UNIVERSITY OF TURKU

REGULATION OF STRESS-INDUCED METABOLIC REARRANGEMENTS IN PLANTS: FROM MODEL SPECIES TO CROPS

Sara Alegre

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. AI OSA – TOM. 639 | ASTRONOMICA – CHEMICA – PHYSICA – MATHEMATICA | TURKU 2020

UNIVERSITY OF TURKU

REGULATION OF STRESS-INDUCED METABOLIC REARRANGEMENTS IN PLANTS: FROM MODEL SPECIES TO CROPS

Sara Alegre

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA - SER. AI OSA – TOM. 639 | ASTRONOMICA – CHEMICA – PHYSICA - MATHEMATICA | TURKU 2020

University of Turku

Faculty of Science and Engineering
Department of Biochemistry
Molecular Plant Biology
Doctoral Programme in Molecular Life Sciences

Supervised by

Dr Saijaliisa Kangasjärvi
Molecular Plant Biology
Department of Biochemistry
University of Turku
Finland

Reviewed by

Dr T. Matthew Robson
Organismal and Evolutionary Biology
University of Helsinki
Finland

Dr Pablo Velasco Pazos
Department of Plant Genetics
Misión Biológica de Galicia (MBG-CSIC)
Spain

Opponent

Dr. Markku Keinänen
Department of Environmental and
Biological Sciences
University of Eastern Finland

ISBN 978-951-29-8287-5 (PRINT)
ISBN 978-951-29-8288-2 (PDF)
ISSN 0082-7002 (Print)
ISSN 2343-3175 (Online)
Painosalama Oy, Turku, Finland 2020

“Science is made up of mistakes, but they are mistakes which it is useful to make, because they lead little by little to the truth.”
Jules Verne, Journey to the Center of the Earth

UNIVERSITY OF TURKU
Faculty of Science and Engineering
Department of Biochemistry
Molecular Plant Biology
SARA ALEGRE: Regulation of stress induced metabolic rearrangements in plants: from model species to crops
Doctoral Dissertation, 133 pp.
Doctoral Programme in Molecular Life Science
December 2020

ABSTRACT

In nature, plants are exposed to continuous changes in the environment. To ensure their growth and survival, plants have evolved intrincate molecular machinery that allows rapid adjustments in cellular functions in response to a variety of biotic and abiotic cues. Mechanisms that integrate stress-induced signals include cascades of protein kinases and phosphatases, which mediate and direct the signals to elicit appropriate cellular responses. Among defensive measures, changes in the contents of specialized metabolites play an important role in plant-environment interactions. Plant-derived specialized metabolites are of great value to humans, since they offer nutritional benefits and a rich resource of raw material for pharmaceutical industries. The aim of this doctoral dissertation was therefore to elucidate how environmental changes affect metabolic rearrangements in the model plant *Arabidopsis thaliana,* and to translate this knowledge into applications on crop species. Work on *Arabidopsis thaliana* identified protein phosphatase 2A as an important regulator of the activated methyl cycle and the associated glucosinolate metabolism, more specifically the formation of a specialized metabolite called 4-methoxy-indol-3-yl-methyl glucosinolate (4MO-I3M GSL). Further analysis with kale (*Brassica oleracea* convar. *acephala*) varieties revealed that formation of specific health-promoting glucosinolates, including 2-phenylethyl glucosinolate (2PE GSL) and 4-methylsulfinyl butenyl (4MSB GSL) could be significantly influenced by light conditions in brassica crops. Collectively, the findings highlighted that plant specialized metabolism is highly responsive to environmental factors, and that exposure to potentially stressful growth light conditions could be utilized to improve the nutritional value of crops.

KEYWORDS: Brassica, high light, metabolites, glucosinolates, transcriptome.

TURUN YLIOPISTO
Luonnontieteiden ja tekniikan tiedekunta
Biokemian laitos
Oppiaine
SARA ALEGRE: Ympäristöstressien aikaansaamat kasvien
Väitöskirja, 133 s.
Molekyylibiotieteiden tohtoriohjelma
marraskuu 2020

TIIVISTELMÄ

Kasvit altistuvat luonnossa kasvaessaan alati vaihteleville kasvuympäristön muutoksille. Kasveille on kuitenkin evoluution kuluessa kehittynyt molekyylitason mekanismeja, joiden avulla ne voivat sopeuttaa aineenvaihduntaansa vallitsevien olosuhteiden mukaisesti. Erityisesti proteiinikinaasien ja -fosfataasien välittämät soluviestinnän signaalit ovat tärkeitä kasvien elintoimintoja sääteleviä tekijöitä. Erilaisten kemiallisten suojayhdisteiden kertyminen kasvin lehtiin taas on tyypillinen sopeutumisvaste, joka havaitaan ympäristöstressille altistuneissa kasveissa. Monet kasvien suojayhdisteet ovat arvokkaita myös ihmiselle, sillä ne voivat vaikuttaa syötävien kasvien ravitsemukselliseen laatuun tai toimivat raaka-aineina lääketeollisuudessa. Tämän väitöskirjan tavoitteena oli kasvibiologian mallikasvina tunnettua lituruohoa (*Arabidopsis thaliana*) hyödyntäen selvittää miten ympäristössä tapahtuvat muutokset vaikuttavat kasvien aineenvaihduntaan. Lisäksi tutkimuksessa etsittiin keinoja soveltaa tutkimustietoa hyötykasvien viljelyyn liittyvissä sovelluksissa. Lituruoholla tehty työ osoitti, että proteiinifosfataasi 2A säätelee kasvien metyylisykliä ja siihen liittyvää glukosinolaattiyhdisteiden biosynteesiä. Lehtikaaleilla (*Brassica oleracea* convar. *acephala*) suoritetut kokeet puolestaan osoittivat, että kasvun aikana vallitsevat valo-olosuhteet muokkaavat kaalikasvien glukosinolaattikoostumusta. Erityisesti kirkkaan valon havaittiin lisäävän tiettyjen terveyttä edistävien glukosinolaattiyhdisteiden kertymistä lehtikaalien lehtiin. Tutkimus osoitti, että kasvien aineenvaihduntakoneisto reagoi herkästi vallitsevissa olosuhteissa tapahtuviin muutoksiin, ja että kasvien tarkoituksellinen altistaminen valostressille voisi toimia työkaluna lehtimäisten vihannesten terveellisyyden parantamiseksi. Tutkimustiedon hyödyntäminen voi tulevaisuudessa edesauttaa kasvien ravitsemuksellisen arvon muokkausta ja ekologisesti kestävien viljelytekniikoiden kehitystä.

ASIASANAT: Kaalikasvit, kirkas valo, aineenvaihdunta, glukosinolaatti, geenitoiminta.

Table of Contents

Abbreviations

2OG	Fe(II)-dependent oxygenase superfamily protein
2PE GSL	2-phenylethyl (or phenethyl) glucosinolate
2PROP GSL	2-propenyl glucosinolate
3MSP GSL	3-methylsulfinylpropyl glucosinolate
4MO-I3M GSL	4-methoxy-indol-3-yl-methyl glucosinolate
4MSB GSL	4-methylsulfinylbutyl
ACC	1-aminocyclopropane-1-carboxylic acid
ADK	Adenosine kinase
AMC	Activated methyl cycle
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BCAT	Branched-chain aminotransferase
CA 1	Carbonic anhydrase 1
CCoAOMT7	Caffeoyl coenzyme A ester O-methyltransferase 7
CHI	Chitinase
CIMS	Cobalamin-independent methionine synthase
CN	Clear native
CoA	Coenzyme A
CRK45	Cys-rich receptor-like protein kinase 45
CWDE	Cell wall degrading enzyme
CYP79	Cytochrome p450 family 79
CYP83	Cytochrome p450 family 83
cytb6f	Cytochrome b6f
DDA	Data dependent acquisition
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EGFP	Enhanced green fluorescent protein
ESI	Electrospray ionisation
ETC	Electron transport chain
GO	Gene onthology
GSL	Glucosinolate

GST	Glutathione S-transferase
HCl	Hydrochloric acid
HL+ET	High light and elevated temperature
I3C	Indole-3-carbinol
IGMT	Indole glucosinolate methyltransferase
IPMDH	Isopropylmalate dehydrogenase
IPMI	Isopropylmalate isomerase
LC	Liquid chromatography
LHC	Light harvesting complex
MAM	Methylthioalkylmalate synthase
MAT	Methionine adenosyltransferase
MEP	2-C-methyl-D-erythritol 4-phosphate
MRM	Multiple Reaction Monitoring
MS	Mass spectrometry
MT	Methyltransferase
MTHF	Methyltetrahydrofolate
NADPH	Adenine dinucleotide phosphate
NF-E2	Nuclear factor erythroid-2
NPQ	Non-photochemical quenching
Nrf2	Nuclear factor erythroid-2 related factor 2
PAGE	Polyacrylamide gel electrophoresis
PAPS	3′-phosphoadenosine 5′-phosphosulfate
PCA	Principal component analysis
PEITC	Phenylethyl (or phenethyl) isothiocyanate
PP2A	Protein phosphatase 2A
PR	Pathogenesis related protein
PSII	Photosystem I
PSII	Photosystem II
PTM	Post-translational modification
PVDF	Polyvinylidene difluoride
RLP23	Receptor-like protein 23
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SABP3	Salicylic acid-binding protein 1
SA	Salicylic acid
SAH	S-adenosyl-L-homocysteine
SAHH	S-adenosyl-L-homocysteine hydrolase
SAM	S-adenosyl-L-methionene
SAMS	S-adenosyl-L-methionine synthase
SDS	Sodium dodecyl sulfate

SOT	Sulfotransferase
SUR1	C-S lyase 1
UGT	Uridine diphosphate glucosyltransferase
UPLC	Ultra-Performance Liquid Chromatography

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Rahikainen M, Trotta A, Alegre S, Pascual J, Vuorinen K, Overmyer K, Moffatt B, Ravanel S, Glawischnig E and Kangasjärvi S. PP2A-B'γ modulates foliar trans-methylation capacity and the formation of 4-methoxy-indol-3-yl-methyl glucosinolate in Arabidopsis leaves. *Plant Journal.* 2017; 89: 112-127.

II Alegre S, Pascual J, Trotta A, Angeleri M, Rahikainen M, Brosche M, Moffatt B and Kangasjärvi S. Evolutionary conservation and post-translational control of S-adenosyl-L-homocysteine hydrolase in land plants. *PLoS ONE*, 2020; issue 15: 1-18.

III Alegre S, Pascual J, Trotta A, Aro EM and Kangasjärvi S. Comparison of transcriptomic responses upon short-term and long-term acclimation to high light in *Arabidopsis thaliana*. Manuscript.

IV Alegre S, Pascual J, Trotta A, Gollan P, Yang W, Yang B, Aro EM, Burow M and Kangasjärvi S. Growth under high light and elevated temperature triggers genotype-dependent metabolic responses in kale. In revision.

1 Introduction

1.1 Photosynthesis supports cell metabolism and life on Earth

Photosynthetic organisms are characterized by their ability to convert sunlight into chemical energy and metabolites (Midgley, 2007). In eukaryotes, photosynthetic light reactions take place within thylakoid membranes inside chloroplasts, where Photosystem II (PSII) and Photosystem I (PSI) with their light-harvesting antenna complexes (LHCs) capture the energy of light. This drives the transfer of electrons from water to nicotinamide adenine dinucleotide phosphate ($NADP^+$) in an electron transport chain (ETC), which is composed of PSII, plastoquinone, cytochrome b6f (cytb6f) complex, plastocyanin, PSI and ferredoxin. Accompanied by the electron flow, protons are translocated into the thylakoid lumen, resulting in formation of a pH gradient across the thylakoid membrane and the subsequent synthesis of adenosine triphosphate (ATP) by the thylakoid ATPase complex. Since light drives the formation of NADPH and ATP, it enables reductive metabolic reactions to be performed, making the chloroplast a hub for various biosynthetic pathways. NADPH and ATP are key determinants of the redox state and energy status of the cell, since they provide energy and reducing power for photosynthetic carbon fixation and other biosynthetic pathways. The ability of photosynthetic machinery to maintain the flux of energy and redox power to the downstream metabolic pathways can significantly affect whole cell metabolism, which has a direct impact on plant growth and reproduction.

Besides carbon fixation, a fraction of the photosynthesis-derived chemical energy is used for the biosynthesis of amino acids (Hildebrandt, 2018). In addition to providing constituents of proteins, amino acids are precursors of a vast number and variety of specialized metabolites. For example, almost 1% of specialized metabolites derive from phenylalanine (Tzin and Galili, 2010). Therefore, it could be considered that amino acids are in a pivotal position between basic carbon metabolism and specialized metabolism, which in turn is a massive energy sink (Pratelli and Pilot, 2014; Hildebrandt *et al.*, 2015).

Plants are able to *de novo* synthetize proteogenic amino acids from inorganic carbon, nitrogen and sulfur compounds, whereas humans and monogastric livestock

can only get the so-called essential amino acids (lysine, methionine, phenylalanine, tryptophan, threonine, valine, leucine, isoleucine and histidine) through the diet (Galili *et al.*, 2016). Photosynthesis operates at the largest scale and is the most efficient method on Earth of harvesting solar energy (Gust *et al.*, 2009). Thus, the rate by which primary producers, including plants and phytoplankton, produce organic matter influences the energy flow in all ecosystems through the trophic chain (Lindeman, 1942).

1.2 Activated methyl cycle and methylation

In plants, methionine is a metabolically important amino acid that acts as a precursor for specialized metabolites including S-Adenosyl-L-methionene (SAM), which is the universal methyl donor within the cell (Figure 1). Trans-methylation is a biological event in which a methyl group ($-CH_3$) is transferred from one compound to another. Thus, the compound gaining the methyl group becomes methylated. Methylation is the fifth most frequent post-translational modification (PTM) occurring on proteins (Huang *et al.*, 2016), but can also be found in other types of molecules, ranging from DNA, RNA, certain cell wall components and a large variety of specialized metabolites (Rahikainen *et al.*, 2018), such as glucosinolates, terpenes and phenylpropanoids (Figure 1). Thus, methylation impacts numerous cellular processes, such as ribosome biogenesis, cell division, cell wall biosynthesis, membrane synthesis, photosynthesis, defence and stress response (Friso and Van Wijk, 2015). DNA and histone methylation also influences gene expression, as it confers an epigenetic level of regulation (Lauria and Rossi, 2011).

The activated methyl cycle (AMC) is the metabolic circuit that ensures the availability of SAM. The methylation by-product S-adenosyl-L-homocysteine (SAH) is rapidly hydrolysed to adenosine and homocysteine to avoid competitive inhibition of SAM-dependent methyltransferase activity (Cantoni, 1975; Moffatt and Weretilnyk, 2001). This key reaction is catalysed by S-adenosyl-L-homocysteine hydrolase to maintain transmethylation potential (SAHH; E.C 3.3.1.1). Adenosine branches out from the AMC in an ATP-dependent reaction catalysed by adenosine kinase (ADK; E.C.2.7.4.3), which generates adenosine monophosphate (AMP). Homocysteine is further converted to methionine in a methyltetrahydrofolate (MTHF)-dependent reaction driven by cobalamin-independent methionine synthase (CIMS; E.C.2.1.1.14). The last cyclic step of SAM regeneration is driven by S-adenosyl-L-methionine synthase (also called methionine adenosyltransferase, SAMS/MAT; E.C.2.5.1.6), which uses methionine as a substrate in an ATP consuming reaction (Figure 1).

