The influence of light and leaf antioxidant status on plant responses to aphids

Brwa Mohammad Ali Rasool

Submitted in accordance with the requirements for the degree of
Doctor of Philosophy

The University of Leeds
Faculty of Biological Sciences
July 2015

The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 4 of the thesis is based on work of jointly-authored publications.

Rasool, B., Karpinska, B., Konert, G., Durian, G., Denessiouk, K., Kangasjärvi, S. and Foyer, C.F. (2014). Effects of light and the regulatory Beta subunit composition of protein phosphatase 2A on the susceptibility of *Arabidopsis thaliana* to aphid (*Myzus persicae*) infestation. Front. Plant Sci. **5: 405**.

This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

© 2015 The University of Leeds Brwa Rasool

Acknowledgements

My infinite thanks to:

Allah, who guided me and gave me patience in completing this project,

My supervisor Christine Foyer for giving me the opportunity to work with her on this project and for her guidance, support and patient in good and bad times during these years,

Barbara Karpinska for her advice, her support and her help,

Rob Hancock for his supervision during my work at the James Hutton Institute and for his support during the analysis of the data,

Jenny Morris and **Pete Hedley** from the James Hutton Institute for processing the microarray and analysing the data,

My family for supporting me during my study.

Abstract

Cross-tolerance to environmental stresses results from the synergistic co-activation of defence pathways that cross biotic-abiotic stress boundaries. However, the signalling mechanisms that underpin such responses remain poorly characterised. The effects of an abiotic stress (high light; HL) on the responses of *Arabidopsis thaliana* and tobacco (*Nicotiana tabacum*) plants to a biotic stress (infestation by the green peach aphid, *Myzus persicae*) were therefore analysed. Particular focus was placed on the role of cellular redox state as a regulator of cross-tolerance phenomena and the identification of signalling pathways that underpin aphid resistance. Aphid fecundity was measured in a range of *A. thaliana* mutants that have defects in non-enzymatic antioxidants (ascorbate and glutathione), enzymatic antioxidants (catalase) or downstream kinase/phosphatase signalling cascades, and in transgenic tobacco lines that have either increased or decreased levels of ascorbate oxidase. A pre-treatment with HL increased the resistance of transgenic tobacco plants with low ascorbate oxidase to aphid infestation. In contrast, the *A. thaliana* ascorbate oxidase knockout mutants did not show the HL-dependent decrease in aphid infestation. Aphid fecundity was decreased on *A. thaliana* mutants that have altered antioxidant (ascorbate, glutathione, catalase) status, or that lack the gamma (γ) subunit of protein phosphatase (PP2A). A pre-treatment with HL increased the resistance of *A. thaliana* plants to aphid infestation in all of the genotypes, except for the *cat2* mutants that lack the photorespiratory form of leaf catalase and glutathione defective mutants. Taken together these findings demonstrate that redox processes and oxidative signalling are important modulators of aphid resistance and the light-aphid interaction. Moreover, the analysis of aphid fecundity on these *A. thaliana* mutants, which also have different levels of leaf camalexin, suggests that the levels of this secondary metabolite alone do not influence aphid infestation. A transcriptome and metabolome profiling analysis of the responses of the different tobacco lines highlights the central role of cell wall modifications/signalling as key components in plant responses to aphid infestation.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ABA	abscisic acid
ABI4	ABA INSENSITIVE 4
AO	ascorbate oxidase
APX	ascorbate peroxidase
AsA	ascorbic acid
CAT	catalase
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
DTT	dithiothreitol
ET	ethylene
GC-MS	gas chromatography - mass spectrometry
GPX	glutathione peroxidases
GR	glutathione reductase
GSH	reduced glutathione
GSSG	glutathione disulphide
H_2O_2	hydrogen peroxide
HL	high light
HR	hypersensitive response
JA	jasmonic acid
LL	low light
MAPK	mitogen-activated protein kinase
NADPH	nicotinamide adenine dinucleotide phosphate
PCR	polymerase chain reaction
PP2A	Protein phosphatase 2A
PR	pathogenesis-related
qRTPCR	quantitative real-time PCR
ROS	reactive oxygen species
SA	salicylic acid
SAR	systemic acquired resistance
SOD	superoxide dismutase
VTC	vitamin C
WT	wild type

Chapter 1. Introduction

1.1 Plant stress responses

Plants are sessile organisms that have to cope with a wide range of biotic threats and unfavourable abiotic stress conditions that adversely influence on plant growth. Such environmental stresses frequently occur simultaneously and in different combinations as well for varying durations. Environmental stresses have a negative impact on current agriculture, where diverse ecosystems have been replaced with monocultures that are much more vulnerable to changing climatic conditions and evolving biotic stresses. Despite crop protection measures, global losses in agriculture due to pathogens and pests are estimated at 25-40% for the major food and cash crops. Moreover, crops chronically attain only about 50% of their potential yield due to the negative effects of environmental stress, with drought stress often considered to be the most important cause of yield decreases (Bray, 1997; Araus et al., 2008).

Stress can be defined as any external factor that has a negative influence on plant growth and/or reproduction (Osmond et al., 1987; Madlung and Comai, 2004). Biotic and abiotic stresses are major components for selection in nature (Wassink and Stolwijk, 1956). Each of the approximate 300,000 plant species living in the world today is exposed to a multitude of other organisms such as microbial pathogens and insect herbivores. In addition, plants have to adapt to extreme weather events and environmental hazards such as high light, high or low temperatures, water-logging and drought, as well as exposure to toxic compounds such as heavy metals and high salinity (Pérez-Clemente et al., 2013).

Plants display a high capacity to respond to diverse stresses through a flexible and finely balanced response network that involves components such as reduction-oxidation (redox) signalling pathways, stress hormones, plant growth regulators and calcium and protein kinase cascades. Numerous genes associated to plant responses to biotic threats and abiotic stress conditions have been identified and characterized in recent years, allowing a deeper understanding of plant stress tolerance traits that can be used in crop breeding programs to improve crop yields (Hirayama and Shinozaki, 2010; Lamb, 2012).

Plants have flexible short-term and long strategies to respond to stress that involves metabolic and physiological adjustments, as well as changes in gene expression (Kilian et al., 2012).

The induction of appropriate responses involves the perception or sensing of stress by primary receptors that activate signalling pathways, including oxidative signals, calcium dependent signals and hormone signalling pathways that alter the expression of stress responsive genes (Fig. 1.1; Atkinson and Urwin, 2012; Kilian et al., 2012).

1.2 Cross tolerance phenomena

Plants continuously monitor their surroundings and adjust their metabolic systems accordingly to optimise metabolism and maintain homeostasis (Pastori and Foyer, 2002). Environmental stresses activate cell signalling pathways, that result in changes in plant hormone levels and signalling, the activation of secondary metabolism leading to the accumulation of low molecular weight metabolites, as well as the synthesis of stress proteins and enhancement of antioxidant capacity and detoxification mechanisms (Cushman and Bohnert, 2000; Fujita et al., 2006).

Cross-tolerance to environmental stresses is a common phenomenon in plants, whereby exposure to one type of stress confers a general increase in resistance to a range of different stresses (Pastori and Foyer, 2002; Mittler, 2006). Cross-tolerance occurs because of synergistic co-activation of non-specific stress-responsive pathways that cross biotic-abiotic stress boundaries (Bostock, 2005). In many cases, cross-tolerance has been linked to enhanced production of reactive oxygen species (ROS) and oxidative signalling. It is now generally accepted that ROS are important signalling molecules in abiotic and biotic stress responses serving as messengers for the activation of defence genes (Foyer and Noctor, 2009). For example, exposure to the atmospheric pollutant ozone generates ROS in the apoplast of plant cells. This response shares many signalling and regulatory response components with ROS-mediated responses to biotic and abiotic stresses (Baier et al., 2005). These responses involve plant hormones such as ethylene (ET), salicylic acid (SA), abscisic acid (ABA) and jasmonates (JA; Fujita et al., 2006). These hormones, which induce tolerance to a wide spectrum of stresses, promote ROS production, often through the activation of NADPH oxidases. These signals ultimately induce expression of specific sub-sets of defence genes that lead to the assembly of the overall defence reaction (Fraire-Velazquez et al., 2011).

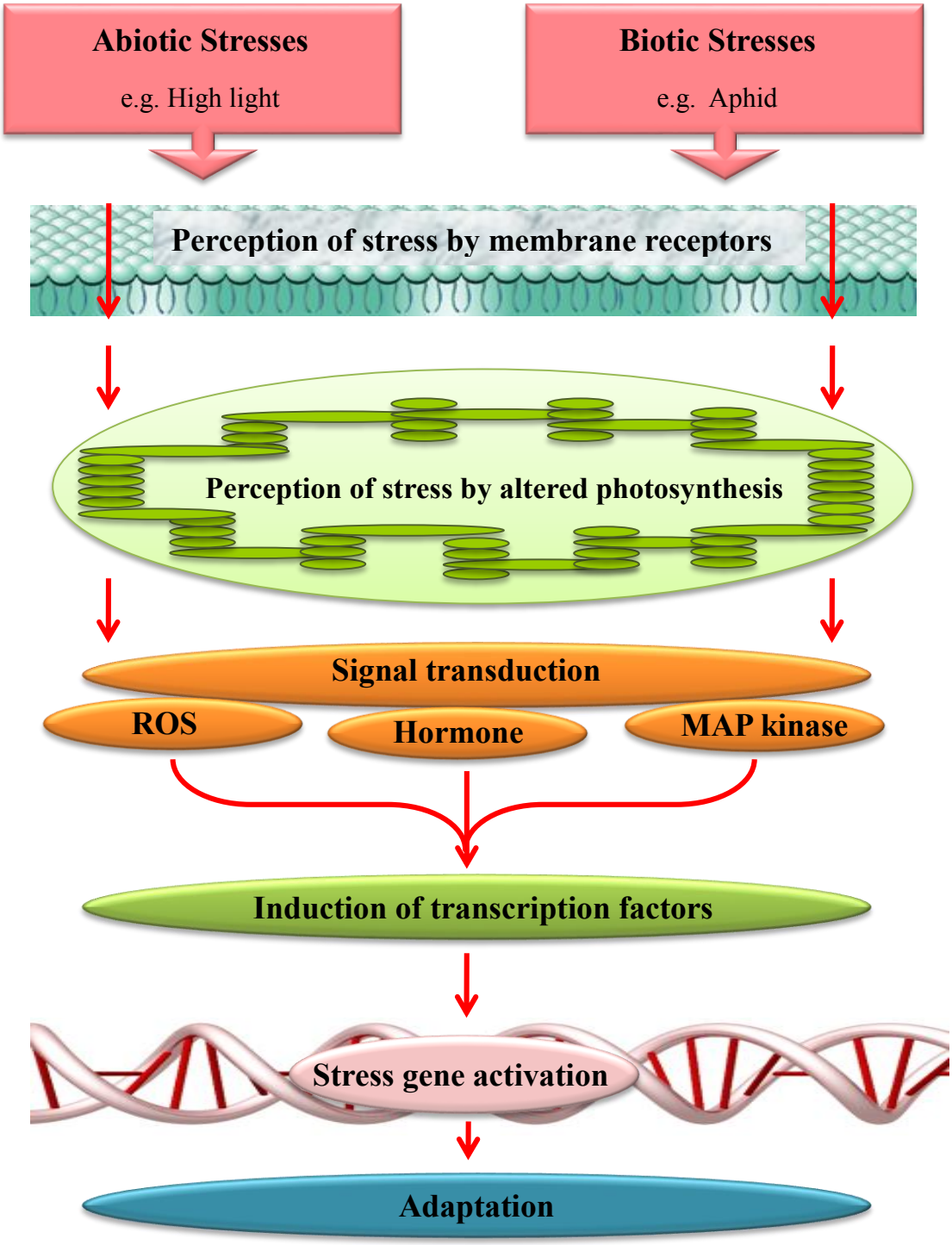


Figure 1.1 Plant stress responses – Cross tolerance phenomena. Cross-tolerance occurs because of synergistic co-activation of non-specific stress-responsive pathways that cross biotic-abiotic stress boundaries. Extensive cross-communication between the different hormone-regulated stress resistance and redox signalling pathways is an important mechanism that triggers the innate immune system leading to a range of adaptive responses (Bostock, 2005; Foyer and Noctor, 2009).

