to stimulate both defence responses and growth in the emerged seedlings, also indicating that direct defence stimulation is not necessarily associated with negative effects on plant growth (Singh, Gupta and Pandey, 2017). However, the opposite can be observed as well, as both BABA and the SA mimic 2,6-dichloroisonicotinic acid and its methyl ester were shown to reduce plant growth and/or yield (Ryals *et al.*, 1996; Cohen, Vaknin and Mauch-Mani, 2016). Finally, it could be hypothesised that the mCCOPE induced accumulation of IAA not only induces defence responses, but also ensures maintenance of the normal growth pattern.

4.5. Potential for application of mCCOPE in agriculture

In this thesis we showed that mCCOPE functions as a non-phytotoxic IR-stimulus and nematicidal/-static plant extract. Such dual action has previously been described for pesticides and plant antipathogenic compounds (Conrath *et al.*, 2015), and can contribute to increased curative or preventive potential. For example, in the field, mCCOPE could be applied in such a way that it also penetrates the soil and thus exerts a dual action on the nematodes. mCCOPE could for example be added to the irrigation system, thus allowing direct contact between mCCOPE and the rice roots and *Mg*. Moreover, it might also protect plants in the field against shoot infecting nematodes (such as *Aphelenchoides besseyi* and *Ditylenchus angustus* for rice) upon foliar application. Further, mCCOPE could also provide protection against other pathogens, as antipathogenic plant extracts often have a broad activity spectrum (Šernaitė, 2017).

The fact that it is forecasted that the global biopesticide market will reach 6.77 billion US dollar by 2026 and will have a growth rate of 116% during the period 2021-2026 (Market Data Forecast, 2020), shows that there is increasing interest in agriculture for IR-stimuli and other biopesticides. Moreover, the plant biostimulant (a term used for commercial products that are marketed as stimulants of natural plant growth and/or abiotic protection) market is forecasted to reach 4.5 billion US dollar by 2027 and will have a growth rate of 11.2% during the period 2020-2027 (Global Industry Analysts Inc., 2020), showing increasing interest in agriculture for stimulating natural plant responses to improve yield. In agriculture, various algae (micro- and macroalgae) based extracts have been commercialised, either for IR-stimulation, antipathogenic properties or growth stimulation. Examples are AMPEP®, Kelpak®, Marinure®, Maxicrop®, Redicrop®, Seamac®, Seasol® and S.M.3® (Craigie, 2011; Ali *et al.*, 2018). Also, higher plant extracts have been commercialised. For example, Trainer® (a legume-derived protein hydrolysate) and Auxym® (a tropical plant extract) are sold on the market as plant growth stimulants that also help alleviate stress (Caruso *et al.*, 2019). The commercial plant extracts Armorex®, Camas® (extract from *Macleaya cordata*), Fungastop®, Neem Azal F®, Nemafric-BL® (extract from *Cucumis africanus*), Nemalan® (extract from *Lantana camara* and *Tulbaghia violacea*), Nemarioc-AL® (extract from *Cucumis myriocarpus*) and TimorexGold® (extract from *Melaleuca alternifolia*) are sold as biopesticides (Abdel-Shafy and Zayed, 2002; Yoon, Cha and Kim, 2013; Zaker, 2016; Mashela *et al.*, 2017). Examples of commercial IR-stimulating plant extracts are Milsana® (extract from giant knotweed), Neudo-Vital® and Bio-S® (mix of several plant extracts) (Fofana *et al.*, 2002; Von Rad, Mueller and Durner, 2005).

Before moving from the lab to the field, negative aspects of mCCOPE should be assessed and alleviated to obtain a practical product. Further, field trails will be required to assure effectivity in non-standardised conditions.

A first potential problem is the shelf-life of the extract. In this thesis it was shown that mCCOPE solution becomes turbid over time, which could potentially affect the application. Furthermore, contamination and spoilage of the extract should be avoided. Moreover, depending on the active compound(s), the activity could become lower over time, also limiting shelf-life. To solve these issues two approaches can be proposed. To start, identifying the active compound(s) would allow us to synthetically produce an IR-stimulus (mixture), which likely has a longer shelf-life due to increased purity. Alternatively, the extract could be concentrated, dried, evaporated or lyophilised, if the active compound(s) would remain active upon such treatment. As drying, evaporation or lyophilisation would result in a stable powder, which would be easy in use and storage, these methods are preferred over alternatives that

stabilise mCCOPE in a liquid form. If this would not be possible, concentration could be an alternative to attempt to stabilise the extract. Similarly, upon concentration, orange juice can be stored for one year at 5°C under aseptic conditions, without major loss in quality (Berk, 2016). This could be done using a falling film evaporator, which is frequently used for sensitive biological compounds (Paramalingam, 2004). As another option, efforts could be made to obtain a more stable formulation, if the above-described alternatives would prove to be impossible. Finally, if none of the above succeeds, the extract could be sterilised by ultra-high temperature (UHT, frequently used for sensitive compounds) and shelf-life should be limited (depending on how quickly the extract loses its activity upon storage). Similarly, UHT treatment of coffee was shown not to affect the chemical composition, while it stabilised and sterilised the coffee (Sopelana *et al.*, 2013).

A second limitation is potential seasonal variability, both in composition and in availability of melon peels. It is likely that the composition of the extract varies over the year, as the melon composition shows seasonal variation (Maietti *et al.*, 2012). Thus, the concentration of our active compound(s) could vary as well (potentially affecting the IR-phenotype (see Section 4.2.4.1.) and nematicidal/-static activity), with the extent likely depending on the nature of these compounds. Moreover, as the extract likely contains multiple synergistic IR-stimuli, this synergism could be affected by such seasonal changes. Also, the melon peel availability is subject to seasonal variation. This would lead to variability in the mCCOPE production line, which is typically associated with increased production costs (Coleman, Smidt and York, 1964; Hennessy and Roosen, 2003). Another source of compositional variation could be the location of melon cultivation. It has been shown that the climatic conditions, the agricultural practices and the soil type can affect the melon composition (Lester and Crosby, 2002; Bernillon *et al.*, 2013). Moreover, the extract is also likely to show batch-to-batch variation, which is a drawback for an industrial product. Again, identifying the active compound(s) and synthetically producing an mCCOPE-equivalent IR-stimulus could be the solution to this problem. However, applying natural mCCOPE could still remain interesting, as it is sustainably based on a natural waste product, and thus perfectly suitable for organic and sustainable agriculture, and a circular economy approach.

Further, at this moment, mCCOPE faces only limited application potential due to volume limitations. Namely, mCCOPE production is limited to the availability of melon peels from the food industry, as due to ethic reasons production of melons only for the sake of peel use would not be accepted by the consumer. Thus, the production of mCCOPE would be limited to the use of eight to twenty million tonnes of melon waste (peels and seeds) per year from food industry worldwide (Rolim, Seabra and de Macedo, 2020). Even though this is a large waste stream that would be validated, it is hardly adequate to support widespread use of mCCOPE in the global agriculture. Again, identification of the active compound(s) could allow to upscale the production and thus the application of this biological IR-stimulus worldwide. Alternatively, it could be investigated if mixing different types of CCOPEs results in similar IR-stimulation as mCCOPE, which would open up the use of additional waste streams and allow upscaling of the production. Even though all studied CCOPEs in the CCOPE patent stimulated IR, mCCOPE was the least phytotoxic CCOPE, which is why it was chosen for further studying and commercialisation (Kyndt *et al.*, 2020). Mixing different CCOPEs with mCCOPE could decrease this phytotoxicity by decreasing the concentration of the phytotoxic component(s), by introducing the IAA stimulating compound(s) in mCCOPE, … Similarly, the commercial Bio-S® is a mixture of multiple plant extracts (Von Rad, Mueller and Durner, 2005).

5. General conclusions

In conclusion, it was shown that mCCOPE acts as an IR-stimulus that directly induces various defence responses. AO and DHA were shown not to be essential for the mCCOPE mediated defence induction. It was argued that likely many different compounds (synergistically) contribute to the here-observed IR-phenotype, with cucurbitacin, DKG, ellagic acid, gallic acid and oxalic acid interesting cucurbit specific candidates.

To elucidate mCCOPE-IR, mRNA-seq and validation assays were conducted. Here, the priming phase was studied, thus the direct changes mediated by mCCOPE were characterised. Based on these observations, the following mode-of-action for systemic mCCOPE mediated IR-stimulation can be hypothesised: The foliar mCCOPE signal is systemically transduced to the rice root by an early ROS wave. Upon transient local ROS-accumulation various signals are locally transduced, including alterations in the glutathione redox balance, RNS signalling, early ET and IAA signalling, and receptor kinase signalling. These local signals result in increased lignin and phytoalexin biosynthesis, and defence protein accumulation.

Further, it was shown that mCCOPE acts as a dual function extract, namely it acts as an IR-stimulus and a nematicidal/-static extract to *Mg*. Such dual function can be of advantage in agricultural applications, by combining a preventive with a curative action, increasing the protective potential.

Finally, it was shown that mCCOPE induces no plant fitness costs upon repeated application throughout the rice life cycle, which is an important requisite for agricultural application of an IR-stimulus. Thus, interestingly, it was shown that the activation of thousands of defence related genes by mCCOPE did not result in allocation costs, which is in conjunction with the new theory on allocation costs for IR-stimuli (De Kesel *et al.*, 2021).

Together, these results indicate that mCCOPE acts as a valuable IR-stimulus in the lab, and thus shows potential for agricultural applications. Further, mCCOPE is a plant waste-based extract that can contribute to ensure global food security and sustainable agriculture. As an alternative for the increasingly banned chemical nematicides, it could help repress the increasing *Mg* yield losses in rice agriculture in a biological, biodegradable and sustainable way. However, to move from the lab to the field certain issues still need to be addressed, including shelf-life, variability in composition and availability of melon peels, limited production potential due to limited waste availability, and lack of full pathogen control. Identifying the active compound(s) in the extract could be a first important step in solving these issues. Further, field trails are needed to assess the consistency of mCCOPE mediated IR in uncontrolled conditions.

6. Recommendations for future research

First, the **contradictory results** observed for ET levels (Figure 3.7) should be further investigated. ET levels could be remeasured at 1 dpt, to provide more information on the trend in ET accumulation at this time point. However, probably more interesting would be an analysis of the ET levels at 6 hpt, 12 hpt, or both. This would allow to elucidate whether the hypothesis of a **feedback inhibition** of the ET accumulation was correct. For consistency and seeing the whole picture, the ABA, IAA, JA and SA levels (Figure 3.8) could be assessed at these earlier timepoints as well. Specifically for JA this could be interesting, as mRNA-seq suggested the involvement of this hormone in mCCOPE-IR, although this was not validated at 1 or 4 dpt. Moreover, JA cooperates with ET in rice root-nematode defence, thus it could be that JA is also involved in the early mCCOPE signalling. For similar reasons as for ET, it seems to be recommendable to also measure ROS (Figure 3.6) at these earlier time points. Moreover, ROS could also be measured in the shoots at these earlier time points, to provide proof for the hypothesis that **ROS** could be responsible for the **systemic signalling**. Further, a repeated measurement of the rice root lignin levels (Figure 3.5) with a higher number of biological replicates should be conducted, to obtain significant results in this validation assay. Additionally, a Wiesner staining experiment could be conducted to **further validate rice root lignification**.

In this thesis we mainly focussed on direct effects induced by mCCOPE treatment. Additionally, the **primed defence** could be assessed in the future. This could be analysed via mRNA-seq, but this time mock and mCCOPE treated plants should be investigated upon subsequent infections with *Mg*. Alternatively, a more directed approach targeting the here-proposed potential primed pathways could be used. It could for example be investigated whether *Mg* infection of mCCOPE treated plants will result in enhanced rice root lignification. Priming could also be assessed using alternative methods. Namely, the proposed **sensitisation** of the rice root immunity could be assessed, for example by applying a dilution series of nematode PAMPs and tracking rice root immunity (PTI) hallmark genes by RT-qPCR, as for example described by De Kesel *et al.* (2020). This way we could see if mCCOPE treatment allows to detect nematode PAMPs and trigger defence at lower infection pressures (PAMP concentrations) than control treated plants, indicating sensitisation of the rice roots. An example of such a PAMP is NemaWater, prepared by overnight nematode soaking in distilled water (De Kesel *et al.*, 2020). The exact active compound(s) in NemaWater remain(s) yet to be identified (Mendy *et al.*, 2017). Additionally, upon NemaWater application, samples could be taken at regularly spaced time points during an early timeframe (for example the first 6 or 12 hpi) and could be analysed via RT-qPCR targeting PTI hallmark genes, to investigate a potential **quicker immune response** to the pathogen by mCCOPE treatment. Finally, it could be investigated whether mCCOPE-mediated defence priming results in a **stronger defence response** upon subsequent challenge. This could be assessed by again using the standardised 'infection pressure' by NemaWater treatment, followed by RT-qPCR of PTI hallmark genes and comparing the activation of defence gene expression.

To further validate the roles and relative importance of the different **mCCOPE mediated signalling** pathways, **mutants** in the different suggested signalling molecules could be subjected to infection assays with *Mg*, to identify essential signalling pathways in mCCOPE-IR. In a next step, the expression patterns induced by mCCOPE treatment in relevant mutants could be assessed to elucidate which signals induce which defence responses or downstream signals. This again could be done in detail, using mRNA-seq, or marker/hallmark genes for pathways of interest (such as lignification, expression of *OsPIP*, …) could be identified and followed up using RT-qPCR. This information could also elucidate

which of the signals are responsible for **systemic signal transduction** and which only participate in local signalling. A systemic signal will be essential for each of the observed defence responses, as without it the signal does not reach the root. Mutants in a local signal will show some of the defence responses, while others will be missing, elucidating the function of the local signal. Particularly for IAA this is an interesting experiment, as IAA is not frequently associated with defence responses and thus the results of such an experiment could provide clarity in its role. Examples of mutants of interest are *Osrbohb* (rice ortholog of *AtRBOHD*, essential for ROS waves), catalase overexpressing lines (with 35S and/or Os03g01700 promotor, a constitutive and root specific promotor, respectively, to distinguish local from systemic H_2O_2 effects), an *ein2b*-RNAi line (mutant in ET signalling), an *OsPINOID* overexpressor (disturbs the polar IAA flow, thus allows detection of a systemic IAA signal), an *miR393* overexpressing line (targets *OsTIR1*, a key positive regulator of IAA signalling), … (Matsumura *et al.*, 2002; Morita and Kyozuka, 2007; Nahar *et al.*, 2011; Y. Li *et al.*, 2019; Zhang *et al.*, 2019; Shi *et al.*, 2020). As an alternative to mutants, **inhibitors of** the **signalling** molecules could be applied. This could be done foliarly, to determine the importance of the signal for systemic signal transduction, or via root drench, to investigate the local importance. Examples are the calcium-channel blocker lanthanum, the calcium-chelator EGTA, the polar IAA transport inhibitor N-1-naphthylphthalamic acid, the ET biosynthesis inhibitor 1-methyl cyclopropane, the H_2O_2 scavengers potassium iodide and potassium benzoate, the RBOH inhibitor diphenylene iodonium-HCl, the peroxidase inhibitor salicylhydroxamic acid, *etc.* (Knight, Trewavas and Knight, 1997; Ella *et al.*, 2003; Liszkay, Van Der Zalm and Schopfer, 2004; Morita and Kyozuka, 2007; Khokon *et al.*, 2010; Wada, Cui and Yoshida, 2019). Additionally, the currently unclear (in)dependence of mCCOPE-IR on JA could be investigated with an infection experiment on the JA biosynthesis mutant *hebiba* (Nahar *et al.*, 2011). Another interesting expression pattern observed upon mCCOPE treatment was the induction of aquaporins, and specifically of the Major Intrinsic Protein PIP family. To assess the role of these aquaporins in the rice H_2O_2 signalling, PIP-mutants could be analysed for their mCCOPE response. It could for example be assessed if they are essential for the redox signalling, or only facilitate it.

In this thesis it was clearly shown that AO and DHA are not the active compounds that induce mCCOPE mediated resistance. The here-proposed **potential IR-stimuli in the extract**, cucurbitacin, DKG, ellagic acid, gallic acid and oxalic acid, could be analysed for their individual contribution to the IR-phenotype. They could be screened via infection experiments for IR-stimulation. Further, some of the typical mCCOPE induced genes could be tracked by RT-qPCR, as these could provide proof for the contribution to the mCCOPE mediated transcriptional changes in rice roots. Examples of these typical genes are *OsPIP1-2* and *OsPIP2-2* for indication of the upregulation of aquaporins, *INDOLE-3-ACETATE O-METHYLTRANSFERASE* and *SAUR36* for indication of the upregulation of IAA signalling, and maybe less specific, but for important mCCOPE mediated processes: *ACC OXIDASE*, *ACC SYNTHASE*, *CINNAMYL ALCOHOL DEHYDROGENASE*, *LACCASE-10*, … Identifying the active compound(s) is particularly interesting from an economic/agricultural perspective (as discussed in Section 4.5.).

Moreover, as AO and DHA are not the active compounds, the used **extraction method** could be adjusted, as it was tailored to the extraction of these two compounds. For example, it could be investigated if a hot extraction provides better results. In the patent describing the use of CCOPE to control nematode infection, this was already investigated and resulted in a small improvement of the activity (Kyndt *et al.*, 2020). As now we know that the active compound(s) are heat stable, further investigation of this method could be conducted. A hot extraction might be able to obtain higher levels of the underlying IR-stimuli in the extract by for example better breaking the cells, thus making better

use of the melon peels. Another alternative is a methanol (or ethanol) based extraction, as this is a frequently applied method in literature to extract plant material to stimulate IR. 100 g melon peels could be mixed in 200 mL methanol and then be allowed to settle for approximately two hours. The supernatant could then be collected and evaporated, and the resulting powder can be reconstituted in water to obtain the final extract (Baysal and Zeller, 2004; Moushib *et al.*, 2013; Shodehinde *et al.*, 2016).

Further experiments could also be conducted to elucidate the **exact negative effect** of mCCOPE **on *Mg* viability**. To distinguish between a potential nematicidal or -static effect, the experiment should be repeated, but this time nematode viability should also be assessed upon transferring the nematodes to water after treatment. This could be done using the sucrose clearing protocol as described for extracting the nematodes from the turbid mCCOPE after 48 hours of treatment. When the nematodes start to move again upon recuperation in water, the extract is said to be nematistatic.

Even though the IR-stimulating mechanism is quite specific for a tissue-plant-pathogen combination, IR-stimuli often induce IR in various plants against various pathogens. For example, BABA and BTH have frequently been described to stimulate **broad-spectrum IR** in various plants (Görlach *et al.*, 1996; Lawton *et al.*, 1996; Jakab *et al.*, 2001; Veronico *et al.*, 2018). Thus, it could be investigated whether mCCOPE stimulates IR in for example other monocot crops, but more interestingly in **dicot crops**, which would be an interesting feature from an agricultural or business perspective. As this is a logical next step to take, these future experiments are currently already being executed in the lab. For example, similar experiments are being conducted on tomato (with the RKN *M. incognita*). Further, the application of mCCOPE against for example **shoot pathogens** is investigated as well. Typical examples for rice are bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), rice blast fungus (*M. oryzae*), white tip disease (*A. besseyi*), … (Ou, 1985). IR-stimuli can also induce acclimation to **abiotic stress** (Martinez-Medina *et al.*, 2016), so in the future it could be investigated if mCCOPE can help rice cope with heat, salt, … stress.

Finally, experiments have to be conducted to improve the application potential in agriculture and to prepare mCCOPE for sale. To ensure the economic value of mCCOPE, **field trails** on large scale and in different environments should be conducted. This will allow to assess the strength and consistency of the IR-phenotype in a real setting. Next, **antipathogenic activity** of mCCOPE to **other pathogens** than *Mg* could be assessed, to increase its protective potential in the field. This way mCCOPE could be used to protect crops from multiple pathogens at once. For rice, nematicidal activity against the shoot infecting *A. besseyi* and *D. angustus* could be assessed, as well as its antipathogenic activity against the shoot infecting fungus *M. oryzae* and the bacterium *X. oryzae* pv. *oryzae*. Further, the **shelf-life** of mCCOPE should be assessed and efforts should be made to process fresh mCCOPE to increase the shelf-life. Shelf-life can be assessed via infection experiments with (processed) mCCOPE that was stored over various time ranges. Ongoing trails for the use of lyophilisation are currently running, but also concentration and drying could be investigated as storage methods. In case these methods would fail, efforts could be made to obtain a stable formulation for mCCOPE. If none of the above would be successful, UHT sterilisation could be assessed to obtain aseptic but not further stabilised mCCOPE. Finally, **variation in mCCOPE composition** could be analysed, due to location, seasonal or batch-to-batch effects. To assess location effects, melon peels from different parts of the world and/or fields with different soil/climatic conditions could be compared in an infection experiment. To assess batch-to-batch effects, melons from a same region and period could be analysed and compared via infection

experiments as well. However, comparing seasonal variation is more difficult, due to confounding effects by for example mCCOPE storage, difference in infection pressure between experiments, ... Thus, a potential solution would be assessing the direct defence responses via RT-qPCR. This would allow to conduct experiments without storage of mCCOPE and to better compare data between different experiments.