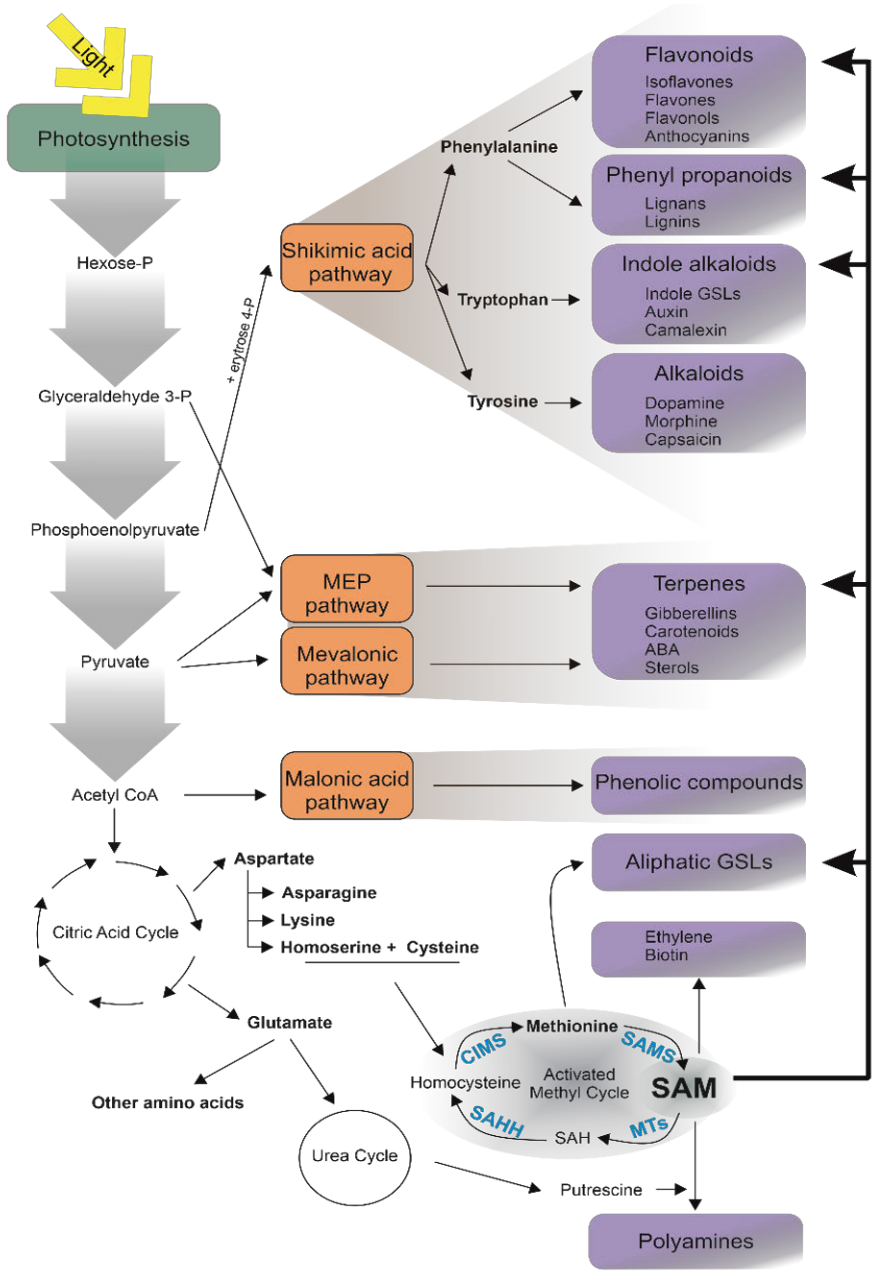


Figure 1. Schematic representation of the major specialized metabolite biosynthetic pathways and their links with SAM metabolism. Thin arrows show where SAM is used as substrate and thick arrows where it is needed it as a methyl donor. Orange boxes represent the four main specialized metabolite biosynthetic pathways and light purple boxes represent groups of specialized metabolites. Amino acids are in bold. Enzymes of the activated methyl cycle are indicated in blue. 2-C-methyl-D-erythritol 4-phosphate (MEP); phosphate (P); coenzyme A (CoA); S-Adenosyl-L-methionine (SAM); S-adenosyl-homocysteine (SAH); glucosinolates (GSLs); S-adenosylhomocysteine hydrolase (SAHH), cobalamin-independent methyl synthase (CIMS); S-Adenosyl-L-methionene synthase (SAMS); methyl transferases (MTs).

Localization studies of AMC enzymes suggest the presence of SAHH, CIMS and SAMS/MAT in both the nucleus and cytosol (Lee *et al.*, 2012). On the contrary, no data has pointed to the presence of AMC components in chloroplasts or mitochondria, which rely on SAM/SAH antiporters that provide SAM as a reaction substrate and remove the inhibitory SAH from the organelles (Palmieri *et al.*, 2006).

Besides its fundamental role as a methyl donor, SAM is also important as a precursor of polyamines, biotin and the phytohormone ethylene (Figure 1). Ethylene biosynthesis from SAM takes place in two enzymatic steps with 1-aminocyclopropane-1-carboxylic acid (ACC) as an intermediate. Biosynthesis of polyamines in turn requires decarboxylated SAM and putrescine as precursors (Chen *et al.*, 2019). Although up to 80% of methionine can be converted to SAM (Giovanelli *et al.*, 1985; Iqbal *et al.*, 2013), adequate SAM and methionine fluxes to each of the above-mentioned metabolic pathways is essential to maintain cell homeostasis (Figure 1).

The *Arabidopsis thaliana* (hereafter Arabidopsis) genome encodes two *SAHH* genes (Rocha *et al.*, 2005; Pereira *et al.*, 2007; Li *et al.*, 2008). A null mutation in SAHH1 (AT4G13940) is lethal to the embryo, while a T-DNA insertion in its closest related gene *SAHH2* (AT3G23810) (*sahh2* mutant) causes no visual phenotype in Arabidopsis (Rocha *et al.*, 2005). A T-DNA insertion in the *SAHH1* promoter (*sahh1* mutant) alters its transcriptional activity, resulting in a lower SAHH1 protein level with otherwise normal functionality (Wu *et al.*, 2009), but has no effect on *SAHH2* expression levels (Ouyang *et al.*, 2012). A point mutation in the *SAHH1* coding sequence (*hog1* mutant, Rocha *et al.*, 2005) leads to sterility, whereas *sahh1* maintains fertility (Wu *et al.*, 2009). Both *SAHH1* mutants exhibit delayed germination and slow growth (Furner *et al.*, 1998; Wu *et al.*, 2009). Characterization of SAHH1 dysfunction mutants further revealed that they exhibit DNA hypomethylation (Rocha *et al.*, 2005; Mull *et al.*, 2006; Ouyang *et al.*, 2012).

SAHH1 is a highly conserved enzyme among both eukaryotic and prokaryotic organisms (Kusakabe *et al.*, 2015). High-resolution crystal structures of bacterial, plant and mammalian enzymes determine that SAHH has dimeric, tetrameric and hexameric conformations (Matuszewska *et al.*, 1987; Tanaka *et al.*, 2004; Reddy *et al.*, 2008; Brzezinski *et al.*, 2012). Besides its polymeric conformation, each SAHH monomer needs a sodium cation close to the active site and a nicotinamide adenine dinucleotide (NAD^+) as cofactor (Ault-Riché *et al.*, 1994). Most bacteria and some eukaryotes, including plants but not fungi or vertebrata, contain a 40 amino acid fragment within the catalytic domain (Stępkowski *et al.*, 2005).

1.3 Plants in a changing environment

1.3.1 Light is a crucial external factor but also a stressor for plants

Although light is essential for primary production, it can cause harmful effects if received in high irradiances or in inappropriate periods of time (Aro *et al.*, 1993; Pascual *et al.*, 2017). Enhanced production of protectant pigments (Chalker-Scott, 1999) and stomatal closure to prevent excessive loss of water are common response to high light (HL) exposure (Lee *et al.*, 2009; Jiang *et al.*, 2011). Dissipation of excess light energy as heat via non-photochemical quenching (NPQ) as well as continuous repair of the PSII core proteins are key processes that maintain the functionality of the photosynthetic apparatus. However, these mechanisms could be insufficient for the plant to cope with the continuously changing light environment in nature. When irradiance exceeds the photosynthetic capacity of a plant, impaired photosynthetic activity can reduce plant productivity (Pandey *et al.*, 2017). Thus, photosynthetic performance needs constant adjustment to the light conditions. Impaired function of the photosynthetic apparatus can trigger retrograde signalling cascades from chloroplast to nucleus to regulate gene expression and promote acclimation on both local and systemic levels (Estavillo *et al.*, 2011; Bartoli *et al.*, 2013). The photosynthetic apparatus, and from a wider perspective the entire chloroplast, is therefore considered to act as a sensor for environmental changes.

Light-induced damage to PSII and the consequent photosynthetic impairment is called photoinhibition, where, although the mechanism is still not fully understood, formation of reactive oxygen species (ROS) has been implicated. Protection of PSII, which is particularly sensitive to oxidative damage in HL (Aro *et al.*, 1993), is linked with the availability of reducing agents, such as ascorbate and glutathione (Müller-Moulé *et al.*, 2002, 2004; Pastori *et al.*, 2003; Pavet *et al.*, 2005). Therefore, photoinhibition of PSII could be partially alleviated by maintaining the cellular redox state.

Besides light stress, plants are also continuously exposed to other abiotic stresses, such as drought, salinity, low/high temperature, and combinations of those. The detrimental effects of abiotic stresses will become intensified by climate change, which has been predicted to result in an increased occurrance of extreme weather events (Fedoroff *et al.*, 2010). The Intergovernmental Panel on Climate Change (Mbow *et al.*, 2019), have declared that the greater frequency of extreme events is already affecting food security.

1.3.2 *Botrytis cinerea* as a biotic plant stressor

In the field, plants are continuously exposed to different types of biotic stresses, including bacterial and fungal plant pathogens. The nature of a plant-pathogen interaction can be diverse depending on the type of pathogen. Among generalist broad host-range species, the necrotrophic fungal plant pathogen *Botrytis cinerea* has drawn attention due to the massive losses it causes in agriculture. *B. cinerea* secretes cell wall degrading enzymes (CWDE) (Łaźniewska *et al.*, 2010) that break down the surface of host plant tissues, as well as specialized phytotoxic metabolites that promote cell death (Howlett, 2006) producing a massive oxidative burst (Govrin and Levine, 2000). Although plant defence mechanisms against *B. cinerea* are not yet fully understood, some components have already been described.

In Arabidopsis, *B. cinerea* infection triggers the production of the phytoalexin camalexin as well as callose deposition through abscisic acid (ABA) and jasmonic acid (JA) signaling (Mol *et al.*, 1996; Kliebenstein *et al.*, 2005). Transcriptomic analysis of mutant lines overexpressing or deficient in the transcription factor WRKY33 demonstrated its role on *B. cinerea* resistance in Arabidopsis (Sham *et al.*, 2017). Salicylic acid (SA) has a key role in mediating defensive responses against biotrophic pathogens, but its contribution to necrotroph resistance, in part through Systemic Acquired Resistance (SAR), is now also increasingly recognized (Govrin and Levine, 2002). Although SA and the JA/ethylene pathways can be antagonistic in plants (Pieterse *et al.*, 2012), synergistic interactions between these pathways have recently been reported (De Vleesschauwer *et al.*, 2014; Caarls *et al.*, 2015; Shigenaga and Argueso, 2016).

It is not yet fully understood how signals arising from pathogen infection are translated into adequate metabolic adjustments in different cell compartments nor how this preserves cell physiology and promotes biotic stress resistance in the entire organism. To elucidate stress-related response mechanisms, biotic and abiotic stress responses have largely been studied each in isolation, but recent studies suggest integrative crosstalk between the regulatory networks (Fujita *et al.*, 2006; Shigenaga and Argueso, 2016; Khan *et al.*, 2020).

1.3.3 PP2A B´γ as a signalling component in plant stress

On a molecular level, stress perception triggers signalling, which is largely relayed as reversible protein phosphorylation cascades generated by the counteracting activity of protein kinases and phosphatases. Protein phosphatase 2A (PP2A) is a trimeric enzyme consisting of a scaffold subunit A, catalytic subunit C and regulatory subunit B. The Arabidopsis genome encodes several isoforms of each of the subunits, of which B subunit is responsible for the target specificity of the holoenzyme.

In Arabidopsis, a specific regulatory subunit PP2A-B'*γ* controls chloroplastic and mitochondrial functions and modulates light-dependent responses to oxidative stress (Rahikainen *et al.*, 2016). Studies on the physiological significance of PP2A-B'*γ* revealed reduced fecundity of green peach aphid in *pp2ab'γ* mutants (Rasool *et al.*, 2014) as well as increased resistance to *B. cinerea* when compared to wild type (Trotta *et al.*, 2011). Recently, the relevance of PP2A-B'*γ* in plant-pathogen interactions was corroborated in crop species. Silencing of wheat (*Triticum aestivum*) *PP2A* conferred resistance against the necrotroph *Rhizoctonia cerealis*, which causes the wheat sharp eyespot disease (Zhu *et al.*, 2018). Arabidopsis *pp2ab'γ* mutants exhibit transcriptional upregulation of SA-related signalling genes such as *PATOGENESIS RELATED PROTEINS 1*, *2* and *5* (*PR1*, *PR2*, and *PR5*), as well as *CHITINASE* (*CHI*), *RECEPTOR LIKE PROTEIN 23* (*RLP23*), *CYS-RICH RECEPTOR-LIKE PROTEIN KINASE 45* (*CRK45*), *2-OXOGLUTARATE* and *FE(II)-DEPENDENT OXYGENASE SUPERFAMILY PROTEIN* (*2OG*), which are involved in fungal stress response (Durian *et al.*, 2020). Despite finding that PP2A-B'*γ* functions as a negative regulator of plants stress defence, the exact molecular mechanisms involved remain to be established.

1.3.4 Impacts of abiotic and biotic stresses on plant productivity in the field

Changes in environmental conditions are the rule in nature. The world's ever-increasing population exceeded 7.5 billion in 2018 (https://www.census.gov/popclock/world). In order to provide adequet nourishment, methods that increase field-yield, such as extensive monoculture and chemical control of unwanted organisms, have dominated farming practises for decades. Well-adapted monocultures growing under good conditions produce high yields. However, sudden and extreme stresses, occurring more frequently nowadays due to the climate change, rinsking entire monocultural fields, which are not very resilient to perturbations (Crews *et al.*, 2018). Moreover, chemical control of unwanted organisms by application of pesticides, fungicides and herbicides is potentially hazardous to humans, other organisms and the environment, since they have been classified as persistent toxic elements due to their low biodegradability.
B. cinerea, by causing the grey mold on horticultural crops, produces annual losses of $10 billion to $100 billion worldwide (Boddy, 2016). The increased societal awareness of the disadvantageous effects of pesticides has limited their usage in the field and promoted the development of alternative means of pathogen control, such as biocontrol, although their use in field is still very limited (Nicot *et al.*, 2013). On the other hand, drought, temperature, flooding and salinity have a relevant impact in yield filed causing up to 70% of crop yield reduction (Francini and Sebastiani, 2019).

Constant changes in light irradiance affect plant productivity by impairing photosynthesis. Moreover, in the field, light irradiance can easily exceed photosynthetic saturation rates only with 25% of the full sunlight (Long et al., 2006). Although triggering NPQ as a photo-protective mechanism under high irradiance may prevent irreversible damage to photosynthetic machinery, especially PSII, the slow relaxation of the NPQ under non-saturating light conditions can also decrease plant productivity, since it limits carbon assimilation (Long and Humphries, 1994). In line with this, recent studies have shown that carbon assimilation can be maximized by acceleration of NPQ relaxation upon transition from HL to shade and theoretically improve productivity up to 20% of their potential yield (Kromdijk et al., 2016).

The effects of concurrent stressors in the field due to climate change will significantly impact crop productivity in the future (Prasch and Sonnewald, 2013; Suzuki *et al.*, 2014; Ramegowda and Senthil-Kumar, 2015). A combination of drought and heat stresses was reported to be more detrimental than each stressor alone (Mittler, 2006; Prasad *et al.*, 2006). In addition, abiotic stressors, such as drought, extreme temperatures and salinity can facilitate plant pathogenesis (Scherm and Coakley, 2003). These examples reflect the complexity and multilevel metabolic regulation required to tolerate harsh natural conditions, where the tightly coordinated pathways of basic carbon metabolism and specialized metabolism need to be steadily tailored according to the external cues.

1.4 Metabolic reprograming as part of the plant stress response

Appropriate stress signalling leads to plant stress responses, which involve metabolic reprogramming. Among protective mechanisms, plants produce an extensive and diverse biochemical group of compounds called specialized metabolites (formely refered to as secondary metabolites). Specialized metabolites form an energetically costly sink for primary metabolites, which derive from the main biosynthetic pathways of basic carbon metabolism (Figure 1). The biochemical complexity and variability of specialized metabolites arises from the wide range of precursors they derive from and their combinatorial assembly into complex chemical structures. In addition, specific modifications, such as methylation, extend their diversity. Therefore, regulatory mechanisms between basic carbon metabolism and specialized metabolism must be tightly coordinated. Independently of their biological roles in plant-environment interactions (Johann *et al.*, 2011; Kappers *et al.*, 2011; Hiltpold and Turlings, 2012), or in reproduction (Luft *et al.*, 2003; Raguso, 2008), specialized metabolites have a significant added value as a raw material for fibre, the pharmacological (Yi *et al.*, 2004; Montanher *et al.*, 2007; Benowitz, 2009; Naveen

and Baskaran, 2018) and food industries (Wurtzel and Kutchan, 2016). Although more than 200 000 specialized metabolites have been described to date, their diversity in plants follows a rather taxa-specific distribution. Moreover, the occurrence of specialized metabolites varies depending on developmental stage, tissue and environment (Fang *et al.*, 2019).

The group of specialized metabolites responsible for the red, purple and blue colours of plant tissues are anthocyanins. The photoinduction of anthocyanin biosynthesis has been extensively studied (Dooner et al., 1991; Mol et al., 1996), and the role of these pigments in both light attenuation and antioxidation in Arabidopsis was demonstrated by Zhang *et al.*, (2018). The accumulation of anthocyanins can however be massively induced by other abiotic stress conditions as well, as demonstrated by cold-stress studies on kale (Zhang *et al.*, 2012). Anthocyanins are flavylium cation derivatives formed from phenylalanine via the phenylpropanoid biosynthesis pathway. The number and position of hydroxylations on the flavylium cation determines the anthocyanin basic agycone structure, which is called anthocyanidin (e.g. Delphinidin, Cyanidin, Petunidin, Peonidin, Malvidin). Anthocyanins are glucosides of anthocyanidines. Cyanidin is the unique aglycone present in Arabidopsis anthocyanins (Figure 2a), which could undergo methylation and several other modifications. Although a number of methylated anthocyanins have been identified, their complete molecular structures have not been revealed (Shi and Xie, 2014) (Figure 2a). Even though anthocyanin methyltransferase genes have been identified in peony and grape vine (Petrussa *et al.*, 2013; Du *et al.*, 2015), Arabidopsis homologues have not to date been identified. Besides their physiological role in plants, a number of potential health benefits to humans have been described (Olivas-Aguirre *et al.*, 2016) mainly due to their antioxidative and antimicrobial activities (Khoo *et al.*, 2017).