1.3 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are produced by metabolism and by the photosynthetic and respiratory electron transport processes in photosynthesis and respiration (Urban et al., 1997), as illustrated in Figure (1.2). ROS include free radicals such as superoxide (O_2^-.) and the hydroxyl radical (HO•), and other active forms of oxygen such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2; Noctor and Foyer, 1998). Singlet oxygen is generated by photosystem II in chloroplasts. In addition, the photosynthetic electron transport chain also generates superoxide and hydrogen peroxide at the level of photosystem I. Hydrogen peroxide is also produced by the photorespiratory pathway which is associated with photosynthesis. During photorespiration, glycollate is produced in peroxisomes and H_2O_2 is generated by the action of glycolate oxidase, as illustrated in Figure (1.2). The mitochondrial electron transport chain also produces superoxide and hydrogen peroxide.

A large number of H_2O_2-producing oxidases, such as the NADPH oxidase family of proteins, are also found in plants. NADPH oxidases (also called respiratory burst oxidase homologues, RBOH), are important in the generation of the oxidative burst, which is part of the hypersensitive response (HR) to pathogen attack (Torres et al., 2006). In the oxidative burst ROS are formed in the apoplast/cell wall compartment of the cell through the activation of NADPH oxidases and other cell wall peroxidases, such as pH-dependent cell wall peroxidases, germin-like oxalate oxidases, and polyamine oxidases (Bolwell et al., 2002; Apel and Hirt, 2004, Foreman et al., 2003; Sierla et al., 2013). H_2O_2 accumulation in the apoplast also occurs in response to hormones such as ABA and auxin, and environmental stress conditions such as drought and salinity (Mittler et al., 2011). The Arabidopsis genome has ten *AtRboh* genes called *ATRBOHA-ATRBOHJ* (Torres and Dangl, 2005). The RbohD and RbohF proteins are considered as the main isoforms during disease resistance reactions to pathogen attack in leaves (Torres et al., 2002). The *AtrbohF* mutants display increased susceptibility to *Pseudomonas syringae* pv. *tomato* DC3000 (Chaouch et al., 2012).

The production of H_2O_2 is essential for many hormone-regulated processes and in the orchestration of plant stress responses. ROS-mediated signalling is controlled by a delicate equilibrium between production and removal (Mittler et al., 2004, 2011). This equilibrium can be shifted towards increased oxidation by stressful environmental conditions (low temperatures, high light, drought, pollution and pathogen attack). For example, in the heat shock response, H_2O_2 is required for the induction of heat shock proteins (Foyer et al., 1997). Hormone-mediated H_2O_2 production is also a key component of plant growth, development and topic responses. For example, ROS production by RbohB plays a role in seed ripening and RbohC is required for root hair tip growth (Monshausen and Gilroy, 2009).

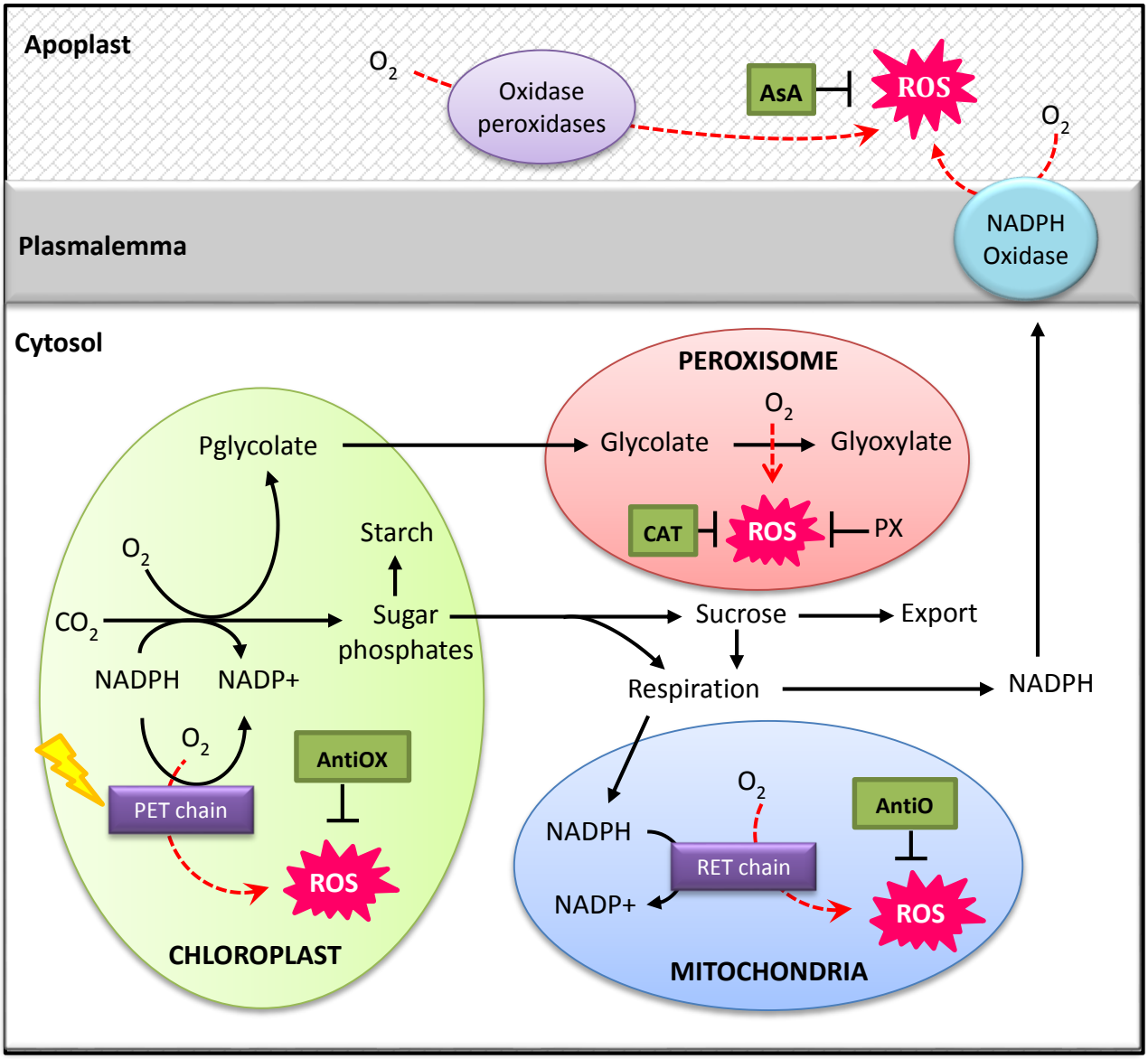


Figure 1.2 Main locations of reactive oxygen species (ROS) generation in the plant cells. AntiOX: antioxidative system; CAT: catalase; PET-RET: photosynthetic-respiratory electron transport; PX: peroxidases; RBOH: respiratory burst oxidase homolog. The figure adapted from (Foyer and Noctor, 2009).

The steady state concentrations of ROS in plant cells are very low because of the presence of a network of low molecular antioxidants and antioxidant enzymes (Foyer and Noctor, 2009). The non-enzymic antioxidants in plant cells include the major cellular redox buffers ascorbic acid, glutathione (*γ*-glutamyl-cysteinyl-glycine) and tocopherols. In addition, other metabolites such as carotenoids and phenolic compounds can also serve an antioxidant function.

Antioxidant enzymes include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), catalase (CAT), peroxiredoxins (PRX) and glutathione *S*-transferase (GST). These enzymes work together with the low molecular weight antioxidants ascorbate and glutathione to scavenge and detoxify $O_2^{\cdot-}$ and H_2O_2 efficiently (Noctor and Foyer, 1998). SOD is often considered to be the first line of defence against oxidative stress (Cadenas, 1989). SOD is encoded by a small gene family that includes chloroplast-localized Fe-containing SODs, Cu/Zn SODs that are found in the chloroplasts, cytosol and a mitochondrial Mn-SOD (Bowler et al., 1991). SOD converts superoxide to H_2O_2, which is removed by catalases, the enzymes of ascorbate-glutathione pathway and other systems (Bowler et al., 1992). These antioxidant defences can be increased in stressful situations to limit the life-time of superoxide and H_2O_2 (Foyer and Noctor, 1998). Green tissues are rich in ascorbate (10–100 mM) and glutathione (1–10 mM) because they have to deal with the very high level of H_2O_2 production by photosynthesis (Noctor et al., 2002). Low molecular weight antioxidants (e.g., ascorbate, glutathione) not only remove ROS but they are also involved in the transmission of redox signals, as illustrated in Figure 1.3 (Foyer and Noctor, 2008).

In contrast to the cytoplasm, the apoplast/cell compartment of the cell has relatively little antioxidant defence. Thus when H_2O_2 is produced in the oxidative burst, a strong oxidative signal is formed on the external face of the plasma membrane. This oxidation can alter ion fluxes, modify plasmalemma-based electron transport systems and cause adjustments in calcium transport and signalling. Moreover, H_2O_2 can be transported into the cytoplasm via the aquaporins, where it is metabolised (Jang et al., 2012).

Catalase (CAT) catalyses a dismutation reaction, in which H_2O_2 is converted to water and oxygen (Zamocky et al., 2008). This reaction occurs mainly in peroxisomes. In leaves, H_2O_2 is generated by several enzymes, the most important of which in relation to photosynthesis is glycolate oxidase, which generates H_2O_2, during the photorespiratory pathway (Corpas et al., 2008). Catalase is distinguished from other antioxidant enzymes because it catalyses a dismutation reaction that removes H_2O_2 without requiring cellular reductant (Mittler and Zilinskas, 1991).

The three catalase genes present in Arabidopsis (*CAT1, CAT2* and *CAT3*) have a high degree of sequence similarity. Each encodes a protein of 492 amino acids (Frugoli et al., 1996; McClung, 1997). CAT2 is the major leaf form of catalase and it is associated with the photorespiratory pathway. Like other enzymes associated with photosynthesis, the expression of *CAT2* is regulated by light, the circadian clock and by leaf age (Zimmermann et al., 2006).

Arabidopsis *cat2* mutants have only about 10% of the catalase activity of the wild type plants (Mhamdi et al., 2010). However, the *cat2* mutants do not have much higher H_2O_2 levels than the wild type, even under photorespiratory conditions (Queval et al., 2007). When the *cat2* mutants were grown air (i.e. photorespiratory conditions) they have a lower rosette biomass than the wild type plants (Queval et al., 2007; Chaouch et al., 2010). The small growth phenotype of *cat2* mutants is linked to an accumulation of glutathione in the leaves and a low ratio of reduced glutathione (GSH) to glutathione disulphide (GSSG) that appears to influence auxin functions (Mhamdi et al., 2010). The *cat2* mutants show lesion development on leaves in a day length-dependent manner; i.e. they have no lesions when grown under short day conditions. Lesions develop on the leaves only when the *cat2* mutants are grown under long day conditions (Queval et al., 2007; Chaouch et al., 2010). The day length-dependent effects on oxidative signalling leading to lesion formation are linked to an accumulation of SA and to a constitutive activation of pathogenesis-related (PR) genes (Li et al., 2013). The *cat2* mutants showed altered responses to pathogens and constitutive resistance to bacterial attack (Chaouch et al., 2010). SA-related responses were absent when the mutants were grown under short day conditions (Queval et al., 2007; Chaouch et al., 2010). The abundance of transcripts encoding heat shock proteins, antioxidant enzymes and anthocyanin-producing enzymes was much higher in *cat2* mutant leaves than those of the wild type, when plants were grown under high light growth conditions (Vandenabeele et al., 2004; Vanderauwera et al., 2005).