7. List of references

Abd El-Rahman, S. S. and Mohamed, H. I. (2014) 'Application of benzothiadiazole and Trichoderma harzianum to control faba bean chocolate spot disease and their effect on some physiological and biochemical traits', *Acta Physiologiae Plantarum*, 36(1), pp. 343–354. doi: 10.1007/s11738-013-1416-5.

Abdel-Shafy, S. and Zayed, A. A. (2002) 'In vitro acaricidal effect of plant extract of neem seed oil (Azadirachta indica) on egg, immature, and adult stages of Hyalomma anatolicum excavatum (Ixodoidea: Ixodidae)', *Veterinary Parasitology*. Elsevier, 106(1), pp. 89–96. doi: 10.1016/S0304-4017(02)00023-7.

Agorio, A. and Vera, P. (2007) 'ARGONAUTE4 is required for resistance to Pseudomonas syringae in Arabidopsis', *Plant Cell*. American Society of Plant Biologists, 19(11), pp. 3778–3790. doi: 10.1105/tpc.107.054494.

Aitchison, J. (2015) *A Concise Guide to Compositional Data Analysis*. 1st edn. Glasgow: Department of Statistics University of Glasgow.

Akimoto, K. *et al.* (2007) 'Epigenetic inheritance in rice plants', *Annals of Botany*. Oxford Academic, 100(2), pp. 205–217. doi: 10.1093/aob/mcm110.

Alexandersson, E. *et al.* (2016) 'Plant resistance inducers against pathogens in Solanaceae species-from molecular mechanisms to field application', *International Journal of Molecular Sciences*. MDPI AG, p. 1673. doi: 10.3390/ijms17101673.

Ali, A. *et al.* (2014) 'Induction of lignin and pathogenesis related proteins in dragon fruit plants in response to submicron chitosan dispersions', *Crop Protection*. Elsevier Ltd, 63, pp. 83–88. doi: 10.1016/j.cropro.2014.05.009.

Ali, M. K. M. *et al.* (2018) 'Impacts of Ascophyllum marine plant extract powder (AMPEP) on the growth, incidence of the endophyte Neosiphonia apiculata and associated carrageenan quality of three commercial cultivars of Kappaphycus', *Journal of Applied Phycology*. Springer Netherlands, 30(2), pp. 1185–1195. doi: 10.1007/s10811-017-1312-2.

Alvarez-Venegas, R. *et al.* (2007) 'Epigenetic control of a transcription factor at the cross section of two antagonistic pathways', *Epigenetics*. Taylor and Francis Inc., 2(2), pp. 106–113. doi: 10.4161/epi.2.2.4404.

Andrews, S. (2010) *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Available at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (Accessed: 25 March 2021).

Anita, B. and Samiyappan, R. (2012) 'Induction of systemic resistance in rice by Pseudomonas fluorescens against rice root knot nematode Meloidogyne graminicola', *JBiopest*, 5, p. 59.

Anupama, R. T. (2020) 'Antimicrobial Compounds (Phytoanticipins and Phytoalexins) and Their Role in Plant Defense', *Springer*, pp. 845–868. doi: 10.1007/978-3-319-96397-6_63.

Apel, K. and Hirt, H. (2004) 'REACTIVE OXYGEN SPECIES: Metabolism, Oxidative Stress, and Signal Transduction', *Annual Review of Plant Biology*. Annual Reviews, 55(1), pp. 373–399. doi: 10.1146/annurev.arplant.55.031903.141701.

Arnaiz, A. *et al.* (2021) 'Nitric Oxide, an Essential Intermediate in the Plant–Herbivore Interaction', *Frontiers in Plant Science*. Frontiers Media S.A., 11. doi: 10.3389/fpls.2020.620086.

Arrigoni, O. and De Tullio, M. C. (2002) 'Ascorbic acid: Much more than just an antioxidant', *Biochimica et Biophysica Acta - General Subjects*. Elsevier, pp. 1–9. doi: 10.1016/S0304-4165(01)00235-5.

Asghar, M. A. *et al.* (2019) 'Crosstalk between Abscisic Acid and Auxin under Osmotic Stress', *Agronomy Journal*. American Society of Agronomy, 111(5), pp. 2157–2162. doi: 10.2134/agronj2018.10.0633.

Astier, J. *et al.* (2019) 'The evolution of nitric oxide signalling diverges between animal and green lineages', *Journal of Experimental Botany*. Oxford University Press, pp. 4355–4364. doi: 10.1093/jxb/erz088.

Azarabadi, S. *et al.* (2017) 'ROS generation, oxidative burst and dynamic expression profiles of ROS-scavenging enzymes of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in response to Erwinia amylovora in pear (Pyrus communis L)', *European Journal of Plant Pathology*. Springer Netherlands, 147(2), pp. 279–294. doi: 10.1007/s10658-016-1000-0.

Baccelli, I. and Mauch-Mani, B. (2016) 'Beta-aminobutyric acid priming of plant defense: the role of ABA and other hormones', *Plant Molecular Biology*. Springer Netherlands, 91(6), pp. 703–711. doi: 10.1007/s11103-015-0406-y.

Badhani, B., Sharma, N. and Kakkar, R. (2015) 'Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications', *RSC Advances*. Royal Society of Chemistry, 5(35), pp. 27540–27557. doi: 10.1039/c5ra01911g.

Baider, A. and Cohen, Y. (2003) 'Synergistic Interaction between BABA and Mancozeb in Controlling Phytophthora infestans in Potato and Tomato and Pseudoperonospora cubensis in Cucumber', *Phytoparasitica*, 31(4), pp. 399–409.

Bailey, T. A. *et al.* (2009) 'Role of Ethylene, Abscisic Acid and MAP Kinase Pathways in Rice Blast Resistance', in *Advances in Genetics, Genomics and Control of Rice Blast Disease*. Springer Netherlands, pp. 185–190. doi: 10.1007/978-1-4020-9500-9_19.

Baljeet, S. Y., Roshanlal, Y. and Ritika, B. Y. (2016) 'Effect of cooking methods and extraction solvents on the antioxidant activity of summer squash (Cucurbita pepo) vegetable extracts', *International Food Research Journal*, 23(4), pp. 1531–1540.

Balmer, A. *et al.* (2015) 'The "prime-ome": Towards a holistic approach to priming', *Trends in Plant Science*. Elsevier Ltd, pp. 443–452. doi: 10.1016/j.tplants.2015.04.002.

Balmer, A. *et al.* (2018) 'Accumulation patterns of endogenous β-aminobutyric acid during plant development and defence in *Arabidopsis thaliana*', *Plant Biology*. Edited by A. Martinez-Medina. Blackwell Publishing Ltd, 21(2), p. plb.12940. doi: 10.1111/plb.12940.

Barnes, W. J. and Anderson, C. T. (2017) 'Acetyl Bromide Soluble Lignin (ABSL) Assay for Total Lignin Quantification from Plant Biomass', *Bio-protocol*, 7(05). doi: 10.21769/BioProtoc.2149.

Bartels, S. and Boller, T. (2015) 'Quo vadis, Pep? Plant elicitor peptides at the crossroads of immunity, stress, and development', *Journal of Experimental Botany*. Oxford University Press, pp. 5183–5193. doi: 10.1093/jxb/erv180.

Barth, C. *et al.* (2004) 'The timing of senescence and response to pathogens is altered in the ascorbate-deficient arabidopsis mutant vitamin c-1', *Plant Physiology*. American Society of Plant Biologists, 134(4), pp. 1784–1792. doi: 10.1104/pp.103.032185.

Bashandy, T. *et al.* (2010) 'Interplay between the NADP-linked thioredoxin and glutathione systems in Arabidopsis auxin signaling', *Plant Cell*. American Society of Plant Biologists, 22(2), pp. 376–391. doi: 10.1105/tpc.109.071225.

Bateman, A. *et al.* (2021) 'UniProt: The universal protein knowledgebase in 2021', *Nucleic Acids Research*. Oxford University Press, 49(D1), pp. D480–D489. doi: 10.1093/nar/gkaa1100.

Baysal, Ö. and Zeller, W. (2004) 'Extract of Hedera helix induces resistance on apple rootstock M26 similar to Acibenzolar-S-methyl against Fire Blight (Erwinia amylovora)', *Physiological and Molecular Plant Pathology*. Academic Press, 65(6), pp. 305–315. doi: 10.1016/j.pmpp.2005.03.003.

Beckers, G. J. M. *et al.* (2009) 'Mitogen-Activated protein kinases 3 and 6 are required for full priming of stress responses in Arabidopsis thaliana', *Plant Cell*. American Society of Plant Biologists, 21(3), pp. 944–953. doi: 10.1105/tpc.108.062158.

Benfey, P. N. and Scheres, B. (2000) 'Root development', *Current Biology*. Cell Press, 10(22), pp. R813–R815. doi: 10.1016/S0960-9822(00)00814-9.

Bentham, A. R. *et al.* (2020) 'A molecular roadmap to the plant immune system', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 295(44), pp. 14916–14935. doi: 10.1074/jbc.REV120.010852.

Bergs, M. *et al.* (2020) 'Comparing chemical composition and lignin structure of: Miscanthus x giganteus and Miscanthus nagara harvested in autumn and spring and separated into stems and leaves', *RSC Advances*. Royal Society of Chemistry, 10(18), pp. 10740–10751. doi: 10.1039/c9ra10576j.

Berk, Z. (2016) 'Shelf life of citrus products: packaging and storage', in *Citrus Fruit Processing*. Haifa: Elsevier, pp. 251–259. doi: 10.1016/b978-0-12-803133-9.00012-6.

Bernier, F. and Berna, A. (2001) 'Germins and germin-like proteins: Plant do-all proteins. But what do they do exactly?', *Plant Physiology and Biochemistry*. ESME - Gauthier-Villars, pp. 545–554. doi: 10.1016/S0981-9428(01)01285-2.

Bernillon, S. *et al.* (2013) 'Metabolomic and elemental profiling of melon fruit quality as affected by genotype and environment', *Metabolomics*. Springer Science and Business Media, LLC, 9(1), pp. 57–77. doi: 10.1007/s11306-012-0429-1.

Berthet, S. *et al.* (2012) 'Role of Plant Laccases in Lignin Polymerization', in *Advances in Botanical Research*. Academic Press Inc., pp. 145–172. doi: 10.1016/B978-0-12-416023-1.00005-7.

Bianchi, G. *et al.* (1993) 'The unusual sugar composition in leaves of the resurrection plant Myrothamnus flabellifolia', *Physiologia Plantarum*. John Wiley & Sons, Ltd, 87(2), pp. 223–226. doi: 10.1111/j.1399-3054.1993.tb00146.x.

Birch, A. N. E. *et al.* (1993) 'DMDP — A Plant-Derived Sugar Analogue with Systemic Activity Against Plant Parasitic Nematodes', *Nematologica*. Brill, 39(1–4), pp. 521–535. doi: 10.1163/187529293X00466.

Biswas, M. S. *et al.* (2019) 'Reactive oxygen species and reactive carbonyl species constitute a feed-forward loop in auxin signaling for lateral root formation', *The Plant Journal*. Blackwell Publishing Ltd, 100(3), pp. 536–548. doi: 10.1111/tpj.14456.

Bolger, A. M., Lohse, M. and Usadel, B. (2014) 'Trimmomatic: a flexible trimmer for Illumina sequence data', *Bioinformatics*. Oxford University Press, 30(15), pp. 2114–2120. doi: 10.1093/bioinformatics/btu170.

Bolwell, G. P. (2002) 'The apoplastic oxidative burst in response to biotic stress in plants: a three-component system', *Journal of Experimental Botany*. Oxford University Press (OUP), 53(372), pp. 1367–1376. doi: 10.1093/jexbot/53.372.1367.

Boubakri, H. *et al.* (2016) 'Vitamins for enhancing plant resistance', *Planta*. Springer Verlag, pp. 529–543. doi: 10.1007/s00425-016-2552-0.

Boughton, A. J., Hoover, K. and Felton, G. W. (2005) 'Methyl jasmonate application induces increased densities of glandular trichomes on tomato, Lycopersicon esculentum', *Journal of Chemical Ecology*. Springer, 31(9), pp. 2211–2216. doi: 10.1007/s10886-005-6228-7.

Bouman, B. A. M. *et al.* (2005) 'Yield and water use of irrigated tropical aerobic rice systems', *Agricultural Water Management*. Elsevier, 74(2), pp. 87–105. doi: 10.1016/j.agwat.2004.11.007.

Boyce, C. K. *et al.* (2004) 'Evolution of xylem lignification and hydrogel transport regulation', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 101(50), pp. 17555–17558. doi: 10.1073/pnas.0408024101.

Bridge, J. and Page, S. L. (1982) 'The rice root-knot nematode, Meloidogyne graminicola, on deep water rice (Oryza sativa subsp. indica).', *Rev. Nematol.*, 5(2), pp.

225–232.

Bridge, J., Plowright, R. and Peng, D. (2005) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. 2nd edn. Edited by L. Michel, R. A. Sikora, and John Bridge. CAB International. Available at: https://books.google.be/books?hl=en&lr=&id=GAdsEt6dEtwC&oi=fnd&pg=PA87&ots=hO8LdUfBAW&sig=zV8U7Wk408zOg8THfLLSbadpEi0&redir_esc=y#v=onepage&q&f=false (Accessed: 21 February 2021).

Brightman, A. O. *et al.* (1988) 'Auxin-Stimulated NADH Oxidase Purified from Plasma Membrane of Soybean', *Plant Physiology*. Oxford University Press (OUP), 86(4), pp. 1264–1269. doi: 10.1104/pp.86.4.1264.

Brotman, Y. *et al.* (2012) 'Transcript and metabolite analysis of the Trichoderma-induced systemic resistance response to Pseudomonas syringae in Arabidopsis thaliana', *Microbiology*. Microbiology Society, 158(1), pp. 139–146. doi: 10.1099/mic.0.052621-0.

Bryan, G. *et al.* (2006) 'Investigations of Globodera pallida invasion and syncytia formation within roots of the susceptible potato cultivar Désirée and resistant species Solanum canasense', *Nematology*, 8(1), pp. 103–110.

Burketova, L. *et al.* (2015) 'Bio-based resistance inducers for sustainable plant protection against pathogens', *Biotechnology Advances*. Elsevier Inc., pp. 994–1004. doi: 10.1016/j.biotechadv.2015.01.004.

Busuioc, A. C. *et al.* (2020) 'Comparative Study of the Chemical Compositions and Antioxidant Activities of Fresh Juices from Romanian Cucurbitaceae Varieties', *Molecules*. NLM (Medline), 25(22), p. 5468. doi: 10.3390/molecules25225468.

Butler, A., Bierman, S. and Marion, G. (2005) 'Statistical methods for environmental risk assessment Compositional data module', *Compositional data module* . Edinburgh: Biomathematics and Statistics Scotland, The University of Edinburgh, pp. 1–45.

Cabrera, C., Giménez, R. and López, M. C. (2003) 'Determination of tea components with antioxidant activity', *Journal of Agricultural and Food Chemistry*. American Chemical Society , 51(15), pp. 4427–4435. doi: 10.1021/jf0300801.

Camejo, D., Guzmán-Cedeño, Á. and Moreno, A. (2016) 'Reactive oxygen species, essential molecules, during plant-pathogen interactions', *Plant Physiology and Biochemistry*. Elsevier Masson SAS, pp. 10–23. doi: 10.1016/j.plaphy.2016.02.035.

Cao, Y. R. *et al.* (2015) 'Tobacco ankyrin protein NEIP2 interacts with ethylene receptor NTHK1 and regulates plant growth and stress responses', *Plant and Cell Physiology*. Oxford University Press, 56(4), pp. 803–818. doi: 10.1093/pcp/pcv009.

Caruso, G. *et al.* (2019) 'Protein hydrolysate or plant extract-based biostimulants enhanced yield and quality performances of greenhouse perennial wall rocket grown in different seasons', *Plants*. MDPI AG, 8(7), p. 208. doi: 10.3390/plants8070208.

Chaouch, S., Queval, G. and Noctor, G. (2012) 'AtRbohF is a crucial modulator of defence-associated metabolism and a key actor in the interplay between intracellular oxidative stress and pathogenesis responses in Arabidopsis', *The Plant Journal*. John Wiley & Sons, Ltd, 69(4), pp. 613–627. doi: 10.1111/j.1365-313X.2011.04816.x.

Chen, C.-W. *et al.* (2006) 'A Novel Function of Abscisic Acid in the Regulation of Rice (Oryza sativa L.) Root Growth and Development', *Plant and Cell Physiology*. Oxford Academic, 47(1), pp. 1–13. doi: 10.1093/pcp/pci216.

Chen, K. *et al.* (2020) 'Abscisic acid dynamics, signaling, and functions in plants', *Journal of Integrative Plant Biology*. Blackwell Publishing Ltd, 62(1), pp. 25–54. doi: 10.1111/jipb.12899.

Choi, H. W. and Klessig, D. F. (2016) 'DAMPs, MAMPs, and NAMPs in plant innate immunity', *BMC Plant Biology*. BioMed Central, 16(1), p. 232. doi: 10.1186/s12870-016-0921-2.

Choi, W. G. *et al.* (2014) 'Salt stress-induced Ca2+ waves are associated with rapid, long-distance root-to-shoot signaling in plants', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 111(17), pp. 6497–6502. doi: 10.1073/pnas.1319955111.

Cohen, Y. and Gisi, U. (1994) 'Systemic translocation of 14C-dl-3-aminobutyric acid in tomato plants in relation to induced resistance against Phytophthora infestans', *Physiological and Molecular Plant Pathology*. Academic Press, 45(6), pp. 441–456. doi: 10.1016/S0885-5765(05)80041-4.

Cohen, Y. R. (2002) 'β-aminobutyric acid-induced resistance against plant pathogens', *Plant Disease*. American Phytopathological Society, pp. 448–457. doi: 10.1094/PDIS.2002.86.5.448.

Cohen, Y., Vaknin, M. and Mauch-Mani, B. (2016) 'BABA-induced resistance: milestones along a 55-year journey', *Phytoparasitica*. Springer Netherlands, pp. 513–538. doi: 10.1007/s12600-016-0546-x.

Coleman, J. R., Smidt, S. and York, R. (1964) 'Optimum Plant Design for Seasonal Production', *Management Science*. Institute for Operations Research and the Management Sciences (INFORMS), 10(4), pp. 778–785. doi: 10.1287/mnsc.10.4.778.

Cong, W. *et al.* (2019) 'Transgenerational memory of gene expression changes induced by heavy metal stress in rice (Oryza sativa L.)', *BMC Plant Biology*. BioMed Central Ltd., 19(1), pp. 1–14. doi: 10.1186/s12870-019-1887-7.

Conrath, U. *et al.* (2006) 'Priming: Getting Ready for Battle Prime-A-Plant Group', *Molecular Plant-Microbe Interactions MPMI*, 19(10), pp. 1062–1071. doi: 10.1094/MPMI.

Conrath, U. *et al.* (2015) 'Priming for Enhanced Defense', *Annual Review of Phytopathology*. Annual Reviews Inc., pp. 97–119. doi: 10.1146/annurev-phyto-080614-120132.

Craigie, J. S. (2011) 'Seaweed extract stimuli in plant science and agriculture', *Journal of Applied Phycology*. Springer, pp. 371–393. doi: 10.1007/s10811-010-9560-4.

D'Hondt, M. *et al.* (2011) 'Dry heat stress stability evaluation of casein peptide mixture', *Food Chemistry*. Elsevier, 128(1), pp. 114–122. doi: 10.1016/j.foodchem.2011.03.004.

Daayf, F., Schmitt, A. and Bélanger, R. R. (1995) 'The effects of plant extracts of Reynoutria sachalinensis on powdery mildew development and leaf physiology of long English cucumber', *Plant Disease*. American Phytopathological Society, 79(6), pp. 577–580. doi: 10.1094/PD-79-0577.