A major group of specialized metabolites in cruciferous plants are a large sulfur- and nitrogen-containing array of compounds called glucosinolates (GSLs) (Bell *et al.*, 2018). Originally, GSLs were described as specialized metabolites involved in pathogen and herbivore defence (Bones and Rossiter, 1996; Wittstock and Burow, 2010; Martínez-Ballesta *et al.*, 2013), but recently a number of abiotic stresses have also been reported to alter GSL profile (Steindal *et al.*, 2015; Burow, 2016). The core GSL structure comprises a central carbon atom that binds a thioglucose and a sulfate group via a nitrogen atom (Figure 2b). GSLs can be grouped based on their precursor amino acid: aliphatic GSLs derive mainly from methionine, indole GSLs from tryptophan and aromatic GSLs from phenylalanine or tyrosine (Blažević *et al.*, 2020).

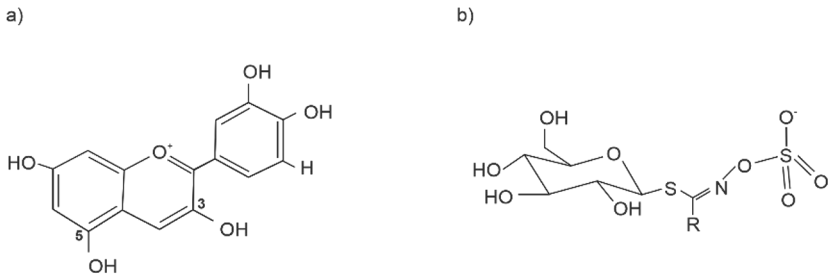


Figure 2. Chemical structure of cyanidin and glucosinolates. a) Schematic representation of cyanidin. Anthocyanins discussed in this thesis are 3 and/or 5 cyanidin glucoside derivatives. b) Glucosinolate squematic structure. Side group R is variable.

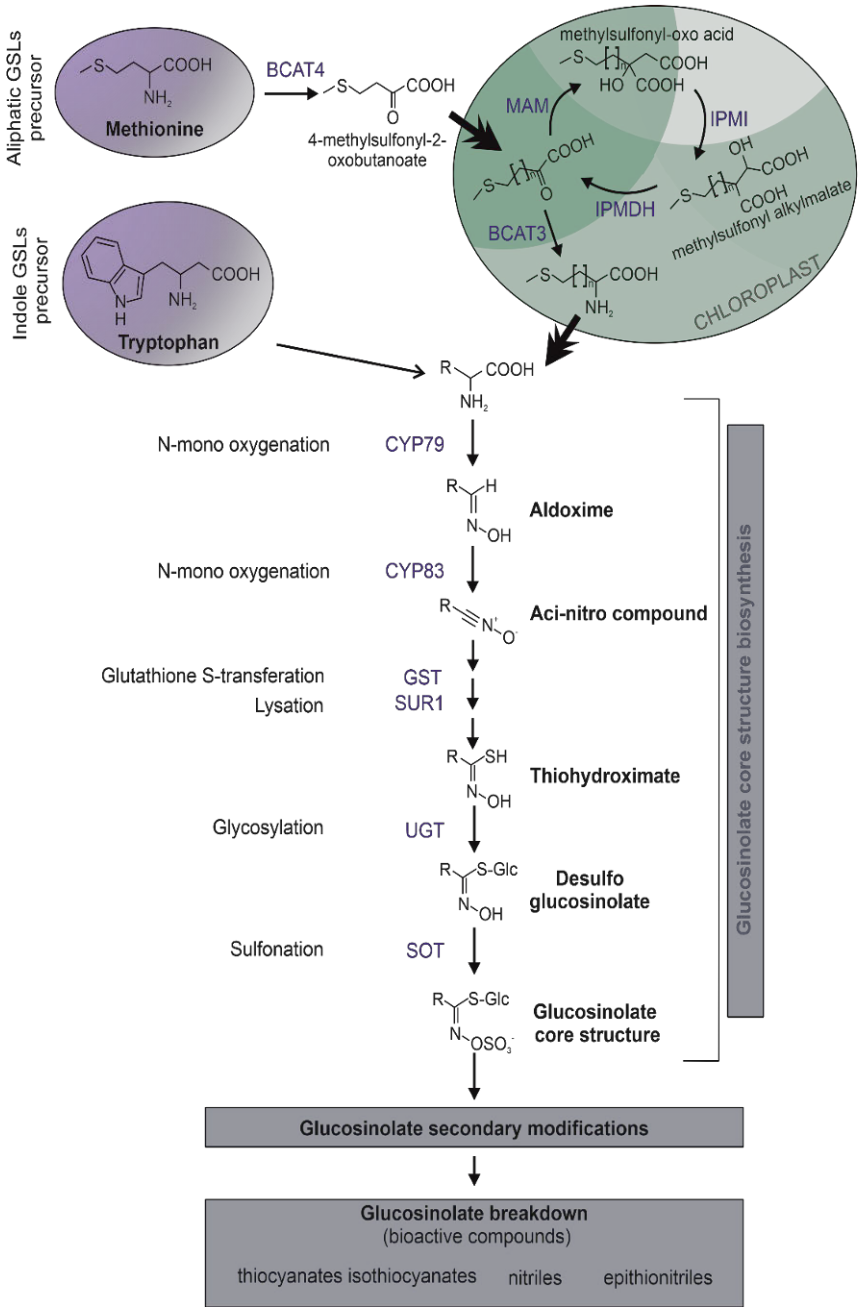
The biosynthesis of all GSLs involves formation of the core GSL structure and side chain modification (Halkier and Gershenzon, 2006; Cartea *et al.*, 2008; Sønderby *et al.*, 2010). Aliphatic GSLs undergo carbon chain elongation where the committed enzyme is methylthioalkylmalate synthase (MAM) (Kroymann *et al.*, 2001, 2003; Benderoth *et al.*, 2009) (Figure 3). In Arabidopsis, three MAM-encoding genes have been identified: MAM1, MAM2 and MAM3 (previously named MAM-L) (Benderoth *et al.*, 2006). *In vitro* and *in planta* experiments on Arabidopsis revealed that MAM2 is predominantly involved in the synthesis of 3-carbon side-chain aliphatic GSLs, whereas MAM1 is responsible for the synthesis of 4-carbon side-chain aliphatic GSLs (Kroymann *et al.*, 2003). In concordance with this, the presence of MAM 1 or MAM2 accounts for the variation related to GSL side-chain length found in Arabidopsis ecotypes. Evolutionary and genetic approaches with 25 Arabidopsis ecotypes have revealed that only one ecotype (named Sorbo) has functional copies of both MAM1 and MAM2 genes (Benderoth *et al.*, 2006). Although it appears that MAM3 contributes to the production of all glucosinolates, their exact role still needs to be clarified (Gershenzon *et al.*, 2007).

During GSL core formation, aldoxime-derived amino acid is oxidized and further conjugated to glutathione (GSH) that works as a sulfur donor. Then, the resulting thiohydroximate is converted to desulfo-GSL by the addition of a S-glucose (Sønderby *et al.*, 2010) (Figure 3). The last step is a sulfonation were 3′-phosphoadenosine 5′-phosphosulfate (PAPS) is the sulfur donor. Downstream of GSL core formation, a number of secondary modifications could take place (Figure 3). Secondary modifications of alipahtic glucosinolates include oxygenation, hydroxylation, alkenylation and benzoylation, while indole GSLs are hydroxylated

and methoxylated (Sønderby *et al.*, 2010). The side carbon group and the subsequent secondary modifications after GSL core formation account for the variability of GSLs. At this stage, GSLs are inactive compounds that are activated upon their breakdown (Wittstock and Burow, 2007). GSL breakdown can generate nitriles, epithionitriles, isothiocyanates, or thiocyanates, which have been demonstrated to have an biological role (Figure 3) (Wittstock and Burow, 2010).

Biosynthesis of GSLs is linked with the availability of AMC intermediates, since methionine is the precursor for aliphatic GSLs biosynthesis and tryptophan-derived GSLs require SAM as a methyl donor in their activation steps (Figures 1 and 3). Besides their biological role, health-promoting benefits of GSLs have been reported with regular consumption of GSLs-rich crops, such as kale or cabbage, in the human diet (Jeschke and Burow, 2018).

Figure 3. Glucosinolate biosynthesis and breakdown pathways. Aliphatic GSLs derive from methionine and undergo a variable side-chain elongation in the chloroplast. Indole GSLs derive mainly from tryptophan. Both amino-acid types undergo successive enzymatic reactions to generate the GSL core structure. Subsequently, secondary modifications (such as hydroxylations or methylations) of these molecules expand their diversity. Enzymatic breakdown of GSLs is necessary to create bioactive compounds (nitriles, epithionitriles, thiocyanates and isothioacyanates). R represents the variable side chain. Enzymes are marked in blue. Branched-chain amino acid aminotransferase (BCAT); methylthioalkylmalate synthase (MAM); isopropylmalate isomerase (IPMI); isopropylmalate dehydrogenase (IPMDH); CYTOCHROME P450 FAMILY 79 (CYP79); CYTOCHROME P450 FAMILY 83 (CYP83); glutathione S-transferase (GST); C-S lyase (SUR1); UDP glucosyltransferase (UGT); sulfotransferase (SOT).


Aliphatic GSLs precursor
Methionine
BCAT4
4-methylsulfonyl-2-oxobutanoate
methylsulfonyl-oxo acid
MAM
IPMI
IPMDH
BCAT3
methylsulfonyl alkylmalate
CHLOROPLAST
Indole GSLs precursor
Tryptophan
N-mono oxygenation
CYP79
Aldoxime
N-mono oxygenation
CYP83
Aci-nitro compound
Glutathione S-transferation
Lysation
GST
SUR1
Thiohydroximate
Glycosylation
UGT
Desulfo glucosinolate
Sulfonation
SOT
Glucosinolate core structure
Glucosinolate core structure biosynthesis
Glucosinolate secondary modifications
Glucosinolate breakdown
(bioactive compounds)
thiocyanates isothiocyanates
nitriles
epithionitriles

1.5 From model to crop

For decades, plant molecular research has been largely performed on the model species *Arabidopsis thaliana*, especially after its genome sequence was released (Initiative, 2000). *A. thaliana* extensive and remarkable genetic tools have allowed advances in the understanding of plant physiology. Nevertheless, physiological biodiversity among the broad plant kingdom cannot be represented only by one species. On the other hand, understanding the physiology of species with an economical value could provide solutions to the predicted impact in plant productivity owing to the climate change. Arabidopsis is phylogenetically related to many crops species such us *Brassica spp, Eruca spp* or *Eutrema japonicum* since all belong to Brassicaceae. This close genetic relation has enhanced the transfer of knowledge and tools from Arabidopsis research to crops science.

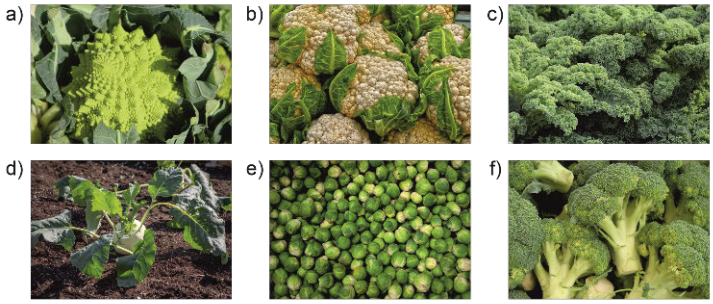


Figure 4. Photographs of *Brassica oleracea* subspecies and varieties. The pictures were obtained from the copyright free server Pixabay. a) Romanesco broccoli; b) Cauliflower; c) Kale; d) Kohlrabi; e) Brussels sprouts; f) Broccoli

Brassica oleracea has been cultivated over two millennia and the long breading time has resulted in varieties with different appearances such as romanesco, cauliflower, kale, kohlrabi, Brussels sprouts and broccoli (Figure 4). Epidemiological studies suggest a strong inverse correlation between cruciferous crops intake in diet and the incidence of cancer (Palmer, 1985; Verhoeven *et al.*, 1996; Boggs *et al.*, 2010; Tang *et al.*, 2010). Moreover, phenylethyl isothiocyanate, an active GSL breakdown product from phenylethyl GSL, prevents carcinogenesis initiation, but also has a role in restraining tumorigenesis (Gupta *et al.*, 2015). Indole-3-carbinol (I3C), breakdown product of 3-indolylmethyl GSL, has a role in colorectal tumour prevention in preclinical models (Megna *et al.*, 2016). These results rise interest in the therapeutic or beneficial properties of GSLs breakdown products, and exploring the accumulation of specific GSLs to enhance the generation of high-value crops.

2 Aims of the study

The overall aim of this thesis was to elucidate metabolic rearrangements and the associated regulatory mechanisms triggered by environmental changes. Furthermore, I explored how this knowledge can be translated from Arabidopsis into applications involving crop species.

The specific aims of this thesis were:

1) To reveal the relevance of protein phoaphatase 2A B'γ-mediated post-translational regulation of the Activated Methyl Cycle and glucosinolate biosynthesis in Arabidopsis (Paper I).

2) To analyse the evolutionary conservation of S-adenosyl-L-homocysteine hydrolase (SAHH) and its responses to stress in land plants (Paper II).

3) To evaluate the impact of high light acclimation on the transcriptomic profile of Arabidopsis (Paper III).

4) To explore how non-invasive treatment with high light and elevated temperature (HL+ET) can be used to increse the accumulation of nutritious metabolites in kale varieties (Paper IV).

3 Materials and Methods

3.1 Biological material and growth conditions

Arabidopsis thaliana wild type (ecotype Columbia-0), homozygote *pp2a-b'γ* mutant line (SALK_039172 for AT4G15415) and a complementation line expressing *PP2A-B'γ* under the 35S-promoter in a genetic *pp2a-b'γ* background (Trotta *et al.*, 2011), transgenic line stably expressing SAHH1P::EGFP-SAHH1 (AT4G13940, Lee *et al.*, 2012), two varieties of *Brassica oleracea* convar. *acephala* (kale) called Black Magic and Half Tall, *B. oleracea* convar. *italica* (broccoli), *Spinacea oleracea* (spinach), *Lupines luteus* (yellow lupin) and *Physcomitrella patens* (spreading earthmoss) comprised the material used to perform this research.

Arabidopsis plants were grown under 8-h light photoperiod at 130 µmol photons m^{-2} sec^{-1}, 22° C and 50% relative humidity for Paper I. Biotic stress was imposed with *B. cinerea* fungus by spraying a suspension of $1x10^6$ spores and maintaining the relative humidity at 100% to facilitate infection. Arabidopsis samples were harvested 24 h post-infection. For Paper II, plants were grown under 8-h or 12-h photoperiod at 130 µmol photons m^{-2} s^{-1} 22° C and 50% relative humidity. *P. patens* was grown on agar plates in minimum media (Alboresia *et al.*, 2010) in a 16-h photoperiod at 45 µmol photons m^{-2} s^{-1} at 24° C. *S. oleracea* and *B. oleracea* convar. *italica* (broccoli) material was obtained from the local market. High light treatments were performed on Arabidopsis (shifted for 2 days to 800 µmol photons m^{-2} s^{-1}, 26°C and 50% relative humidity.) and on *P. patens* (shifted for 2 days to 500 µmol photons $m^{-2}s^{-1}$). For paper III Arabidopsis was grown in 8-h light photoperiod under 130 µmol photons m^{-2} sec^{-1}, 22°C and 50% relative humidity (growth condition) and treated for two weeks with 800 µmol photons m^{-2} s^{-1}, 28°C and 50% relative humidity (HL condition) and collected at 4 weeks of age for transcriptomic analysis. For Paper IV, the two varieties of *B. oleracea* were germinated and grown at 12-h light photoperiod either at growth light (130 µmol photons $m^{-2}s^{-1}$, 22°C and 50% relative humidity) or high light and elevated temperature (800 µmol photons m^{-2} s^{-1}, 26°C and 50% relative humidity; HL+ET).

3.2 Protein analysis

3.2.1 Extraction of proteins

Leaves of Arabidopsis, *B. oleracea* convar. *acephala* (Half Tall and Black Magic), *B. oleracea* convar. *italica*, *L. luteus* and *S. oleracea*, and aerial parts of *P. patens* were ground in liquid nitrogen and mixed with extraction buffer (10 mM HEPES-KOH pH 7.5; 10 mM $MgCl_2$) supplemented with protease and phosphatase inhibitors. After centrifugation at 18 000 ***g*** for 15 min, protein soluble fractions were obtained for further analysis.

3.2.2 Polyacrylamide Gel Electrophoresis

To study proteins, the soluble extracts were run on a variety of polyacrylamide gel electrophoresis (PAGE), which were used in a broad and combinatorial manner. Denaturing gels (sodium dodecyl sulfate SDS), non-denaturing gels (Clear Native) and Phostag gels were used either as a single first dimension (1D) or in a combinatorial way to run second (2D) and third (3D) dimension experiments. Fixed acrylamide concentration or different ranges of acrylamide concentrations (gradient gels) were used in different experiments (papers I, II and IV). Further specifications are detailed in Materials and Methods sections of each paper.