The overexpression of maize *CAT2* in transgenic tobacco plants led to a significant increase in plants susceptibility to bacterial infection compared to wild type plants (Polidoros et al., 2001). This sensitivity to bacterial attack was linked to higher capacity of the transgenic lines to remove H_2O_2, and hence limit its action as a signalling molecule (Polidoros et al., 2001). However, the enhnaced susceptibility to bacterial infection was not observed in the *CAT2* over-expressers, when the plants were grown under high light growth conditions (Polidoros et al., 2001). In other transgenic tobacco plants that were deficient in catalase (Cat1AS), the abundance of H_2O_2, SA, ET and PR proteins were increased when the plants were exposed to short term high light stress (Chamnongpol et al., 1998).

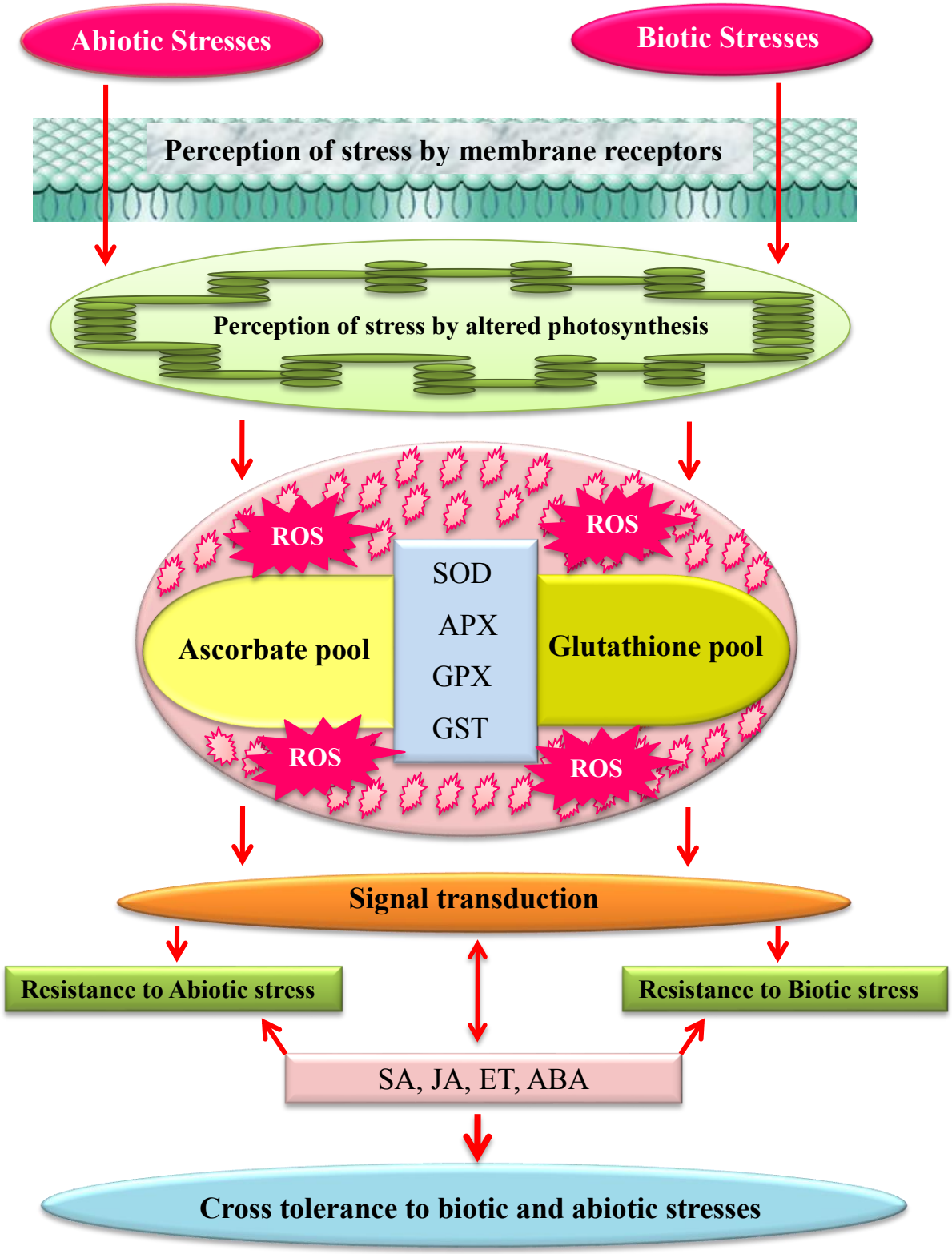


Figure 1.3 Integration of metabolic and stress signalling by produced reactive oxygen species (ROS) and their scavengers. The redox state of antioxidants ascorbate and glutathione is determined by environmental and metabolic signalling. The altered redox state induces signalling cascades that activate signal transducers such as kinases, ROS and hormones that result in the induction of sets of defence-related genes and then leads to tolerance to a wide range of stresses (Foyer et al., 2012).

ROS-dependent regulation of gene expression

The concept that ROS production by metabolism and during biotic and abiotic stress responses is important in the regulation of gene expression is widely accepted. ROS signalling facilitates enhanced stress tolerance and is an important factor contributing to cross tolerance phenomena because of interactions with the hormone-signalling network (Xia et al., 2015).

H_2O_2 produced during photorespiration can act as a local and systemic signal that is important in increasing plant resistance to biotic and abiotic stresses (Chamnongpol et al., 1998). The ability of ROS to trigger cross tolerance to different stresses occurs because plants rely on a basal defence mechanism that operates by recognizing pathogen associated molecular patterns (PAMPs) in order to prevent the penetration and restrict the growth of pathogens. PAMP perception initiates innate immune responses, called PAMP-triggered immunity (PTI). PTI acts as a wide-spectrum defence and is sufficient to prevent attack by most micro-organisms.

Successful pathogens can overcome this type of defence by using effectors to interfere with PTI, leading to effector-triggered susceptibility (ETS). However, some plants have evolved specific resistance proteins (Hammond-Kosack and Jones, 1997; Dangl and Jones, 2001) which can recognize these effectors to initiate effector-triggered immunity (ETI; Tao et al., 2003). ETI is an amplified version of PTI and usually involves a HR with cell death in response to the pathogen infection (Jones and Dangl, 2006). HR at the infection site can also activate systemic acquired resistance (SAR; Fu and Dong, 2013), which confers immunity to a broad-range of pathogens throughout the plant. There is now considerable molecular genetic evidence in support of the crucial function of RBOH in the pathogen-induced oxidative burst (Torres et al., 2002).

Ectopic expression of antioxidant enzymes has been used to confer a higher degree of abiotic stress tolerance in plants. For example, transgenic tobacco plants over-expressing chloroplastic Cu/Zn-SOD were shown to have an increased resistance to the oxidative stress caused by exposure to low temperatures and high light (Gupta et al., 1993a). Moreover, transgenic tobacco plants expressing Mn-SOD showed a reduction in leaf injury upon exposure to ozone relative to wild type plants (Van Camp et al., 1996). Similarly, ozone-induced foliar necrosis was partially reduced in the transgenic tobacco plants over-expressing Cu/Zn-SOD (Van Camp et al., 1996). The over-expression of encoded cDNA for an enzyme with both glutathione peroxidase (GPX) and glutathione S transferase (GST) activity in the transgenic tobacco plants resulted in the faster growth than control plants following cold or high salinity stress (Roxas et al., 1997).

1.4 Ascorbic acid (AsA)

Ascorbic acid vitamin C (L-ascorbic acid) is the most abundant low molecular weight antioxidant in plants. Together with the low molecular weight thiol, glutathione, ascorbate plays a central role in H_2O_2 detoxification in plants (Zheng and Vanhuystee, 1992; Noctor and Foyer, 1998). Oxidation of ascorbate by the enzyme APX in order to remove H_2O_2 is the first step of the ascorbate-glutathione cycle. The ascorbate peroxidase reaction produces monodehydroascorbate (MDHA), which can then either be reduced by MDHA reductase, or be further oxidised to dehydroascorbate (DHA). DHA is then reduced to ascorbate by the enzyme DHA reductase (DHAR) via ascorbate-glutathione cycle (AsA-GSH; Foyer and Halliwell, 1976). There is a chain of of enzymatic and non-enzymatic reactions in AsA-GSH cycle that regulate oxidative stress signalling through H_2O_2 (Fig 1.4; Foyer and Noctor, 2011).

1.4.1 Functions of AsA

In addition to its antioxidant role, ascorbate is also an important co-factor for many enzymes. For example, ascorbate is a cofactor for violaxanthin de-epoxidase which has important role in the xanthophyll cycle and regeneration of vitamin E (Foyer et al., 1996). Furthermore, the activity of the 2-oxoacid-dependent dioxygenase enzymes, which plays important role in the biosynthetic pathways of some hormones such as ABA and gibberellic acid (GA), requires ascorbate (Mirica and Klinman, 2008). Moreover, ascorbate is also important in glucosinolate and anthocyanin biosynthetic pathways (Turnbull et al., 2004).

The essential role of ascorbate in plant growth and development was demonstrated by knockout mutants defective in ascorbate synthesis, which were embryo lethal (Dowdle et al., 2007). Other mutants that have less severe mutations in enzymes of the ascorbate synthesis pathway have been useful in characterizing the functions of ascorbate in plants. For example, the *Arabidopsis thaliana* vitamin C-defective (*vtc*) mutants, *vtc1* and *vtc2-1,* which have only about 30% of the wild type ascorbate levels in leaves have a slow growth phenotype and they show hypersensitivity to abiotic stresses such as ozone and UV-B radiation (Conklin et al., 1999; Pastori et al., 2003). These mutants show an increased resistance to biotrophic pathogens that is linked to higher ABA and SA levels and constitutive up-regulation of SA-mediated resistance (Pastori et al., 2003; Kerchev et al., 2013).

1.4.2 AsA biosynthesis in leaves

Although a number of different pathways for ascorbate biosynthesis have now been identified in plants (Maruta et al., 2010; Badejo et al., 2012), molecular genetic evidence has shown that the D-mannose/ L-galactose is the major ascorbate biosynthetic pathway in leaves (Wheeler et al., 1998; Dowdle et al., 2007). In this pathway D-fructose-6-phosphate is converted to GDP-D-mannose by the activity of phosphomannose isomerase, phosphomannomutase, and GDP-D-mannose pyrophosphorylase (Fig. 1.4). GDP-D-mannose is then converted to ascorbate through intermediate steps including production of GDP-L-galactose, L-galactose-1-phosphate, L-galactose and L-galactono-1,4-lactone (Smirnoff, 2011). The last enzyme of the ascorbate biosynthesis pathway, L-galactono-1,4-lactone dehydrogenase, is localised in the mitochondria. This enzyme uses cytochrome C as an electron carrier and is associated with Complex I in the inner mitochondrial membrane (Fig. 1.4; Bartoli et al., 2000; Millar et al., 2003).

Ascorbate fulfils important roles in plant growth, particularly in the regulation of cell expansion (Kato and Esaka, 2000). In contrast to quiescent plant organs, which often have low tissue ascorbate contents (Potters et al., 2002), growing tissues have large amounts of ascorbate (Smirnoff, 2000; Kato and Esaka, 2000; Pellny et al., 2009). Ascorbate is present in most if not all of the intracellular compartments such as chloroplasts, mitochondria, cytosol, peroxisomes, and it is also present in the cell wall/ apoplast. The highest ascorbate concentrations have been reported in chloroplasts (2-25 mM). In contrast, relatively little ascorbate is found in the apoplast (~ 1 mM; Takahama, 1993; Foyer and Lelandais, 1996; Sanmartin et al., 2003; Pignocchi et al., 2003). This is perhaps because of the activity of the enzyme ascorbate oxidase (AO), which is localized in the apoplast. AO is considered to catalyse the first step in the pathway of ascorbate degradation, a process that controls the ascorbate content of the apoplast because this compartment contains few enzymes that can regenerate reduced ascorbate from its oxidised forms, monodehydroascorbate (MDHA) and dehydroascorbate (DHA; Vanacker et al., 1998).