Daayf, F., Schmitt, A. and Bélanger, R. R. (1997) 'Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of Reynoutria sachalinensis', *Plant Physiology*. American Society of Plant Biologists, 113(3), pp. 719–727. doi: 10.1104/pp.113.3.719.

Datta, R. *et al.* (2015) 'Glutathione regulates 1-aminocyclopropane-1-carboxylate synthase transcription via WRKY33 and 1-aminocyclopropane-1-carboxylate oxidase by modulating messenger RNA stability to induce ethylene synthesis during stress', *Plant Physiology*. American Society of Plant Biologists, 169(4), pp. 2963–2981. doi: 10.1104/pp.15.01543.

Daudi, A. *et al.* (2012) 'The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of pattern-triggered immunity', *Plant Cell*. American Society of Plant Biologists, 24(1), pp. 275–287. doi: 10.1105/tpc.111.093039.

Davey, M. W. *et al.* (2000) 'PlantL-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing', *Journal of the Science of Food and Agriculture*. John Wiley and Sons Ltd, 80(7), pp. 825–860. doi: 10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6.

Davin, L. B. *et al.* (1997) 'Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center', *Science*. American Association for the Advancement of Science, 275(5298), pp. 362–366. doi: 10.1126/science.275.5298.362.

Deborah, S. D. *et al.* (2001) 'Time-course study of the induction of defense enzymes, phenolics and lignin in rice in response to infection by pathogen and non-pathogen', *Journal of Plant Diseases and Protection*. Oxford University Press (OUP), 108(2), pp. 204–216. doi: 10.1104/pp.91.3.889.

Deng, Y. *et al.* (2020) 'Molecular Basis of Disease Resistance and Perspectives on Breeding Strategies for Resistance Improvement in Crops', *Molecular Plant*. Cell Press, pp. 1402–1419. doi: 10.1016/j.molp.2020.09.018.

Deutsch, J. C. (2000) 'Dehydroascorbic acid', *Journal of Chromatography A*. Elsevier, pp. 299–307. doi: 10.1016/S0021-9673(00)00166-7.

Devireddy, A. R. *et al.* (2018) 'Coordinating the overall stomatal response of plants: Rapid leaf-to-leaf communication during light stress', *Science Signaling*. American Association for the Advancement of Science, 11(518). doi: 10.1126/scisignal.aam9514.

Devireddy, A. R., Arbogast, J. and Mittler, R. (2020) 'Coordinated and rapid whole-plant systemic stomatal responses', *New Phytologist*. Blackwell Publishing Ltd, 225(1), pp. 21–25. doi: 10.1111/nph.16143.

Dewhirst, R. A. and Fry, S. C. (2018) 'The oxidation of dehydroascorbic acid and 2,3-diketogulonate by distinct reactive oxygen species', *The Biochemical journal*. NLM (Medline), 475(21), pp. 3451–3470. doi: 10.1042/BCJ20180688.

Dhakshinamoorthy, S. *et al.* (2014) 'Phenols and lignin are involved in the defence response of banana (Musa) plants to Radopholus similis infection', *Nematology*, 16(5), pp. 565–576. Available at: https://doi.org/10.1163/15685411-00002788 (Accessed: 10 March 2020).

Diallinas, G. *et al.* (1997) 'Melon ascorbate oxidase: Cloning of a multigene family, induction during fruit development and repression by wounding', *Plant Molecular Biology*. Kluwer Academic Publishers, 34(5), pp. 759–770. doi: 10.1023/A:1005851527227.

Ding, H. *et al.* (2020) 'The pivotal function of dehydroascorbate reductase in glutathione homeostasis in plants', *Journal of Experimental Botany*. Oxford University Press (OUP), 71(12), pp. 3405–3416. doi: 10.1093/jxb/eraa107.

Dixon, D. P. *et al.* (2005) 'Stress-induced protein S-glutathionylation in arabidopsis', *Plant Physiology*. American Society of Plant Biologists, 138(4), pp. 2233–2244. doi: 10.1104/pp.104.058917.

Dobin, A. *et al.* (2013) 'STAR: Ultrafast universal RNA-seq aligner', *Bioinformatics*. Bioinformatics, 29(1), pp. 15–21. doi: 10.1093/bioinformatics/bts635.

Dubreuil-Maurizi, C. *et al.* (2010) 'β-Aminobutyric acid primes an NADPH oxidase-dependent reactive oxygen species production during grapevine-triggered immunity', *Molecular Plant-Microbe Interactions*. The American Phytopathological Society , 23(8), pp. 1012–1021. doi: 10.1094/MPMI-23-8-1012.

Dunwell, J. M. *et al.* (2008) 'Germin and Germin-like Proteins: Evolution, Structure, and Function', *Critical Reviews in Plant Sciences*. Taylor & Francis Group , 27(5), pp. 342–375. doi: 10.1080/07352680802333938.

El-Soud, W. A. *et al.* (2013) 'Ability of ellagic acid to alleviate osmotic stress on chickpea seedlings', *Plant Physiology and Biochemistry*. Elsevier Masson, 71, pp. 173–183. doi: 10.1016/j.plaphy.2013.07.007.

Elbadri, G. A. *et al.* (2008) 'Evaluation of various plant extracts for their nematicidal efficacies against juveniles of Meloidogyne incognita', *Journal of Asia-Pacific Entomology*. Elsevier, 11(2), pp. 99–102. doi: 10.1016/j.aspen.2008.04.004.

Ella, E. S. *et al.* (2003) 'Blocking ethylene perception enhances flooding tolerance in rice seedlings', *Functional Plant Biology*. CSIRO PUBLISHING, 30(7), pp. 813–819. doi: 10.1071/FP03049.

Eman, A. A. and El-Nuby, A. S. M. (2019) 'Phytochemical and Antinematodal Screening on Water Extracts of Some Plant Wastes against Meloidogyne incognita',

International Journal of Chemical and Pharmaceutical Sciences, 10(4). Available at: www.ijcps.com (Accessed: 18 March 2021).

Van Der Ent, S. *et al.* (2009) 'Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: Differences and similarities in regulation', *New Phytologist*. John Wiley & Sons, Ltd, 183(2), pp. 419–431. doi: 10.1111/j.1469-8137.2009.02851.x.

Espinas, N. A., Saze, H. and Saijo, Y. (2016) 'Epigenetic Control of Defense Signaling and Priming in Plants', *Frontiers in Plant Science*. Frontiers Media S.A., 7(AUG2016), p. 1201. doi: 10.3389/fpls.2016.01201.

European and Mediterranean Plant Protection Organization (2020) *EPPO Alert List – Meloidogyne graminicola Rice root-knot nematode*. Available at: https://www.eppo.int/ACTIVITIES/plant_quarantine/alert_list_nematodes/meloidogyne_graminicola (Accessed: 23 March 2021).

Eyheraguibel, B. *et al.* (2010) 'Photoprotection by plant extracts: A new ecological means to reduce pesticide photodegradation', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 58(17), pp. 9692–9696. doi: 10.1021/jf101792h.

Ezzat, S. M. *et al.* (2019) 'In vivo anti-inflammatory activity and UPLC-MS/MS profiling of the peels and pulps of Cucumis melo var. cantalupensis and Cucumis melo var. reticulatus', *Journal of Ethnopharmacology*. Elsevier Ireland Ltd, 237, pp. 245–254. doi: 10.1016/j.jep.2019.03.015.

Fan, L. *et al.* (2006) 'Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stelar accumulation of wall phenolics', *Plant Physiology*. American Society of Plant Biologists, 140(2), pp. 603–612. doi: 10.1104/pp.105.073130.

FAOSTAT (2021) *Food and Agriculture Organization of the United Nations.*, *FAOSTAT Database*. Available at: http://www.fao.org/faostat/en/#data/QC (Accessed: 11 February 2021).

Fayle, S. E. *et al.* (2000) 'Crosslinkage of proteins by dehydroascorbic acid and its degradation products', *Food Chemistry*. Elsevier, 70(2), pp. 193–198. doi: 10.1016/S0308-8146(00)00077-7.

Feige, M. J. and Hendershot, L. M. (2011) 'Disulfide bonds in ER protein folding and homeostasis', *Current Opinion in Cell Biology*. Elsevier Current Trends, pp. 167–175. doi: 10.1016/j.ceb.2010.10.012.

Fernandez, L., Cabasan, M. T. N. and De Waele, D. (2014) 'Life cycle of the rice root-knot nematode Meloidogyne graminicola at different temperatures under non-flooded and flooded conditions', *Archives of Phytopathology and Plant Protection*. Taylor and Francis Ltd., 47(9), pp. 1042–1049. doi: 10.1080/03235408.2013.829627.

Ferreira, F. J. and Kieber, J. J. (2005) 'Cytokinin signaling', *Current Opinion in Plant Biology*. Elsevier Current Trends, pp. 518–525. doi: 10.1016/j.pbi.2005.07.013.

Fichman, Y., Miller, G. and Mittler, R. (2019) 'Whole-Plant Live Imaging of Reactive Oxygen Species', *Molecular Plant*. Cell Press, 12(9), pp. 1203–1210. doi: 10.1016/j.molp.2019.06.003.

Fichman, Y. and Mittler, R. (2020) 'Rapid systemic signaling during abiotic and biotic stresses: is the ROS wave master of all trades?', *The Plant Journal*. Blackwell Publishing Ltd, 102(5), pp. 887–896. doi: 10.1111/tpj.14685.

Fofana, B. *et al.* (2002) 'Milsana-induced resistance in powdery mildew-infected cucumber plants correlates with the induction of chalcone synthase and chalcone isomerase', *Physiological and Molecular Plant Pathology*. Elsevier BV, 61(2), pp. 121–132. doi: 10.1006/pmpp.2002.0420.

Foyer, C. H., Kyndt, T. and Hancock, R. D. (2020) 'Vitamin C in Plants: Novel Concepts, New Perspectives, and Outstanding Issues', *ANTIOXIDANTS & REDOX SIGNALING*, 32(7), pp. 463–485. doi: 10.1089/ars.2019.7819.

Foyer, C. H. and Noctor, G. (2005) 'Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses', *Plant Cell*. American Society of Plant Biologists, pp. 1866–1875. doi: 10.1105/tpc.105.033589.

Foyer, C. H. and Noctor, G. (2011) 'Ascorbate and glutathione: The heart of the redox hub', *Plant Physiology*. Oxford Academic, pp. 2–18. doi: 10.1104/pp.110.167569.

de Freitas, M. B. and Stadnik, M. J. (2015) 'Ulvan-induced resistance in Arabidopsis thaliana against Alternaria brassicicola requires reactive oxygen species derived from NADPH oxidase', *Physiological and Molecular Plant Pathology*. Academic Press, 90, pp. 49–56. doi: 10.1016/j.pmpp.2015.03.002.

Fry, S. C. (1979) 'Phonolic components of the primary cell wall and their possible rôle in the hormonal regulation of growth', *Planta*. Springer-Verlag, 146(3), pp. 343–351. doi: 10.1007/BF00387807.

Fry, S. C. (1986) 'Cross-Linking of Matrix Polymers in the Growing Cell Walls of Angiosperms', *Annual Review of Plant Physiology*. Annual Reviews, 37(1), pp. 165–186. doi: 10.1146/annurev.pp.37.060186.001121.

Fujikawa, I. *et al.* (2021) 'Magnesium oxide induces immunity against Fusarium wilt by triggering the jasmonic acid signaling pathway in tomato', *Journal of Biotechnology*. Elsevier B.V., 325, pp. 100–108. doi: 10.1016/j.jbiotec.2020.11.012.

Fujimoto, T. *et al.* (2015) 'Sclareol Induces Plant Resistance to Root-Knot Nematode Partially Through Ethylene-Dependent Enhancement of Lignin Accumulation', *Molecular Plant-Microbe Interactions*. American Phytopathological Society, 28(4), pp. 398–407. doi: 10.1094/MPMI-10-14-0320-R.

Fujiwara, A. *et al.* (2016) 'Ascorbic acid accumulates as a defense response to *Turnip mosaic virus* in resistant *Brassica rapa* cultivars', *Journal of Experimental Botany*. Oxford University Press, 67(14), pp. 4391–4402. doi: 10.1093/jxb/erw223.

Fukushima, R. S. and Dehority, B. A. (2000) 'Feasibility of using lignin isolated from forages by solubilization in acetyl bromide as a standard for lignin analyses', *Journal of Animal Science*. American Society of Animal Science, 78(12), pp. 3135–3143. doi: 10.2527/2000.78123135x.

Gad, S. B. *et al.* (2018) 'In vivo and In vitro Inhibition of Three Plants Water Extracts on Meloidogyne incognita (Meloidogynidae)', *Indian Journal of Nematology*, 48(1), pp. 77–83. Available at: https://www.indianjournals.com/ijor.aspx?target=ijor:ijn&volume=48&issue=1&article=015 (Accessed: 21 April 2021).

Gallie, D. R. (2013) 'The role of l-ascorbic acid recycling in responding to environmental stress and in promoting plant growth', *Journal of Experimental Botany*. Oxford Academic, 64(2), pp. 433–443. doi: 10.1093/jxb/ers330.

Gamir, J., Sánchez-Bel, P. and Flors, V. (2014) 'Molecular and physiological stages of priming: how plants prepare for environmental challenges', *Plant Cell Reports*. Springer Verlag, pp. 1935–1949. doi: 10.1007/s00299-014-1665-9.

Gao, Y. *et al.* (2020) 'Antagonistic activity against rice blast disease and elicitation of host-defence response capability of an endophytic *Streptomyces albidoflavus* OsiLf-2', *Plant Pathology*. Blackwell Publishing Ltd, 69(2), pp. 259–271. doi: 10.1111/ppa.13118.

García, T., Veloso, J. and Díaz, J. (2018) 'Vanillyl nonanoate induces systemic resistance and lignification in pepper plants', *Journal of Plant Physiology*. Elsevier GmbH, 231, pp. 251–260. doi: 10.1016/j.jplph.2018.10.002.

Gaujoux, R. and Seoighe, C. (2010) 'A flexible R package for nonnegative matrix factorization', *BMC Bioinformatics*. BioMed Central, 11(1), p. 367. doi: 10.1186/1471-2105-11-367.

Gauthier, A. *et al.* (2014) 'The Sulfated Laminarin Triggers a Stress Transcriptome before Priming the SA- and ROS-Dependent Defenses during Grapevine's Induced Resistance against Plasmopara viticola', *PLoS ONE*. Edited by M. Gijzen. Public Library of Science, 9(2), p. e88145. doi: 10.1371/journal.pone.0088145.

Giacomelli, L., Rudella, A. and Van Wijk, K. J. (2006) 'High light response of the thylakoid proteome in Arabidopsis wild type and the ascorbate-deficient mutant vtc2-2. A comparative proteomics study', *Plant Physiology*. American Society of Plant Biologists, 141(2), pp. 685–701. doi: 10.1104/pp.106.080150.

Gil, M. I. *et al.* (2000) 'Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing', *Journal of Agricultural and Food Chemistry*. American Chemical Society , 48(10), pp. 4581–4589. doi: 10.1021/jf000404a.

Gilroy, S. *et al.* (2014) 'A tidal wave of signals: Calcium and ROS at the forefront of rapid systemic signaling', *Trends in Plant Science*. Elsevier Ltd, pp. 623–630. doi: 10.1016/j.tplants.2014.06.013.

Global Industry Analysts Inc. (2020) *Plant Biostimulants - Global Market Trajectory & Analytics.*, *StrategyR*. Available at: https://www.strategyr.com/market-report-plant-biostimulant-forecasts-global-industry-analysts-inc.asp (Accessed: 22 April 2021).

Gomes, D. G. *et al.* (2021) 'Seed priming with copper-loaded chitosan nanoparticles promotes early growth and enzymatic antioxidant defense of maize (*Zea mays* L.) seedlings', *Journal of Chemical Technology & Biotechnology*. John Wiley and Sons Ltd, p. jctb.6738. doi: 10.1002/jctb.6738.

Gómez-García, R. *et al.* (2020) 'Valorization of melon fruit (Cucumis melo L.) by-products: Phytochemical and Biofunctional properties with Emphasis on Recent Trends and Advances', *Trends in Food Science and Technology*. Elsevier Ltd, pp. 507–519. doi: 10.1016/j.tifs.2020.03.033.

González-Bosch, C. (2018) 'Priming plant resistance by activation of redox-sensitive genes', *Free Radical Biology and Medicine*. Elsevier Inc., 122, pp. 171–180. doi: 10.1016/j.freeradbiomed.2017.12.028.

Görlach, J. *et al.* (1996) 'Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat', *Plant Cell*. American Society of Plant Biologists, 8(4), pp. 629–643. doi: 10.1105/tpc.8.4.629.

Groß, F., Durner, J. and Gaupels, F. (2013) 'Nitric oxide, antioxidants and prooxidants in plant defence responses', *Frontiers in Plant Science*. Frontiers Research Foundation, p. 419. doi: 10.3389/fpls.2013.00419.

Grunewald, W. *et al.* (2009) 'Expression of the *Arabidopsis* jasmonate signalling repressor *JAZ1* / *TIFY10A* is stimulated by auxin', *EMBO reports*. John Wiley & Sons, Ltd, 10(8), pp. 923–928. doi: 10.1038/embor.2009.103.

Grzybek, M. *et al.* (2016) 'Evaluation of anthelmintic activity and composition of pumpkin (Cucurbita pepo L.) seed extracts—in vitro and in vivo studies', *International Journal of Molecular Sciences*. MDPI AG, 17(9). doi: 10.3390/ijms17091456.

Haeck, A. *et al.* (2018) 'Trace analysis of multi-class phytohormones in Oryza sativa using different scan modes in high-resolution Orbitrap mass spectrometry: method validation, concentration levels, and screening in multiple accessions', *Analytical and Bioanalytical Chemistry*. Springer Verlag, 410(18), pp. 4527–4539. doi: 10.1007/s00216-018-1112-9.

Häkkinen, S. H. *et al.* (2000) 'Ellagic acid content in berries: Influence of domestic processing and storage', *European Food Research and Technology*. Springer Verlag, 212(1), pp. 75–80. doi: 10.1007/s002170000184.

Han, B. *et al.* (2007) 'Rice functional genomics research in China', *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society, 362(1482), pp. 1009–1021. doi: 10.1098/rstb.2007.2030.

Han, Y., Chaouch, S., *et al.* (2013) 'Functional analysis of arabidopsis mutants points to novel roles for glutathione in coupling H2O2 to activation of salicylic acid accumulation and signaling', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA , 18(16), pp. 2106–

2121. doi: 10.1089/ars.2012.5052.

Han, Y., Mhaldi, A., *et al.* (2013) 'Regulation of basal and oxidative stress-triggered jasmonic acid-related gene expression by glutathione', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 36(6), pp. 1135–1146. doi: 10.1111/pce.12048.

Hasanuzzaman, M. *et al.* (2019) 'Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress', *Antioxidants*. MDPI AG, p. 384. doi: 10.3390/antiox8090384.

Hennessy, D. A. and Roosen, J. (2003) 'A cost-based model of seasonal production with application to milk policy', *Journal of Agricultural Economics*. Agricultural Economics Society, 54(2), pp. 285–312. doi: 10.1111/j.1477-9552.2003.tb00064.x.

Hentrich, M. *et al.* (2013) 'The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of *YUCCA8* and *YUCCA9* gene expression', *The Plant Journal*. John Wiley & Sons, Ltd, 74(4), pp. 626–637. doi: 10.1111/tpj.12152.

Hoagland, D. R. and Arnon, D. I. (1950) *The water-culture method for growing plants without soil.* 2nd edn, *Circular. California Agricultural Experiment Station*. 2nd edn. Berkeley, Calif. : College of Agriculture, University of California.

Hoffmann, D., Vierheilig, H. and Schausberger, P. (2011) 'Mycorrhiza-induced trophic cascade enhances fitness and population growth of an acarine predator', *Oecologia*, 166, pp. 141–149. doi: 10.1007/s00442-010-1821-z.

Holbein, J., Grundler, F. M. W. and Siddique, S. (2016) 'Plant basal resistance to nematodes: an update', *Journal of Experimental Botany*. Oxford Academic, 67(7), pp. 2049–2061. doi: 10.1093/jxb/erw005.