3.2.3 Protein identification by Mass Spectrometry

In combination with gel-based electrophoresis, mass spectrometry analysis was performed for protein identification as in Li *et al.*, 2014 (Paper I). Data Dependent Acquisition (DDA) methods either with protein bands from 1D gels or protein spots from 3D-gel approaches were used for MS analysis. In-gel-trypsin digestion was applied to bands or spots excised from the gels. Protein band analysis was performed with nLC/ESI-MS/MS into Q-Exactive (Thermo Scientific) and using Uniprot Arabidopsis (https://www.uniprot.org/) as database. Protein spot analysis was performed with LC/ESI-MS/MS into Q-TOF Elite (AB Sciex) and using TAIR 10 (https://www.arabidopsis.org/index.jsp) database.

3.3 Analysis of metabolites

3.3.1 Glucosinolate analysis

In Paper I, GSLs from non-infected and *B. cinerea*-infected Arabidopsis plants were used for GSL content determination following Mikkelsen and Halkier, 2003. In

short, after homogenization of freeze dried leaves, 70% (v/v) methanol and diethylaminoethyl Sephadex CL-6B (Amersham Pharmacia Biotech) in columns (Polyprep; Bio-Rad, Hercules, CA) were used to extract GSLs. Combination of results from HPLC system and LC-MS analysis allowed identification and quantification of GSLs. Internal standards were used for quantification.

In Paper IV, per analysed condition, eight biological replicates were used. Each replicate consisted of the longitudinal leaf halves of two kale plants. After homogenization, 1 mL of ice-cold 85% (v/v) methanol containing, internal standard, 20 nmol p-hydroxybenzyl glucosinolate (pOHb) was centrifuged (10 min, 13 000 ***g***, 4° C) and supernantant was divided for both GSLs and amino acid extraction (detailed in next section). Desulfo-GSLs were analysed as in Crocoll *et al.*, 2016 by Multiple Reaction Monitoring (MRM) on a LC-MS/MS described as follows. Advance UHPLC system (Bruker) with C18 column (Kinetex 1.7 u XB-C18, 10 cm x 2.1 mm, 1.7 µm particle size, Phenomenex) in tandem with an EVOQ Elite TripleQuad mass spectrometer (Bruker) rigged with an electrospray ionisation source (ESI). The separation gradient was composed of water/0.05% (v/v) formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.4 mL/min. Quantification of individual GSLs was calculated based on the internal standard (pOHB).

3.3.2 Analysis of amino acids

In Paper I, Arabidopsis leaves were ground to fine powder in liquid nitrogen and amino acids were extracted with 25 mM HCl in 50% (v/v) acetonitrile. After centrifugation, extracts were derivatized in a column in tandem with HPLC as described in Kreft *et al.*, (2003).

In Paper III, per analysed condition, eigth biological replicates were used. Each replicate consisted of the longitudinal leaf halves of two kale plants. Amino acids were extracted as described above (GSLs section), the supernatant was mixed 1:10 (v/v) with an internal standard (13C-, 15N-labelled amino acids from Algal amino acids 13C, 15N, Isotec). After filtration (Durapore® 0.22µm PVDF filters, Merck Millipore), samples were analysed by MRM as in Docimo *et al.*, (2012) and in Petersen *et al.*, (2019) on a LC-MS/MS with advance UHPLC system (Bruker) with a Zorbax Eclipse XDB-C18 column (100×3.0 mm, 1.8 µm, Agilent Technologies) in tandem with an EVOQ Elite Triple Quad mass spectrometer (Bruker) rigged with an ESI. Separation gradient was composed of water/0.05% (v/v) formic acid (solvent A) and acetonitrile/0.05% (v/v) formic acid (solvent B) at a flow rate of 500 µL/min.

3.3.3 Analysis of anthocyanins

Four biological replicates were used per analysed condition. Each replicate consisted of the longitudinal leaf halves of two kale plants. After homogenization, 45 mL of acidified methanol (0.1% HCl, v/v) were centrifuged and the collected supernatant was dried on a vacuum rotary evaporator to dissolve the sample on 1 mL acidified methanol (1% HCl, v/v).

Qualitative analysis of anthocyanins was performed using a Waters Acquity ultra-high-performance liquid chromatography (UPLC) system (Waters Corp.) tandem to Waters Quattro Premier Tandem Quadrupole mass spectrometer (Waters Corp.) rigged with electrospray ionization (ESI) as described in Yang *et al.*, 2018. The separation gradient was composed of 5% formic acid/95% water (v/v) (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min.

Quantitative analysis of anthocyanins was performed using a Shimadzu Nexera UHPLC system (Shimadzu Corp.). The chromatographic conditions were as described above. Cyanidin 3-O-glucoside was used as external standard for the quantification and total content of anthocyanins was calculated as the sum of the area under the peaks of the chromatogram.

3.4 Amino acid alignment and construction of the phylogenic tree

Arabidopsis (AT4G13940; www.arabidopsis.org), *L. luteus* (Q9SP37; https://www.uniprot.org), *S. oleracea* (A0A0K9RFV6; https://www.uniprot.org), *P. patens* (Pp3c19_13810V3.1; https://phytozome-next.jgi.doe.gov/) and *B. oleracea* convar. *capitata*, (Bol033424; https://phytozome.jgi.doe.gov/pz/portal.html) SAHH1 amino acid sequences were selected for the following analysis: amino acid alignment using ClustalW (Thompson *et al.*, 1994); pairwise comparison using BLOSUM62 matrix in BioEdit Version 7.2.5 and generation of phylogenetic tree with Neighbor-Joining method (Saitou and Nei, 1987) in MEGA7. Human SAHH amno acid sequence was included in the phylogenetic tree analisys.

3.5 Transcriptomic analysis

3.5.1 Arabidopsis

Four-week-old Arabidopsis rosettes grown under GL or long-term acclimated to HL were collected for transcriptomic analysis as described in Konert *et al.*, (2015). Arabidopsis short-term HL treatment transcriptomes, publicly available from the Genevestigator database (Hruz *et al.*, 2008), were compared with those from long-

term HL treatments. After processing, to enable comparison among the selected data and long-term HL data (details in Paper III), the transcript profiles were hierarchically clustered (Kolde, 2019) and a Venn diagram was generated with VennDiagram (Chen and Boutros, 2011) in R environment (R Core, 2018). Generated transcript clusters were further analysed by Gene Ontology enrichment with ShinyGO v0.60 (Ge and Jung, 2018).

3.5.2 *Brassica oleracea* convar. *acephala* RNAseq

Half Tall and Black Magic transcriptomic analysis was performed at Novogen UK (https://en.novogene.com/). Per analysed condition, four biological replicates were used and RNA purity, integrity and quantity were evaluated (Paper IV). Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) and quality control of the raw-data was performed by Novogen using in their custom-developed scripts (https://en.novogene.com/).

The *de novo* transcriptome assembly was conducted with Trinity (details in http://trinityrnaseq.github.io), and CORSET tool to avoid redundant assemblies. (https://github.com/Oshlack/Corset/wiki). The following seven databases were used for annotation: Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), Pfam (Protein families database), KOG/COG (Clusters of Orthologous Groups of proteins), Swiss-Prot (a manually annotated and reviewed protein sequence database), KO (KEGG Ortholog database), GO (Gene Ontology) and PlabiDB (Schwacke *et al.*, 2019). Quantification of the aligned reads was performed with RSEM (Li and Dewey, 2011).

R package DESeq2 (Love *et al.*, 2014) was used to evaluate the differential expression among kale varieties (four biological replicates per group) and *P*-values were adjusted by Benjamin and Hochberg's approach to control the False Discovery Rate (FDR). Genes with a $P < 0.05$ were assigned as differentially expressed. GO enrichment analysis and PCA were conducted in R environment.

4 Results

4.1 *Botrytis cinerea* infection involves AMC and glucosinolates as defence mechanisms in Arabidopsis

4.1.1 4MO-I3M GSL abundance depends on PP2A-B'*γ* in Arabidopsis

Yeast two-hybrid screening identified INDOLE GLUCOSINOLATE METHYLTRANSFERASE 4 (IGMT4, AT1G21130) as a candidate interactor for PP2A-B'*γ*. Further support for the physical interaction between PP2A-B'*γ* and both IGMT1 and 4 was obtained by bimolecular fluorescence complementation BiFC (Paper I). IGMTs form a highly conserved family of proteins, which share 95% amino acid identity and catalyse an O-methylation reaction in the indole GSL biosynthesis pathway by converting hydroxyl-indole GSL intermediates into methoxy-indole-methyl-GSL. Specifically, IGMTs convert I3M to 4-methoxy-indol-3-yl-methyl GSL (4MO-I3M). To elucidate the significance of PP2A-B'*γ*-IGMT interactions, GSL profiles were analysed in Arabidopsis wild type, *pp2a-b'γ* and *pp2a-b'γ 35S::PP2A-B'γ* complementation line. Since foliar GSL content is known to vary between different developmental stages, and their biosynthesis becomes enhanced under biotic stress, healthy Arabidopsis rosettes of two different developmental stages (19 and 33 day-old plants) were compared with *B. cinerea* infected samples. Five aliphatic and three indole GSL species were detected and quantified in the analysed conditions (Paper I).

Developmental stage did not affect the accumulation of 4MO-I3M GSL in *pp2a-b'γ*, but *B. cinerea* infection enhanced the accumulation of 4MO-I3M in *pp2a-b'γ*. To underline the specific regulation of 4MO-I3M GSL accumulation by PP2A-B'*γ,* it should be noted that none of the other seven GSLs detected showed differential accumulation in *pp2a-b'γ* or in the *35S::PP2A-B'γ* complementation line. Altogether, these results suggested that PP2A-B'*γ* is required to control the abundance of 4MO-I3M (Paper I).

4.1.2 The abundance of SAHH complexes is regulated by PP2A-B'*γ*

Since PP2A-B'*γ* affected the formation of 4MO-I3M GSL, possibly through IGMT regulation, we explored the contents of SAM and its methylation by-product SAH in Arabidopsis wild type, *pp2a-b'γ* and *pp2a-b'γ 35S::PP2A-B'γ* complementation line. Higher SAM/SAH ratios were observed in *pp2a-b'γ*, owing to the content of SAM increasing over a constant SAH level. This result, together with previous reports describing SAHH1 as a phospho-protein (Trotta *et al.*, 2011; Li *et al.*, 2014), prompted us to further investigate the role of PP2A-B'*γ* in the regulation of AMC.

Clear native gel electrophoresis, followed by immunoblotting, revealed six oligomeric complexes when detected with anti-SAHH antibody. The most intense band in our gel-based system was the fourth band, named complex 4, which accumulated in *pp2a-b'γ*. Based on size, the results suggested that complex 4 could correspond to a SAHH tetramer, while complexes 5 and 6 would correspond to a SAHH dimer and SAHH monomer, respectively. The higher-molecular-weight complexes (complex 1 and 2) were detected as two adjacent migrating bands, while complex 3 was located very close to complex 4 in our gel system (Paper I). The identity of these complexes remains to be determined.

4.2 SAHH is evolutionarily conserved in land plants

To analyse the evolutionary conservation of SAHH, amino acid sequences from representative photosynthetic organisms were studied in Paper II. The species selected for analysis were: the model species Arabidopsis; *L. luteu*s, the only plant with a crystalized SAHH structure; the highly economically valuable crops *B. oleracea* and *S. oleracea,* and the moss *P. patens*. Amino acid sequence comparison indicated similarities ranging from 90 to 99% on amino-acid level with no less than 84% of identity (Paper II). Of note, conservation of the previously described phosphorylation sites (Rahikainen *et al.*, 2018) suggested conserved PTM regulation mechanisms in these photosynthetic species.

To investigate if the level of amino-acid conservation is reflected in protein complex conformation, Clear Native gel electrophoresis and subsequent immunoblotting with an anti-SAHH antibody was performed with Arabidopsis, *L. luteus*, *P. patens*, *S. oleracea* and two varieties of *B. oleracea* convar. *acephala* called Half Tall and Black Magic. A band coincident with Arabidopsis complex 4, hypothesized to be a tetramer (Paper I), was found in all the species analysed (Paper II). Even *L. luteus*, in which SAHH was proposed to be functional as a dimer based on crystal structure (Brzezinski *et al.*, 2012), the corresponding complex 4 band was the most abundant. Furthermore, bands corresponding to the other five SAHH protein complexes in Arabidopsis were found in all the analysed species. These

results indicated that the organization of SAHH in protein complexes is highly conserved in land plants (Paper II).

4.3 Biochemical characterization of SAHH

4.3.1 Characterization of SAHH complexes

In Paper I, a 3D clear native gel electrophoresis-based approach in tandem with MS analysis identified carbonic anhydrase 1 (CA1; previously identified as the chloroplastic SALICYLIC ACID-BINDING PROTEIN 1, SABP3; Slaymaker *et al.*, 2002) as a SAHH complex 4 co-migrating protein. To verify whether CA1 was a SAHH-complex 4 component, biochemical treatments with dithiothreitol (DTT) and different concentrations of SDS were used in Arabidopsis leaf soluble protein extracts. While 10 mM DTT did not affect the stability or the amount of SAHH complex 4, treatment with 1% SDS decreased the amount of complex 4 when compared with non treated extracts (Paper II). Therefore a milder SDS treatment (0.25%) was applied to maintain SAHH complex 4 intact. When assessed on a 2D clear-native gel, the CA1-containing protein spot completely disappeared from the 0.25% SDS-treated samples, but SAHH complex 4 remained unaffected. This was a clear indication that CA1 was not a stoichiometric component of SAHH complex 4. These results in Paper II further supported the idea of SAHH complex 4 being a tetramer, as suggested in Paper I.

A SAHH immunoblot based on a 2D gel approach with isoelectric focusing followed by SDS-PAGE revealed that SAHH run at three different molecular masses over a wide range of isoelectric points. This result suggested a high variety of possible combinatorial PTMs on SAHH. In the same line, SAHH immunoblotting based on a 2D gel approach, consisting of clear native followed by Phostag gel, revealed slower-migrating spots from complexes 3, 4 and 5, indicating the existence of phosphorylated forms of the protein in all these complexes (Paper II).

4.3.2 Light-induced adjustments of SAHH in Arabidopsis and *Physcomitrella patens*

As the phylogenetically most distant species included in this thesis, Arabidopsis and *P. patens* were selected to investigate the conservation of the SAHH-complex response to short HL treatment. SAHH-complex formation turned out to be sensitive to short HL treatment in both species, while the total abundance of SAHH remained unaltered. Arabidopsis exhibited a slight decrease in the abundance of SAHH complex 2 with a concomitant increase in the abundance of complexes 5 and 6. *P. patens*, in turn, exhibited increased abundance of the complex corresponding to the

Arabidopsis SAHH complex 2. Moreover, light-dependent phosphoregulation of SAHH was evident in both species (Paper II).

4.4 Long-term acclimation to high light and elevated temperature impacts the plant transcriptome and metabolome

4.4.1 Long-term high light leads to specific transcriptomic reprogramming in Arabidopsis

To investigate long-term acclimation, the transcriptomic profile of Arabidopsis grown and acclimated for two-weeks under HL+ET was studied. The HL+ET Arabidopsis phenotype, with visually evident accumulation of protective pigments, was endorsed by transcriptomic data, since Gene Ontology (GO) enrichment analysis of differentially expressed genes revealed up-regulation of anthocyanin biosynthesis, flavonoid metabolism and abiotic stress related GO categories. In contrast, genes related to photosynthesis and light harvesting , as well as genes related to defence responses, SA signalling and indole GSL metabolism, were down-regulated in the long-term HL+ET treated plants (Paper III).

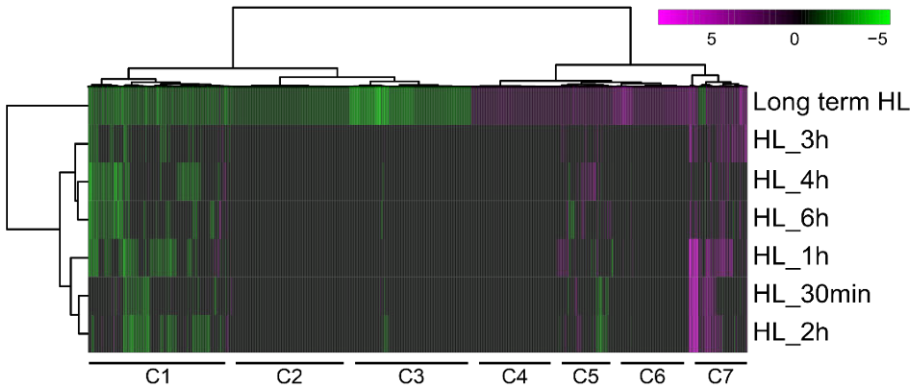


Figure 5. Hierarchical clustering and heatmap representation of differentially expressed genes (*p-value* <0.05) in long-term high light-acclimated Arabidopsis Col-0 compared with different short-term HL shift experiments. The long-term HL acclimation dataset was obtained experimentally, while the other sets of data were gathered from AtGenExpress and Gene Expression Omnibus. Purple and gren colours represent transcript abundance upregulation and downregulation respectively. HL, high light; C, Cluster.