1.5 Apoplast

The extra protoplastic matrix of plant cells is called the apoplast. This compartment plays a crucial role in many plant functions including growth, nutrient transport, defence and signal transduction (Sakurai, 1998). The apoplast may be considered as a metabolic bridge between the environment surrounding the cell and the symplast that passes information from external environment to the cell (Sakurai, 1998). The apoplast has many proteins including hydrolases and peroxidases, as well as metabolites such as sugars and cell wall related components including polysaccharides, glycoproteins and celluloses (Dietz, 1997; Sattelmacher, 2001). Unlike other low molecular weight antioxidants, ascorbate is abundant in the apoplast, where it fulfils important roles in redox buffering (Foyer and Noctor, 2000). Moreover, apoplastic ascorbate levels are tightly controlled in relation to cell growth and expansion (Horemans et al., 2000).

Ascorbate is transported from cytosol to apoplast through the AsA/ DHA transporter that is localized in the plasmalemma (Fig. 1.4; Horemans et al., 2000). Similarly, DHA in the apoplast can be transported into the cytosol to be recycled to ascorbate in the ascorbate-glutathione cycle (Fig. 1.4; Foyer and Halliwell, 1976). This is important because NADPH, which is required for the recycling of ascorbate, is largely absent from the apoplast (Horemans et al., 2000; Pignocchi et al., 2003). Ascorbate in the apoplast is oxidised to DHA by enzymatic and non- enzymatic reactions (Dumville and Fry, 2003; Green and Fry, 2005) as follows:

- Non-enzymatic oxidation of AsA (Plochl et al., 2000)

$$AsA + O_2 \rightarrow H_2O_2 + DHA$$

- Non-enzymatic oxidation of AsA in the presence of Cu^{2+} (Fry, 1998)

$$\tfrac{1}{2}AsA + Cu^{2+} \rightarrow Cu^{+} + \tfrac{1}{2}DHA + H^{+}$$

- Enzymatic oxidation of AsA by AO (Pignocchi et al., 2003)

$$2AsA + O_2 \rightarrow 2H_2O + 2DHA$$

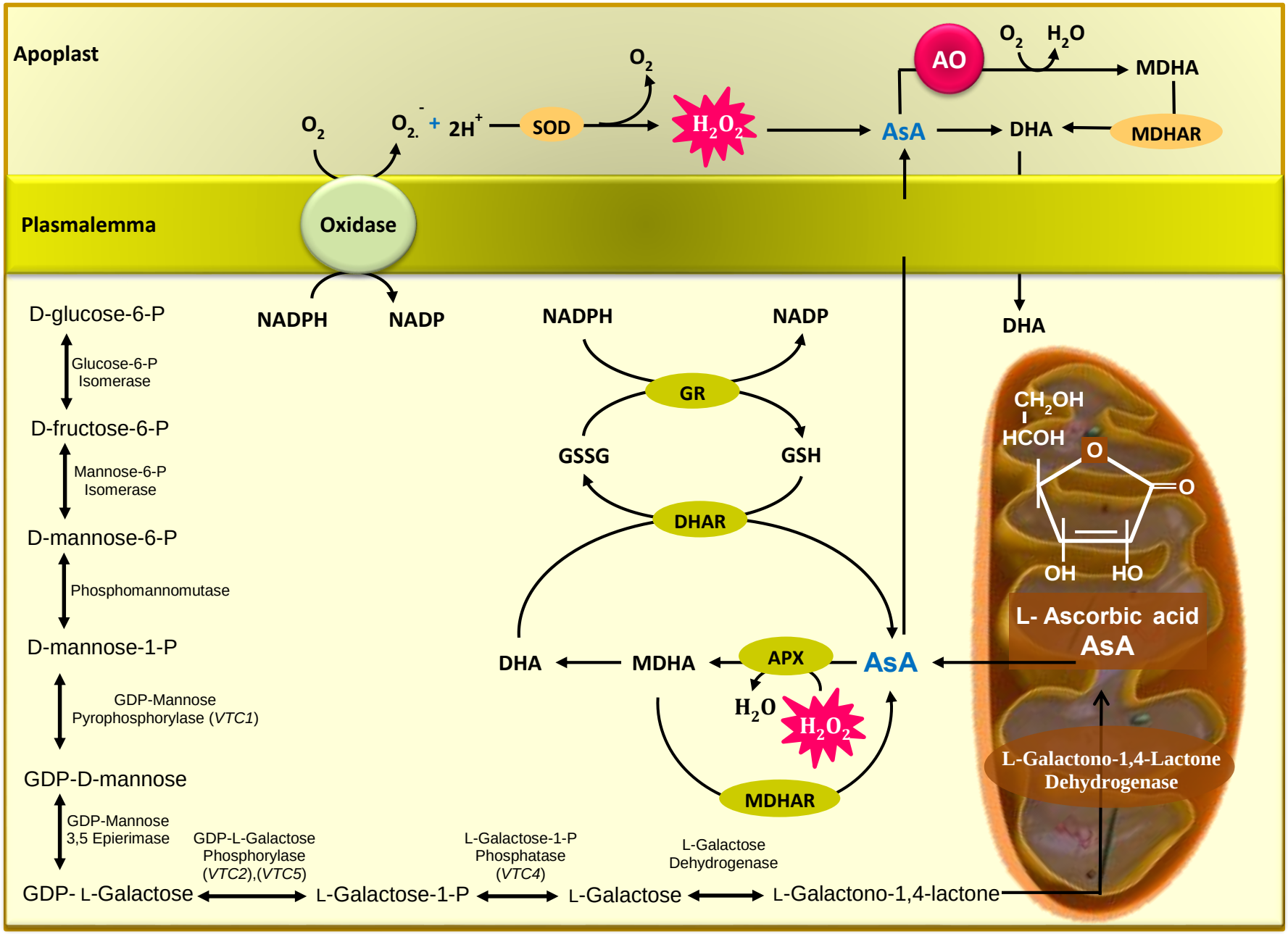


Figure 1.4 Biosynthesis of ascorbate in plant cells. GR: glutathione reductase; DHAR: dehydroascorbate reductase; MDHAR: monodehydroascorbate reductase; APX: ascorbate peroxidase; AO: ascorbate oxidase; SOD: superoxide dismotase.

1.6 Ascorbate oxidase (AO)

Ascorbate oxidase (AO) is a copper protein catalysing dioxygen reduction to water using ascorbate as the electron donor. AO activities are high in the rapid-growing plant tissues such as fruits and germinating seeds (Suzuki and Ogiso, 1973; Kato and Esaka, 1999). This enzyme catalyses ascorbate oxidation to MDHA and DHA in the apoplast/cell wall compartment of the cell, after which other metabolites such as tartrate and oxalate are formed (Pignocchi and Foyer, 2003).

AO functions in the apoplast/cell wall have largely been characterized by the analysis of transgenic plants with altered AO expression. AO is considered to have a role in cell elongation (Kato and Esaka, 2000) and this was confirmed by studies using ectopic expression of a pumpkin AO in tobacco BY-2 cells, which showed increased cell elongation rates. Expression of a melon AO gene in tobacco led to oxidation of the ascorbate pool in the apoplast and enhanced sensitivity to ozone (Sanmartin et al., 2003). Moreover, the expression and activities of several ascorbic acid- related enzymes was altered by the ectopic expression of the melon AO in tobacco (Sanmartin et al., 2003; Fotopoulos et al., 2006). Moreover, the expression of PR1a was decreased in the transgenic plants, which were more susceptible to infection by the necrotrophic fungus *Botrytis cinerea* than the wild type (Fotopoulos et al., 2006). The expression of a pumpkin AO gene in tobacco led to the 40-fold increase in the apoplastic AO activity and a 3.5-fold increase in the DHA content of the apoplast (Pignocchi et al., 2003). The increase in the apoplastic AO activity in the AO-over-expressing tobacco lines led to the activation of defence gene expression and increased susceptibility to biotrophic pathogens (Pignocchi et al., 2006). Antisense AO expression in transgenic tobacco plants resulted in 2.5-fold decrease in AO activity and over a 40 % increase in the apoplastic pool of reduced ascorbate (Pignocchi et al., 2003). The antisense AO plants had higher germination rates and an altered sensitivity to auxin (Pignocchi et al., 2003; 2006). Low AO activities were associated with an increased resistance to salt stress (Yamamoto et al., 2005).

High AO activities in rapidly-growing plant tissues have been linked to a high level of ascorbate oxidation and an increase in auxin-mediated cell elongation (Arrigoni, 2003; Potters et al., 2010). Root meristems elongation was enhanced in onion by the addition of DHA (Hidalgo et al., 1989). The overexpression of a pumpkin AO in transgenic tobacco lines resulted in an acceleration of protoplast expansion relative to wild type controls (Kato and Esaka, 2000). Young leaves tend to have a higher AO content than older leaves (Yamamoto et al., 2005). Moreover, the abundance of AO transcripts was higher in rapid-growing tobacco tissues (Kato and Esaka, 1996).

Studies on transgenic tobacco plants showed that auxin stimulated shoot growth only when the apoplastic ascorbate pool was reduced in wild type or AO antisense lines (Pignocchi et al., 2006). Oxidation of apoplastic AsA in AO sense lines was associated with loss of the auxin response (Pignocchi et al., 2006).

In Arabidopsis *AO* is encoded by a small gene family comprising three *AO* genes, called *AO1* (At4g39830), *AO2* (At5g21105) and *AO3* (At5g21100) respectively (Lim, 2012). The Arabidopsis *ao3* mutant was first described by Yamamoto et al. (2005). Although the mutant plants have less than 20% of the wild type AO levels, they have no significant phenotype relative to the wild type plants, except for a higher capacity for seed production than the wild type plants under salt stress conditions (Yamamoto et al., 2005). In contrast, the *ao1ao2* double mutants had a smaller growth phenotype than the wild type plants with delayed flowering (Lee et al., 2011). The *ao1* has a similar level of AO activity to that of wild type but like the *ao3* mutants the *ao1ao3* double mutants have about 10-20% of wild type AO levels (Lim, 2012). No phenotypic differences were observed in any of the AO mutant lines relative to the wild type and all lines showed similar responses to high light or drought stress (Lim, 2012). In other studies, the *AO1*, *AO2*, and *AO3* genes were silenced using microRNA technology in transgenic Arabidopsis lines (*amiR-AO*; Lim, 2012). AO activities were below the levels of detection in the transgenic amiR-*AO* lines, which had larger rosettes than wild type plants under either optimal or stress conditions (Lim, 2012).

1.7 Protein kinases and MAP-kinase cascades

Protein phosphorylation and de-phosphorylation, catalyzed by protein kinases and phosphatases respectively, serve as an "on-off" switch to regulate many biological processes. Within this system, the transduction of oxidative signals is often mediated by protein phosphorylation systems that involve mitogen-activated protein (MAP) kinase (MAPK) cascades that comprise of MAPK, MAPK kinase (MAPKK/MKK) and MAPKK kinase (MAPKKK/MEKK; Nakagami et al., 2005). The MAPK/MPK cascades are highly conserved central regulators of diverse cellular processes, such as differentiation, proliferation, growth, death and stress responses.

Many different MAPK cascades can be activated following H_2O_2 accumulation but in Arabidopsis, the MEKK1–MKK1/2–MPK4 pathway is a central regulator of H_2O_2 homeostasis. In this pathway, MAP kinase kinase 1 (MEKK1) serves as an activator of MAPKs MKK1 and MKK2, which function upstream of MPK4 (Xing et al., 2008). MEKK1 is regulated by various stresses and H_2O_2 in a proteasome-dependent manner (Pitzschke et al., 2009a).

MEKK1 can activate the downstream kinases MPK3, MPK4 and MPK6. However, H_2O_2-induced MEKK1 is only required for activation of MPK4, but not MPK3 or MPK6 (Nakagami et al., 2005). Gene expression analysis identified 32 distinct transcription factors that respond to multiple ROS-promoting conditions, 20 of them are regulated preferentially via the MEKK1–MKK1/2–MPK4 pathway (Pitzschke et al., 2009a).