Horemans, N., Asard, M. and Caubergs, R. J. (1997) 'The ascorbate carrier of higher plant plasma membranes preferentially translocates the fully oxidized (dehydroascorbate) molecule', *Plant Physiology*. American Society of Plant Biologists, 114(4), pp. 1247–1253. doi: 10.1104/pp.114.4.1247.

Hu, X. *et al.* (2003) 'Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower', *Plant Physiology*. American Society of Plant Biologists, 133(1), pp. 170–181. doi: 10.1104/pp.103.024026.

Hu, X. *et al.* (2005) 'Nitric oxide mediates gravitropic bending in soybean roots', *Plant Physiology*. American Society of Plant Biologists, 137(2), pp. 663–670. doi: 10.1104/pp.104.054494.

Huang, B. L. *et al.* (2019) 'Transcriptomic analysis of Eruca vesicaria subs. sativa lines with contrasting tolerance to polyethylene glycol-simulated drought stress', *BMC Plant Biology*. BioMed Central Ltd., 19(1), pp. 1–11. doi: 10.1186/s12870-019-1997-2.

Huang, W. K. *et al.* (2016) 'Thiamine-induced priming against root-knot nematode infection in rice involves lignification and hydrogen peroxide generation', *Molecular Plant Pathology*. Blackwell Publishing Ltd, 17(4), pp. 614–624. doi: 10.1111/mpp.12316.

Huang, Y. *et al.* (2019) 'OsNCED5, a 9-cis-epoxycarotenoid dioxygenase gene, regulates salt and water stress tolerance and leaf senescence in rice', *Plant Science*. Elsevier Ireland Ltd, 287, p. 110188. doi: 10.1016/j.plantsci.2019.110188.

Ibrahim, T. A., El-Hefnawy, H. M. and El-Hela, A. A. (2010) 'Antioxidant potential and phenolic acid content of certain cucurbitaceous plants cultivated in Egypt', *Natural Product Research*. Taylor & Francis Group , 24(16), pp. 1537–1545. doi: 10.1080/14786419.2010.489049.

Jakab, G. *et al.* (2001) 'β-aminobutyric acid-induced resistance in plants', *European Journal of Plant Pathology*. Springer, 107(1), pp. 29–37. doi: 10.1023/A:1008730721037.

Jaskiewicz, M., Conrath, U. and Peterhänsel, C. (2011) 'Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response', *EMBO reports*. John Wiley & Sons, Ltd, 12(1), pp. 50–55. doi: 10.1038/embor.2010.186.

Ji, H. *et al.* (2013) 'Transcriptional analysis through RNA sequencing of giant cells induced by Meloidogyne graminicola in rice roots', *Journal of Experimental Botany*. Oxford Academic, 64(12), pp. 3885–3898. doi: 10.1093/jxb/ert219.

Ji, H. *et al.* (2015) 'β-aminobutyric acid-induced resistance against root-knot nematodes in rice is based on increased basal defense', *Molecular Plant-Microbe Interactions*. American Phytopathological Society, 28(5), pp. 519–533. doi: 10.1094/MPMI-09-14-0260-R.

Jian, C. C. *et al.* (2005) 'Cucurbitacins and cucurbitane glycosides: Structures and biological activities', *Natural Product Reports*. Royal Society of Chemistry, pp. 386–399. doi: 10.1039/b418841c.

Jiang, J. *et al.* (2017) 'WRKY transcription factors in plant responses to stresses', *Journal of Integrative Plant Biology*. Blackwell Publishing Ltd, 59(2), pp. 86–101. doi: 10.1111/jipb.12513.

Jiang, K., Meng, Y. L. and Feldman, L. J. (2003) 'Quiescent center formation in maize roots is associated with an auxin-regulated oxidizing environment', *Development*. The Company of Biologists Ltd, pp. 1429–1438. doi: 10.1242/dev.00359.

Jin, H. (2008) 'Endogenous small RNAs and antibacterial immunity in plants', *FEBS Letters*. No longer published by Elsevier, pp. 2679–2684. doi: 10.1016/j.febslet.2008.06.053.

Jones, J. D. G. and Dangl, J. L. (2006) 'The plant immune system', *Nature*. Nature Publishing Group, pp. 323–329. doi: 10.1038/nature05286.

Jones, J. T. *et al.* (2013) 'Top 10 plant-parasitic nematodes in molecular plant pathology', *Molecular Plant Pathology*. John Wiley & Sons, Ltd, 14(9), pp. 946–961. doi: 10.1111/mpp.12057.

Kadota, Y. *et al.* (2014) 'Direct Regulation of the NADPH Oxidase RBOHD by the PRR-Associated Kinase BIK1 during Plant Immunity', *Molecular Cell*. Cell Press, 54(1), pp. 43–55. doi: 10.1016/j.molcel.2014.02.021.

Kalaskar, M. G. and Surana, S. J. (2014) 'Free radical scavenging, immunomodulatory activity and chemical composition of luffa acutangula var. amara (cucurbitaceae) pericarp', *Journal of the Chilean Chemical Society*. Sociedad Chilena de Quimica, 59(1), pp. 2299–2302. doi: 10.4067/s0717-97072014000100012.

Kano, A. *et al.* (2013) 'The rare sugar d-allose acts as a triggering molecule of rice defence via ROS generation', *Journal of Experimental Botany*. Oxford University Press, 64(16), pp. 4939–4951. doi: 10.1093/jxb/ert282.

Kärkönen, A. *et al.* (2017) 'Metabolites of 2,3-diketogulonate delay peroxidase action and induce non-enzymic H2O2 generation: Potential roles in the plant cell wall', *Archives of Biochemistry and Biophysics*. Academic Press Inc., 620, pp. 12–22. doi: 10.1016/j.abb.2017.03.006.

Kende, H., Van Knaap, E. Der and Cho, H. T. (1998) 'Deepwater rice: A model plant to study stem elongation', *Plant Physiology*. American Society of Plant Biologists, 118(4), pp. 1105–1110. doi: 10.1104/pp.118.4.1105.

Kerchev, P. I. *et al.* (2013) 'Vitamin C and the abscisic acid-insensitive 4 transcription factor are important determinants of aphid resistance in arabidopsis', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA , 18(16), pp. 2091–2105. doi: 10.1089/ars.2012.5097.

Kerk, N. M., Jiang, K. and Feldman, L. J. (2000) 'Auxin metabolism in the root apical meristem', *Plant Physiology*. American Society of Plant Biologists, 122(3), pp. 925–932. doi: 10.1104/pp.122.3.925.

De Kesel, J. *et al.* (2020) 'The use of PTI-marker genes to identify novel compounds that establish induced resistance in rice', *International Journal of Molecular Sciences*. MDPI AG, 21(1), p. 317. doi: 10.3390/ijms21010317.

De Kesel, J. *et al.* (2021) 'The Induced Resistance Lexicon: Do's and Don'ts', *Trends in Plant Science*. Elsevier Ltd. doi: 10.1016/j.tplants.2021.01.001.

Key, J. L. (1962) 'Changes in Ascorbic Acid Metabolism Associated With Auxin-Induced Growth', *Plant Physiology*. Oxford University Press (OUP), 37(3), pp. 349–356. doi: 10.1104/pp.37.3.349.

Khan, S. R. (1995) *Calcium Oxalate in Biological Systems*. 1st edn. Edited by S. R. Khan. New York: CRC Press.

Khokon, M. A. R. *et al.* (2010) 'Yeast Elicitor-Induced Stomatal Closure and Peroxidase-Mediated ROS Production in Arabidopsis', *Plant and Cell Physiology*. Oxford Academic, 51(11), pp. 1915–1921. doi: 10.1093/pcp/pcq145.

Kiddle, G. *et al.* (2003) 'Effects of leaf ascorbate content on defense and photosynthesis gene expression in Arabidopsis thaliana', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc. , 5(1), pp. 23–32. doi: 10.1089/152308603321223513.

Kimura, S. *et al.* (2017) 'Bound by fate: The role of reactive oxygen species in receptor-like kinase signaling', *Plant Cell*. American Society of Plant Biologists, pp. 638–654. doi: 10.1105/tpc.16.00947.

Knight, H., Trewavas, A. J. and Knight, M. R. (1997) 'Calcium signalling in Arabidopsis thaliana responding to drought and salinity', *The Plant Journal*. Blackwell Publishing Ltd, 12(5), pp. 1067–1078. doi: 10.1046/j.1365-313X.1997.12051067.x.

Kollist, H. *et al.* (2019) 'Rapid Responses to Abiotic Stress: Priming the Landscape for the Signal Transduction Network', *Trends in Plant Science*. Elsevier Ltd, pp. 25–37. doi: 10.1016/j.tplants.2018.10.003.

Koprivova, A., Mugford, S. T. and Kopriva, S. (2010) 'Arabidopsis root growth dependence on glutathione is linked to auxin transport', *Plant Cell Reports*. Springer, 29(10), pp. 1157–1167. doi: 10.1007/s00299-010-0902-0.

Koshiishi, I. and Imanari, T. (1997) 'Measurement of ascorbate and dehydroascorbate contents in biological fluids', *Analytical Chemistry*. American Chemical Society , 69(2), pp. 216–220. doi: 10.1021/ac960704k.

Krzyzaniak, Y. *et al.* (2018) 'A Plant Extract Acts Both as a Resistance Inducer and an Oomycide Against Grapevine Downy Mildew', *Frontiers in Plant Science*. Frontiers Media S.A., 9, p. 1085. doi: 10.3389/fpls.2018.01085.

Kukawka, R. *et al.* (2018) 'New ionic liquids based on systemic acquired resistance inducers combined with the phytotoxicity reducing cholinium cation', *New Journal of Chemistry*. Royal Society of Chemistry, 42(14), pp. 11984–11990. doi: 10.1039/C8NJ00778K.

Kumar, A. *et al.* (2014) 'Histopathology of the rice root-knot nematode, Meloidogyne graminicola, on Oryza sativa and O. glaberrima', *Nematology*. Brill, 16(1), pp. 73–81. doi: 10.1163/15685411-00002746.

Kusajima, M. *et al.* (2018) 'Involvement of ethylene signaling in Azospirillum sp. B510-induced disease resistance in rice', *Bioscience, Biotechnology, and Biochemistry*. Japan Society for Bioscience Biotechnology and Agrochemistry, 82(9), pp. 1522–1526. doi: 10.1080/09168451.2018.1480350.

Kuźniak, E. *et al.* (2015) 'Salicylic acid and cysteine contribute to arbutin-induced alleviation of angular leaf spot disease development in cucumber', *Journal of Plant Physiology*. Urban und Fischer Verlag GmbH und Co. KG, 181, pp. 9–13. doi: 10.1016/j.jplph.2015.03.017.

Kuźnicki, D. *et al.* (2019) 'BABA-induced DNA methylome adjustment to intergenerational defense priming in potato to Phytophthora infestans', *Frontiers in Plant Science*. Frontiers Media S.A., 10. doi: 10.3389/fpls.2019.00650.

Kwang, J. W. (2004) *International Review of Cytology: A Survey of Cell Biology*. Edited by J. W. Kwang. London: Elsevier Inc. Available at: https://books.google.be/books?hl=nl&lr=&id=11-yw92c13kC&oi=fnd&pg=PA1&dq=leucine+rich+repeat+receptor+kinases+pathogen+type+III&ots=jrRtgdt7S4&sig=yck-WiJd3ZJ3ZipFKckEn2lLYjw#v=onepage&q&f=false (Accessed: 15 April 2021).

Kyndt, T. *et al.* (2012) 'Transcriptional reprogramming by root knot and migratory nematode infection in rice', *New Phytologist*. John Wiley & Sons, Ltd, 196(3), pp. 887–900. doi: 10.1111/j.1469-8137.2012.04311.x.

Kyndt, T. *et al.* (2013) 'Nematode feeding sites: Unique organs in plant roots', *Planta*. Springer, pp. 807–818. doi: 10.1007/s00425-013-1923-z.

Kyndt, T. *et al.* (2020) 'Plant extract for controlling parasitic nematodes'. *Patent WO2021/009164A1*. Belgium: World Intellectual Property Organisation. Available at: https://patents.google.com/patent/WO2021009164A1/en?oq=WO2021%2F009164+A1 (Accessed: 28 April 2021).

Kyndt, T., Fernandez, D. and Gheysen, G. (2014) 'Plant-Parasitic Nematode Infections in Rice: Molecular and Cellular Insights', *Annual Review of Phytopathology*. Annual Reviews Inc., 52(1), pp. 135–153. doi: 10.1146/annurev-phyto-102313-050111.

Lawrence, M. *et al.* (2013) 'Software for Computing and Annotating Genomic Ranges', *PLoS Computational Biology*. Edited by A. Prlic. Public Library of Science, 9(8), p. e1003118. doi: 10.1371/journal.pcbi.1003118.

Lawton, K. A. *et al.* (1996) 'Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway', *The Plant Journal*. Blackwell Publishing Ltd, 10(1), pp. 71–82. doi: 10.1046/j.1365-313X.1996.10010071.x.

Lee, S. C. and Hwang, B. K. (2005) 'Induction of some defense-related genes and oxidative burst is required for the establishment of systemic acquired resistance in Capsicum annuum', *Planta*. Springer, 221(6), pp. 790–800. doi: 10.1007/s00425-005-1488-6.

Lester, G. E. and Crosby, K. M. (2002) 'Ascorbic acid, folic acid, and potassium content in postharvest green-flesh honeydew muskmelons: Influence of cultivar, fruit size, soil type, and year', *Journal of the American Society for Horticultural Science*. American Society for Horticultural Science, 127(5), pp. 843–847. doi: 10.21273/jashs.127.5.843.

Levine, A. *et al.* (1996) 'Calcium-mediated apoptosis in a plant hypersensitive disease resistance response', *Current Biology*. Cell Press, 6(4), pp. 427–437. doi: 10.1016/S0960-9822(02)00510-9.

Li, H. *et al.* (2009) 'The Sequence Alignment/Map format and SAMtools', *Bioinformatics*. Bioinformatics, 25(16), pp. 2078–2079. doi: 10.1093/bioinformatics/btp352.

Li, T. *et al.* (2019) 'β-Aminobutyric Acid Priming Acquisition and Defense Response of Mango Fruit to Colletotrichum gloeosporioides Infection Based on Quantitative Proteomics', *Cells*. NLM (Medline), 8(9), p. 1029. doi: 10.3390/cells8091029.

Li, Y. *et al.* (2019) 'Over-expression of OsPT2 under a rice root specific promoter Os03g01700', *Plant Physiology and Biochemistry*. Elsevier Masson SAS, 136, pp. 52–57. doi: 10.1016/j.plaphy.2019.01.009.

Liszkay, A., Van Der Zalm, E. and Schopfer, P. (2004) 'Production of reactive oxygen intermediates (O2.-, H2O2, and .OH) by maize roots and their role in wall loosening and elongation growth', *Plant Physiology*. American Society of Plant Biologists, 136(2), pp. 3114–3123. doi: 10.1104/pp.104.044784.

Liu, C. J. (2012) 'Deciphering the enigma of lignification: Precursor transport, oxidation, and the topochemistry of lignin assembly', in *Molecular Plant*. Oxford University Press, pp. 304–317. doi: 10.1093/mp/ssr121.

Liu, X. yu, Ou, H. and Gregersen, H. (2020) 'Ultrasound-assisted supercritical CO2 extraction of cucurbitacin E from Iberis amara seeds', *Industrial Crops and Products*. Elsevier B.V., 145, p. 112093. doi: 10.1016/j.indcrop.2020.112093.

Liu, Y. and Zhang, S. (2004) 'Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in arabidopsis', *Plant Cell*. American Society of Plant Biologists, 16(12), pp. 3386–3399. doi: 10.1105/tpc.104.026609.

Lopez, M. G. and Feather, M. S. (1992) 'The production of threose as a degradation product from l-ascorbic acid1', *Journal of Carbohydrate Chemistry*. Taylor & Francis Group, 11(6), pp. 799–806. doi: 10.1080/07328309208020093.

López Sánchez, A. *et al.* (2016) 'The role of DNA (de)methylation in immune responsiveness of Arabidopsis', *The Plant Journal*. Blackwell Publishing Ltd, 88(3), pp. 361–374. doi: 10.1111/tpj.13252.

López Sánchez, A. *et al.* (2021) 'Costs and Benefits of Transgenerational Induced Resistance in Arabidopsis', *Frontiers in Plant Science*. Frontiers Media S.A., 12, p. 644999. doi: 10.3389/fpls.2021.644999.

Lori, M. *et al.* (2015) 'Evolutionary divergence of the plant elicitor peptides (Peps) and their receptors: interfamily incompatibility of perception but compatibility of downstream signalling', *Journal of Experimental Botany*. Oxford University Press, 66(17), pp. 5315–5325. doi: 10.1093/jxb/erv236.

Love, M. I., Huber, W. and Anders, S. (2014) 'Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2', *Genome Biology*. BioMed Central Ltd., 15(12), p. 550. doi: 10.1186/s13059-014-0550-8.

Lu, K. *et al.* (2016) 'Overexpression of an Arabidopsis cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance', *Journal of Experimental Botany*. Oxford University Press, 67(17), pp. 5009–5027. doi: 10.1093/jxb/erw266.

Luc, M., Bridge, J. and Sikora, R. A. (2005) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. 2nd edn. Edited by M. Luc, J. Bridge, and R. A. Sikora. Wallingford: CABI. Available at: https://books.google.be/books?hl=nl&lr=&id=GAdsEt6dEtwC&oi=fnd&pg=PR7&ots=hO8NbPcIAR&sig=NQgUcgAh5kkGRqL97GZsL8WIlOo&redir_esc=y#v=onepage&q=bermann&f=false (Accessed: 13 March 2021).

Luna, E. *et al.* (2012) 'Next-generation systemic acquired resistance', *Plant Physiology*. American Society of Plant Biologists, 158(2), pp. 844–853. doi: 10.1104/pp.111.187468.

Luna, E. and Ton, J. (2012) 'The epigenetic machinery controlling transgenerational systemic acquired resistance', *Plant Signaling & Behavior*. Taylor & Francis, 7(6), pp. 615–618. doi: 10.4161/psb.20155.

Ma, F. and Peterson, C. A. (2003) 'Current insights into the development, structure, and chemistry of the endodermis and exodermis of roots', *Canadian Journal of Botany*. NRC Research Press Ottawa, Canada , 81(5), pp. 405–421. doi: 10.1139/b03-042.

Maietti, A. *et al.* (2012) 'Analytical Traceability of Melon (Cucumis Melo Var Reticulatus): Proximate Composition, Bioactive Compounds, and Antioxidant Capacity in Relation to Cultivar, Plant Physiology State, and Seasonal Variability', *Journal of Food Science*. John Wiley & Sons, Ltd, 77(6), pp. C646–C652. doi: 10.1111/j.1750-3841.2012.02712.x.

Mandal, S. *et al.* (2013) 'Elicitor-induced defense responses in solanum lycopersicum against Ralstonia solanacearum', *The Scientific World Journal*, 2013. doi: 10.1155/2013/561056.

Mangano, S. *et al.* (2017) 'Molecular link between auxin and ROS-mediated polar growth', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 114(20), pp. 5289–5294. doi: 10.1073/pnas.1701536114.

Mantelin, S., Bellafiore, S. and Kyndt, T. (2017) 'Meloidogyne graminicola: a major threat to rice agriculture', *Molecular Plant Pathology*. Blackwell Publishing Ltd, 18(1), pp. 3–15. doi: 10.1111/mpp.12394.

Marcec, M. J. *et al.* (2019) 'Mutual interplay of Ca 2+ and ROS signaling in plant immune response', *Plant Science*. Elsevier Ireland Ltd, pp. 343–354. doi: 10.1016/j.plantsci.2019.03.004.

Marinho, H. S. *et al.* (2014) 'Hydrogen peroxide sensing, signaling and regulation of transcription factors', *Redox Biology*. Elsevier B.V., pp. 535–562. doi: 10.1016/j.redox.2014.02.006.

Marjamaa, K., Kukkola, E. M. and Fagerstedt, K. V. (2009) 'The role of xylem class III peroxidases in lignification', *Journal of Experimental Botany*. Oxford Academic, 60(2), pp. 367–376. doi: 10.1093/jxb/ern278.

Market Data Forecast (2020) *BioPesticides Market | Growth, Trends, and Forecast 2021-2026*. Available at: https://www.marketdataforecast.com/market-reports/bio-pesticide-market (Accessed: 22 May 2021).

Marrè, E. and Arrigoni, O. (1957) 'Metabolic Reactions to Auxin I. The Effects of Auxin on Glutathione and the Effects of Glutathione on Growth of Isolated Plant Parts', *Physiologia Plantarum*, 10(2), pp. 289–301. doi: 10.1111/j.1399-3054.1957.tb06951.x.