Transcriptomic data from six different short-term HL experiments in Arabidopsis were gathered from public repositories and compared to the data from the long-term HL experiment (Paper III). The analysis revealed 1469 genes were differentially expressed uniquely in the long-term treatment, in contrast with the 49 genes that were common to all HL treatments. Among the set of long-term HL-specific up-regulated genes, GO categories related to positive regulation of transcription, membrane transport, or regulation of biosynthetic processes and biosynthesis of flavonoids were enriched. Whereas, GO enrichment analysis of the long-term HL downregulated genes revealed enrichment of GO categories related to photosynthetic light harvesting, chlorophyll biosynthesis, DNA integrity and biotic stress responses (Paper III).

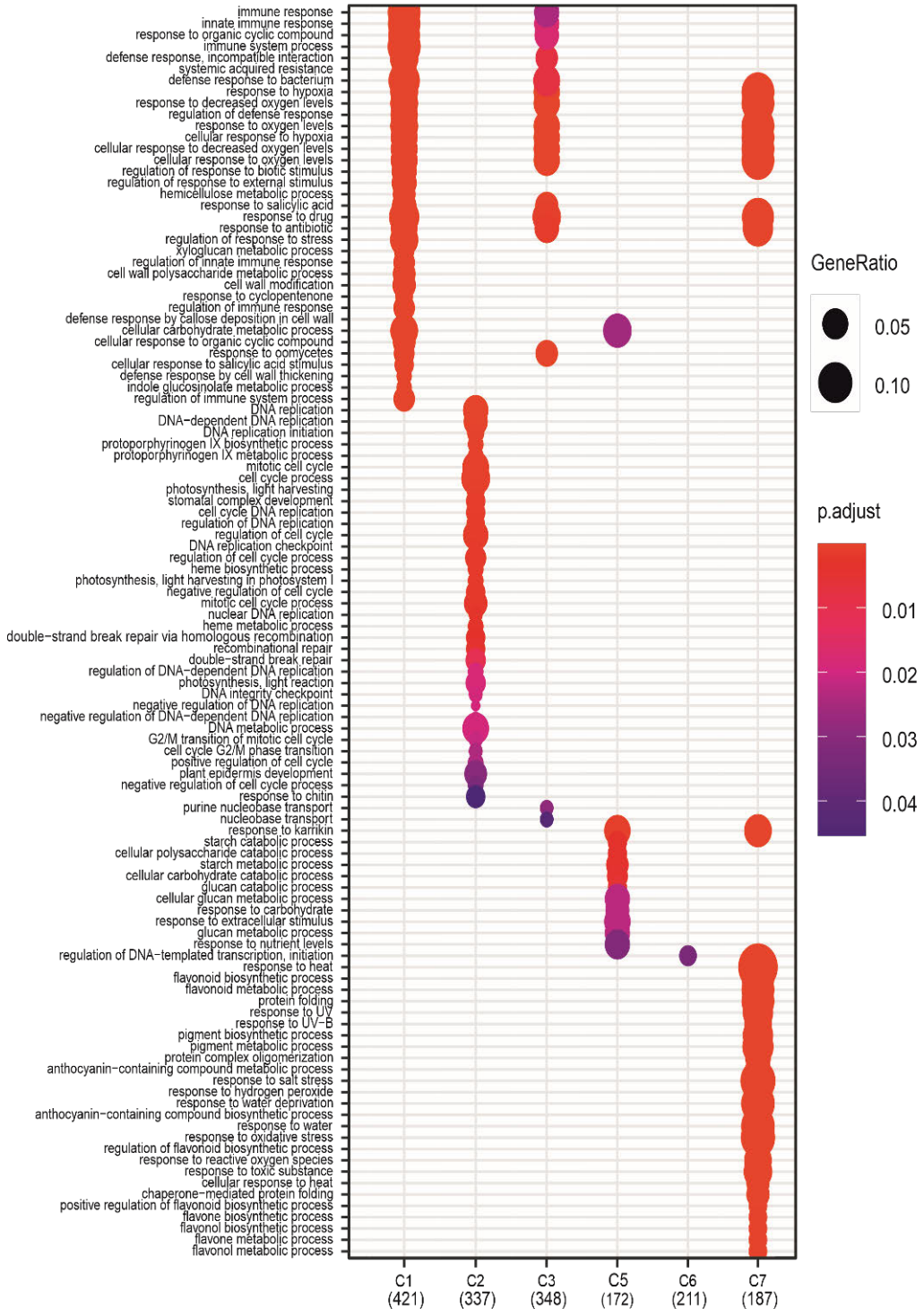

immune response
innate immune response
response to organic cyclic compound
immune system process
defense response, incompatible interaction
systemic acquired resistance
defense response to bacterium
response to hypoxia
response to decreased oxygen levels
regulation of defense response
response to oxygen levels
cellular response to hypoxia
cellular response to decreased oxygen levels
cellular response to oxygen levels
regulation of response to biotic stimulus
regulation of response to external stimulus
hemicellulose metabolic process
response to salicylic acid
response to drug
response to antibiotic
regulation of response to stress
xyloglucan metabolic process
regulation of innate immune response
cell wall polysaccharide metabolic process
cell wall modification
response to cyclopentenone
regulation of immune response
defense response by callose deposition in cell wall
cellular carbohydrate metabolic process
cellular response to organic cyclic compound
response to oomycetes
cellular response to salicylic acid stimulus
defense response by cell wall thickening
indole glucosinolate metabolic process
regulation of immune system process
DNA replication
DNA−dependent DNA replication
DNA replication initiation
protoporphyrinogen IX biosynthetic process
protoporphyrinogen IX metabolic process
mitotic cell cycle
cell cycle process
photosynthesis, light harvesting
stomatal complex development
cell cycle DNA replication
regulation of DNA replication
regulation of cell cycle
DNA replication checkpoint
regulation of cell cycle process
heme biosynthetic process
photosynthesis, light harvesting in photosystem I
negative regulation of cell cycle
mitotic cell cycle process
nuclear DNA replication
heme metabolic process
double−strand break repair via homologous recombination
recombinational repair
double−strand break repair
regulation of DNA−dependent DNA replication
photosynthesis, light reaction
DNA integrity checkpoint
negative regulation of DNA replication
negative regulation of DNA−dependent DNA replication
DNA metabolic process
G2/M transition of mitotic cell cycle
cell cycle G2/M phase transition
positive regulation of cell cycle
plant epidermis development
negative regulation of cell cycle process
response to chitin
purine nucleobase transport
nucleobase transport
response to karrikin
starch catabolic process
cellular polysaccharide catabolic process
starch metabolic process
cellular carbohydrate catabolic process
glucan catabolic process
cellular glucan metabolic process
response to carbohydrate
response to extracellular stimulus
glucan metabolic process
response to nutrient levels
regulation of DNA−templated transcription, initiation
response to heat
flavonoid biosynthetic process
flavonoid metabolic process
protein folding
response to UV
response to UV−B
pigment biosynthetic process
pigment metabolic process
protein complex oligomerization
anthocyanin−containing compound metabolic process
response to salt stress
response to hydrogen peroxide
response to water deprivation
anthocyanin−containing compound biosynthetic process
response to water
response to oxidative stress
regulation of flavonoid biosynthetic process
response to reactive oxygen species
response to toxic substance
cellular response to heat
chaperone−mediated protein folding
positive regulation of flavonoid biosynthetic process
flavone biosynthetic process
flavonol biosynthetic process
flavone metabolic process
flavonol metabolic process
C1 (421)
C2 (337)
C3 (348)
C5 (172)
C6 (211)
C7 (187)
GeneRatio
0.05
0.10
p.adjust
0.01
0.02
0.03
0.04

Figure 6. Gene ontology enrichment analysis of the differentially expressed transcripts including in each cluster in Figure 5. The 35 most significantly enriched biological processes are presented. Cluster 4 was omitted from the figure as it did not show significant enrichment for any GO category. The dot colour code depicts the adjusted *p*-values of the GO enrichment analysis, while their sizes account for the gene ratio of the GO enrichment. The numbers in brackets represent the numbers of transcripts in each cluster. C: cluster.

To further study the differential responses to short-term and long-term HL, hierarchical clustering analysis was performed. Long-term HL treatment branched out from all short-term HL treatments. Short-term HL treatments clustered into two sub-cluster based on the length of the HL applied treatment (30 min, 1 h and 2 h treatment separated from 3h, 4h and 6 h treatment) (Figure 5). These results emphasise the differential response mechanism between short-term and long-term HL treatments (Paper III). A hierarchical clustering heatmap defined seven clusters (Figure 5; Paper III). Clusters 1 and 7 comprised genes that responded in all the analysed conditions, by decreased and increased transcript abundance, respectively. On the contrary, clusters 2, 3 and 4 mainly responded exclusively to long-term conditions. Cluster 5 comprised a set of genes accumulated in long-term HL, but with a variety of responses among the short-term HL experiments. The main enriched GO categories in the above-mentioned clusters were biotic stress and immunity responses (cluster 1), DNA regulation and replication (cluster 2), biotic stimulus (cluster 3), karrikin and carbohydrate monomer metabolism (cluster 5), DNA and RNA processes (cluster 6), flavonoid metabolism and stress-related genes (cluster 7). Cluster 4 was omitted from the analysis since it did not show significant GO enrichment (Figures 5 and 6; Paper III).

4.4.2 Long-term acclimation to high light and elevated temperature modulates the accumulation of nutritionally valuable specialized metabolites in kale

Two different varieties of the economically important crop kale, Half Tall and Black Magic, were selected to study their GSL pattern when grown under HL+ET. Kale indole GSLs were mainly accumulated in the form of I3M, a precursor of both NMO-I3M and 4MO-I3M. Half Tall did not differ in its accumulation of I3M under HL+ET, whereas Black Magic decreased its level of I3M when grown under HL+ET. Notably, both varieties exhibited a decrease in 4MO-I3M under HL+ET (Paper IV), this was opposite to the trend that was previously described in Arabidopsis under *B. cinerea* infection (Paper I).

Although cultivar-dependent differences were observed in the profile of aliphatic kale GSLs, HL+ET independently enhanced their accumulation. Aliphatic GSLs can be grouped based on their lateral chain length, and they vary depending on which MAM enzyme isoform is present (Benderoth et al., 2009). Thus, 3-carbon side chain (C3) 3-methylsulfinylpropyl (3MSP) and 2-propenyl (2PROP) GSLs were the predominant aliphatic GSLs in the Half Tall variety, but nearly undetectable in Black Magic. In contrast, aliphatic GSLs content in Black Magic was predominantly increased in the C4 forms of 4MSB. The only detectable aromatic GSL, 2-phenylethyl (2PE GSL), was uniquely present in Black Magic and HL+ET enhanced its accumulation (Paper IV).

The two selected kale varieties differed in their pigmentation pattern, Half Tall being a pale green variety and Black Magic exhibiting darker green and more red-range-related colours. When grown under HL+ET conditions, kale pigment accumulation responses were also different. Half Tall did not show any colour change, while Black Magic accumulated purple pigments under HL+ET. To evaluate pigment accumulation on the two kales, anthocyanin profiling was performed.

Half Tall anthocyanins were only detectable in trace amounts. On the contrary, ten different anthocyanin compounds were detected in Black Magic, always as acylated forms. Eight of them were identified based on mass and UV spectra (Table 1). Interestingly, sinapic, ferulic, caffeic and p-coumaric acids are the principal acyl donors of the acylated anthocyanins found. The most abundant antocyanins, cyanidin-3-feruloyl-sinapoyl-diglucoside-5-glucoside and cyanidin-3-disinapoyl-diglucoside-5-glucoside, were also the most highly differentially accumulated in HL+ET. Nonetheless, the data suggested a general accumulation of anthocyanins in HL+ET.

Table 1. Identification of anthocyanins from Black Magic kale leaf extracts.

Peak number	Tentative compound
1	cyanidin-3-sinapoyl-diglucoside-5-glucoside
2	cyanidin-3-feruloyl-diglucoside-5-glucoside
3	unknown
4	cyanidin-3-sinapoyl-caffeoyl-diglucoside-5-glucoside I
5	cyanidin-3-sinapoyl-caffeoyl-diglucoside-5-glucoside II
6	cyanidin-3-caffeoyl-feruloyl-diglucoside
7	cyanidin-3-sinapoyl-p-coumaroyl-diglucoside-5-glucoside
8	unknown
9	cyanidin-3-feruloyl-sinapoyl-diglucoside-5-glucoside
10	cyanidin-3-disinapoyl-diglucoside-5-glucoside

5 Discussion

Photosynthetic organisms undergo coordinated adjustments in gene expression, metabolism and growth to optimize their fitness in the prevailing growth environment. Under natural conditions, fluctuations in light irradiance and the occurrence of plant pathogens are common environmental stress factors, while in greenhouses alterations in growth conditions can be used to artificially manipulate plant metabolomes to enhance the production of desired end products. However, the basic understanding of how plants ward off pathogenic microbes or respond to abiotic environmental factors is still limited. In this PhD thesis, I aimed towards understanding how biotic and abiotic factors affect specialized metabolism in cruciferous plants, and how these metabolic interactions are regulated on a molecular level.

5.1 Post-translational control allows tight regulation of metabolic processes

Posttranslational modifications allow a wide range of regulatory mechanisms in metabolism and thereby support the basic mechanisms of life. A diversity of PTMs occurs in metabolic enzymes and their upstream regulators, altering protein interactions, enzymatic kinetics, and stabilization/degradation ratios (Huber and Hardin, 2004). The majority of PTMs are reversible and energetically inexpensive. Also, the same amino acid residue may potentially undergo several different PTMs depending on cellular conditions. Since one PTM could influence the occurrence of other PTMs, protein diversity is massively extended by PTMs, resulting in a blast of possible proteomes with different regulatory interactions (Friso and Van Wijk, 2015). This thesis partially focused on the post-translational regulation of the AMC and its effects on the formation of methoxylated specialized metabolites.

In the plant kingdom, the occurrence of methylation reactions is particularly widespread, and extends far beyond the well-known DNA and histone methylation and its impacts on epigenetic regulation of gene expression. In addition to enzymes related to carbon fixation, other metabolic pathways and cellular signalling (Houtz *et al.*, 1989), a large number of specialized metabolites undergo methylation (Wang

et al., 2019). Methyl transferases catalyse the transmethylation reactions using SAM as a methyl donor.

To date, the Arabidopsis genome database harbours nearly 400 different methyl transferases (https://www.uniprot.org/), which reflects their high-substrate specificity (Rahikainen *et al.*, 2018). The wide occurrence of methylation in different molecules necessitates a tight regulation mechanism that ensures suitable availability of SAM. Every methylation produces a reaction by-product, SAH, which inhibits methyltransferases and should therefore be rapidly hydrolysed by SAHH (Rahikainen *et al.*, 2018). Therefore, adequate function of SAHH maintains the methylation capacity in the cell by ensuring the conversion of SAH to homocysteine within the SAM-regenerating AMC. Thus, regulation of SAHH could impact transmethylation capacity, and therefore SAHH activity affects processes such as defence and development (Rahikainen *et al.*, 2018).

The evolutionary conservation of SAHH1 has been demonstrated at amino acid level (Turner *et al.*, 2000; Kusakabe *et al.*, 2015; Paper II). Concretely, even the two phylogenetically most distant species studied in Paper II, Arabidopsis and *P. patens*, exhibited a high level of amino acid conservation. Furthermore, the amino acids known to carry PTMs in Arabidopsis turned out to be highly conserved as well (Paper II). Structural studies on different taxa postulated SAHH as an enzymatic complex composed by 2, 4 or 6 SAHH monomers (Matuszewska *et al.*, 1987; Tanaka *et al.*, 2004; Reddy *et al.*, 2008; Brzezinski *et al.*, 2012). The only crystal structure of SAHH in plants was reported for *L. luteus*, and it proposed SAHH to be active as a dimer (Brzezinski *et al.*, 2012). However, the data from Paper I presented evidence indicating that the predominant form of SAHH in Arabidopsis would correspond to a tetramer. This conclusion was reinforced by the results in Paper II, where comparative analysis by clear native PAGE detected protein complexes that co-migrated with the Arabidopsis SAHH tetramer in *B. oleracea* convar *italica* (broccoli), two varieties of *B. oleracea* convar *acephala* (kale), *S. oleracea* (spinach), *P. patens*, and notably also in *L. luteus*.

The absence of post-translational modifications on the recombinant *L. luteus* SAHH used in the crystallographic studies (Brzezinski *et al.*, 2012) could explain the difference between the results arising from the previous studies and those obtained in my research. Most likely, formation of the more abundant SAHH complex 4, which is likely to be a tetramer in all the analysed species (Papers I and II) is PTM-dependent, although the required pattern of modifications that would support the tetrameric complex is still elusive. The amino acid sequence conservation of SAHH among different species would allow equivalent complex formation. Likewise, it would support the existence of evolutionarily conserved post-translational regulation mechanisms.

Another aspect that can significantly affect SAHH function is its sub-cellular localization, but whether SAHH moves in monomeric or oligomeric compositions is currently not known. DNA and histone methylation rely on the correct SAHH translocation into the nucleus to prevent the inhibitory effect of methylation by-product on methyl transferases. Arabidopsis SAHH1 contains a nuclear targeting 40-amino-acid sequence (Stępkowski *et al.*, 2005). Although localization studies revealed SAHH1 in multiple sub-cellular compartments, chloroplast and mitochondria are devoid in SAHH1 (Lee *et al.*, 2012; Paper II). Presumably, these organelles depend on SAH transporters for its detoxification, which are supported by SAHH1 accumulation in the chloroplast periphery (Paper II). Thus, even though trans-methylation reactions are ubiquitously distributed within the cell, their regulation mechanisms could differ. Besides, SAHH1 was localized in vesicular and reticulate structures, the plasma membrane, and also proven to persistently move along cytosolic strands (Paper II) although the exact mechanisms that govern the sub-cellular motion require further investigation.