MEKK1 is transcriptionally induced by cold, salt, drought, touch, and wounding. The MEKK1–MAPK kinase 2 (MKK2) MPK4/MPK6 cascade functions as part of cold and salt stress signalling (Teige et al., 2004), whereas the MEKK1–MKK4/MKK5–MPK3/MPK6 cascades have been reported to regulate the pathogen defence response pathway via the expression of WRKY22 and WRKY29 (Asai et al., 2002). MPK3 and MPK6 are also activated by abiotic stresses and involved in hormone signalling pathways. MPK3 has been shown to function in ABA signalling at the post-germination stage (Lu et al., 2002).

In Arabidopsis, H_2O_2 activates MPK3 and MPK6 through ANP1 (ANP, Arabidopsis NPK1-like protein kinase, in which NPK is a *Nicotiana* protein kinase). Over-expression of *ANP1* leads to enhanced tolerance to heat shock, freezing and salt stress in plants (Kovtun et al., 2000). Oxidative signal inducible 1 (OXI1) protein kinase is also an upstream mediator of MPK3 and MPK6. OXI1 kinase activity is induced by phosphatidic acid (Anthony et al., 2004) and by H_2O_2 (Rentel et al., 2004).

The o*xi1* null mutants are hypersensitive to infection by virulent fungal pathogens and are compromised in the activation of MPK3 and MPK6 under oxidative stress (Rentel et al., 2004). *MPK9* and *MPK12*, which are preferentially expressed in guard cells, function downstream of ROS and cytosolic Ca^{2+} and upstream of anion channels as positive regulators in ABA signalling in guard cells.

The activity of MPK12 is increased by both ABA and H_2O_2 treatments (Jammes et al., 2009). MPK9 and MPK12 also play a role in basal defence to pathogens (Jammes et al., 2011).

1.8 Protein phosphatases in plants

Like protein kinases, protein phosphatases also function in cell signalling and plant stress responses (Luan, 2003). Protein phosphatases are classified into families of serine/threonine (Ser/Thr) phosphatase, tyrosine phosphatase (PTP) and dual specificity phosphatase (DSP) which catalyses the dephosphorylation of serine, threonine and tyrosine residues. Ser/Thr phosphatase consists of serine/threonine specific phosphatase (PPP) and metal ion-dependent protein phosphatase (PPM). PPM consists of PP2C and other Mg^{2+}-dependent phosphatases (Luan, 2003).

PP2C is a negative regulator of ABA signalling that can bind the ABA receptor pyrabactin resistant 1 (PYR1)/PYR1-like (PYL)/regulatory component of ABA response 1 (RCAR1; Cutler et al., 2010). PPP can be further divided into PP1, PP2A, PP2B, PP4, PP5, PP6, PP7 and protein phosphatases with kelch-repeat domains (Luan, 2003). PP1 and PP2A do not require divalent cations for activity, whereas the activities of PP2B and PP2C are dependent on Ca^{2+} (PP2B) and Mn^{2+}/Mg^{2+} (Smith and Walker, 1996). Many protein phosphatases are sensitive to inhibitors such as okadaic acid and cyclosporin A. While PP1 and PP2A are inhibited by these inhibitors, but PP2B and PP2C are insensitive to inhibition by these compounds (Mackintosh et al., 1994; Luan, 2003).

1.8.1 Protein phosphatases 2A (PP2A)

PP2A is a major phosphatase that accounts for about 25% of the total protein phosphatase activity in crude homogenates from several plants (MacKintosh and Cohen, 1989). A catalytic subunit C (36 kDa), a scaffold subunit A (65 kDa), and a highly variable regulatory subunit B constitute the trimeric form of the holoenzyme PP2A (Figure 1.5; Mayer-Jaekel and Hemmings, 1994). The B subunits, which influence the structural conformation and determine the substrate specificity and subcellular localization of PP2As, include B (or B55; 55 kDa), B' (54-74 kDa), and B" (72-130 kDa) subfamilies.

The B-family are encoded by four related genes (Bα, Bβ, Bγ, Bδ), the B' family are derived from different related genes (B'α, B'β, B'γ, B'ζ, B'δ and B'ε) and The B" family contains three related genes encoding (PR48, PR59, and PR72/130; Janssens and Goris, 2001).

The Arabidopsis genome has five genes encoding catalytic PP2A-C subunits, three genes encoding PP2A-A subunits and seventeen genes encoding B subunits, theoretically accounting for 255 different heterotrimer combinations (Zhou et al., 2004).

The Arabidopsis genome encodes three functionally different A subunits: PP2A-A1, PP2A-A2 and PP2A-A3 (Zhou et al., 2004). The characterization of the functions of these subunits has been greatly aided by the characterisation of PP2A mutants. For example, PP2A-A1 was first identified as RCN1, the gene encodes a regulatory α-subunit of PP2A, which shows a curled root phenotype when exposed to naphthylphthalamic acid (NPA), an inhibitor of polar auxin transport and gravitropism (Garbers et al., 1996). RCN1 is a major determinant of phosphatase activity. The *pp2a-a2* and *pp2a-a3* mutations have little effect except in the absence of RCN1 (Zhou et al., 2004).

In addition to increased sensitivity to NPA, the *rcn1* mutant shows an approximately two-fold reduction of PP2A activity compared with wild type and exhibits strong defects in root curling, apical hypocotyl hook formation, and differential cell elongation (Garbers et al., 1996). Recent studies also have shown other roles of RCN1-dependent phosphatase activity, such as in ABA, JA and ET signalling.

The A subunits act as a scaffold that allows combination of the different subunits of the PP2A holoenzyme, PP2A-A subunits also interact with other proteins. For example, PP2A-A subunits interact with the PP6 catalytic subunits FyPP1 and FyPP3 to form a new holoenzyme complex to dephosphorylate PIN proteins and direct auxin distribution (Dai et al., 2012). PP2A-A subunits also compete with regulatory 14-3-3 protein for binding to the same position in the C-terminus of the plasma membrane H1-ATPase in plants, whose activities are dependent on phosphorylation status and influenced by interactions with 14-3-3 proteins (Fuglsang et al., 2006).

Recently, novel roles for a gene encoding a specific PP2A-B'γ subunit were described (Trotta et al., 2011a, b). Loss of function mutants for PP2A-B'γ were shown to constitutively activate pathogenesis responses, an effect linked to enhanced ROS signals (Trotta et al., 2011a). The *pp2a-b'γ* mutant shows constitutive expression of defence related genes and premature yellowing in leaves (Trotta et al., 2011a). The premature yellowing observed in the *pp2a-b'γ* mutant has been linked to hyper-phosphorylation of calreticulin 1, the endoplasmic reticulum-resident chaperonin, which is involved in the unfolded protein response, leading to endoplasmic reticulum stress (Trotta et al., 2011b).

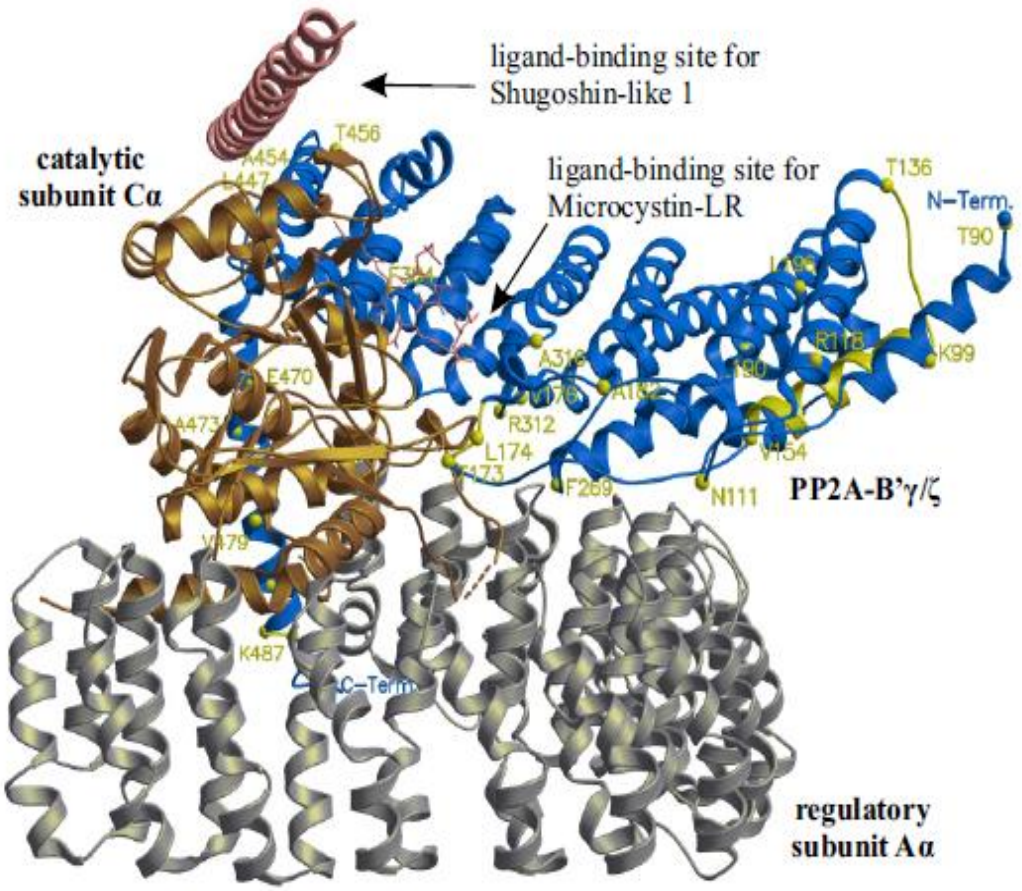


Figure 1.5 Structural model of a trimeric PP2A complex containing Arabidopsis PP2A-B'γ (blue), mouse PP2A-Aα (silver) and human PP2A-Cα (gold). Sites of amino acid differences between PP2A-B'γ and PP2A-B'ζ are mapped in yellow. The N-terminal segment M1-V88 of PP2A-B'γ is not present in the structural model. All amino acid labels are as in PP2A-B'γ. Sites known to recognize the Shugoshin-like 1 and Microcystin-LR molecules in 3FGA are indicated (Rasool et al., 2014).

The lack of regulatory B'γ subunit of PP2A in *pp2a-b'γ* mutant resulted in increased resistance against virulent *Pseudomonas syringae* pv *tomato* DC3000 and *Botrytis cinerea* compared with wild type. In *pp2a-b'γ* mutant 11 common SA-related genes showed higher expression level compared with wild type plant, even though no changes in the ratio of SA/ JA is detected in the mutant. Nevertheless, a slight reduction in the level of DNA methylation and the increased ratio of S-adenosyl-homocysteine-hydrolase (SAHH1) and adenosine kinase (ADK) of the methionine-salvage (Met-salvage) pathway, suggest a changing in the balance of cellular methylation reactions in *pp2a-b'γ* mutant. Biosynthesis of toxic compounds such as aliphatic glucosinolates is strongly connected with the maintenance and *de novo* synthesis of Met, which produce toxic compounds via the activity of myrosinase during plant responses to biotic stresses (Fig. 1.6; Hirai et al., 2007; Fan et al., 2011).