Martinez-Medina, A. *et al.* (2016) 'Recognizing Plant Defense Priming', *Trends in Plant Science*. Elsevier Ltd, pp. 818–822. doi: 10.1016/j.tplants.2016.07.009.

Mashela, P. W. *et al.* (2017) 'Alternative nematode management strategies', in *Nematology in South Africa: A View from the 21st Century*. Springer International Publishing, pp. 151–181. doi: 10.1007/978-3-319-44210-5_7.

Mashela, P. W. and Shokoohi, E. (2021) 'Morphometric and total protein responses in Meloidogyne incognita second-stage juveniles to Nemafric-BL phytonematicide', *Scientific Reports*. Nature Research, 11(1), p. 1135. doi: 10.1038/s41598-020-80210-7.

Mata-Pérez, C. and Spoel, S. H. (2019) 'Thioredoxin-mediated redox signalling in plant immunity', *Plant Science*. Elsevier Ireland Ltd, pp. 27–33. doi: 10.1016/j.plantsci.2018.05.001.

Matamoros, M. A. *et al.* (2006) 'Biosynthesis of ascorbic acid in legume root nodules', *Plant Physiology*. American Society of Plant Biologists, 141(3), pp. 1068–1077. doi: 10.1104/pp.106.081463.

Matsumura, T. *et al.* (2002) 'Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress', *Physiologia Plantarum*. John Wiley & Sons, Ltd, 116(3), pp. 317–327. doi: 10.1034/j.1399-3054.2002.1160306.x.

Matzke, M. A. and Mosher, R. A. (2014) 'RNA-directed DNA methylation: An epigenetic pathway of increasing complexity', *Nature Reviews Genetics*. Nature Publishing Group, pp. 394–408. doi: 10.1038/nrg3683.

Mauch-Mani, B. *et al.* (2017) 'Defense Priming: An Adaptive Part of Induced Resistance', *Annual Review of Plant Biology*. Annual Reviews Inc., 68(1), pp. 485–512. doi: 10.1146/annurev-arplant-042916-041132.

De Medeiros, H. A. *et al.* (2017) 'Tomato progeny inherit resistance to the nematode Meloidogyne javanica linked to plant growth induced by the biocontrol fungus Trichoderma atroviride', *Scientific Reports*. Nature Publishing Group, 7(1), pp. 1–13. doi: 10.1038/srep40216.

Melillo, M. T., Leonetti, P. and Veronico, P. (2014) 'Benzothiadiazole effect in the compatible tomato-Meloidogyne incognita interaction: changes in giant cell development and priming of two root anionic peroxidases', *Planta*. Springer Verlag, 240(4), pp. 841–854. doi: 10.1007/s00425-014-2138-7.

Mendy, B. *et al.* (2017) 'Arabidopsis leucine-rich repeat receptor–like kinase NILR1 is required for induction of innate immunity to parasitic nematodes', *PLOS Pathogens*. Edited by D. Mackey. Public Library of Science, 13(4), p. e1006284. doi: 10.1371/journal.ppat.1006284.

Meng, F. *et al.* (2019) 'Molecular Mechanisms of Root Development in Rice', *Rice*. Springer New York LLC, pp. 1–10. doi: 10.1186/s12284-018-0262-x.

Meng, X. and Zhang, S. (2013) 'MAPK cascades in plant disease resistance signaling', *Annual Review of Phytopathology*. Annual Reviews , 51, pp. 245–266. doi: 10.1146/annurev-phyto-082712-102314.

Mhamdi, A. and Van Breusegem, F. (2018) 'Reactive oxygen species in plant development', *Development (Cambridge)*. Company of Biologists Ltd, 145(15). doi: 10.1242/dev.164376.

Miller, G. *et al.* (2009) 'The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli', *Science Signaling*. American Association for the Advancement of Science, 2(84), pp. ra45–ra45. doi: 10.1126/scisignal.2000448.

Mirica, L. M. and Klinman, J. P. (2008) 'The nature of O2 activation by the ethylene-forming enzyme 1-aminocyclopropane-1-carboxylic acid oxidase', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 105(6), pp. 1814–1819. doi: 10.1073/pnas.0711626105.

Mitkowski, N. and Abawi George (2003) 'Reproductive fitness on lettuce of populations of Meloidogyne hapla from New York State vegetable fields', *Nematology*, 5(1), pp. 77–83. Available at: https://brill.com/view/journals/nemy/5/1/article-p77_9.xml (Accessed: 20 March 2020).

Mittler, R. *et al.* (2004) 'Reactive oxygen gene network of plants', *Trends in Plant Science*. Elsevier Current Trends, pp. 490–498. doi: 10.1016/j.tplants.2004.08.009.

Miyazawa, J., Kawabata, T. and Ogasawara, N. (1998) 'Induction of an acidic isozyme of peroxidase and acquired resistance to wilt disease in response to treatment of tomato roots with 2-furoic acid, 4-hydroxybenzoic hydrazide or salicylic hydrazide', *Physiological and Molecular Plant Pathology*. Academic Press, 52(2), pp. 115–126. doi: 10.1006/pmpp.1997.0141.

Mochizuki, S. *et al.* (2020) 'The rare sugar d-tagatose protects plants from downy mildews and is a safe fungicidal agrochemical', *Communications Biology*. Nature Research, 3(1), pp. 1–15. doi: 10.1038/s42003-020-01133-7.

Moghaddam, M. R. B. and Van Den Ende, W. (2012) 'Sugars and plant innate immunity', *Journal of Experimental Botany*. Oxford Academic, pp. 3989–3998. doi: 10.1093/jxb/ers129.

Momma, K. *et al.* (2008) 'Direct interaction of Cucurbitacin E isolated from Alsomitra macrocarpa to actin filament', *Cytotechnology*, 56, pp. 33–39. doi: 10.1007/s10616-007-9100-5.

Morita, Y. and Kyozuka, J. (2007) 'Characterization of OsPID, the Rice Ortholog of PINOID, and its Possible Involvement in the Control of Polar Auxin Transport', *Plant and Cell Physiology*. Oxford Academic, 48(3), pp. 540–549. doi: 10.1093/pcp/pcm024.

Morkunas, I. and Ratajczak, L. (2014) 'The role of sugar signaling in plant defense responses against fungal pathogens', *Acta Physiologiae Plantarum*. Polish Academy of Sciences, pp. 1607–1619. doi: 10.1007/s11738-014-1559-z.

Moroz, N., Huffaker, A. and Tanaka, K. (2017) 'Extracellular Alkalinization Assay for the Detection of Early Defense Response', *Current Protocols in Plant Biology*. Blackwell Publishing Ltd, 2(3), pp. 210–220. doi: 10.1002/cppb.20057.

Mosery, O. and Kanellis, A. K. (1994) 'Ascorbate oxidase of *Cucumis melo* L. var. reticulatus: purification, characterization and antibody production', *Journal of Experimental Botany*. Oxford Academic, 45(6), pp. 717–724. doi: 10.1093/jxb/45.6.717.

Moushib, L. I. *et al.* (2013) 'Sugar beet extract induces defence against Phytophthora infestans in potato plants', *European Journal of Plant Pathology*. Kluwer Academic Publishers, 136(2), pp. 261–271. doi: 10.1007/s10658-012-0160-9.

Mucharromah, E. and Kuc, J. (1991) 'Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber', *Crop Protection*. Elsevier, 10(4), pp. 265–270. doi: 10.1016/0261-2194(91)90004-B.

Müller-Moulé, P., Golan, T. and Niyogi, K. K. (2004) 'Ascorbate-deficient mutants of Arabidopsis grow in high light despite chronic photooxidative stress', *Plant Physiology*. American Society of Plant Biologists, 134(3), pp. 1163–1172. doi: 10.1104/pp.103.032375.

Muthayya, S. *et al.* (2014) 'An overview of global rice production, supply, trade, and consumption', *Ann. N.Y. Acad. Sci*, (1324), pp. 7–14. doi: 10.1111/nyas.12540.

Nagahara, N. (2011) 'Intermolecular disulfide bond to modulate protein function as a redox-sensing switch', *Amino Acids*. Springer, pp. 59–72. doi: 10.1007/s00726-010-0508-4.

Nahar, K. *et al.* (2011) 'The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice', *Plant Physiology*. American Society of Plant Biologists, 157(1), pp. 305–316. doi: 10.1104/pp.111.177576.

Nahar, K. *et al.* (2012) 'Abscisic acid interacts antagonistically with classical defense pathways in rice-migratory nematode interaction', *New Phytologist*. John Wiley & Sons, Ltd, 196(3), pp. 901–913. doi: 10.1111/j.1469-8137.2012.04310.x.

Naseem, M., Kaltdorf, M. and Dandekar, T. (2015) 'The nexus between growth and defence signalling: Auxin and cytokinin modulate plant immune response pathways', in *Journal of Experimental Botany*. Oxford University Press, pp. 4885–4896. doi: 10.1093/jxb/erv297.

Nguyen, D. M. C. *et al.* (2013) 'Nematicidal activity of gallic acid purified from Terminalia nigrovenulosa bark against the root-knot nematode Meloidogyne incognita', *Nematology*. Brill, 15(5), pp. 507–518. doi: 10.1163/15685411-00002696.

Nguyễn, P. V. *et al.* (2014) 'Meloidogyne incognita - rice (Oryza sativa) interaction: a new model system to study plant–root-knot nematode interactions in monocotyledons', *Rice*. Springer New York LLC, 7(1), p. 23. doi: 10.1186/s12284-014-0023-4.

Nicol, J. M. *et al.* (2011) 'Current Nematode Threats to World Agriculture', in *Genomics and Molecular Genetics of Plant-Nematode Interactions*. Springer Netherlands, pp. 21–43. doi: 10.1007/978-94-007-0434-3_2.

Niederhuth, C. E. and Schmitz, R. J. (2014) 'Covering your bases: Inheritance of DNA methylation in plant genomes', *Molecular Plant*. Oxford University Press, pp. 472–480. doi: 10.1093/mp/sst165.

Noctor, G., Reichheld, J. P. and Foyer, C. H. (2018) 'ROS-related redox regulation and signaling in plants', *Seminars in Cell and Developmental Biology*. Elsevier Ltd, pp. 3–12. doi: 10.1016/j.semcdb.2017.07.013.

Ogasawara, Y. *et al.* (2008) 'Synergistic activation of the arabidopsis NADPH oxidase AtrbohD by Ca 2+ and phosphorylation', *Journal of Biological Chemistry*. Elsevier, 283(14), pp. 8885–8892. doi: 10.1074/jbc.M708106200.

Oka, Y. (2020) 'From Old-Generation to Next-Generation Nematicides', *Agronomy*. MDPI AG, 10(9), p. 1387. doi: 10.3390/agronomy10091387.

Okuma, E. *et al.* (2011) 'Negative regulation of abscisic acid-induced stomatal closure by glutathione in Arabidopsis', *Journal of Plant Physiology*. Urban & Fischer, 168(17), pp. 2048–2055. doi: 10.1016/j.jplph.2011.06.002.

Olatunji, D., Geelen, D. and Verstraeten, I. (2017) 'Control of Endogenous Auxin Levels in Plant Root Development', *International Journal of Molecular Sciences*. MDPI AG, 18(12), p. 2587. doi: 10.3390/ijms18122587.

Omokhua-Uyi, A. G. and Van Staden, J. (2020) 'Phytomedicinal relevance of South African Cucurbitaceae species and their safety assessment: A review', *Journal of Ethnopharmacology*. Elsevier Ireland Ltd, p. 112967. doi: 10.1016/j.jep.2020.112967.

Orozco-Cardenas, M. and Ryan, C. A. (1999) 'Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 96(11), pp. 6553–6557. doi: 10.1073/pnas.96.11.6553.

Ortwerth, B. J. *et al.* (1994) 'Ascorbic Acid Glycation: the Reactions of l-Threose in Lens Tissue', *Experimental Eye Research*. Academic Press, 58(6), pp. 665–674. doi: 10.1006/exer.1994.1064.

Ou, S. H. (1985) *Rice Diseases*. 2nd edn. Edited by D. L. Hawksworth. Aberystwyth: CABI. Available at: https://books.google.be/books?hl=nl&lr=&id=-k3mewv9nMoC&oi=fnd&pg=PR1&dq=rice+disease+white+tip+rice+blast+bacterial+blight&ots=ZmiZxABhfg&sig=juDYCU3LeSOzAJsqgL4cUO3NM5U#v=snippet&q=Xanthomonas &f=false (Accessed: 16 April 2021).

Overvoorde, P., Fukaki, H. and Beeckman, T. (2010) 'Auxin control of root development.', *Cold Spring Harbor perspectives in biology*. Cold Spring Harbor Laboratory Press, p. a001537. doi: 10.1101/cshperspect.a001537.

Owino, W. O. *et al.* (2002) 'Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocados', *Journal of the American Society for Horticultural Science*. American Society for Horticultural Science, 127(4), pp. 520–527. doi: 10.21273/jashs.127.4.520.

Papademetriou, M. K., Dent, F. J. and Herath, E. M. (1999) *Bridging the rice yield gap in the asia-pacific region*. Rome. Available at: http://www.fao.org/3/X6905e/x6905e00.htm#Contents (Accessed: 13 March 2021).

Paramalingam, S. (2004) *Modelling, Optimisation and Control of a Falling-Film Evaporator*. Massey University.

Pasternak, T., Palme, K. and Paponov, I. A. (2020) 'Glutathione enhances auxin sensitivity in arabidopsis roots', *Biomolecules*. MDPI AG, 10(11), pp. 1–24. doi: 10.3390/biom10111550.

Pastor, V. *et al.* (2013) 'Fine tuning of reactive oxygen species homeostasis regulates primed immune responses in arabidopsis', *Molecular Plant-Microbe Interactions*. The American Phytopathological Society , 26(11), pp. 1334–1344. doi: 10.1094/MPMI-04-13-0117-R.

Pastor, V. *et al.* (2014) 'Preparing to fight back: Generation and storage of priming compounds', *Frontiers in Plant Science*. Frontiers Research Foundation, 5(JUN). doi: 10.3389/fpls.2014.00295.

Pastrana-Bonilla, E. *et al.* (2003) 'Phenolic content and antioxidant capacity of muscadine grapes', *Journal of Agricultural and Food Chemistry*. American Chemical Society , 51(18), pp. 5497–5503. doi: 10.1021/jf030113c.

Patra, F., Patel, A. and Shah, N. (2017) 'Microbial Production of Low-Calorie Sugars', in *Microbial Production of Food Ingredients and Additives*. Elsevier, pp. 259–290. doi: 10.1016/b978-0-12-811520-6.00009-x.

Paul, P. K. and Sharma, P. D. (2002) 'Azadirachta indica leaf extract induces resistance in barley against leaf stripe disease', *Physiological and Molecular Plant Pathology*. Academic Press, 61(1), pp. 3–13. doi: 10.1006/pmpp.2002.0412.

Pavet, V. *et al.* (2005) 'Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis', *Plant Physiology*. American Society of Plant Biologists, 139(3), pp. 1291–1303. doi: 10.1104/pp.105.067686.

Pavet, V. *et al.* (2006) 'Arabidopsis displays centromeric DNA hypomethylation and cytological alterations of heterochromatin upon attack by Pseudomonas syringae', *Molecular Plant-Microbe Interactions*. The American Phytopathological Society , 19(6), pp. 577–587. doi: 10.1094/MPMI-19-0577.

Peck, S. C. and Kende, H. (1998) 'Differential regulation of genes encoding 1-aminocyclopropane-1-carboxylate (ACC) synthase in etiolated pea seedlings: Effects of indole-3-acetic acid, wounding, and ethylene', *Plant Molecular Biology*. Springer, 38(6), pp. 977–982. doi: 10.1023/A:1006033030081.

Peer, W. A., Cheng, Y. and Murphy, A. S. (2013) 'Evidence of oxidative attenuation of auxin signalling', *Journal of Experimental Botany*. Oxford Academic, 64(9), pp. 2629–2639. doi: 10.1093/jxb/ert152.

Peiffer, M. *et al.* (2009) 'Plants on Early Alert: Glandular Trichomes as Sensors for Insect Herbivores', *The New Phytologist*, 184(3), pp. 644–656. Available at: https://www.jstor.org/stable/27735815?seq=1#metadata_info_tab_contents (Accessed: 30 August 2020).

Perold, G. W., Beylis, P. and Howard, A. S. (1973) 'Metabolites of proteaceae. Part VIII. The occurrence of (+)-D-allose in nature: Rubropilosin and pilorubrosin from protea rubropilosa beard', *Journal of the Chemical Society, Perkin Transactions 1*. The Royal Society of Chemistry, (0), pp. 643–649. doi: 10.1039/P19730000643.

Petrov, V. *et al.* (2015) 'ROS-mediated abiotic stress-induced programmed cell death in plants', *Frontiers in Plant Science*. Frontiers Research Foundation, 6(FEB), p. 69. doi: 10.3389/fpls.2015.00069.

Pieterse, C. M. J. *et al.* (2012) 'Hormonal Modulation of Plant Immunity', *Annual Review of Cell and Developmental Biology*. Annual Reviews , 28(1), pp. 489–521. doi: 10.1146/annurev-cellbio-092910-154055.

Pieterse, C. M. J. (2012) 'Prime time for transgenerational defense', *Plant Physiology*. American Society of Plant Biologists, p. 545. doi: 10.1104/pp.112.900430.

Pignocchi, C. *et al.* (2003) 'The function of ascorbate oxidase in tobacco', *Plant Physiology*. American Society of Plant Biologists, 132(3), pp. 1631–1641. doi: 10.1104/pp.103.022798.

Pignocchi, C. *et al.* (2006) 'Ascorbate oxidase-dependent changes in the redox state of the apoplast modulate gene transcript accumulation leading to modified hormone signaling and orchestration of defense processes in tobacco', *Plant Physiology*. American Society of Plant Biologists, 141(2), pp. 423–435. doi: 10.1104/pp.106.078469.

Pignocchi, C. and Foyer, C. H. (2003) 'Apoplastic ascorbate metabolism and its role in the regulation of cell signalling', *Current Opinion in Plant Biology*. Elsevier Ltd, pp. 379–389. doi: 10.1016/S1369-5266(03)00069-4.

Pohjamo, S. P. *et al.* (2003) 'Phenolic extractives in Salix caprea wood and knots', *Phytochemistry*. Elsevier Ltd, 63(2), pp. 165–169. doi: 10.1016/S0031-9422(03)00050-5.

Pokholok, D. K. *et al.* (2005) 'Genome-wide map of nucleosome acetylation and methylation in yeast', *Cell*. Cell Press, 122(4), pp. 517–527. doi: 10.1016/j.cell.2005.06.026.

Ponzio, C. *et al.* (2016) 'Compatible and incompatible pathogen–plant interactions differentially affect plant volatile emissions and the attraction of parasitoid wasps', *Functional Ecology*. Edited by S. Rasmann. Blackwell Publishing Ltd, 30(11), pp. 1779–1789. doi: 10.1111/1365-2435.12689.

Popko, J. *et al.* (2010) 'The role of abscisic acid and auxin in the response of poplar to abiotic stress', *Plant Biology*. John Wiley & Sons, Ltd, 12(2), pp. 242–258. doi: 10.1111/j.1438-8677.2009.00305.x.

Portillo, M. *et al.* (2013) 'Distinct and conserved transcriptomic changes during nematode-induced giant cell development in tomato compared with Arabidopsis: A functional role for gene repression', *New Phytologist*. John Wiley & Sons, Ltd, 197(4), pp. 1276–1290. doi: 10.1111/nph.12121.

Priyadarsini, K. I. *et al.* (2002) 'Free radical studies of ellagic acid, a natural phenolic antioxidant', *Journal of Agricultural and Food Chemistry*. American Chemical Society , 50(7), pp. 2200–2206. doi: 10.1021/jf011275g.

Prot, J. C. and Matias, D. M. (1995) 'Effects of water regime on the distribution of meloidogyne graminicola and other root-parasitic nematodes in a rice field toposequence and pathogenicity of M. graminicola on rice cultivar upl r15', *Nematologica*. Brill Academic Publishers, 41(1–4), pp. 219–228. doi: 10.1163/003925995X00189.

Puthur, S. *et al.* (2019) 'Synergistic control of storage pest rice weevil using Hypericum japonicum and deltamethrin combinations: a key to combat pesticide resistance', *Environmental Sustainability*. Springer Science and Business Media LLC, 2(4), pp. 411–417. doi: 10.1007/s42398-019-00086-w.