PTM-catalyzing enzymes are also subject of PMT regulation themselves, which illustrates their complex multilayer and cross-level metabolic regulation networks. For example, SAHH1 is regulated by PTMs among other mechanisms. Although an extensive number of PTMs, including phosphorylation, S-nitrosylation, acetylation and ubiquitination, have been empirically identified on SAHH1 (list of all known SAHH PTMs is detailed in Rahikainen *et al.*, 2018), their biological significance has not yet been clarified. Likewise, the enzymes driving these modifications are still poorly understood.

PP2A-B′*γ* has been characterized as negative defence regulator in plants (Li *et al.*, 2014; Konert *et al.*, 2015). Moreover, PP2A-B′*γ* is necessary to control defence-related signalling as well as to prevent cell death during developmental leaf senescence, in both cases in relation to salicylic acid signalling (Durian *et al.*, 2020), but the exact mechanisms remain to be established. The abundant SAHH complex 4 was accumulated in *pp2a-b′γ*, which phenocopies constitutive stress responses (Paper I) and therefore SAHH function is likely to be modulated by PP2A-B′*γ*-mediated dephosphorylation. The location of S20 and T44 in the surface of the folded protein (previously reported to be phosphorylated) on SAHH1, together with PP2A-B′*γ*-SAHH physical interaction (demonstrated in Paper I) suggest that PP2A-B′*γ* could target surface phosphorylation sites of SAHH. This, in turn, could affect SAHH complex 4 abundance, and therefore SAHH activity. However, the exact mechanism that governs this de-phosphorylation is not fully clarified.

5.2 The biosynthesis of specifically modified indole glucosinolates is regulated by PP2A-B′γ

GSLs have been well studied due to their role as potential repellents for insects as pathogens (Halkier and Gershenzon, 2006; Hopkins *et al.*, 2009), although abiotic stressors have recently also been reported to induce the accumulation and activation of GSLs (Engelen-Eigles *et al.*, 2006; Khan *et al.*, 2010; Steindal *et al.*, 2015).

The chemical diversity of GSLs arises from the secondary modifications that occur after the GSL core structure is formed (either aliphatic or indole GSLs). Thus, the enzymes responsible for these modifications, and their regulation have drawn scientific attention (Sønderby *et al.*, 2010; Nour-Eldin *et al.*, 2012). Environmentally induced adjustments in GSL formation are highly stress and species specific (Wittstock and Burow, 2010; Jensen *et al.*, 2015; Blažević *et al.*, 2020). Arabidopsis GSL methyltransferases, IGMT1–IGMT3 are transcriptionally activated upon green peach aphid infestation (De Vos and Jander, 2009), whereas *B. cinerea* infection increases transcript abundance of *IGMT1* and *IGMT2* (Xu *et al.*, 2016). IGMT family members drive the methylation of I3M GSL to generate 4MO-I3M. The specific antimicrobial and insect repellent role of 4MO-I3M in plants (Frerigmann *et al.*, 2016), and its influence on lepidopteran oviposition preferences (Kroymann, 2011), in addition to the induction of defences by 4MO-I3M-degradation products (Bednarek *et al.*, 2009; Clay *et al.*, 2009), directly impact plant-environment interactions in nature. Altogether, this underscores the tight interconnection between methionine metabolism and the ecological plant response through production of specialized metabolites. *B. cinerea* infection leads to 4MO-I3M accumulation, likely due to enhanced IGMT activity (Paper I).

Since IGMT phosphopetides were not detected in Paper I, it remains unclear whether PP2A-B′γ directly regulates the activity of IGMTs by dephosphorylating the enzymes. Even so, the proposed regulation mechanism suggests transient interactions among PP2A-B′γ, SAHH1 and IGMTs, where phosphoregulation of SAHH1 by PP2A-B′γ could indirectly influence IGMT activity (Paper I). In line with this model, multiple MTs have been reported to establish dynamic interactions with SAHH, presumably to ensure maintenance of their trans-methylation activity (Rahikainen *et al.*, 2018). Moreover, in the cell wall ferulate biosynthesis pathway, SAHH isoforms interacte with Caffeoyl coenzyme A ester O-methyltransferase 7 (CCoAOMT7) *in vivo*, which allow SAMS to bind in the complex (Yang *et al.*, 2019) to control the process.

5.3 Long-term high light and elevated temperature induces specific regulation of gene expression and metabolism in plants

5.3.1 Comparison of transcript profiles leads to identification of specific transcriptomic regulation upon long-term acclimation to high light

High light conditions can cause impaired photosynthetic capacity in unacclimated plants, and result in accumulation of ROS with a consequent damage to photosynthetic protein complexes and impairing of metabolic processes (Aro *et al.*, 1993; Muller, 2001; Miyake, 2010; Kono *et al.*, 2014; Tiwari *et al.*, 2016; Gu *et al.*, 2017). To diminish the effect of the ever-changing light conditions, plants have evolved coordinated mechanisms to avoid light-induced impairment. A vast number of studies aiming to understand HL stress responses in plants have focused on short-term exposure to excess irradiance, whereas studies on long-term HL acclimation are scarce. Therefore, the long-term HL transcriptome of Arabidopsis was evaluated in comparison with control conditions and six published short-term HL experiments (Paper III).

The hierarchical clustering heatmap analysis underlined the extensive transcriptomic regulation that was specifically triggered by long-term HL (Figure 2 of the Paper III). Among transcripts that were differentially abundant after long-term HL acclimation, those corresponding to GO categories related to DNA regulation and replication appeared to be slightly down-regulated, whereas processes related to DNA and RNA were up-regulated (Paper III). Such differential regulation of genes related to DNA metabolism suggested that unique mechanisms become triggered by the long-term HL. Most likely, DNA damage is caused by ROS accumulation during the stress period. Since ROS act as signalling components in many different stresses in plants, their effects on HL stress response has been specifically reported (Willems *et al.*, 2016; Pascual *et al.*, 2017). ROS accumulation leads to DNA damage at multiple molecular levels (Halliwell, 2006), however, the organelle and nuclear DNA undergoes different ROS-induced damage due to their different folding and the large extent of ROS production in the organelles (Das and Roychoudhury, 2014; Rahikainen *et al.*, 2016). Thus, to be able to sustain ROS scavenging mechanisms under long-term HL stress and counteracting their damaging effects requires intricate transcriptomic regulation and a constant mechanism for correct DNA replication (Paper III).

The long-term HL Arabidopsis transcriptome should be translated into a proteome and metabolome to determine the actual response to the environment, which could be plant acclimation or cell death. Particularly, the results obtained from

long-term HL acclimated Arabidopsis indicated accumulation of transcripts related to protectant pigments, like flavonoids and anthocyanins, as a consequence of HL acclimation (Chalker-Scott, 1999; Paper III). GSL-related genes are among the processes that undergo a downregulation at transcriptomic level as consequence of long-term HL (Paper III). Specialized metabolites and correct redox balance would determine actual plant survival.

5.3.2 Leaf GSL content is substantially altered by high light and elevated temperature in two varieties of kale

Abiotic stresses provoked by light or temperature conditions, as well as UV-B, drought or high ozone levels have been reported to impact the GSL content of cruciferous plants (Khan *et al.*, 2010; Khaling *et al.*, 2015; Steindal *et al.*, 2015). This raises a question, whether long-term HL+ET could affect GSL accumulation in high-value Brassica crops. In this scenario, we analysed the transcriptome and GSL profiles of two varieties of kale, the Half Tall and Back Magic.

Regarding aliphatic GSLs, Half Tall variety is prominent in the formation of 3-carbon side-chain aliphatic GSLs, while 4-carbon side chain aliphatic GSLs are more prominent in Black Magic. The aliphatic chain length is enzymatically controlled by an early step of GSL biosynthesis, even before GSL core formation, by MAM, and, although the length is settled, after GSL core formation different enzymes can further modify and increase GSL diversity (Sønderby *et al.*, 2010). Different MAM isoforms are responsible for the side chain length of aliphatic GSLs (Kroymann *et al.*, 2003; Benderoth *et al.*, 2006), so the differential GSL profiles in the kale varieties stem from genetic versatility.

On the other hand, both kale varieties also differ in the extent to which different chemical species are accumulated during the subsequent enzymatic modifications that indole GSLs could undergo. Black Magic heavily accumulated the methylsulfinyl form of the 4-carbon side chain 4-methylsulfinylbutyl GSL (4MSB GSL), whereas Half Tall accumulated the next modification step form, 2PROP GSL. Altogether, the GSL profiles indicate that the varieties have different enzymatic isoforms and/or regulation mechanisms in the GSL biosynthesis pathways (Figure 4 and Paper IV)

6 Conclusions and future perspectives: high light stress as a non-invasive means for cultivation of healthier plants

Plants are the best chemists. Basic carbon metabolism is universal in all plant species, and even other kingdoms. However, plants have a stunning feature, shared with fungi and bacteria, of being capable of synthetizing an enormous assortment of specialized metabolites (Wang *et al.*, 2019). Thus, it has been reckoned that across plant kingdom no less than 200 000, but up to $1x10^6$, specialized metabolites may be generated (Dixon and Strack, 2003; Rai *et al.*, 2017; Fang *et al.*, 2019). Although this remarkable chemical diversity is due to evolutionary pressure in response to variation in the environment, specialized metabolites have an immense impact on human life.

GSLs are formed in a family of plants that are used as nourishment, such as kales, broccoli, sprouts and cabbage. Epidemiological studies have correlated a diet rich in GSL-containing vegetables with reduced incidence and/or progression of a wide range of cancers, including, lung, bowel, kidney, breast, prostate, stomach and colorectal (Lin *et al.*, 1998; Seow *et al.*, 2002; Hsu *et al.*, 2007; Kirsh *et al.*, 2007; Zeng *et al.*, 2010; Bosetti *et al.*, 2012). Moreover, evidence has also accumulated for protective effects against chronic inflammation, neurodegeneration (Cartea *et al.*, 2008; Angelino and Jeffery, 2014) and type II diabetes (Kurotani *et al.*, 2013).

The actual bioactive molecules are the GSL breakdown products, nitriles, epithionitriles, thiocyanates and isothiocyanates. Specific studies on the bioactive products of 4MSB and 2PE GSLs, named sulforaphane and phenylethyl isothiocyanate (PEITC), respectively, have revealed their multifaceted involvement in preventing cancer.

Long-term oxidative stress has been proven to trigger carcinogenesis in humans, due to DNA damage and inflammation (Narendhirakannan and Hannah, 2013). Sulforaphane strongly activates the nuclear factor erythroid-2 (NF-E2) and related factor 2 (Nrf2), which has a critical role in oxidative stress response (Yamagishi and Matsui, 2016). The Nrf2-mediated oxidative stress response includes the activation

of a positive feedback loop/mechanism that maintains this response activated, therefore this pathway has a crucial impact in preventing carcinogenesis. In addition, sulforaphane is involved in the epigenetic control of carcinogenesis progression. It inhibits histone deacetylases promoting the expression of the tumour suppressor gene p21 and the proapoptotic protein Bax (Singh *et al.*, 2005). Interestingly, sulforaphane has an effect over histone H1 phosphorylation, positively correlated to bladder cancer progression, through its PP2A and PP1β enhancing role (Su *et al.*, 2018). On the other hand, PEITC has been proposed as anti-metastatic agent by blocking the migration and tissue invasion mechanisms (Soundararajan and Kim, 2018). PEITC causes cancer cell oxidative damage by ROS induction within tumour cells (Gupta *et al.*, 2015) that among many other consequences, promotes cancer cell apoptosis. Also, PEITC anti-inflamatory effects have been described in prostate cancer (Tang *et al.*, 2011). Altogether Sulforaphane and PEITC play an important role in both cancer prevention and progression.

Both kale varieties studied in this thesis produce 4MSB and 2PE, and even more importantly, their accumulation could be enhanced by growing the plants under HL+ET (Paper IV). Although Half Tall contains these two GSLs, which respond to a HL+ET treatment, their total accumulation is low in Half Tall in comparison with Black Magic (Paper IV). 2PE is the second most abundant GSL species accumulated in Black Magic, where HL+ET promoted a 162% increase, to reach 191.54 nmol g^{-1} FW. Additionally, in Black Magic it was possible to detect ten different anthocyanin species, which were impossible to trace in Half Tall. Altogether, the results of this thesis indicate the high nutritional value of Black Magic. In the case of 4MSB GSL from Black Magic, HL+ET enhanced its accumulation by 265% leading to a total of 101.34 nmol g^{-1} FW. As a precursor of sulforaphane, 4MSB-rich crops have drawn special interest in nutritional breading programs. Classical breeding program using broccoli and *Brassica villosa* led to the commercialization of Beneforte™, which contains at least two times more 4MSB GSL than other broccoli varieties (Faulkner *et al.*, 1998; Mithen *et al.*, 2003). Field experiments showed that Beneforte™ lines are able to accumulate between 1500-2500 nmol g^{-1} FW (Traka *et al.*, 2013). Despite the significantly different quantities in already commercialized crops like Beneforte™ and the findings in this thesis, Black Magic could be an excellent candidate for breading programs to enhance nutritional potential.

The results of this thesis emphasize the potential of HL for modulate the plant metabolome (Papers III and IV). The current global change affects biotic conditions all over the world. In many Mediterranean regions where these Brassicas are important field crops, solar irradiance has increased due to climate change over the last few decades. Understanding how the long-term HL leads to the unique regulation of metabolic networks in plants is crucial in this context (Paper III). Moreover,

determining the impact of light on the nutritional value of crop species would ensure the quality of the foodstuff.

Acknowledgements

This work was carried out at the unit of Molecular Plant Biology, Department of Biochemistry. The work was financially supported by CoE Molecular Biology of Primary Producers, Academy of Finland and University of Turku Graduate School Doctoral Programme in Molecular Life Sciences. Prof. Eva-Mari Aro is warmly thanked for the opportunity to work in an exquisite and well equipped laboratory.

I want to thank my supervisor Dr. Saijaliisa Kangasjärvi for support and guidance during these years. Moreover, I want to thank the current and former group members which have contributed to my scientific development.

I am grateful for Prof. Eevi Rintamäki for the help and instructions during my PhD studies and as member of my scientific advisory committee. In the same way, I thank Dr. Petri Kouvonen and Dr. Kirk Overmyer for advice and encouragement as scientific advisory committee members. I thank Dr. T. Matthew Robson and Dr. Pablo Velasco Pazos for critically reviewing this PhD thesis and Dr. Markku Keinänen for kindly agreeing to be my opponent. I also would like to express my gratitude to all the co-authors for their contribution to the research presented in this PhD thesis. Moreover, I want to thank Eve, Anniina, Essi, Mika and Tapio for all the help in daily practical and technical issues.

I want to give thanks to my workmates who have created the friendly and supportive environment. Specially, Marjaana (who never complain for share office with a talk-alone-me), Mika (for morning chats in different languages), Julia and Martina (for travel with me and become my true friends), and Chus (for uncountable scientific and friendship related issues). Thanks to everybody at the Molecular Plant Biology!

On the personal level, I want to thank my friends who have my back through these years. Thank you Marta, Mara, Laura, Juan and Paula; you have cut out the distance! I would like to express my gratitude to my food-family in Finland, Barajas-Sánchez and Pascual-Alfonso. Also, I need to mention the person who enjoy the most

by doing science, and this joy is contagious around him, Dr. Valledor. Finally, I am thankful for the support that my family has always given me, which is unspeakable. Gracias mamá, papá, Lucía y María por creer que podría cuando yo misma dudaba.

December 2020

Sara

List of References

Alboresia A, Gerottob C, Giacomettib GM, Bassia R, Morosinotto T. 2010. *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. Proceedings of the National Academy of Sciences of the United States of America **107**, 11128–11133.

Angelino D, Jeffery E. 2014. Glucosinolate hydrolysis and bioavailability of resulting isothiocyanates: Focus on glucoraphanin. Journal of Functional Foods **7**, 67–76.

Aro EM, Virgin I, Andersson B. 1993. Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. BBA - Bioenergetics **1143**, 113–134.

Ault-Riché DB, Yuan CS, Borchardt RT. 1994. A single mutation at lysine 426 of human placental S-adenosylhomocysteine hydrolase inactivates the enzyme. Journal of Biological Chemistry **269**, 31472–31478.

Bar-On YM, Phillips R, Milo R. 2018. The biomass distribution on Earth. Proceedings of the National Academy of Sciences of the United States of America **115**, 6506–6511.

Bartoli CG, Casalongué CA, Simontacchi M, Marquez-Garcia B, Foyer CH. 2013. Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. Environmental and Experimental Botany **94**, 73–88.

Bednarek P, Piślewska-Bednarek M, Svatoš A, *et al.* 2009. A Glucosinolate Metabolism Pathway in Living Plant Cells Mediates Broad-Spectrum Antifungal Defense. Plant Cell **323**, 101–106.

Bell L, Oloyede OO, Lignou S, Wagstaff C, Methven L. 2018. Taste and Flavor Perceptions of Glucosinolates, Isothiocyanates, and Related Compounds. Molecular nutrition & food research **62**, e1700990.

Benderoth M, Pfalz M, Kroymann J. 2009. Methylthioalkylmalate synthases: Genetics, ecology and evolution. Phytochemistry Reviews **8**, 255–268.