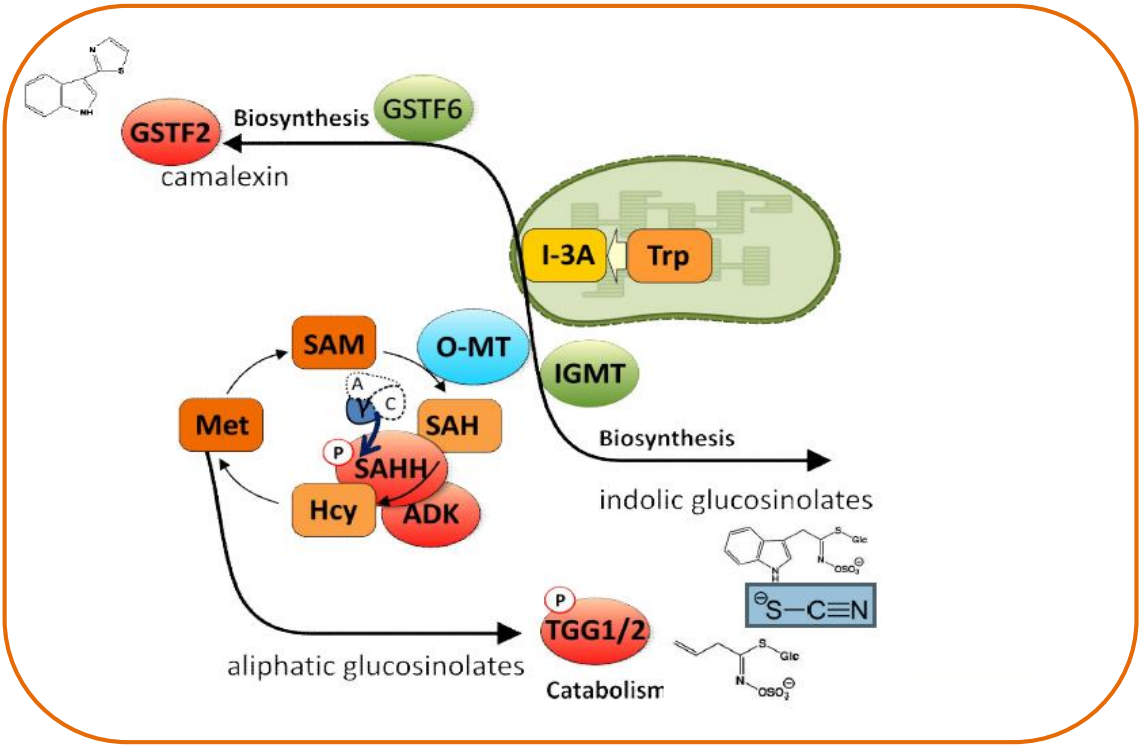


Figure 1.6 Secondary metabolites in *pp2a-b'γ* mutant and responses to pathogen infection. O-MT: O-methyltransferase; SAM: S-adenosyl-methionine; SAH: S-adenosyl-homocysteine; Hcy: homocysteine; SAHH: S-adenosyl-homocysteine-hydrolase;
Met: methionine; Trp: tryptophan; I-3A: indole-3-acetaldoxime; ADK: adenosine kinase; IGMT: indoleglucosinolate O-methyltransferase; TGG1/2: thioglucoside glucosidase (myrosinase); GST: glutathione S-transferase; C: catalytic subunit of PP2A; A: scaffold subunit A of PP2A; **γ**: gamma subunit of regulatory subunit B of PP2A (Trotta et al., 2011a).

1.9 Plant stress hormones linked to redox metabolism

ROS signalling is highly integrated with hormonal signalling networks to regulate plant growth and defence pathways. Plants synthesize a diverse range of hormones including abscisic acid (ABA), auxin, cytokinins (CKs), gibberellins (GAs), salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Hormone signalling pathways regulate plant growth and development, and they play crucial role in responses to biotic and abiotic stimuli. Of the many hormones that mediate plant growth and defence responses, SA, ABA and JA are considered to be particularly important in responses to herbivory (De Vos et al., 2005).

Salicylic acid

Salicylic acid plays a key role in ROS signalling transduction cascades that regulate plant defence mechanisms against biotic and abiotic stresses (Vlot et al., 2009). Increased ROS lead to SA accumulation and induce the expression of SA-dependent pathogenesis related (PR) genes (Chen et al., 1993; Chamnongpol et al., 1998). SA is required for the induction of effective defence against biotrophic and hemi-biotrophic pathogens. Plants challenged by pathogens and herbivores accumulate SA and PR proteins such as β-1,3-glucanase (BGL2; Patrick and Gary, 2001). The SA dependent pathways caused the establishment of systemic acquired resistance (SAR) that alleviates enhanced resistance to broad range pathogens. Mutations that cause reduced SA production or perception enhance susceptibility to pathogen (Loake and Grant, 2007). SAR is regulated or preceded by an increase in SA (Silverman et al., 1995). However, SA does not provide an effective defence against necrotrophic pathogens (Coquoz et al., 1995; Yu et al., 1997).

Jasmonic acid

Jasmonic acid and related compounds, including methyl jasmonate (MeJA) and jasmonoyl-isoleucine (JA-Ile), regulate plant responses to wounding, necrotrophic pathogens and herbivores (Creelman and Mullet, 1992; 1995; Devoto and Turner, 2005). Interactions between the JA and SA-mediated signalling pathways are complex. Although many studies show that JA and SA act in an antagonistic manner in the regulation of plant defences (Spoel et al., 2003), oxidative stress can act to induce both pathways in parallel (Han et al., 2013b). JA also interacts with ABA-dependent signalling pathways (Birkenmeier and Ryan, 1998).

Abscisic acid

Abscisic acid is involved in the regulation of many aspects of plant growth and development and also is the major hormone that controls plant responses to abiotic stresses (Wasilewska et al., 2009). ABA also plays an important role in inducing stomatal closure by increasing cytosolic Ca^{2+} concentration of the guard cells, for example by enhancing Ca^{2+} influx from the extracellular space (Pei et al., 2000) and Ca^{2+} release from intracellular stores (Garcia-Mata et al., 2003). The flux of Ca^{2+} from the extracellular space is mediated by Ca^{2+} permeable channels in the plasma membrane (Pei et al., 2000). ABA-mediated activation of NADPH oxidases is also required for stomatal closure (Kwak et al., 2003). ROS formation and protein phosphorylation are required for the activation of Ca^{2+} permeable channels (Pei et al., 2000; Murata et al., 2001; Köhler and Blatt, 2002; Kwak et al., 2003).

ABA-induced closure of stomata is inhibited if H_2O_2 production is blocked (Pei et al., 2000; Petrov and Van Breusegem, 2012). The *rcn1* mutation impairs ABA-induced stomatal closure and attenuates activation of anion channels (Kwak et al., 2002). Hence, RCN1 is considered to act upstream of cytosolic Ca^{2+} elevation in ABA signal transduction and downstream of PP2C (Kwak et al., 2002). ABA is therefore central in protection against stresses that involve water-limitation such as drought, salt stress and cold (Xiong et al., 2002).

It is widely accepted that ABA is an important modulator of hormone-regulated defence, for instance mutants defective in ABA biosynthesis such as *aba2*, failed to accumulate JA or associated oxylipins following challenge with the oomycete *Pythium irregulare* (Adie et al., 2007) but the role of ABA in the orchestration of plant defences is complex and poorly understood (Ton et al., 2009).

1.10 Transcription factors in stress responses

Plant stress responses are regulated by multiple signalling pathways that activate gene transcription and its downstream machinery. Plant genomes contain a large number of transcription factors (TFs) which are involved in responses against biotic and abiotic stresses, and they play an essential role in regulation of plant adaptation to environmental changes. The majority of these transcription factors belong to a few large multi gene families, for example AP2/EREBP, MYB, bZIP and WRKY (Kreps et al., 2002).

Relatively few TFs have been documented to involve in the crosstalk between abiotic and biotic stress signalling networks. The basic helix-loop-helix (bHLH) domain-containing transcription factor AtMYC2 is a positive regulator of ABA signalling.

The genetic lesion of AtMYC2 results in elevated levels of basal and activated transcription from JA-ethylene responsive defence genes (Anderson et al., 2004). MYC2 distinctively modifies two types of JA mediated responses, it regulates positively wound-responsive genes, including LOX3, VSP2 and TAT, but represses the expression of pathogen-responsive genes such as PR4, PR1, and PDF1,2. These compound interactions are co-mediated by the ethylene-responsive transcription factor ERF1. ABA signalling plays a crucial role in plant stress responses as evidenced by the fact that many of the drought-inducible genes studied to date are also induced by ABA. Two TF groups, bZIP and MYB, are taking part in ABA signalling and its gene activation (Lorenzo et al., 2004).

Several transcription factors which constitute the components of ABA signalling pathways have been shown to regulate a large number of downstream target genes involved in plant responses to abiotic and biotic stresses. Three well-characterized positive regulators of ABA signaling are the transcription factors encoded by ABSCISIC ACID (ABA)-INSENSITIVE-3, 4 and 5 (ABI3, ABI4 and ABI5) which were initially identified in screens for mutants exhibiting the phenotype insensitive to inhibiting effect of ABA on seed germination. These proteins are members of the B3-, APETALA2- (AP2), and basic leucine zipper-(bZIP) domain families, respectively, and regulate overlapping subsets of seed-specific and/or ABA-inducible genes. The *abi* mutants are impaired in a range of physiological responses to stress including the ABA effect on the stomata closure (Finkelstein and Lynch, 2000a).

The ABI4 protein belongs to the DREBA3 sub group of a large family of plant specific transcription factors known as AP2/EREBP (Sakuma et al., 2002). The *A. thaliana* genome encodes 147 AP2/EREBP members and many of them are of particular interest because they are implicated in many signalling processes, including biotic and abiotic stress responses (Mizoi et al., 2012).

ABI4 has emerged as a central player in many signalling processes during plant development. For example, ABI4 is important in the coordination of metabolic and environmental signals, particularly involving sugar and nitrogen responses that control plant development, as well as organellar retrograde signalling pathways (Kaliff et al., 2007).

The lack of the ABI4 function in (*abi4-102*) mutant and in vitamin C synthesis in (*vtc2-1*) mutant on plant resistance to aphids was studied by Kerchev et al. (2011). In the *abi4* mutants aphid fecundity was higher than that of wild type, explaining the central role of ABI4 signalling pathways in the regulation of aphid resistance. In contrast, in *vtc2-1* mutants, decrease in aphid fecundity was observed.

The small phenotype and aphid resistance in *vtc2-1* mutants could be reversed through loss of ABI4 function in the (*abi4vtc2*) double mutants (Kerchev et al., 2013). This shows that low ascorbate activates ABA-dependent signaling pathways that control plant growth and resistance to aphid through ABI4 transcription factor (Kerchev et al., 2011; 2013).

1.11 Plant-aphid interactions

1.11.1 Plant responses to aphid attack

Aphids are the biggest group of phloem feeding insects. They are major agricultural pests causing extensive damage to crop and garden plants in three ways. Firstly, the photo assimilates of the host plants that go for aphid feeding can be otherwise used in growth and development. Secondly, aphid feeding on host plants results in reduction of photosynthetic efficiency via secretion of honeydew that negatively affects CO_2 uptake through leaf stomata and enhance leaf infection with fungus (Hogenhout et al., 2008). The third negative impact of aphid attack on plant productivity is via transmitting of viruses as they are vectors for more than 100 disease-causing viruses such as potato leaf roll virus and cucumber or cauliflower mosaic virus (Van Emden et al., 1969; Ng and Perry, 2004; Braendle et al., 2006).

The green peach aphid (*Myzus persicae*) is considered as “generalist" feeder because it can colonise on more than 30 plant families and transmits over 100 viruses (Van Emden et al., 1969).

1.11.2 Aphid feeding

Generally, aphids induced damage results in decreases in crop yield of up to 15% (Leather et al., 1989) and especially, cause wheat yield loss either directly (35-40%) by sucking the sap of the plants or indirectly (20-80%) by transmitting viral and fungal diseases (Aslam et al., 2005). Aphids penetrate plant tissues by probing between the cells in the epidermal and mesophyll layers with their piercing-sucking mouthparts called stylets to feed on photo-assimilates translocated in the phloem sieve elements (Fig. 1.7).

The epidermal and mesophyll cells are not punctured by stylet activity because it is probing between the cells on its way to reach the phloem. The rapidly-gelling called "sheath-saliva" is secreted during stylets puncturing activity (Tjallingii and Hogen, 1993). As a result, the direct interaction of the chitinous stylet and the infested plant tissue is prevented. While, the presence of several metabolites in the sheath saliva such as conjugated carbohydrates, phospholipids, pectinases, phgenoloxidases and β-glucosidases have potential to induce plant responses against aphid infestation (Miles, 1999).