Qi, L. *et al.* (2012) '*Arabidopsis thaliana* plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*', *New Phytologist*. John Wiley & Sons, Ltd, 195(4), pp. 872–882. doi: 10.1111/j.1469-8137.2012.04208.x.

Queval, G. *et al.* (2011) 'Increased intracellular H2O2 availability preferentially drives glutathione accumulation in vacuoles and chloroplasts', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 34(1), pp. 21–32. doi: 10.1111/j.1365-3040.2010.02222.x.

Von Rad, U., Mueller, M. J. and Durner, J. (2005) 'Evaluation of natural and synthetic stimulants of plant immunity by microarray technology', *New Phytologist*. John Wiley & Sons, Ltd, 165(1), pp. 191–202. doi: 10.1111/j.1469-8137.2004.01211.x.

Rahman, M. L. (1990) 'EFFECT OF DIFFERENT CROPPING SEQUENCES ON ROOT .KNOT NEMATODE, MELOIDOGYNE GRAMINICOLA, AND YIELD OF DEEPWATER RICE', *Nematol. medit.*, 18, pp. 213–217.

Rajasree, R. S., Francis, F. and William, H. (2016) 'Phytochemicals of Cucurbitaceae Family-A Review', *International Journal of Pharmacognosy and Phytochemical Research*, 8(1), pp. 113–123. Available at: www.ijppr.com (Accessed: 10 April 2021).

Rasmann, S. *et al.* (2012) 'Herbivory in the previous generation primes plants for enhanced insect resistance', *Plant Physiology*. American Society of Plant Biologists, 158(2), pp. 854–863. doi: 10.1104/pp.111.187831.

Ratiu, I. A. *et al.* (2019) 'Simultaneous Determination of Cyclitols and Sugars Following a Comprehensive Investigation of 40 Plants', *Food Analytical Methods*. Springer New York LLC, 12(6), pp. 1466–1478. doi: 10.1007/s12161-019-01481-z.

Raudvere, U. *et al.* (2019) 'G:Profiler: A web server for functional enrichment analysis and conversions of gene lists (2019 update)', *Nucleic Acids Research*. Oxford University Press, 47(W1), pp. W191–W198. doi: 10.1093/nar/gkz369.

Rawson, A. *et al.* (2013) 'Effect of boiling and roasting on the polyacetylene and polyphenol content of fennel (Foeniculum vulgare) bulb', *Food Research International*. Elsevier, 50(2), pp. 513–518. doi: 10.1016/j.foodres.2011.01.009.

Reuveni, M., Zahavi, T. and Cohen, Y. (2001) 'Controlling Downy Mildew (Plasmopara viticola) in Field-grown Grapevine with/3-Aminobutyric Acid (BABA)', *Phytoparasitica*, 29(2), pp. 125–133.

Ribera, A. E. and Zuñiga, G. (2012) 'Induced plant secondary metabolites for phytopatogenic fungi control: A review', *Journal of Soil Science and Plant Nutrition*. Sociedad Chilena de la Ciencia del Suelo, 12(4), pp. 893–911. doi: 10.4067/s0718-95162012005000040.

Rich, J. R. *et al.* (2009) 'WEED SPECIES AS HOSTS OF MELOIDOGYNE : A REVIEW', *Nematropica*, 39(2), pp. 157–185. Available at: https://journals.flvc.org/nematropica/article/view/64478 (Accessed: 26 August 2020).

del Río, L. A. (2015) 'ROS and RNS in plant physiology: an overview', *Journal of Experimental Botany*. Oxford University Press, 66(10), pp. 2827–2837. doi: 10.1093/jxb/erv099.

Riov, J. and Yang, S. F. (1982) 'Effects of Exogenous Ethylene on Ethylene Production in Citrus Leaf Tissue', *Plant Physiology*. Oxford University Press (OUP), 70(1), pp. 136–141. doi: 10.1104/pp.70.1.136.

Rizvi, T. S. and Fayyaz, S. (2014) 'Nematicidal activity of Citrullus colocynthis extracts against root-knot nematodes Screening of the genetic diversity of wheat germplasm against cyst nematodes View project', *Pakistan Journal of Nematology* , 32(1), pp. 101–112. Available at: https://www.researchgate.net/publication/275037899 (Accessed: 21 April 2021).

Roberts, D. A. (1983) 'Acquired resistance to Tobacco mosaic virus transmitted to the progeny of hypersensitive Tobacco', *Virology*. Academic Press, 124(1), pp. 161–163. doi: 10.1016/0042-6822(83)90299-4.

Rockel, P. *et al.* (2002) 'Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro', *Journal of Experimental Botany*. Oxford University Press (OUP), 53(366), pp. 103–110. doi: 10.1093/jexbot/53.366.103.

Rodrigues, O. *et al.* (2017) 'Aquaporins facilitate hydrogen peroxide entry into guard cells to mediate ABA- and pathogen-triggered stomatal closure', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 114(34), pp. 9200–9205. doi: 10.1073/pnas.1704754114.

Rolim, P. M. *et al.* (2018) 'Phenolic profile and antioxidant activity from peels and seeds of melon (Cucumis melo L. var. reticulatus) and their antiproliferative effect in cancer cells', *Brazilian Journal of Medical and Biological Research*. Associacao Brasileira de Divulgacao Cientifica, 51(4). doi: 10.1590/1414-431x20176069.

Rolim, P. M., Seabra, L. M. J. and de Macedo, G. R. (2020) 'Melon By-Products: Biopotential in Human Health and Food Processing', *Food Reviews International*. Taylor and Francis Inc., pp. 15–38. doi: 10.1080/87559129.2019.1613662.

Roos, G. and Messens, J. (2011) 'Protein sulfenic acid formation: From cellular damage to redox regulation', *Free Radical Biology and Medicine*. Pergamon, pp. 314–326. doi: 10.1016/j.freeradbiomed.2011.04.031.

Roy, S. *et al.* (2016) 'Use of plant extracts for tea pest management in India', *Applied Microbiology and Biotechnology*. Springer Verlag, pp. 4831–4844. doi: 10.1007/s00253-016-7522-8.

Rumer, S., Gupta, K. J. and Kaiser, W. M. (2009) 'Plant cells oxidize hydroxylamines to NO', *Journal of Experimental Botany*. Oxford University Press, 60(7), pp. 2065–2072. doi: 10.1093/jxb/erp077.

Růžička, K. *et al.* (2015) 'Xylem development - from the cradle to the grave', *New Phytologist*. Blackwell Publishing Ltd, 207(3), pp. 519–535. doi: 10.1111/nph.13383.

Ryals, J. A. *et al.* (1996) 'Systemic acquired resistance', *Plant Cell*. American Society of Plant Physiologists, 8(10), pp. 1809–1819. doi: 10.1105/tpc.8.10.1809.

Ryan, C. A. and Pearce, G. (2003) 'Systemins: A functionally defined family of peptide signals that regulate defensive genes in Solanaceae species', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 100(24), pp. 14577–14580. doi: 10.1073/pnas.1934788100.

Sakai, H. *et al.* (2013) 'Rice annotation project database (RAP-DB): An integrative and interactive database for rice genomics', *Plant and Cell Physiology*. Plant Cell Physiol, 54(2). doi: 10.1093/pcp/pcs183.

Sanchez Carranza, A. P. *et al.* (2016) 'Hydrolases of the ILR1-like family of Arabidopsis thaliana modulate auxin response by regulating auxin homeostasis in the endoplasmic reticulum', *Scientific Reports*. Nature Publishing Group, 6(1), pp. 1–11. doi: 10.1038/srep24212.

Sandroni, M. *et al.* (2020) 'Plant resistance inducers (PRIs): perspectives for future disease management in the field', *CAB Reviews*, 15(1). doi: 10.1079/PAVSNNR202015001.

Sanmartin, M. *et al.* (2007) 'Differential expression of the ascorbate oxidase multigene family during fruit development and in response to stress', *Planta*. Springer, 225(4), pp. 873–885. doi: 10.1007/s00425-006-0399-5.

Sasaki, M., Yamamoto, Y. and Matsumoto, H. (1996) 'Lignin deposition induced by aluminum in wheat (Triticum aestivum) roots', *Physiologia Plantarum*. Blackwell Publishing Ltd, 96(2), pp. 193–198. doi: 10.1111/j.1399-3054.1996.tb00201.x.

Sass, R. L. *et al.* (1992) 'Methane emission from rice fields: The effect of floodwater management', *Global Biogeochemical Cycles*. John Wiley & Sons, Ltd, 6(3), pp. 249–262. doi: 10.1029/92GB01674.

Sawamura, M. *et al.* (1994) 'Identification of Two Degradation Products from Aqueous Dehydroascorbic Acid', *Journal of Agricultural and Food Chemistry*. American Chemical Society, 42(5), pp. 1200–1203. doi: 10.1021/jf00041a028.

Schillheim, B. *et al.* (2018) 'Sulforaphane modifies histone H3, unpacks chromatin, and primes defense', *Plant Physiology*. American Society of Plant Biologists, 176(3), pp. 2395–2405. doi: 10.1104/pp.17.00124.

Schwarzenbacher, R. E. *et al.* (2020) 'The IBI1 Receptor of β-Aminobutyric Acid Interacts with VOZ Transcription Factors to Regulate Abscisic Acid Signaling and Callose-Associated Defense', *Molecular Plant*. Cell Press, 13(10), pp. 1455–1469. doi: 10.1016/j.molp.2020.07.010.

Seck, P. A. *et al.* (2012) 'Crops that feed the world 7: Rice', *Food Security*. Springer Science and Business Media LLC, 4(1), pp. 7–24. doi: 10.1007/s12571-012-0168-1.

Seo, S. (2002) *A Review and Comparison of Methods for Detecting Outliers in Univariate Data Sets*. University of Pittsburgh.

Serk, H. *et al.* (2015) 'Cooperative lignification of xylem tracheary elements', *Plant Signaling and Behavior*. Taylor and Francis Inc., 10(4), pp. 1–5. doi: 10.1080/15592324.2014.1003753.

Šernaitė, L. (2017) 'Plant extracts: antimicrobial and antifungal activity and appliance in plant protection (Review)', *Sodininkystės ir daržininkystė*, 36(3–4), pp. 58–68.

Sharma, R. *et al.* (2013) 'Recent advances in dissecting stress-regulatory crosstalk in rice', *Molecular Plant*. Oxford University Press, pp. 250–260. doi: 10.1093/mp/sss147.

Shi, Y. *et al.* (2020) 'OsRbohB-mediated ROS production plays a crucial role in drought stress tolerance of rice', *Plant Cell Reports*. Springer Science and Business Media Deutschland GmbH, 39(12), pp. 1767–1784. doi: 10.1007/s00299-020-02603-2.

Shimamoto, K. and Kyozuka, J. (2002) 'Rice as a model for comparative genomics of plants', *Annual Review of Plant Biology*. Annual Reviews Inc., pp. 399–419. doi: 10.1146/annurev.arplant.53.092401.134447.

Shodehinde, S. A. *et al.* (2016) 'Phenolic Composition and Evaluation of Methanol and Aqueous Extracts of Bitter Gourd (Momordica charantia L) Leaves on Angiotensin-I-Converting Enzyme and Some Pro-oxidant-Induced Lipid Peroxidation In Vitro.', *Journal of Evidence-Based Complementary & Alternative Medicine*. SAGE Publications Ltd, 21(4), pp. NP67–NP76. doi: 10.1177/2156587216636505.

Shoresh, M. *et al.* (2006) 'Characterization of a mitogen-activated protein kinase gene from cucumber required for trichoderma-conferred plant resistance', *Plant Physiology*. American Society of Plant Biologists, 142(3), pp. 1169–1179. doi: 10.1104/pp.106.082107.

Shukla, N. *et al.* (2017) 'Transcriptome analysis of root-knot nematode (Meloidogyne incognita)-infected tomato (Solanum lycopersicum) roots reveals complex gene expression profiles and metabolic networks of both host and nematode during susceptible and resistance responses', *Molecular Plant Pathology*. John Wiley & Sons, Ltd, 19(3), pp. 615–633. doi: 10.1111/mpp.12547.

Siddique, S. *et al.* (2014) 'Myo-inositol oxygenase is important for the removal of excess myo-inositol from syncytia induced by Heterodera schachtii in Arabidopsis roots', *New Phytologist*. John Wiley & Sons, Ltd, 201(2), pp. 476–485. doi: 10.1111/nph.12535.

Simpson, G. L. W. and Ortwerth, B. J. (2000) 'The non-oxidative degradation of ascorbic acid at physiological conditions', *Biochimica et Biophysica Acta - Molecular Basis of Disease*. Elsevier, 1501(1), pp. 12–24. doi: 10.1016/S0925-4439(00)00009-0.

Singh, A., Gupta, R. and Pandey, R. (2017) 'Exogenous application of rutin and gallic acid regulate antioxidants and alleviate reactive oxygen generation in Oryza sativa L.', *Physiology and Molecular Biology of Plants*, 23(2), pp. 301–309. doi: 10.1007/s12298-017-0430-2.

Singh, P. and Roberts, M. R. (2015) 'Keeping it in the family: Transgenerational memories of plant defence', *CAB Reviews*, 10(26), pp. 1–6.

Singh, R. R., Nobleza, N., *et al.* (2020) 'Ascorbate Oxidase Induces Systemic Resistance in Sugar Beet Against Cyst Nematode Heterodera schachtii', *Frontiers in Plant Science*. Frontiers Media S.A., 11. doi: 10.3389/fpls.2020.591715.

Singh, R. R., Verstraeten, B., *et al.* (2020) 'Ascorbate oxidation activates systemic defence against root-knot nematode Meloidogyne graminicola in rice', *Journal of Experimental Botany*. Oxford University Press, 71(14), pp. 4271–4284. doi: 10.1093/jxb/eraa171.

Singh, U. B. *et al.* (2019) 'Trichoderma harzianum-and methyl jasmonate-induced resistance to bipolaris sorokiniana through enhanced phenylpropanoid activities in bread wheat (Triticum aestivum L.)', *Frontiers in Microbiology*. Frontiers Media S.A., 10, p. 1697. doi: 10.3389/fmicb.2019.01697.

Slaughter, A. *et al.* (2012) 'Descendants of primed Arabidopsis plants exhibit resistance to biotic stress', *Plant Physiology*. American Society of Plant Biologists, 158(2), pp. 835–843. doi: 10.1104/pp.111.191593.

Slight, S. H., Feather, M. S. and Ortwerth, B. J. (1990) 'Glycation of lens proteins by the oxidation products of ascorbic acid', *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*. Elsevier, 1038(3), pp. 367–374. doi: 10.1016/0167-4838(90)90250-J.

Slusarenko, A. J., Fraser, R. S. and van Loon, L. C. (2012) *Mechanisms of Resistance to Plant Diseases*. Edited by A. J. Slusarenko, R. S. Fraser, and L. C. van Loon. Berlin: Springer Science & Business Media. Available at: https://books.google.be/books?hl=nl&lr=&id=Lo1qCQAAQBAJ&oi=fnd&pg=PA324&dq=%22phytoanticipins+are+found+in+many+different+plant+families+and+chemical+classes%22&ots=-dw3HsUg_k&sig=7WzwaEeWZaeJI5oKK1mdcTw325w#v=onepage&q=%22phytoanticipins are found in many different plant families and chemical classes%22&f=false (Accessed: 8 May 2021).

De Smet, I. *et al.* (2006) 'A novel role for abscisic acid emerges from underground', *Trends in Plant Science*. Elsevier Current Trends, pp. 434–439. doi: 10.1016/j.tplants.2006.07.003.

Smirnoff, N. (2000) 'Ascorbic acid: metabolism and functions of a multi-facetted molecule', *Current Opinion in Plant Biology*. Elsevier BV, 3(3), pp. 229–235. doi: 10.1016/s1369-5266(00)80070-9.

Smirnoff, N. and Arnaud, D. (2019) 'Hydrogen peroxide metabolism and functions in plants', *New Phytologist*. Blackwell Publishing Ltd, 221(3), pp. 1197–1214. doi: 10.1111/nph.15488.

Smirnoff, N. and Wheeler, G. L. (2000) 'Ascorbic acid in plants: Biosynthesis and function', *Critical Reviews in Plant Sciences*. Taylor & Francis Group , 19(4), pp. 267–290. doi: 10.1080/07352680091139231.

Smit, F. and Dubery, I. A. (1997) 'Cell wall reinforcement in cotton hypocotyls in response to a Verticillium dahliae elicitor', *Phytochemistry*. Pergamon, 44(5), pp. 811–815. doi: 10.1016/S0031-9422(96)00595-X.

Song, Y. *et al.* (2015) 'Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus', *Frontiers in Plant Science*. Frontiers Research Foundation, 6(September), p. 786. doi: 10.3389/fpls.2015.00786.

Song, Y. Y. *et al.* (2013) 'Priming of Anti-Herbivore Defense in Tomato by Arbuscular Mycorrhizal Fungus and Involvement of the Jasmonate Pathway', *Journal of Chemical Ecology*. Springer, 39(7), pp. 1036–1044. doi: 10.1007/s10886-013-0312-1.

Sopelana, P. *et al.* (2013) 'Effect of ultra high temperature (UHT) treatment on coffee brew stability', *Food Research International*. Elsevier, 50(2), pp. 682–690. doi: 10.1016/j.foodres.2011.07.038.

Soriano, I. R. and Reversat, G. (2003) 'Management of Meloidogyne graminicola and yield of upland rice in South-Luzon, Philippines', *Nematology*. Brill, 5(6), pp. 879–884. doi: 10.1163/156854103773040781.

Speeckaert, N. *et al.* (2020) 'Characterization of the udp-glycosyltransferase ugt72 family in poplar and identification of genes involved in the glycosylation of monolignols', *International Journal of Molecular Sciences*. MDPI AG, 21(14), pp. 1–24. doi: 10.3390/ijms21145018.

Sripinyowanich, S. *et al.* (2013) 'Exogenous ABA induces salt tolerance in indica rice (Oryza sativa L.): The role of OsP5CS1 and OsP5CR gene expression during salt stress', *Environmental and Experimental Botany*. Elsevier, 86, pp. 94–105. doi: 10.1016/j.envexpbot.2010.01.009.

Stassen, J. H. M. *et al.* (2018) 'The relationship between transgenerational acquired resistance and global DNA methylation in Arabidopsis', *Scientific Reports*. Nature Publishing Group, 8(1), p. 14761. doi: 10.1038/s41598-018-32448-5.

Stevens, R. *et al.* (2018) 'Ascorbate oxidase in plant growth, Development, and stress tolerance', in *Ascorbic Acid in Plant Growth, Development and Stress Tolerance*. Springer International Publishing, pp. 273–295. doi: 10.1007/978-3-319-74057-7_11.

Sultana, R., Nahar, K. and Bachar, S. C. (2018) 'In-vitro membrane stabilizing, thrombolytic, antioxidant and antimicrobial activities of Bangladeshi origin Coccinia indica (Cucurbitaceae)', *African Journal of Pharmacy and Pharmacology*, 12(16), pp. 188–192. doi: 10.5897/AJPP2018.4913.

Sun, J. *et al.* (2009) 'Arabidopsis ASA1 is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation', *Plant Cell*. American Society of Plant Biologists, 21(5), pp. 1495–1511. doi: 10.1105/tpc.108.064303.

Suzuki, N. *et al.* (2012) 'ROS and redox signalling in the response of plants to abiotic stress', *Plant, Cell & Environment*. John Wiley & Sons, Ltd, 35(2), pp. 259–270. doi: 10.1111/j.1365-3040.2011.02336.x.

Suzuki, N. *et al.* (2013) 'Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants', *Plant Cell*. American Society of Plant Biologists, 25(9), pp. 3553–3569. doi: 10.1105/tpc.113.114595.

Tada, Y. *et al.* (2008) 'Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins', *Science*. American Association for the Advancement of Science, 321(5891), pp. 952–956. doi: 10.1126/science.1156970.

Taheri, P. and Tarighi, S. (2010) 'Riboflavin induces resistance in rice against Rhizoctonia solani via jasmonate-mediated priming of phenylpropanoid pathway', *Journal of Plant Physiology*. Urban & Fischer, 167(3), pp. 201–208. doi: 10.1016/j.jplph.2009.08.003.

Tamaoki, D. *et al.* (2006) 'Effects of hypergravity conditions on elongation growth and lignin formation in the inflorescence stem of Arabidopsis thaliana', *Journal of Plant Research*. Springer, 119(2), pp. 79–84. doi: 10.1007/s10265-005-0243-1.