Benderoth M, Textor S, Windsor AJ, Mitchell-Olds T, Gershenzon J, Kroymann J. 2006. Positive selection driving diversification in plant secondary metabolism. Proceedings of the National Academy of Sciences of the United States of America **103**, 9118–9123.

Benowitz NL. 2009. Pharmacology of nicotine: Addiction, smoking-induced disease, and therapeutics. Annual Review of Pharmacology and Toxicology **49**, 57–71.

Blažević I, Montaut S, Burčul F, Olsen CE, Burow M, Rollin P, Agerbirk N. 2020. Glucosinolate structural diversity, identification, chemical synthesis and metabolism in plants. Phytochemistry **169**, 112100.

Boddy L. 2016. *Pathogens of Autotrophs*. Elsevier Ltd.

Boggs DA, Palmer JR, Wise LA, Spiegelman D, Stampfer MJ, Adams-Campbell LL, Rosenberg L. 2010. Fruit and vegetable intake in relation to risk of breast cancer in the black women's health study. American Journal of Epidemiology **172**, 1268–1279.

Bones AM, Rossiter JT. 1996. The myrosinase-glucosinolate system, its organisation and biochemistry. Physiologia Plantarum **97**, 194–208.

Bosetti C, Filomeno M, Riso P, Polesel J, Levi F, Talamini R, Montella M, Negri E, Franceschi S, La Vecchia C. 2012. Cruciferous vegetables and cancer risk in a network of case-control studies. Annals of Oncology **23**, 2198–2203.

Brzezinski K, Dauter Z, Jaskolski M. 2012. High-resolution structures of complexes of plant S -adenosyl- L -homocysteine hydrolase (*Lupinus luteus*). Acta Crystallographica **D68**, 218–231.

Burow M. 2016. Chapter Two - Complex Environments Interact With Plant Development to Shape Glucosinolate Profiles. In: Kopriva SBT-A in BR, ed. Glucosinolates. Academic Press, 15–30.

Caarls L, Pieterse CMJ, Van Wees SCM. 2015. How salicylic acid takes transcriptional control over jasmonic acid signaling. Frontiers in Plant Science **6**, 1–11.

Cantoni GL. 1975. Biological methylation: selected aspects. Annual review of biochemistry **44**, 435–451.

Cartea ME, Velasco P, Obregón S, Padilla G, de Haro A. 2008. Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. Phytochemistry **69**, 403–410.

Chalker-Scott L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochemistry and Photobiology **70**, 1–9.

Chen H, Boutros PC. 2011. VennDiagram: A package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics **12**, 35.

Chen D, Shao Q, Yin L, Younis A, Zheng B. 2019. Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. Frontiers in Plant Science **9**, 1–13.

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM. 2009. Glucosinolate Metabolites required for and Arabidopsis innate immune response. Science **323**, 95–101.

Crews TE, Carton W, Olsson L. 2018. Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. Global Sustainability **1**.

Crocoll C, Halkier BA, Burow M. 2016. Analysis and quantification of glucosinolates. Current protocols in plant biology **1**, 385–409.

Das K, Roychoudhury A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science **2**, 1–13.

Dixon RA, Strack D. 2003. Phytochemistry meets genome analysis, and beyond. Phytochemistry **62**, 815–816.

Docimo T, Reichelt M, Schneider B, Kai M, Kunert G, Gershenzon J, D'Auria JC. 2012. The first step in the biosynthesis of cocaine in *Erythroxylum coca*: the characterization of arginine and ornithine decarboxylases. Plant molecular biology **78**, 599–615.

Du H, Wu J, Ji KX, Zeng QY, Bhuiya MW, Su S, Shu QY, Ren HX, Liu ZA, Wang LS. 2015. Methylation mediated by an anthocyanin, O-methyltransferase, is involved in purple flower coloration in *Paeonia*. Journal of Experimental Botany **66**, 6563–6577.

Durian G, Jeschke V, Rahikainen M, *et al.* 2020. PROTEIN PHOSPHATASE 2A-B'γ Controls *Botrytis cinerea* Resistance and Developmental Leaf Senescence. Plant Physiology **182**, 1161–1181.

Engelen-Eigles G, Holden G, Cohen JD, Gardner G. 2006. The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.). Journal of Agricultural and Food Chemistry **54**, 328–334.

Estavillo GM, Crisp P a., Pornsiriwong W, *et al.* 2011. Evidence for a SAL1-PAP Chloroplast Retrograde Pathway That Functions in Drought and High Light Signaling in Arabidopsis. The Plant Cell **23**, 3992–4012.

Fang C, Fernie AR, Luo J. 2019. Exploring the Diversity of Plant Metabolism. Trends in Plant Science **24**, 83–98.

Faulkner K, Mithen R, Williamson G. 1998. Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. Carcinogenesis **19**, 605–609.

Fedoroff N V., Battisti DS, Beachy RN, *et al.* 2010. Radically rethinking agriculture for the 21st century. Science **327**, 833–834.

Francini A, Sebastiani L. 2019. Abiotic stress effects on performance of horticultural crops. Horticulturae **5**.

Frerigmann H, Piślewska-Bednarek M, Sánchez-Vallet A, Molina A, Glawischnig E, Gigolashvili T, Bednarek P. 2016. Regulation of Pathogen-Triggered Tryptophan Metabolism in *Arabidopsis thaliana* by MYB Transcription Factors and Indole Glucosinolate Conversion Products. Molecular Plant **9**, 682–695.

Friso G, Van Wijk KJ. 2015. Posttranslational protein modifications in plant metabolism. Plant Physiology **169**, 1469–1487.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Current Opinion in Plant Biology **9**, 436–442.

Furner IJ, Sheikh MA, Collett CE. 1998. Gene silencing and homology-dependent gene silencing in Arabidopsis: Genetic modifiers and DNA methylation. Genetics **149**, 651–662.

Galili G, Amir R, Fernie AR. 2016. The Regulation of Essential Amino Acid Synthesis and Accumulation in Plants. Annual Review of Plant Biology **67**, 153–178.

Ge S, Jung D. 2018. ShinyGO: a graphical enrichment tool for animals and plants. bioRxiv, 315150.

Gershenzon J, Textor S, Tokuhisa JG, de Kraker J-W, Hause B. 2007. MAM3 Catalyzes the Formation of All Aliphatic Glucosinolate Chain Lengths in Arabidopsis. Plant Physiology **144**, 60–71.

Giovanelli J, Mudd SH, Datko AH. 1985. Quantitative analysis of pathways of methionine metabolism and their regulation in *Lemna*. Plant Physiology **78**, 555–560.

Govrin EM, Levine A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Current Biology **10**, 751–757.

Govrin EM, Levine A. 2002. Infection of Arabidopsis with a necrotrophic pathogen, *Botrytis cinerea*, elicits various defense responses but does not induce systemic acquired resistance (SAR). Plant Molecular Biology **48**, 267–276.

Gu J, Zhou Z, Li Z, Chen Y, Wang Z, Zhang H, Yang J. 2017. Photosynthetic Properties and Potentials for Improvement of Photosynthesis in Pale Green Leaf Rice under High Light Conditions. Frontiers in Plant Science **8**, 1–14.

Gupta P, Wright SE, Kim S-H, Srivastava SK. 2015. Phenethyl Isothiocyanate: A comprehensive review of anti- cancer mechanisms. **1846**, 405–424.

Gust D, Moore TA, Moore AL. 2009. Solar fuels via artificial photosynthesis. Accounts of Chemical Research **42**, 1890–1898.

Halkier BA, Gershenzon J. 2006. Biology and Biochemistry of Glucosinolates. Annual Review of Plant Biology **57**, 303–333.

Halliwell B. 2006. Reactive Species and Antioxidants. Redox Biology Is.pdf. Plant Physology **141**, 312–322.

Hildebrandt TM. 2018. Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. Plant Molecular Biology **98**, 121–135.

Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP. 2015. Amino Acid Catabolism in Plants. Molecular Plant **8**, 1563–1579.

Hiltpold I, Turlings TCJ. 2012. Manipulation of Chemically Mediated Interactions in Agricultural Soils to Enhance the Control of Crop Pests and to Improve Crop Yield. Journal of Chemical Ecology **38**, 641–650.

Hopkins RJ, van Dam NM, van Loon JJA. 2009. Role of Glucosinolates in Insect-Plant Relationships and Multitrophic Interactions. Annual Review of Entomology **54**, 57–83.

Houtz RL, Stults JT, Mulligan RM, Tolbert NE. 1989. Post-translational modifications in the large subunit of ribulose bisphosphate carboxylase/oxygenase. Proceedings of the National Academy of Sciences of the United States of America **86**, 1855–1859.

Howlett BJ. 2006. Secondary metabolite toxins and nutrition of plant pathogenic fungi. Current Opinion in Plant Biology **9**, 371–375.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P. 2008. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Advances in bioinformatics **2008**, 420747.

Hsu CC, Chow WH, Boffetta P, *et al.* 2007. Dietary risk factors for kidney cancer in Eastern and Central Europe. American Journal of Epidemiology **166**, 62–70.

Huang KY, Su MG, Kao HJ, Hsieh YC, Jhong JH, Cheng KH, Huang H Da, Lee TY. 2016. dbPTM 2016: 10-year anniversary of a resource for post-translational modification of proteins. Nucleic Acids Research **44**, D435–D446.

Huber SC, Hardin SC. 2004. Numerous posttranslational modifications provide opportunities for the intricate regulation of metabolic enzymes at multiple levels. Current Opinion in Plant Biology **7**, 318–322.

Initiative TAG. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408**, 796–815.

Iqbal N, Masood A, Khan MIR, Asgher M, Fatma M, Khan NA. 2013. Cross-talk between sulfur assimilation and ethylene signaling in plants. Plant Signaling and Behavior **8**, 104–112.

Jensen LM, Jepsen HSK, Halkier BA, Kliebenstein DJ, Burow M. 2015. Natural variation in cross-talk between glucosinolates and onset of flowering in Arabidopsis. Frontiers in Plant Science **6**, 1–10.

Jeschke V, Burow M. 2018. Glucosinolates. eLS, 15–30.

Jiang CD, Wang X, Gao HY, Shi L, Chow WS. 2011. Systemic regulation of leaf anatomical structure, photosynthetic performance, and high-light tolerance in sorghum. Plant Physiology **155**, 1416–1424.

Johann S, Mendes BG, Missau FC, de Resende MA, Pizzolatti MG. 2011. Antifungal activity of five species of *Polygala*. Brazilian Journal of Microbiology **42**, 1065–1075.

Kappers IF, Hoogerbrugge H, Bouwmeester HJ, Dicke M. 2011. Variation in Herbivory-induced Volatiles Among Cucumber (*Cucumis sativus* L.) Varieties has Consequences for the Attraction of Carnivorous Natural Enemies. Journal of Chemical Ecology **37**, 150–160.

Khaling E, Papazian S, Poelman EH, Holopainen JK, Albrectsen BR, Blande JD. 2015. Ozone affects growth and development of *Pieris brassicae* on the wild host plant *Brassica nigra*. Environmental Pollution **199**, 119–129.

Khan N, Bano A, Ali S, Babar MA. 2020. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regulation **90**, 189–203.

Khan MAM, Ulrichs C, Mewis I. 2010. Influence of water stress on the glucosinolate profile of Brassica oleracea var. italica and the performance of *Brevicoryne brassicae* and *Myzus persicae*. Entomologia Experimentalis et Applicata **137**, 229–236.

Khoo HE, Azlan A, Tang ST, Lim SM. 2017. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & Nutrition Research **61**, 1361779.

Kirsh VA, Peters U, Mayne ST, Subar AF, Chatterjee N, Johnson CC, Hayes RB. 2007. Prospective study of fruit and vegetable intake and risk of prostate cancer. Journal of the National Cancer Institute **99**, 1200–1209.

Kliebenstein DJ, Rowe HC, Denby KJ. 2005. Secondary metabolites influence *Arabidopsis/Botrytis* interactions: Variation in host production and pathogen sensitivity. Plant Journal **44**, 25–36.

Kolde R. 2019. pheatmap: Pretty Heatmaps. R package version 1.0. 12.

Konert G, Rahikainen M, Trotta A, Durian G, Salojärvi J, Khorobrykh S, Tyystjärvi E, Kangasjärvi S. 2015. Subunits B′ γ and B′ ζ of protein phosphatase 2A regulate photo-oxidative stress responses and growth in *Arabidopsis thaliana*. Plant, Cell & Environment, n/a-n/a.

Kono M, Noguchi K, Terashima I. 2014. Roles of the cyclic electron flow around PSI (CEF-PSI) and O_2-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in *Arabidopsis thaliana*. Plant and Cell Physiology **55**, 990–1004.

Kreft O, Hoefgen R, Hesse H. 2003. Functional analysis of cystathionine γ-synthase in genetically engineered potato plants. Plant Physiology **131**, 1843–1854.

Kromdijk J, Głowacka K, Leonelli L, Gabilly ST, Iwai M, Niyogi KK, Long SP. 2016. Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Plant Science **354**, 857–862.

Kroymann J. 2011. Natural diversity and adaptation in plant secondary metabolism. Current Opinion in Plant Biology **14**, 246–251.

Kroymann J, Donnerhacke S, Schnabelrauch D, Mitchell-Olds T. 2003. Evolutionary dynamics of an Arabidopsis insect resistance quantitative trait locus. Proceedings of the National Academy of Sciences **100**, 14587–14592.

Kroymann J, Textor S, Tokuhisa JG, Falk KL, Bartram S, Gershenzon J, Mitchell-Olds T. 2001. A Gene Controlling Variation in Arabidopsis Glucosinolate Composition Is Part of the Methionine Chain Elongation Pathway. Plant Physiology **127**, 1077–1088.

Kurotani K, Nanri A, Goto A, Mizoue T, Noda M, Kato M, Inoue M, Tsugane S, Group for the JPHCPS. 2013. Vegetable and fruit intake and risk of type 2 diabetes: Japan Public Health Center-based Prospective Study. British Journal of Nutrition **109**, 709–717.

Kusakabe Y, Ishihara M, Umeda T, Kuroda D, Nakanishi M, Kitade Y, Gouda H, Nakamura KT, Tanaka N. 2015. Structural insights into the reaction mechanism of S-adenosyl-L-homocysteine hydrolase. Scientific Reports **5**, 1–16.

Lauria M, Rossi V. 2011. Epigenetic control of gene regulation in plants. Biochimica et Biophysica Acta - Gene Regulatory Mechanisms **1809**, 369–378.

Łaźniewska J, Macioszek VK, Lawrence CB, Kononowicz AK. 2010. Fight to the death: *Arabidopsis thaliana* defense response to fungal necrotrophic pathogens. Acta Physiologiae Plantarum **32**, 1–10.

Lee S, Doxey AC, Mcconkey BJ, Moffatt BA. 2012. Nuclear Targeting of Methyl-Recycling Enzymes in *Arabidopsis thaliana* Is Mediated by Specific Protein Interactions. Molecular Plant **5**, 231–248.

Lee SC, Lan W, Buchanan BB, Luan S. 2009. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. Proceedings of the National Academy of Sciences of the United States of America **106**, 21419–24.

Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics **12**, 323.

Li S, Mhamdi A, Trotta A, Kangasjärvi S, Noctor G. 2014. The protein phosphatase subunit PP2A-B'γ is required to suppress day length-dependent pathogenesis responses triggered by intracellular oxidative stress. New Phytologist **202**, 145–160.

Li CH, Yu N, Jiang SM, Shangguan XX, Wang LJ, Chen XY. 2008. Down-regulation of S-adenosyl-L-homocysteine hydrolase reveals a role of cytokinin in promoting transmethylation reactions. Planta **228**, 125–136.

Lin HJ, Probst-Hensch NM, Louie AD, Kau IH, Witte JS, Ingles SA, Frankl HD, Lee ER, Haile RW. 1998. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. Cancer Epidemiology Biomarkers and Prevention **7**, 647–652.

Lindeman R. 1942. The Trophic Dynamic of Ecology. Ecology **23**, 399–417.

Long SP, Humphries S. 1994. Photoinhibition of. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45**, 633–662.

Long SP, Zhu XG, Naidu SL, Ort DR. 2006. Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment **29**, 315–330.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology **15**, 1–21.

Luft S, Curio E, Tacud B. 2003. The use of olfaction in the foraging behaviour of the golden-mantled flying fox, Pteropus pumilus, and the greater musky fruit bat, *Ptenochirus jagori* (Megachiroptera: Pteropodidae). Naturwissenschaften **90**, 84–87.

Martínez-Ballesta M del C, Moreno DA, Carvajal M. 2013. The physiological importance of glucosinolates on plant response to abiotic stress in *Brassica*. International Journal of Molecular Sciences **14**, 11607–11625.

Matuszewska B, Borchardt RT, Muto N, Tsujino M, Sudate Y, Hayashi M, Antibiot MOJ. 1987. The Mechanism of Inhibition of *Alcaligenes faecalis* Hydrolase by Neplanocin A. **256**, 50–55.

Mbow C, Rosenzweig C, Barioni LG, *et al.* 2019. Climate change and land. Chapter 5: Food Security. IPCC SPECIAL REPORT Global Warming of 1.5 ºC, 1–200.

Megna BW, Carney PR, Nukaya M, Geiger P, Kennedy GD. 2016. Indole-3-carbinol induces tumor cell death: Function follows form. Journal of Surgical Research **204**, 47–54.