However, the stylet might puncture epidermal and mesophyll cells occasionally during probing process (Martin et al., 1997). There is another type of saliva that is secreted by aphids at the puncture points and feeding locations called "watery saliva" (Prado and Tjallingii, 1994; Tjallingii, 2006). The watery saliva prevents the induction of wound responses of the penetrated tissues that usually try to repair any damaged tissues (Will et al., 2009). This mechanism helps the aphid for feeding on a single sieve element of the phloem for a long period of time (Will et al., 2006).

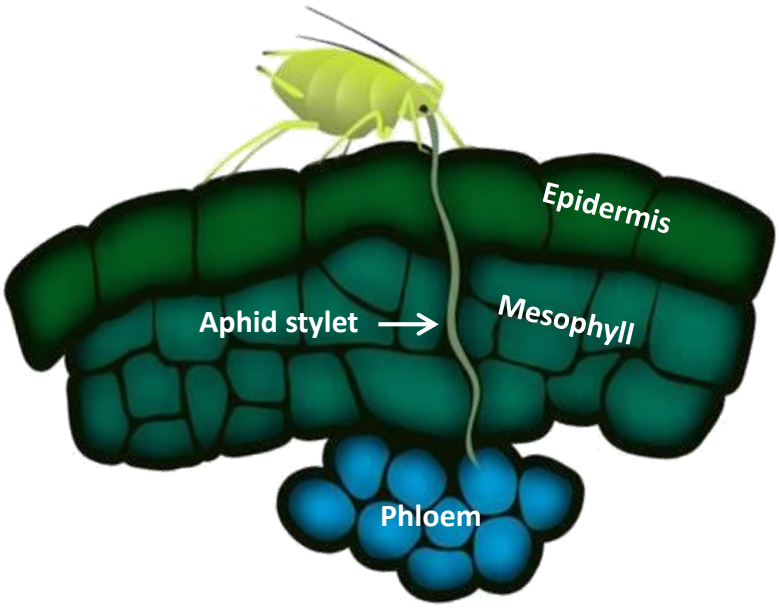


Figure 1.7 Aphid feeding on plant cell. Aphids use their mouthparts stylet to reach the photo-assimilates in the sieve elements. While, epidermal and mesophyll cells are not punctured by the stylet activity as it probes between the cells to reach the phloem (Tjallingii and Hogen, 1993).

1.11.3 Aphid life cycle

Aphids have primary hosts for oviposition and secondary hosts for feeding. The secondary hosts usually includes wider group of families than the primary hosts. Most of aphids have parthenogenic life cycle via different sexual and asexual reproductive stages (Blackman & Eastop, 2000).

Having different reproductive strategies is important for non-tolerant species to cold and need primary host to lay eggs in autumn to survive overwinter, as well as for producing a big population in a short period of time. In spring female aphids hatch from overwintered eggs which only giving birth by parthenogenesis into a high fecund offspring called nymphs. The newly-born nymphs are already pregnant with the embryos of the next generation. During spring, depending on the population density, aphids can stay as highly fecund wingless morphs or produce winged progeny to spread and colonise secondary hosts then give birth to new nymphs on the new host plants by parthenogenesis. At the end of summer, the changes in temperature and day length can trigger physical and behavioural changes in aphids which lead to production of winged female and winged or wingless male progenies to return back to the sexual phase. Winged progeny leave the summer hosts and lay eggs on primary hosts overwinter (Fig. 1.8; Fenton et al., 1998; Tagu et al., 2005; Braendle et al., 2006).

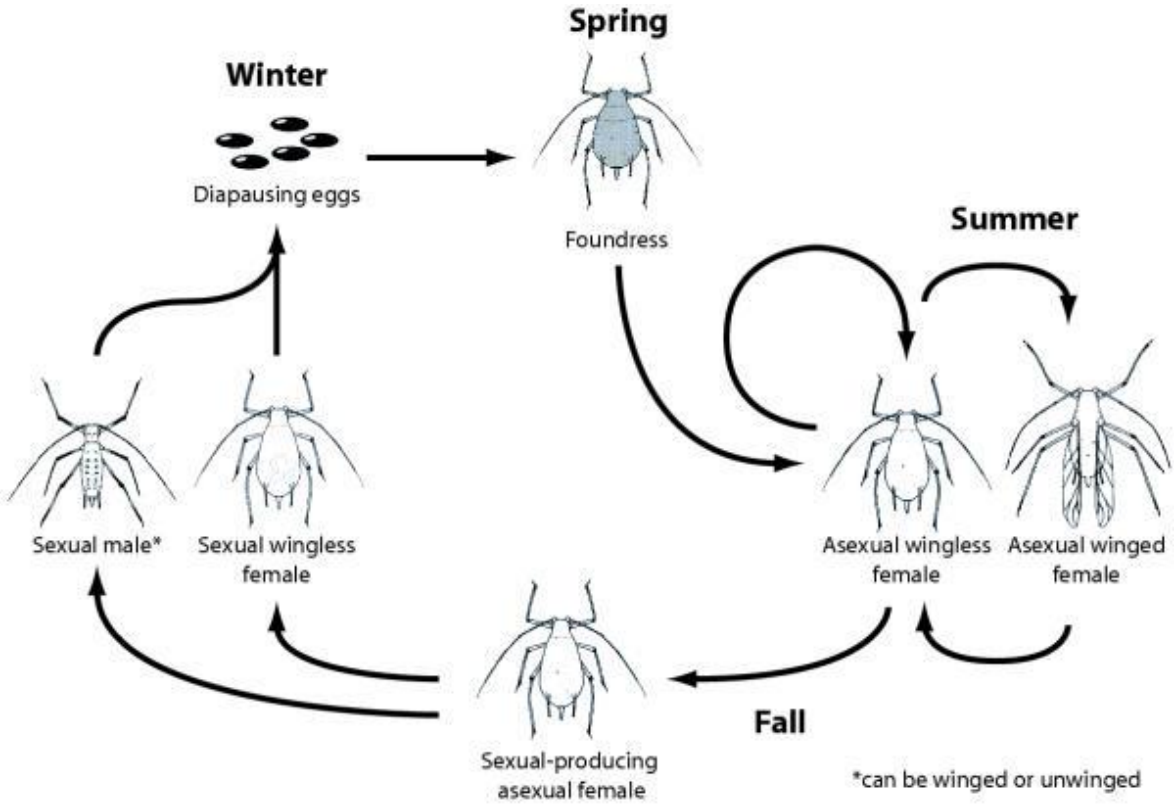


Figure 1.8 Aphid life cycle. In spring female aphids hatch from overwintered eggs. During summer aphids stay as wingless high fecund progeny and propagate by parthenogenesis. However, when the population is overcrowded aphids produce winged progeny to spread to another places and find another host plants then give birth to new nymphs by parthenogenesis. In autumn when the weather is changed aphids produce winged female and winged or wingless male progenies to lay eggs and stay survival overwinter (Fenton et al., 1998; Tagu et al., 2005; Braendle et al., 2006).

1.11.4 Plant responses to aphid attack

In contrast to chewing insects, aphid infestation does not lead to the induction of wound responses as it does not cause extensive wounding or damage. However, infested plant tissues perceive aphid probing in a way that results in significant transcriptome changes, hence induction of defence responses (De Vos et al., 2005). Little is known about the perception mechanisms of aphid attack by plants. Similarly, the induction of plants defence responses and the elicitors that involve in this induction is poorly understood. The metabolites and proteins that synthesized by the aphids or by the endosymbiotic bacteria that exist in the aphid and released with the secreted saliva into the plant tissues are considered as the potential elicitors (Urbanska et al., 1998; Miles, 1999; Forslund et al., 2000). Moreover, the wound-like responses might be induced via activation of mechanoreceptors in the plant cells by probing activity of the stylet or cell wall puncturing (Hamel and Beaudoin, 2010; Perfect and Green, 2001). The hyphae of fungal pathogens grow through intercellular spaces, a process that could be compared to the aphid stylet probing (Hamel and Beaudoin, 2010; Perfect and Green, 2001). Plants perceive fungal pathogen attack through the protein elicitor called chitin that is localized in the cell walls of fungus. Chitin is elicitor for induction of plant microbe-associated molecular patterns (MAMP) signalling (Wan et al., 2008). MAMPs play important roles in the perception of pathogen attack (Boller and Felix, 2009). Therefore, it is likely that MAMP are also involved in plant perception for aphid attack following its activation by aphid feeding that might be similar to those used to perceive fungal pathogens.

During penetration and feeding, aphids produce the “gelling” and “watery” saliva. Although little is known about the function of the aphid saliva, programmed cell death could be triggered by the watery saliva. Defence signalling in Arabidopsis could be induced by a proteinaceous elicitor present in the saliva of green peach aphid (De Vos and Jander, 2009). Furthermore, the chlorosis and local cell death were induced in *Nicotiana benthamiana* by the effect of (Mp10) elicitor present in green peach aphid saliva (Bos et al., 2010). In addition, defence signalling against aphid infestation is activated by some elicitors of plant origin that release from plant cell in response to the released enzyme by the aphid. For example, secretions of enzymes by the stylet sheath lead to an induction of the plant cell walls to release oligogalacturonides which involves in the induction of defence responses (Heil, 2009).

1.11.5 Plant defence mechanisms against aphid attack

In order to affect aphid physiology and reduce its growth and reproductive performance, plants manipulate the advantage of constitutive and inducible defence mechanisms. For example, leaf structure, the wax layer that cover leaf surfaces and presence or density of trichomes on the leaf surface can be considered as the constitutive defence mechanisms that negatively affect aphid ability on settling and feeding on the leaves. In addition, some plant proteins such as protease inhibitors and lectins also affect aphid reproductive performance (Dutta et al., 2005; Rahbe et al., 2003).

The glucosinolates are plant secondary metabolites and their hydrolysis by the activity of myrosinases produces numerous compounds that play important roles in constitutive and inducible defences. The myrosinase and its substrate glucosinolates are located in different tissue types. (Thangstad et al., 2004). The produced compounds from the hydrolysis of glucosinolates have negative impact on insect herbivores (Kliebenstein et al., 2005) and previously published studies have reported that glucosinolates regulate plant defence responses against aphid attack (Levy et al., 2005; Mewis et al., 2005). For example, Arabidopsis mutants lacking CYP81F2, which is required for indole-3-yl-methyl glucosinolate to 4-hydroxyindole-3-yl methyl glucosinolate, were more sensitive to green peach aphid infestation than the wild type plants (Pfalz et al., 2009). Furthermore, it has been shown that JA treatment and wounding led to an increase in the abundance of genes that are implicated in glucosinolate biosynthesis and increased leaf indole glucosinolate content (Mikkelsen et al., 2000).

A range of volatile compounds are released by plants tissues upon aphid feeding and some of them negatively affect aphid fecundity (Hildebrand et al., 1993, Hardie et al., 1994). For example, transgenic potato plants that are impaired in hydroperoxide lyase, required for synthesis of volatile C6 aldehydes, were more susceptible to aphid infestation compared to wild type plants (Vancanneyt et al., 2001). On the other hand, emission of volatile compounds in response to aphid infestation might attract aphid natural enemies and indirectly affect aphid infestation. It was previously observed that infested potato plants with *M. persicae* released some volatile compounds (Gosset et al., 2009).

The production of volatiles does not have a significant effect on plant growth and development (Fritzsche et al., 2001). For example, the reduction in growth was not observed in the plants that constitutively contained high abundance of volatile compounds (Lücker et al., 2001; Ohara et al., 2003).

The hypersensitive response (HR) is another important mechanism in plant responses to aphid infestation. The plant disease resistance (R) genes, which can recognize the products of avirulence genes in the pest and lead to induction of incompatible plant-pathogen interactions, have trigger function in hypersensitive response.