Tanaka, K. and Heil, M. (2021) 'Damage-Associated Molecular Patterns (DAMPs) in Plant Innate Immunity: Applying the Danger Model and Evolutionary Perspectives', *Annual Review of Phytopathology*, 59(3), pp. 1–23. doi: 10.1146/annurev-phyto-082718.

Tanaka, Y. (2006) 'Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in Arabidopsis', *Journal of Experimental Botany*. Oxford Academic, 57(10), pp. 2259–2266. doi: 10.1093/jxb/erj193.

Tannin-Spitz, T. *et al.* (2007) 'Growth inhibitory activity of cucurbitacin glucosides isolated from Citrullus colocynthis on human breast cancer cells', *Biochemical Pharmacology*. Elsevier, 73(1), pp. 56–67. doi: 10.1016/j.bcp.2006.09.012.

Tannin-Spitz, T., Bergman, M. and Grossman, S. (2007) 'Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities', *Biochemical and Biophysical Research Communications*. Academic Press, 364(1), pp. 181–186. doi: 10.1016/j.bbrc.2007.09.075.

Tao, J. J. *et al.* (2015) 'Tobacco translationally controlled tumor protein interacts with ethylene receptor tobacco histidine kinase1 and enhances plant growth through promotion of cell proliferation', *Plant Physiology*. American Society of Plant Biologists, 169(1), pp. 96–114. doi: 10.1104/pp.15.00355.

Tateda, C. *et al.* (2014) 'Salicylic acid regulates Arabidopsis microbial pattern receptor kinase levels and signaling', *Plant Cell*. American Society of Plant Biologists, 26(10), pp. 4171–4187. doi: 10.1105/tpc.114.131938.

Tayeh, C. *et al.* (2014) 'Exogenous trehalose induces defenses in wheat before and during a biotic stress caused by powdery mildew', *Phytopathology*. The American Phytopathological Society , 104(3), pp. 293–305. doi: 10.1094/PHYTO-07-13-0191-R.

Terrile, M. C. *et al.* (2012) 'Nitric oxide influences auxin signaling through S-nitrosylation of the Arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor', *The Plant Journal*. John Wiley & Sons, Ltd, 70(3), pp. 492–500. doi: 10.1111/j.1365-313X.2011.04885.x.

Tian, S. *et al.* (2016) 'Plant aquaporin AtPIP1;4 links apoplastic H2O2 induction to disease immunity pathways', *Plant Physiology*. American Society of Plant Biologists, 171(3), pp. 1635–1650. doi: 10.1104/pp.15.01237.

TNAU (2016) *Cereals :: Rice :: Aerobic*. Available at: https://agritech.tnau.ac.in/agriculture/agri_cropproduction_cereals_rice_aerobic_weed_mgmnt.html (Accessed: 23 March 2021).

Tobias, C. M. and Chow, E. K. (2005) 'Structure of the cinnamyl-alcohol dehydrogenase gene family in rice and promoter activity of a member associated with lignification', *Planta*. Springer, 220(5), pp. 678–688. doi: 10.1007/s00425-004-1385-4.

Tomás-Barberán, F. A. and Clifford, M. N. (2000) 'Dietary hydroxybenzoic acid derivatives-nature, occurrence and dietary burden', *Journal of the Science of Food and Agriculture*, 80(7), pp. 1024–1032.

Ton, J., Flors, V. and Mauch-Mani, B. (2009) 'The multifaceted role of ABA in disease resistance', *Trends in Plant Science*. Elsevier Current Trends, 14(6), pp. 310–317. doi: 10.1016/j.tplants.2009.03.006.

Torres, M. A., Jones, J. D. G. and Dangl, J. L. (2006) 'Reactive oxygen species signaling in response to pathogens', *Plant Physiology*. American Society of Plant Biologists, pp. 373–378. doi: 10.1104/pp.106.079467.

Trdá, L. *et al.* (2019) 'Dual Mode of the Saponin Aescin in Plant Protection: Antifungal Agent and Plant Defense Elicitor', *Frontiers in Plant Science*. Frontiers Media S.A., 10, p. 1448. doi: 10.3389/fpls.2019.01448.

Triantaphyllou, A. C. (1969) 'Gametogenesis and the Chromosomes of Two Root-knot Nematodes, Meloidogyne graminicola and M. naasi.', *Journal of nematology*. Society of Nematologists, 1(1), pp. 62–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19325656 (Accessed: 26 August 2020).

Truffault, V. *et al.* (2014) 'Variation in tomato fruit ascorbate levels and consequences of manipulation of ascorbate metabolism on drought stress tolerance', *Acta Horticulturae*. International Society for Horticultural Science, 1048(1), pp. 75–84. doi: 10.17660/ActaHortic.2014.1048.8.

Tupe, S. B. *et al.* (2013) 'PHYTOCHEMICAL SCREENING IN SOME CUCURBITACEAE MEMBERS', *International Research Journal of Pharmaceutical and Applied Sciences*, 3(1), pp. 49–51. Available at: https://scienztech.org/irjpas/article/view/396/324 (Accessed: 10 April 2021).

Turnbull, J. J. *et al.* (2004) 'Mechanistic studies on three 2-oxoglutarate-dependent oxygenases of flavonoid biosynthesis: Anthocyanidin synthase, flavonol synthase, and flavanone 3β-hydroxylase', *Journal of Biological Chemistry*. Elsevier, 279(2), pp. 1206–1216. doi: 10.1074/jbc.M309228200.

Underwood, W. (2012) 'The Plant Cell Wall: A Dynamic Barrier Against Pathogen Invasion', *Frontiers in Plant Science*. Frontiers Research Foundation, 3(MAY), p. 85. doi: 10.3389/fpls.2012.00085.

Usadel, B. *et al.* (2005) 'Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of coresponding genes, and comparison with known responses', *Plant Physiology*. Plant Physiol, pp. 1195–1204. doi: 10.1104/pp.105.060459.

UTZ (2015) *LIST OF BANNED PESTICIDES AND PESTICIDES WATCHLIST*. Amsterdam. Available at: www.utz.org/resource-library.org (Accessed: 22 November 2020).

Väisänen, E. E. *et al.* (2015) 'Coniferyl alcohol hinders the growth of tobacco BY-2 cells and Nicotiana benthamiana seedlings', *Planta*. Springer Verlag, 242(3), pp. 747–760. doi: 10.1007/s00425-015-2348-7.

Valderrama, R. *et al.* (2007) 'Nitrosative stress in plants', *FEBS Letters*. No longer published by Elsevier, 581(3), pp. 453–461. doi: 10.1016/j.febslet.2007.01.006.

Vandenbussche, F. *et al.* (2012) 'Ethylene in vegetative development: A tale with a riddle', *New Phytologist*. John Wiley & Sons, Ltd, pp. 895–909. doi: 10.1111/j.1469-8137.2012.04100.x.

Ventura, W. *et al.* (1981) 'Involvement of nematodes in the soil sickness of a dryland rice-based cropping system', *Soil Science and Plant Nutrition*. Taylor & Francis Group , 27(3), pp. 305–315. doi: 10.1080/00380768.1981.10431285.

Veronico, P. *et al.* (2018) 'Changes in lignin biosynthesis and monomer composition in response to benzothiadiazole and root-knot nematode Meloidogyne incognita infection in tomato', *Journal of Plant Physiology*. Elsevier GmbH, 230, pp. 40–50. doi: 10.1016/j.jplph.2018.07.013.

de Vleesschauwer, D. *et al.* (2010) 'Abscisic acid-induced resistance against the brown spot pathogen Cochliobolus miyabeanus in rice involves MAP kinase-mediated repression of ethylene signaling', *Plant Physiology*. American Society of Plant Biologists, 152(4), pp. 2036–2052. doi: 10.1104/pp.109.152702.

De Vleesschauwer, D., Gheysen, G. and Höfte, M. (2013) 'Hormone defense networking in rice: Tales from a different world', *Trends in Plant Science*. Elsevier Current Trends, pp. 555–565. doi: 10.1016/j.tplants.2013.07.002.

Vogel-Adghough, D. *et al.* (2013) 'Pipecolic acid enhances resistance to bacterial infection and primes salicylic acid and nicotine accumulation in tobacco', *Plant Signaling & Behavior*. Taylor & Francis, 8(11), p. e26366. doi: 10.4161/psb.26366.

Vos, C. M. *et al.* (2012) 'Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode Meloidogyne incognita and the migratory nematode Pratylenchus penetrans', *Applied Soil Ecology*. Elsevier, 61, pp. 1–6. doi: 10.1016/j.apsoil.2012.04.007.

Wada, S., Cui, S. and Yoshida, S. (2019) 'Reactive Oxygen Species (ROS) Generation Is Indispensable for Haustorium Formation of the Root Parasitic Plant Striga hermonthica', *Frontiers in Plant Science*. Frontiers Media S.A., 10, p. 328. doi: 10.3389/fpls.2019.00328.

De Waele, D. and Elsen, A. (2007) 'Challenges in Tropical Plant Nematology', *Annual Review of Phytopathology*. Annual Reviews, 45(1), pp. 457–485. doi: 10.1146/annurev.phyto.45.062806.094438.

Walters, D. R., Havis, N. D., Paterson, L., *et al.* (2011) 'Cultivar effects on the expression of induced resistance in spring barley', *Plant Disease*. The American Phytopathological Society , 95(5), pp. 595–600. doi: 10.1094/PDIS-08-10-0577.

Walters, D. R., Havis, N. D., Sablou, C., *et al.* (2011) 'Possible trade-off associated with the use of a combination of resistance elicitors', *Physiological and Molecular Plant Pathology*. Academic Press, 75(4), pp. 188–192. doi: 10.1016/j.pmpp.2011.02.001.

Walters, D. R. and Fountaine, J. M. (2009) 'Practical application of induced resistance to plant diseases : an appraisal of effectiveness under field conditions', *Journal of Agricultural Science*, 147, pp. 523–535. doi: 10.1017/S0021859609008806.

Walters, D. R. and Paterson, L. (2012) 'Parents lend a helping hand to their offspring in plant defence', *Biology Letters*. Royal Society, 8(5), pp. 871–873. doi: 10.1098/rsbl.2012.0416.

Wang, G. *et al.* (2019) 'Systemic Root-Shoot Signaling Drives Jasmonate-Based Root Defense against Nematodes', *Current Biology*. Cell Press, 29(20), pp. 3430-3438.e4. doi: 10.1016/j.cub.2019.08.049.

Wang, Y. *et al.* (2013) 'Plant cell wall lignification and monolignol metabolism', *Frontiers in Plant Science*. Frontiers Research Foundation, p. 220. doi: 10.3389/fpls.2013.00220.

Wang, Y. *et al.* (2014) 'Transcriptome analysis of early responsive genes in rice during Magnaporthe oryzae infection', *Plant Pathology Journal*. Korean Society of Plant Pathology, 30(4), pp. 343–354. doi: 10.5423/PPJ.OA.06.2014.0055.

Wasternack, C. and Feussner, I. (2018) 'The Oxylipin Pathways: Biochemistry and Function', *Annual Review of Plant Biology*. Annual Reviews Inc., pp. 363–386. doi: 10.1146/annurev-arplant-042817-040440.

Weijers, D. and Wagner, D. (2016) 'Transcriptional Responses to the Auxin Hormone', *Annual Review of Plant Biology*. Annual Reviews Inc., 67(1), pp. 539–574. doi: 10.1146/annurev-arplant-043015-112122.

Wen (2011) *Rice Paddy Fields @ Ubud, Bali*. Available at: https://wensdelight.blogspot.com/2012/08/rice-paddy-fields-ubud-bali.html (Accessed: 23 March 2021).

Wesemael, W., Viaene, N. and Moens, M. (2011) 'Root-knot nematodes (Meloidogyne spp.) in Europe', *Nematology*, 13(1), pp. 3–16.

Wilkinson, S. W. *et al.* (2019) 'Surviving in a Hostile World: Plant Strategies to Resist Pests and Diseases', *Annual Review of Phytopathology*. Annual Reviews Inc., pp. 505–529. doi: 10.1146/annurev-phyto-082718-095959.

Win, P. P. *et al.* (2013) 'Population dynamics of Meloidogyne graminicola and Hirschmanniella oryzae in a double rice-cropping sequence in the lowlands of Myanmar', *Nematology*. Brill, 15(7), pp. 795–807. doi: 10.1163/15685411-00002719.

Windram, O. *et al.* (2012) 'Arabidopsis defense against Botrytis cinerea: Chronology and regulation deciphered by high-resolution temporal transcriptomic analysis', *Plant Cell*. Oxford Academic, 24(9), pp. 3530–3557. doi: 10.1105/tpc.112.102046.

Wolucka, B. A., Goossens, A. and Inzé, D. (2005) 'Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions', *Journal of Experimental Botany*. Oxford Academic, 56(419), pp. 2527–2538. doi: 10.1093/jxb/eri246.

Wong, H. L. *et al.* (2007) 'Regulation of rice NADPH oxidase by binding of Rac GTPase to its N-terminal extension', *Plant Cell*. American Society of Plant Biologists, 19(12), pp. 4022–4034. doi: 10.1105/tpc.107.055624.

Wuyts, N. (2006) 'Nematode infection and reproduction in transgenic and mutant Arabidopsis and tobacco with an altered phenylpropanoid metabolism', *Journal of Experimental Botany*. Oxford Academic, 57(11), pp. 2825–2835. doi: 10.1093/jxb/erl044.

Yang, D. L., Yang, Y. and He, Z. (2013) 'Roles of plant hormones and their interplay in rice immunity', *Molecular Plant*. Oxford University Press, pp. 675–685. doi: 10.1093/mp/sst056.

Yassin, M. *et al.* (2021) 'The rise, fall and resurrection of chemical-induced resistance agents', *Pest Management Science*. John Wiley and Sons Ltd, p. ps.6370. doi: 10.1002/ps.6370.

Yen, G. C., Duh, P. Der and Tsai, H. L. (2002) 'Antioxidant and pro-oxidant properties

of ascorbic acid and gallic acid', *Food Chemistry*. Elsevier, 79(3), pp. 307–313. doi: 10.1016/S0308-8146(02)00145-0.

Yoda, H., Hiroi, Y. and Sano, H. (2006) 'Polyamine oxidase is one of the key elements for oxidative burst to induce programmed cell death in tobacco cultured cells', *Plant Physiology*. American Society of Plant Biologists, 142(1), pp. 193–206. doi: 10.1104/pp.106.080515.

Yoo, S. H. *et al.* (2014) 'Estimating water footprint of paddy rice in Korea', *Paddy and Water Environment*. Springer Verlag, 12(1), pp. 43–54. doi: 10.1007/s10333-013-0358-2.

Yoon, M. Y., Cha, B. and Kim, J. C. (2013) 'Recent trends in studies on botanical fungicides in agriculture', *Plant Pathology Journal*. The Korean Society of Plant Pathology, 29(1), pp. 1–9. doi: 10.5423/PPJ.RW.05.2012.0072.

Yoshioka, K. *et al.* (2008) 'Probenazole induces systemic acquired resistance in Arabidopsis with a novel type of action', *The Plant Journal*. Wiley, 25(2), pp. 149–157. doi: 10.1111/j.1365-313X.2001.00952.x.

Yu, A. *et al.* (2013) 'Dynamics and biological relevance of DNA demethylation in Arabidopsis antibacterial defense', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 110(6), pp. 2389–2394. doi: 10.1073/pnas.1211757110.

Yu, X. *et al.* (2013) 'Plastid-localized glutathione reductase2-regulated glutathione redox status is essential for Arabidopsis root apical meristem maintenance', *Plant Cell*. American Society of Plant Biologists, 25(11), pp. 4451–4468. doi: 10.1105/tpc.113.117028.

Yu, Y. *et al.* (2019) 'Ascorbic acid integrates the antagonistic modulation of ethylene and abscisic acid in the accumulation of reactive oxygen species', *Plant Physiology*. American Society of Plant Biologists, 179(4), pp. 1861–1875. doi: 10.1104/pp.18.01250.

Yuan, R. Q. *et al.* (2019) 'Cucurbitacins extracted from Cucumis melo L. (CuEC) exert a hypotensive effect via regulating vascular tone', *Hypertension Research*. Nature Publishing Group, 42(8), pp. 1152–1161. doi: 10.1038/s41440-019-0258-y.

Zacheo, G. *et al.* (1995) 'The association between heat-induced susceptibility of tomato to Meloidogyne incognita and peroxidase activity', *Physiological and Molecular Plant Pathology*. Academic Press, 46(6), pp. 491–507. doi: 10.1006/pmpp.1995.1037.

Zaffagnini, M. *et al.* (2007) 'The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3-phosphate dehydrogenase is selectively regulated by glutathionylation', *FEBS Journal*. John Wiley & Sons, Ltd, 274(1), pp. 212–226. doi: 10.1111/j.1742-4658.2006.05577.x.

Zaker, M. (2016) 'Natural Plant Products as Eco-friendly Fungicides for Plant Diseases Control- A Review', *The Agriculturists*. Bangladesh Journals Online (JOL), 14(1), pp. 134–141. doi: 10.3329/agric.v14i1.29111.

Zandalinas, S. I. *et al.* (2019) 'Identification and characterization of a core set of ROS wave-associated transcripts involved in the systemic acquired acclimation response of Arabidopsis to excess light', *The Plant Journal*. Blackwell Publishing Ltd, 98(1), pp. 126–141. doi: 10.1111/tpj.14205.

Zandalinas, S. I. and Mittler, R. (2018) 'ROS-induced ROS release in plant and animal cells', *Free Radical Biology and Medicine*. Elsevier Inc., pp. 21–27. doi: 10.1016/j.freeradbiomed.2017.11.028.

Zehra, A. *et al.* (2017) 'Activation of defense response in tomato against Fusarium wilt disease triggered by Trichoderma harzianum supplemented with exogenous chemical inducers (SA and MeJA)', *Revista Brasileira de Botanica*. Springer International Publishing, 40(3), pp. 651–664. doi: 10.1007/s40415-017-0382-3.

Zhang, H. *et al.* (2019) 'Suppression of auxin signalling promotes rice susceptibility to *Rice black streaked dwarf virus* infection', *Molecular Plant Pathology*. Blackwell Publishing Ltd, 20(8), pp. 1093–1104. doi: 10.1111/mpp.12814.

Zhang, K. *et al.* (2021) 'Genome-Wide Characterization of HSP90 Gene Family in Cucumber and Their Potential Roles in Response to Abiotic and Biotic Stresses', *Frontiers in Genetics*. Frontiers Media S.A., 12, p. 584886. doi: 10.3389/fgene.2021.584886.

Zhang, L., Du, L. and Poovaiah, B. W. (2014) 'Calcium signaling and biotic defense responses in plants', *Plant Signaling and Behavior*. Landes Bioscience, 9(11). doi: 10.4161/15592324.2014.973818.

Zhang, M. *et al.* (2018) 'The MAP4 Kinase SIK1 Ensures Robust Extracellular ROS Burst and Antibacterial Immunity in Plants', *Cell Host and Microbe*. Cell Press, 24(3), pp. 379-391.e5. doi: 10.1016/j.chom.2018.08.007.

Zhang, X. *et al.* (2006) 'Genome-wide High-Resolution Mapping and Functional Analysis of DNA Methylation in Arabidopsis', *Cell*. Cell Press, 126(6), pp. 1189–1201. doi: 10.1016/j.cell.2006.08.003.

Zheng, Q. L., Nakatsuka, A. and Itamura, H. (2006) 'Involvement of negative feedback regulation in wound-induced ethylene synthesis in "Saijo" persimmon', *Journal of Agricultural and Food Chemistry*. American Chemical Society , 54(16), pp. 5875–5879. doi: 10.1021/jf060048h.

Zhou, F. *et al.* (1998) 'Molecular characterization of the oxalate oxidase involved in the response of barley to the powdery mildew fungus', *Plant Physiology*. American Society of Plant Biologists, 117(1), pp. 33–41. doi: 10.1104/pp.117.1.33.

Zhou, G. *et al.* (2011) 'Genome-wide transcriptional changes and defence-related chemical profiling of rice in response to infestation by the rice striped stem borer Chilo suppressalis', *Physiologia Plantarum*. John Wiley & Sons, Ltd, 143(1), pp. 21–40. doi: 10.1111/j.1399-3054.2011.01483.x.