Midgley GF. 2007. Plant Physiological Responses to Climate and Environmental Change. Encyclopedia of Life Sciences, 1–11.

Mikkelsen MD, Halkier BA. 2003. Metabolic engineering of valine- and isoleucine-derived glucosinolates in Arabidopsis expressing CYP79D2 from cassava. Plant Physiology **131**, 773–779.

Mithen R, Faulkner K, Magrath R, Rose P, Williamson G, Marquez J. 2003. Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. Theoretical and Applied Genetics **106**, 727–734.

Mittler R. 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science **11**, 15–19.

Miyake C. 2010. Alternative electron flows (water-water cycle and cyclic electron flow around PSI) in photosynthesis: Molecular mechanisms and physiological functions. Plant and Cell Physiology **51**, 1951–1963.

Moffatt B, Weretilnyk E. 2001. Sustaining S-adenosyl-L-methionine-dependent methyltransferase activity in plant cells. Physiologia Plantarum **113**, 435–442.

Mol J, Jenkins G, Schäfer E, Weiss D, Walbot V. 1996. Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Critical Reviews in Plant Sciences **15**, 525–557.

Montanher AB, Zucolotto SM, Schenkel EP, Fröde TS. 2007. Evidence of anti-inflammatory effects of *Passiflora edulis* in an inflammation model. Journal of Ethnopharmacology **109**, 281–288.

Mull L, Ebbs ML, Bender J. 2006. A histone methylation-dependent DNA methylation pathway is uniquely impaired by deficiency in Arabidopsis S-adenosylhomocysteine hydrolase. Genetics **174**, 1161–1171.

Müller-Moulé P, Conklin PL, Niyogi KK. 2002. Ascorbate deficiency can limit violaxanthin de-epoxidase activity in vivo. Plant Physiology **128**, 970–977.

Müller-Moulé P, Golan T, Niyogi KK. 2004. Ascorbate-deficient mutants of Arabidopsis grow in high light despite chronic photooxidative stress. Plant Physiology **134**, 1163–1172.

Muller P. 2001. Non-Photochemical Quenching. A Response to Excess Light Energy. Plant Physiology **125**, 1558–1566.

Narendhirakannan RT, Hannah MAC. 2013. Oxidative stress and skin cancer: An overview. Indian Journal of Clinical Biochemistry **28**, 110–115.

Naveen J, Baskaran V. 2018. Antidiabetic plant-derived nutraceuticals: a critical review. European Journal of Nutrition **57**, 1275–1299.

Nicot P, Bardin M, Debruyne F, Duffaud M, Lecompte F, Neu L, Pascal M. 2013. Effect of nitrogen fertilisation of strawberry plants on the efficacy of defence-stimulating biocontrol products against *Botrytis cinerea*. IOBC-WPRS Bulletin **88**, 39–42.

Nour-Eldin HH, Andersen TG, Burow M, Madsen SR, Jørgensen ME, Olsen CE, Dreyer I, Hedrich R, Geiger D, Halkier BA. 2012. NRT/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds. Nature **488**, 531–534.

Olivas-Aguirre FJ, Rodrigo-García J, Martínez-Ruiz NDR, Cárdenas-Robles AI, Mendoza-Díaz SO, Álvarez-Parrilla E, González-Aguilar GA, De La Rosa LA, Ramos-Jiménez A, Wall-Medrano A. 2016. Cyanidin-3-O-glucoside: Physical-chemistry, foodomics and health effects. Molecules **21**, 1–30.

Ouyang B, Fei Z, Joung JG, *et al.* 2012. Transcriptome profiling and methyl homeostasis of an Arabidopsis mutant deficient in S-adenosylhomocysteine hydrolase1 (SAHH1). Plant Molecular Biology **79**, 315–331.

Palmer S. 1985. Diet, nutrition, and cancer. Progress in food & nutrition science **9**, 283–341.

Palmieri L, Arrigoni R, Blanco E, Carrari F, Zanor MI, Studart-Guimaraes C, Fernie AR, Palmieri F. 2006. Molecular identification of an Arabidopsis S-adenosylmethionine transporter. analysis of organ distribution, bacterial expression, reconstitution into liposomes, and functional characterization. Plant Physiology **142**, 855–865.

Pandey P, Irulappan V, Bagavathiannan M V., Senthil-Kumar M. 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Frontiers in Plant Science **8**, 1–15.

Pascual J, Rahikainen M, Kangasjärvi S. 2017. Plant Light Stress. eLS, 1–6.

Pastori GM, Kiddle G, Antoniw J, Bernard S, Veljovic-Jovanovic S, Verrier PJ, Noctor G, Foyer CH. 2003. Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell **15**, 939–951.

Pavet V, Olmos E, Kiddle G, Mowla S, Kumar S, Antoniw J, Alvarez E, Foyer CH. 2005. Ascorbic Acid Deficiency Activates Cell Death and Disease Resistance Responses in Arabidopsis. Society **139**, 1291–1303.

Pereira LAR, Todorova M, Cai X, Makaroff CA, Emery RJN, Moffatt BA. 2007. Methyl recycling activities are co-ordinately regulated during plant development. Journal of Experimental Botany **58**, 1083–1098.

Petersen A, Crocoll C, Halkier BA. 2019. De Novo production of benzyl glucosinolate in *Escherichia coli*. Metabolic engineering.

Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S, Vianello A. 2013. Plant flavonoids-biosynthesis, transport and involvement in stress responses. International Journal of Molecular Sciences **14**, 14950–14973.

Pieterse CMJ, Van Der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM. 2012. Hormonal modulation of plant immunity. Annual Review of Cell and Developmental Biology **28**, 489–521.

Prasad PVV, Boote KJ, Allen LH. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. Agricultural and Forest Meteorology **139**, 237–251.

Prasch CM, Sonnewald U. 2013. Simultaneous application of heat, drought, and virus to Arabidopsis plants reveals significant shifts in signaling networks. Plant Physiology **162**, 1849–1866.

Pratelli R, Pilot G. 2014. Regulation of amino acid metabolic enzymes and transporters in plants. Journal of Experimental Botany **65**, 5535–5556.

R Core T. 2018. R: A language and environment for statistical computing.

Raguso RA. 2008. Wake up and smell the roses: The ecology and evolution of floral scent. Annual Review of Ecology, Evolution, and Systematics **39**, 549–569.

Rahikainen M, Alegre S, Trotta A, Pascual J, Kangasjärvi S. 2018. Trans-methylation reactions in plants: focus on the activated methyl cycle. Physiologia Plantarum **162**, 162–176.

Rahikainen M, Pascual J, Alegre S, Durian G, Kangasjärvi S. 2016. PP2A Phosphatase as a Regulator of ROS Signaling in Plants. Antioxidants **5**, 8.

Rai A, Saito K, Yamazaki M. 2017. Integrated omics analysis of specialized metabolism in medicinal plants. Plant Journal **90**, 764–787.

Ramegowda V, Senthil-Kumar M. 2015. The interactive effects of simultaneous biotic and abiotic stresses on plants: Mechanistic understanding from drought and pathogen combination. Journal of Plant Physiology **176**, 47–54.

Rasool B, Karpinska B, Konert G, Durian G, Denessiouk K, Kangasjärvi S, Foyer CH. 2014. Effects of light and the regulatory B-subunit composition of protein phosphatase 2A on the

susceptibility of *Arabidopsis thaliana* to aphid (*Myzus persicae*) infestation. Frontiers in plant science **5**, 405.

Reddy MCM, Kuppan G, Shetty ND, Owen JL, Ioerger TR, Sacchettini JC. 2008. Crystal structures of *Mycobacterium tuberculosis* S-adenosyl-L-homocysteine hydrolase in ternary complex with substrate and inhibitors . Protein Science **17**, 2134–2144.

Rocha PSCF, Sheikh M, Melchiorre R, Fagard M, Boutet S, Loach R, Moffatt B, Wagner C, Vaucheret H, Furner I. 2005. The Arabidopsis HOMOLOGY-DEPENDENT GENE SILENCING1 gene codes for an S-adenosyl-L-homocysteine hydrolase required for DNA methylation-dependent gene silencing. Plant Cell **17**, 404–417.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4**, 406–425.

Scherm H, Coakley SM. 2003. Plant pathogens in a changing world. Australasian Plant Pathology **32**, 157–165.

Schwacke R, Ponce-Soto GY, Krause K, Bolger AM, Arsova B, Hallab A, Gruden K, Stitt M, Bolger ME, Usadel B. 2019. MapMan4: A Refined Protein Classification and Annotation Framework Applicable to Multi-Omics Data Analysis. Molecular Plant **12**, 879–892.

Seow A, Yuan J, Sun C, Berg D Van Den, Lee H, Yu MC. 2002. Dietary isothiocyanates , glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study been inversely related to colorectal cancer risk , and this degradation products such as isothiocyanates (ITCs). These con. Carcinogenesis **23**, 2055–2061.

Sham A, Moustafa K, Al-Shamisi S, Alyan S, Iratni R, AbuQamar S. 2017. Microarray analysis of Arabidopsis WRKY33 mutants in response to the necrotrophic fungus *Botrytis cinerea*. PLoS ONE **12**, 1–20.

Shi M-Z, Xie D-Y. 2014. Biosynthesis and Metabolic Engineering of Anthocyanins in *Arabidopsis thaliana*. Recent Patents on Biotechnology **8**, 47–60.

Shigenaga AM, Argueso CT. 2016. No hormone to rule them all: Interactions of plant hormones during the responses of plants to pathogens. Seminars in Cell and Developmental Biology **56**, 174–189.

Singh S V., Srivastava SK, Choi S, *et al.* 2005. Sulforaphane-induced cell death in human prostate cancer cells is initiated by reactive oxygen species. Journal of Biological Chemistry **280**, 19911–19924.

Slaymaker DH, Navarre DA, Clark D, Del Pozo O, Martin GB, Klessig DF. 2002. The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. Proceedings of the National Academy of Sciences of the United States of America **99**, 11640–11645.

Sønderby IE, Geu-Flores F, Halkier BA. 2010. Biosynthesis of glucosinolates - gene discovery and beyond. Trends in Plant Science **15**, 283–290.

Soundararajan P, Kim JS. 2018. Anti-carcinogenic glucosinolates in cruciferous vegetables and their antagonistic effects on prevention of cancers. Molecules **23**.

Steindal ALH, Rdven R, Hansen E, Mlmann J. 2015. Effects of photoperiod, growth temperature and cold acclimatisation on glucosinolates, sugars and fatty acids in kale. Food Chemistry **174**, 44–51.

Stępkowski T, Brzeziński K, Legocki AB, Jaskólski M, Béna G. 2005. Bayesian phylogenetic analysis reveals two-domain topology of S-adenosylhomocysteine hydrolase protein sequences. Molecular Phylogenetics and Evolution **34**, 15–28.

Su X, Jiang X, Meng L, Dong X, Shen Y, Xin Y. 2018. Anticancer activity of sulforaphane: The epigenetic mechanisms and the Nrf2 signaling pathway. Oxidative Medicine and Cellular Longevity **2018**.

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R. 2014. Abiotic and biotic stress combinations. New Phytologist **203**, 32–43.

Tanaka N, Nakanishi M, Kusakabe Y, Shiraiwa K, Yabe S, Ito Y, Kitade Y, Nakamura KT. 2004. Crystal structure of S-Adenosyl-l-Homocysteine hydrolase from the human malaria parasite *Plasmodium falciparum*. Journal of Molecular Biology **343**, 1007–1017.

Tang NY, Huang YT, Yu CS, *et al.* 2011. Phenethyl isothiocyanate (PEITC) promotes G2/M phase arrest via p53 expression and induces apoptosis through caspase- and mitochondria-dependent signaling pathways in human prostate cancer DU 145 cells. Anticancer Research **31**, 1691–1702.

Tang L, Zirpoli GR, Guru K, Moysich KB, Zhang Y, Ambrosone CB, McCann SE. 2010. Intake of cruciferous vegetables modifies bladder cancer survival. Cancer Epidemiology Biomarkers and Prevention **19**, 1806–1811.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research **22**, 4673–4680.

Tiwari A, Mamedov F, Grieco M, Suorsa M, Jajoo A, Styring S, Tikkanen M, Aro EM. 2016. Photodamage of iron-sulphur clusters in photosystem i induces non-photochemical energy dissipation. Nature Plants **2**.

Traka MH, Saha S, Huseby S, *et al.* 2013. Genetic regulation of glucoraphanin accumulation in Beneforté® broccoli. New Phytologist **198**, 1085–1095.

Trotta A, Wrzaczek M, Scharte J, *et al.* 2011. Regulatory subunit B'gamma of protein phosphatase 2A prevents unnecessary defense reactions under low light in Arabidopsis. Plant physiology **156**, 1464–1480.

Turner MA, Yang X, Yin D, Kuczera K, Borchardt RT, Howell PL. 2000. Structure and function of S-adenosylhomocysteine hydrolase. Cell Biochemistry and Biophysics **33**, 101–125.

Tzin V, Galili G. 2010. New Insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Molecular Plant **3**, 956–972.

Verhoeven DTH, Goldbohm RA, Van Poppel G, Verhagen H, Van Den Brandt PA. 1996. Epidemiological studies on Brassica vegetables and cancer risk. Cancer Epidemiology Biomarkers and Prevention **5**, 733–748.

De Vleesschauwer D, Xu J, Höfte M. 2014. Making sense of hormone-mediated defense networking: From rice to Arabidopsis. Frontiers in Plant Science **5**, 1–15.

De Vos M, Jander G. 2009. *Myzus persicae* (green peach aphid) salivary components induce defence responses in *Arabidopsis thaliana*. Plant, Cell and Environment **32**, 1548–1560.

Wang S, Alseekh S, Fernie AR, Luo J. 2019. The Structure and Function of Major Plant Metabolite Modifications. Molecular Plant **12**, 899–919.

Willems P, Mhamdi A, Stael S, Storme V, Kerchev P, Noctor G, Gevaert K, Van Breusegem F. 2016. The ROS wheel: Refining ROS transcriptional footprints. Plant Physiology **171**, 1720–1733.

Wittstock U, Burow M. 2007. Tipping the scales - Specifier proteins in glucosinolate hydrolysis. IUBMB Life **59**, 744–751.

Wittstock U, Burow M. 2010. Glucosinolate breakdown in Arabidopsis: mechanism, regulation and biological significance. The Arabidopsis book **8**, e0134.

Wu X, Li F, Kolenovsky A, Caplan A, Cui Y, Cutler A, Tsang EWT. 2009. A mutant deficient in S-adenosylhomocysteine hydrolase in Arabidopsis shows defects in roothair development. Botany **87**, 571–584.

Wurtzel ET, Kutchan TM. 2016. Plant metabolism, the diverse chemistry set of the future. Science **353**, 1232–1236.

Xu J, Meng J, Zhao Y, Sun T, Liu J, Meng X, Liu Y, Zhang S, Wang Q. 2016. Pathogen-Responsive MPK3 and MPK6 Reprogram the Biosynthesis of Indole Glucosinolates and Their Derivatives in Arabidopsis Immunity. The Plant Cell **28**, 1144–1162.

Yamagishi SI, Matsui T. 2016. Protective role of sulphoraphane against vascular complications in diabetes. Pharmaceutical Biology **54**, 2329–2339.

Yang W, Kortesniemi M, Yang B, Zheng J. 2018. Enzymatic Acylation of Anthocyanins Isolated from Alpine Bearberry (*Arctostaphylos alpina*) and Lipophilic Properties, Thermostability, and

Antioxidant Capacity of the Derivatives. Journal of Agricultural and Food Chemistry **66**, 2909–2916.

Yang SX, Wu TT, Ding CH, Zhou PC, Chen ZZ, Gou JY. 2019. SAHH and SAMS form a methyl donor complex with CCoAOMT7 for methylation of phenolic compounds. Biochemical and Biophysical Research Communications **520**, 122–127.

Yi Z, Wang Z, Li H, Liu M. 2004. Inhibitory effect of tellimagrandin I on chemically induced differentiation of human leukemia K562 cells. Toxicology Letters **147**, 109–119.

Zeng XQ, Chow WS, Su LJ, Peng XX, Peng CL. 2010. Protective effect of supplemental anthocyanins on Arabidopsis leaves under high light. Physiologia Plantarum **138**, 215–225.

Zhang B, Hu Z, Zhang Y, Li Y, Zhou S, Chen G. 2012. A putative functional MYB transcription factor induced by low temperature regulates anthocyanin biosynthesis in purple kale (*Brassica oleracea* var. *acephala* f. *tricolor*). Plant Cell Reports **31**, 281–289.

Zhang XH, Zheng XT, Sun BY, Peng CL, Chow WS. 2018. Over-expression of the CHS gene enhances resistance of Arabidopsis leaves to high light. Environmental and Experimental Botany **154**, 33–43.

Zhu X, Wang Y, Su Z, Lv L, Zhang Z. 2018. Silencing of the wheat protein phosphatase 2A catalytic subunit TaPP2AC enhances host resistance to the necrotrophic pathogen *Rhizoctonia cerealis*. Frontiers in Plant Science **871**, 1–13.

UNIVERSITY OF TURKU

Painosalama Oy, Turku, Finland 2020

ISBN 978-951-29-8287-5 (PRINT)
ISBN 978-951-29-8288-2 (PDF)
ISSN 0082-7002 (Print)
ISSN 2343-3175 (Online)