Zhou, J. *et al.* (2018) 'Heat shock factor HsfA1a is essential for R gene-mediated nematode resistance and triggers H2O2 production', *Plant Physiology*. American Society of Plant Biologists, 176(3), pp. 2456–2471. doi: 10.1104/pp.17.01281.

Zhu, L. *et al.* (2021) 'Pseudomonas fluorescens DN16 Enhances Cucumber Defense Responses Against the Necrotrophic Pathogen Botrytis cinerea by Regulating Thermospermine Catabolism', *Frontiers in Plant Science*. Frontiers Media S.A., 12, p. 645338. doi: 10.3389/fpls.2021.645338.

8. Supplementary

Supplementary table S.1: Composition of Hoagland solution. The table shows the concentration of the elements present in Hoagland solution in parts per million (ppm). (Hoagland and Arnon, 1950)

Concentration (ppm)	
K	235
N	210
Ca	200
S	64
Mg	48.6
P	31
Fe	2.9
Na	1.2
Cl	0.65
B	0.5
Mn	0.5
Zn	0.05
Mo	0.05
Cu	0.02

Supplementary table S.2: Annotated 'top genes' 1 dpt with mCCOPE. The table includes the genes that were annotated with the 30 highest (upregulated) and lowest (downregulated) log2FC values, 1 dpt upon mCCOPE treatment relative to the mock treatment.

Gene name	log2FC	Annotation
Upregulated:		
Os05g0460000	2.817	Cellular response to unfolded protein, refolding, heatshock protein binding, ATPase
Os08g0448000	2.620	Probable 4-coumarate—coenzyme A ligase 5: part of phytoalexin biosynthesis/lignin biosynthesis
Os02g0770800	2.584	Nitrate reductase
Os08g0189500	2.563	Plays role in broad-spectrum disease resistance, Germin-like protein 8-6
Os01g0609300	2.546	ABC transporter G family member 36/Pleiotropic drug resistance protein 9
Os03g0273200	2.528	Laccase-10: lignin degradation and detoxification of lignin-derived products
Os02g0767800	2.507	DNA-binding transcription factor activity
Os11g0592200	2.500	PR4: defence to bacteria and fungi
Os09g0341500	2.420	F-box protein
Os04g0419900	2.345	Non-specific serine/threonine protein kinase
Os06g0302000	2.313	BURP domain-containing protein 11
Os04g0617900	2.304	Germin-like protein 4-1, nutrient reservoir activity
Os05g0470000	2.297	(xylan) O-acetyltransferase activity
Os06g0649000	2.278	WRKY transcription factor (WRKY28): activation of defence-related genes
Os04g0412300	2.276	Glycosidase: carbohydrate metabolic process
Os09g0425200	2.257	Fibroin heavy chain (Fib-H)-like protein
Os03g0225900	2.256	Allene oxide synthase 2: biosynthesis of JA
Os02g0712500	2.241	Glycosyltransferase family 92 protein
Os03g0313300	2.229	Polynucleotide adenylyltransferase
Os01g0389700	2.222	Endomembrane system organization
Os12g0478400	2.217	Serine/threonine-protein kinase, polysaccharide binding/EGF-type aspartate/asparagine hydroxylation site domain containing protein.
Os02g0803300	2.202	SNARE binding, clathrin-dependent endocytosis
Os07g0416900	2.198	Lipid metabolism, oxidoreductase activity
Os05g0149400	2.182	ACC oxidase: ET biosynthesis
Os08g0277200	2,068	Similar to Cinnamoyl-CoA reductase
Os04g0597600	2.040	Nitrate transporter
Downregulated:		
Os11g0444700	-2.020	Transcription regulation
Os01g0733200	-2.002	Heat stress transcription factor C-1b: binds heat shock promoter elements, response to heat, stress, osmotic stress
Os02g0818000	-1.987	Putative brown planthopper-induced resistance protein 1
Os11g0444900	-1.962	Transcription regulation
Os04g0665200	-1.915	Indole-3-acetate O-methyltransferase 1: converts IAA to IAA methyl ester (MeIAA). Regulates IAA activities by IAA methylation, methylation of IAA plays an important role in regulating plant development and auxin homeostasis. MeIAA seems to be an inactive form of IAA.
Os12g0617400	-1.899	9-cis-epoxycarotenoid dioxygenase NCED5 (chloroplastic): first step of ABA biosynthesis from carotenoids
Os01g0687300	-1.895	Regulation of photomorphogenesis
Os06g0716100	-1.883	Similar to Chaperone protein dnaJ.
Os09g0545300	-1.815	Auxin-responsive protein SAUR36: negative regulation of organ growth, may act as a negative regulator of auxin synthesis and transport
Os03g0711100	-1.805	CCT motif family protein(member of the CONSTANS-like (COL) family)
Os01g0102900	-1.786	Cellular response to light stimulus, circadian rhythm
Os12g0120100	-1.757	Putative eukaryotic translation initiation factor 2B family protein
Os07g0687900	-1.696	Galactinol synthase: glycosyl transferase activity, induced by water and salt stress
Os06g0681200	-1.672	Electron transfer activity

Supplementary table S.3: Annotated 'top genes' 4 dpt with mCCOPE. The table includes the genes that were annotated with the 30 highest (upregulated) and lowest (downregulated) log2FC values, 4 dpt upon mCCOPE treatment relative to the mock treatment.

Gene name	log2FC	Annotation
Upregulated:		
Os03g0313300	2.977	Polynucleotide adenylyltransferase
Os06g0253100	2.660	Heat shock protein (peroxisomal): protein complex oligomerization, protein folding; response to heat, hydrogen peroxide, reactive oxygen species, salt stress
Os02g0677300	2.611	Dehydration-responsive element-binding protein 1G: mediates high salinity- and dehydration-inducible transcription
Os10g0418100	2.610	Calcium-transporting ATPase 7 (plasma membrane-type)
Os07g0174900	2.539	Lipid transport
Os07g0542900	2.535	Electron transfer activity
Os03g0368000	2.500	Peroxidase
Os02g0767800	2.486	Transcription factor activity
Os03g0836800	2.387	IAA-amino acid hydrolase ILR1-like 3: hydrolyses certain amino acid conjugates of IAA (IAA-Ala)
Os03g0155900	2.386	Expansin-A18
Os11g0595200	2.370	Harpin binding protein 1
Os05g0135000	2.365	Peroxidase
Os07g0639400	2.364	Peroxidase
Os01g0772100	2.264	Hormone-mediated signalling pathway
Os04g0447100	2.247	Lipoxygenase 5
Os07g0129200	2.223	Pathogenesis-related 1a protein
Os08g0457400	2.196	Similar to Avr9/Cf-9 induced kinase 1
Os02g0676800	2.190	Similar to Dehydration responsive element binding protein 1E
Os11g0117500	2.176	WRKY domain containing protein
Os07g0638600	2.175	Peroxidase
Os11g0151400	2.166	Cytochrome P450 family protein: oxidoreductase activity
Os02g0112100	2.161	High-affinity nitrate transporter, nitrate uptake
Os05g0134800	2.108	Peroxidase
Downregulated:		
Os04g0688300	-3.053	Peroxidase
Os07g0129700	-2.578	Homeobox protein knotted-1-like 12: probable transcription factor in shoot formation during embryogenesis, repression of lignin biosynthesis
Os11g0168500	-2.380	Transcription factor activity
Os02g0649300	-2.202	Homeobox-leucine zipper protein HOX24: transcription factor activity, stress response, panicle development
Os01g0102900	-2.005	Cellular response to light stimulus, circadian rhythm
Os06g0142350	-1.989	Similar to Early nodulin
Os01g0733200	-1.983	Heat stress transcription factor C-1b: response to abscisic acid, osmotic stress
Os09g0255400	-1.813	Indole-3-glycerol-phosphate synthase: synthesizes L-tryptophan from chorismate, part of amino-acid biosynthesis
Os11g0125900	-1.764	Similar to GDA1/CD39 family protein
Os08g0203201	-1.760	Similar to SHR5-receptor-like kinase
Os04g0580800	-1.755	Ubiquitin protein ligase activity
Os05g0525900	-1.745	Zinc finger CCCH domain-containing protein 37
Os06g0129400	-1.742	SPX domain-containing membrane protein: transmembrane transporter activity, Pi homeostasis
Os09g0536400	-1.728	Defence response
Os02g0678200	-1.727	SPX domain-containing membrane protein: transmembrane transporter activity, Pi homeostasis
Os03g0674700	-1.681	Similar to Growth-regulating factor 3

Supplementary table S.4: Pathway analysis of mCCOPE treated plants at 1 and 4 dpt with GO and MapMan. This table shows all significant pathways for 1 dpt (left side) and 4 dpt (right side) with mCCOPE. The red asterisks show pathways that were also detected in the analysis of 0.15 mM DHA treatment at the same time point. The p-values can be found between brackets. For MapMan, Benjamini-Hochberg p-value correction was used.

1 dpt	4 dpt
Upregulated:	
GO:	
Cellulose metabolism (6.78e-5)	Hydrogen peroxide metabolic process (6.32e-8)
Monocarboxylic acid biosynthetic process (2.54e-3)	Reactive oxygen species metabolism (3.74e-7)
Phenylpropanoid metabolic process (4.21e-3)	Hydrogen peroxide catabolic process* (4.79e-7)
Beta-glucan metabolic process (5.81e-4)	Response to oxidative stress* (2.38e-4)
Cell wall biogenesis and organisation (1.00e-2)	Nitrate transport (2.46e-4)
Secondary metabolic process (8.87e-3)	Cellular oxidant detoxification (3.60e-4)
Oxylipin biosynthetic process (9.87e-3)	Plant-type cell wall organisation (8.19e-4)
	Nitrate assimilation/reactive nitrogen species metabolism (2.74e-2)
	Water transport (3.26e-2)
Mapman:	
Cell wall (<1e-20)	Cell wall (1.85e-9)
Cell wall degradation and modification (1.55e-2 and 6.03e-2, respectively)	Cell wall degradation and modification (6.66e-2 and 1.58e-3, respectively)
Phenylpropanoid pathway: lignin biosynthesis (6.55e-6)	Lipid metabolism (3.03e-5)
Phenylpropanoid pathway (6.81e-8)	Phenylpropanoid pathway (2.85e-5)
Cellulose synthesis (2.98e-4)	Phenylpropanoid pathway: lignin biosynthesis (1.70e-3)
Glutathione *S*-transferases (4.38e-3)	Cytochrome P450 (5.16e-12)
Peroxidases (6.51e-6)	Secondary metabolism: simple phenols (8.26e-5)
Ascorbate and glutathione (3.13e-2)	Transport (1.06e-6)
Major Intrinsic Proteins PIP (1.44e-3)	Nitrate transport (3.97e-3)
Secondary metabolism (1.25e-8)	Ammonium transport (4.39e-3)
Secondary metabolism: simple phenols (2.11e-4)	Major Intrinsic Proteins (1.45e-3), specifically PIP (1.67e-4)
Cytochrome P450 (2.86e-2)	Glutathione *S*-transferases (1.75e-7)
Lipid metabolism (1.57e-5)	Peroxidases (6.63e-10)
Amino acid metabolism (3.42e-2)	Biotic stress (3.97e-3)
Biotic stress response (1.56e-4)	Abiotic stress (2.25e-3)
Abiotic stress response (1.69e-3)	ET synthesis and degradation (4.04e-2)
Hormone metabolism (4.10e-3)	Posttranslational modifications (6.67e-7)
ET synthesis and degradation (1.85e-2)	Receptor kinases (3.14e-7)
JA synthesis and degradation (1.69e-2)	Receptor like cytoplasmatic kinase VII (4.17e-13)
Receptor kinase signalling (receptor like cytoplasmic kinase VII, wheat LRK10 like, leucine rich repeat III and S-locus glycoprotein like) (4.35e-9)	AP2/EREBP, APETALA2/ET-responsive element binding protein family (9.66e-3)
Protein modifications (1.14e-2)	WRKY domain transcription factor family (2.85e-5)
WRKY transcription factor family (4.37e-3)	Calcium signalling (9.46e-5)
PHOR1 transcription factor (1,34e-2)	Amino acid degradation: aromatic tyrosine (5.43e-2)
Calcium signalling (1.67e-6)	Gluco-, galacto- and monnosidases (7.99e-4)
UDP glucosyl and glucoronyl transferases (1.25e-8)	GSDL-motif lipase (1.97e-6)
Gluco-, galacto- and monnosidases (2.14e-4)	UDP glucosyl and glucoronyl transferases (8.69e-10)
Downregulated:	
GO:	
NA	Galactose binding (9.24e-2)
Mapman:	
NA	ARR transcription factor family (8.08e-3)

Supplementary table S.5: Pathway analysis of 0.15 mM DHA treated plants at 1 and 4 dpt with GO. This table shows all significant pathways for 1 dpt (left side) and 4 dpt (right side) with 0.15 mM DHA. Only pathways obtained from GO are shown, as no biologically significant pathways were detected using MapMan. The p-values can be found between brackets.

1 dpt	4 dpt
	Upregulated:
Response to heat (4.00e-2)	Hydrogen peroxide catabolic process and response to oxidative stress (4.40e-6 and 9.10e-4, respectively) Glutathione metabolic process (8.32e-4) Negative regulation of protein metabolic process (4.16e-2) Carbohydrate metabolic process (1.20e-2)
	Downregulated:
NA	Protein folding (1.00e-6)

Supplementary table S.6: Overlapping differentially expressed annotated genes between 0.15 mM DHA and mCCOPE treatment at 1 dpt. The table includes the overlapping differentially expressed genes (relative to the mock treatment) between 0.15 mM DHA and mCCOPE at 1 dpt that were annotated.

Gene name	Annotation
Upregulated:	
Os03g0266300	Class I heat shock protein: unfolded protein binding, response to heat, hydrogen peroxide, reactive oxygen species and salt
Os01g0838600	Zinc-finger domain, C2H2-like domain containing protein
Os09g0526600	Heat stress transcription factor B-2c
Os05g0149400	ACC oxidase: ET biosynthesis
Os12g0478400	Protein serine/threonine kinase activity and polysaccharide binding

Supplementary table S.7: Overlapping differentially expressed annotated genes between 0.15 mM DHA and mCCOPE treatment at 4 dpt. The table includes the overlapping differentially expressed genes (relative to the mock treatment) between 0.15 mM DHA and mCCOPE at 4 dpt that were annotated.

Gene name	Annotation
Upregulated:	
Os08g0189200	Germin-like protein 8-3
Os08g0137800	Electron transfer activity
Os08g0137900	Electron transfer activity
Os06g0274800	Peroxidase
Os12g0548700	Serine-type endopeptidase inhibitor activity: response to wounding
Os01g0962700	Peroxidase
Os04g0629200	Electron transfer activity
Os09g0491100	Beta-glucosidase 30
Os01g0827300	Laccase-3
Os12g0135800	Cell death associated protein, hydrolase activity
Os06g0275800	Amino acid transport
Os07g0599600	Early nodulin 75-like protein
Os10g0552200	Cortical cell delineating protein
Os03g0218400	Sugar transport protein MST4
Os01g0594300	Extensin-like
Os11g0306400	O-methyltransferase activity: protein dimerization activity
Os05g0134800	Peroxidase
Os03g0767800	Cold acclimation protein, WCOR413-like protein
Os01g0697100	UDP-glycosyltransferase activity
Os06g0228200	MIP/aquaporin (TC 1.A.8) family: aquaporin NIP2-2
Os04g0474800	Beta-glucosidase 12
Os08g0114300	L-gulonolactone oxidase
Os04g0495400	Cytochrome b561 and DOMON domain-containing protein
Os03g0836800	IAA-amino acid hydrolase ILR1-like 3
Os03g0370400	Endomembrane system organization
Os07g0287400	Systemic acquired resistance
Os01g0505600	Probable calcium-binding protein CML11
Os11g0644700	Dirigent protein
Os01g0620800	UDP-glycosyltransferase activity
Os08g0482600	Electron transfer activity
Os10g0553300	Probable trehalose-phosphate phosphatase 2
Os02g0720900	Aspartic-type endopeptidase activity
Os08g0557600	Monodehydroascorbate reductase 4 (cytosolic)
Os01g0668100	Arabinogalactan protein-like
Os07g0542900	Electron transfer activity
Os07g0142500	Early nodulin 75-like protein
Os04g0535600	Carbohydrate metabolic process
Os08g0155400	Proton-dependent oligopeptide transporter (POT/PTR) (TC 2.A.17) family
Os01g0248900	Expansin-A8
Os02g0485000	Transferring acyl groups other than amino-acyl groups
Os11g0307300	O-methyltransferase family protein: aromatic compound biosynthetic process

Os07g0251200	Putative cortical cell delineating protein
Os07g0638400	1-Cys peroxiredoxin B
Os12g0159000	Harpin-induced protein 1 containing protein
Os10g0416800	Chitinase 1
Os01g0233000	Salt stress root protein RS1
Os07g0142300	Early nodulin 75-like protein
Os04g0659300	DUF26-like protein
Os03g0368000	Peroxidase
Os10g0416100	Chitinase 2
Os11g0702100	Chitinase III
Os04g0462500	14-3-3-like protein GF14-B
Os03g0841600	UDP-glycosyltransferase activity
Os10g0527400	Transferase activity
Os03g0368900	Peroxidase
Os01g0170000	Galactinol-sucrose galactosyltransferase
Os07g0553700	Mitochondrial pyruvate carrier
Os01g0720400	Phosphatase activity
Os03g0310800	EF hand family protein: potential calcium sensor
Os06g0547400	Peroxidase
Os03g0220100	3-ketoacyl-CoA synthase
Os04g0554500	Lipid transfer protein-like protein
Os01g0713200	Beta-1,3-glucanase
Os10g0530200	Putative glutathione *S*-transferase GSTU6
Os03g0749300	Glycosyl hydrolase family 3 N terminal domain containing protein
Os07g0677200	Peroxidase
Os06g0306300	Peroxidase
Os04g0652700	Aspergillus nuclease S(1)
Os12g0541700	Calcium-mediated signalling
Os05g0595100	UDP-glucose 4-epimerase 1
Os10g0444700	Probable inorganic phosphate transporter 1-8
Os10g0185400	Negative regulation of catalytic activity, enzyme inhibitor
Os10g0486100	Cytochrome P450 family protein
Os08g0297800	Sulfotransferase
Os05g0135000	Peroxidase
Os02g0580900	Nitrate transmembrane transporter activity, nitrate response
Os05g0499300	Peroxidase 1
Os06g0470000	Glycosyl transferase
Os07g0599500	Early nodulin 75-like protein
Os01g0946700	Endo-1,3-beta-glucanase
Os11g0591200	Very-long-chain 3-oxoacyl-CoA synthase, fatty acid biosynthetic process
Os03g0628800	Kinase activity
Os10g0528400	Glutathione *S*-transferase GSTU6
Os07g0539100	Glucan endo-1,3-beta-D-glucosidase
Os09g0399800	Probable cinnamyl alcohol dehydrogenase 8A
Os11g0614400	Patatin
Os03g0339400	Peroxidase

Os06g0215600	Putative 12-oxophytodienoate reductase 5
Os11g0507200	Transferring acyl groups other than amino-acyl groups
Os03g0661300	Tubulin beta-8 chain
Os07g0630400	Ribonuclease T2 activity
Os06g0648500	Asymmetric cell division
Os05g0186300	Malic enzyme
Os06g0336200	Probable aquaporin TIP2-2
Os05g0542800	Polygalacturonase activity
Os01g0369700	Glutathione transferase
Os01g0220100	Endoglucanase 2
Os05g0501300	NAD(P)H dehydrogenase (quinone)
Os06g0513943	Acidic protein, toxin activity and defence response
Os12g0548401	Serine-type endopeptidase inhibitor activity
Os07g0574800	Tubulin alpha-1 chain
Downregulated:	
Os03g0352300	Nucleolar protein Nop56 (putative): snoRNA binding, ribosome biogenesis
Os03g0166000	RNA-binding
Os08g0278900	Stromal cell-derived factor 2-like protein
Os07g0636000	Pseudouridine synthase activity: RNA-binding (box H/ACA snoRNA 3'-end processing)
Os08g0490900	Histone H2B.2
Os05g0541900	Structural constituent of ribosome
Os03g0370500	Putative H/ACA ribonucleoprotein complex subunit 4: pseudouridine synthase activity (box H/ACA snoRNA 3'-end processing)
Os11g0235200	Nitrate transporter NTL1 (putative)
Os01g0191100	Structural constituent of ribosome
Os10g0203000	Putative endonuclease/exonuclease/phosphatase family protein