Most of the (R) genes are encoding nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins that involve in the detection of the plant proteins that targeted by pathogen (McHale et al., 2006). For example, the incompatible interaction in the infested tomato leaves with potato aphid (*Macrosiphum euphorbiae*) was triggered by the NBS-LRR gene that increased plants resistance to aphid which is characterized by reduction in aphid growth and reproductive performance (Martin et al., 2003; Rossi et al., 1998). Likewise, the NBS-LRR protein, which is encoded by *Vat* gene, was implicated in the resistance response of infested melon plants with the aphid *Aphis gossypii* (Villada et al., 2009). In addition, this NBS-LRR-mediated resistance response was accompanied by apoplastic callose or lignin degradation and lesions formation in the infested leaves (Villada et al., 2009).

Little is known about the resistance responses that are mediated by the (R) genes. However, the signalling mechanisms that induced by the (R) genes may include the production of reactive oxygen species (ROS) and pathogenesis-related (PR) proteins or changes in calcium fluxes (Smith and Boyko, 2007).

1.12 High light stress

Photosynthesis is considered as the most crucial biological process on the earth by releasing oxygen and consuming carbon dioxide via converting light energy to chemical energy. Although, the essential energy source for this vital process is light, excess light has harmful impacts on plants and affect the efficiency of photosynthesis (Osakabe and Osakabe, 2012). Excess light of plant's photosynthetic capacity can cause damage to the photosynthetic apparatus, which can function optimally within a range of light intensities, and photooxidation of chlorophyll. Furthermore, the generation of reactive oxygen species (ROS) is another responsible reason that directly causes photoinhibition and reduction in the overall efficiency of photosynthesis or indirectly induces transcriptional changes (Suzuki et al., 2012). Hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are generated in photosynthetic apparatus in response to exposure to high light intensities from three sites; the reaction centres of photosystem II (PSII), light-harvesting complex of PSII and acceptor sites of photosystem I (PSI; Fig. 1.9; Niyogi, 1999). The produced ROS can cause the oxidation not only of chlorophyll but of lipids, proteins and the required enzymes for performing chloroplast functions or the whole cell (Foyer et al., 1994).

Photoinhibition can be defined as a light-dependent reduction of the photosynthetic quantum yield due to plant exposure to excess light over the saturation of photosynthesis capacity (Niyogi, 1999). The absorbed light energy by chlorophyll has three main destinies. Either the absorbed energy is; transported to adjacent chlorophyll and reaches the photochemical reaction centres (PSI and PSII) where the energy is utilized in photosynthesis process which known as photochemical quenching; or the absorbed energy is converted or dissipated in the form of heat through photoprotective non-photochemical quenching (NPQ) mechanism; or the absorbed energy is emitted in the form of photon by chlorophyll fluorescence (Horton and Ruban, 2004).

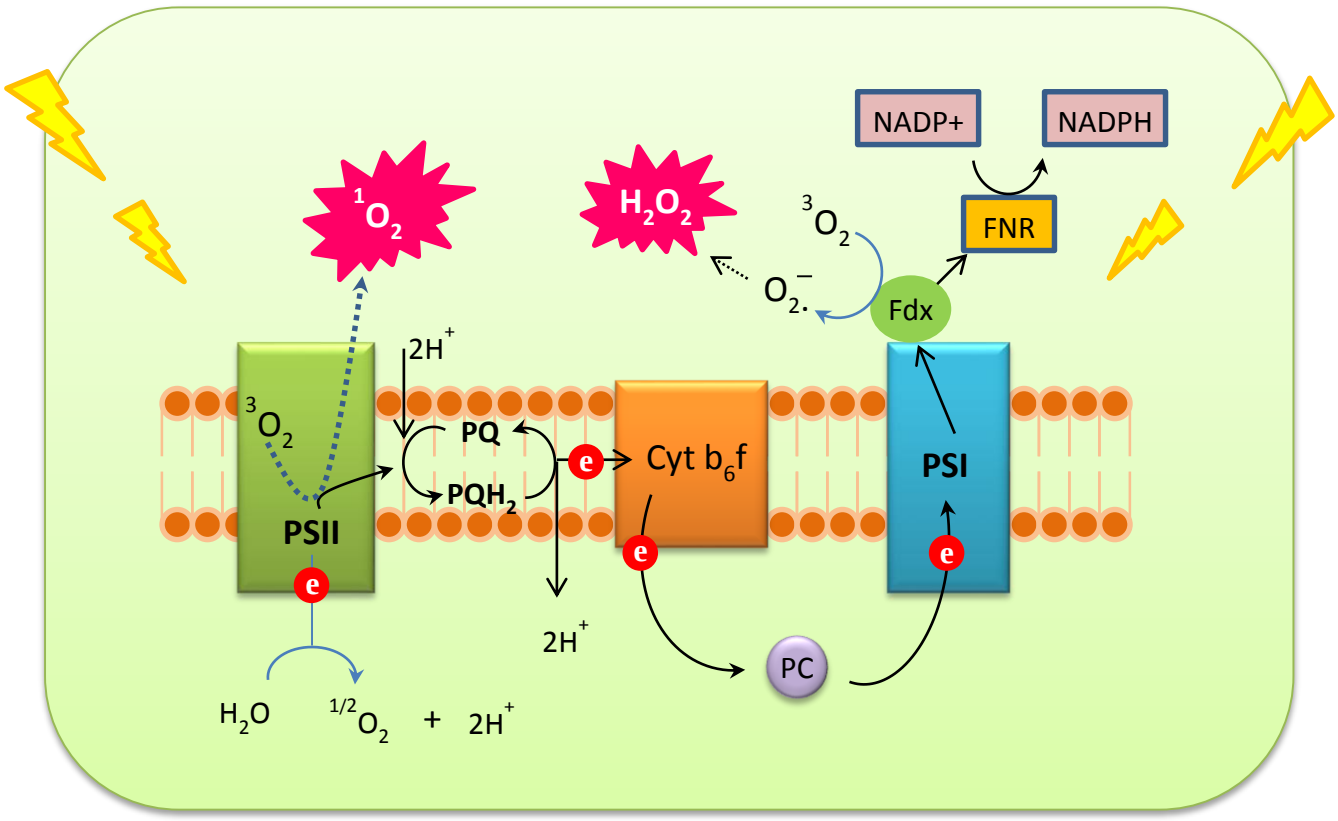


Figure 1.9 Generation of reactive oxygen species (ROS) in chloroplast. Singlet oxygen ($^1O_2$) is generated by the reaction centres and light-harvesting complex of photosystem II (PSII). Superoxide ($O_2^{\cdot -}$) is generated by the acceptor sites of photosystem I (PSI), which is converted to the hydrogen peroxide (H_2O_2), in response to exposure to high light intensities (Niyogi, 1999).

1.12.1 Plant responses to high light stress

Dissipation and conversion of the excess excitation light energy in the form of heat through non-photochemical quenching (NPQ) is one of photoprotective mechanisms in plants in order to respond to high light (HL) stress (Becker et al., 2006). Different conformational alterations involve in NPQ mechanism. The change in pigment interactions through the conformational change in light harvesting proteins of PSII leads to development of energy traps. This conformational change is derived by the transmembrane proton gradient. The changes are also induced by the PSII subunit S (PsbS) and the conversion of carotenoid violaxanthin to zeaxanthin which is re-transformed to violaxanthin again in the dark condition via the xanthophyll cycle. The change in redox state of thioredoxin/glutathione and the plastoquinone (PQ) pool under HL condition are the suggested sensors of excess light. PQ pool is the transporter of the electron from photosystem II through thylakoid discs to the cytochrome b_6f in the electron transport chain (Fig. 1.9; Becker et al., 2006).

1.12.2 Cross talk between light stress and pathogen

Photosynthesis is a major source of ROS and has a strong influence on plant stress responses (Karpinski et al., 2003; Foyer and Noctor 2009; Sierla et al., 2013). Retrograde signals arising in the chloroplast transmit information to the nucleus in order to regulate gene expression (Karpinski and Szechyńska-Hebda, 2012). For example, Arabidopsis mutants lacking FLU, which contain high levels of the chlorophyll precursor protochlorophyllide, have been instrumental in defining $^1O_2$ signalling pathways leading to defence responses (Lee et al., 2007). Moreover, cell death programmes induced in response to HL involve the same genes that are induced during SAR responses (Luna et al. 2012; Luna and Ton 2012) and the HR responses (Chang et al. 2009; Frenkel et al. 2009). High light induces the expression of genes involved in plant defence responses by pathways that involve ROS generated by the chloroplasts and regulation by Ca^{2+} signalling (Nomura et al., 2012). Such processes may also form the basis for the induction of systemic acquired acclimation (SAA), which is induced by HL (Mateo et al. 2004; Rossel et al. 2007). For example, HL-treated plants were found to have increased resistance to virulent *Pseudomonas syringae* pv *tomato* DC3000 (Karpinski et al., 2012).

HL stress also induces the expression of genes encoding proteins involved in ROS scavenging, as well as SA, ABA and auxin synthesis/signalling (Mühlenbock et al. 2008; Fini et al. 2012). When Arabidopsis mutants lacking antioxidants such as APX are exposed to HL they show enhanced expression of heat shock proteins (HSP) such as HSP70, which is typically also induced in response to viral infections (Pnueli et al., 2003).

High light intensities increase production of secondary metabolites that play key roles in plant stress responses (Edreva et al., 2008; Zavala and Ravetta, 2001; Coelho et al., 2007). For example, Arabidopsis leaves grown under HL (600 μmol m^{-2} s^{-1}) have higher levels of sinapinic acid and threonic acid than the leaves grown under LL (30 μmol m^{-2} s^{-1}; Jänkänpää et al., 2012).

The abundance of raffinose, polyamines and glutamate are also increased in Arabidopsis leaves under HL (Wulff-Zottele et al., 2010). It is possible that some members of the raffinose family such as stachyose, which is induced by ROS production, might fulfil antioxidant functions under stress conditions (Wienkoop et al., 2008; Widodo et al., 2009). Light quality also exerts a strong influence on leaf metabolite composition. For example, broccoli (*Brassica oleacea*) shoots exposed to (350 μmol m^{-2} s^{-1}) red and blue LED light for 5 days contained higher levels of β-carotene, violaxanthin and aliphatic glucosinolates than plants grown under (41 μmol m^{-2} s^{-1}) blue LED light (Kopsell and Sams, 2013). Glucosinolates are secondary metabolites that have a negative impact on plant pathogens and herbivores (Huseby et al., 2013).

1.12.3 Light memory

Several studies have shown that exposure to HL triggers both local and systemic signals (Karpinski et al., 2012). Moreover, plants pre-treated with HL retain a "memory" of the HL stress that persists when plants are returned to LL conditions (Karpinski et al., 2012). These pathways of light signalling trigger both abiotic and biotic plant defence responses. For example, a pre-exposure of Arabidopsis plants to HL altered responses to the biotrophic pathogen *Pseudomonas syringae* pv *tomato* DC3000 (Karpinski et al., 2012). Plants grown under LL were more susceptible to *P. syringae* than those grown under HL. However, if the plants were grown under LL (100 μmol m^{-2} s^{-1}) and then exposed to HL (1500 μmol m^{-2} s^{-1}) for 8 or 24h prior to the infection, plant resistance to the pathogen was significantly increased (Karpinski et al., 2012).

Similarly, a pre HL-treatment, (white light 1500 μmol m^{-2} s^{-1} for 1h) or (red light 120 μmol m^{-2} s^{-1} for 4h) led to a significant decrease in *P. syringae* infection on wild type Arabidopsis and in transgenic lines over expressing phyB::YFP fusion protein (Zhao et al., 2014). Growth under HL conditions for 4h resulted in a significant increase in the abundance of PR1 compared to plants grown under LL (100 μmol m^{-2} s^{-1}) conditions. The leaves of HL treated-plants had higher MPK3 and MPK6 activities, as well as higher lipoxygenase (LOX) transcript levels (Zhao et al., 2014).

Short term exposures to HL can trigger a "light memory" that can last for several days (Karpinski et al., 2012). Even a 1h HL treatment resulted in a "light memory" that lasted for several days (Szechyńska-Hebda et al., 2010). The nature of this "light memory" is complex and poorly understood but it is thought to involve a network of signalling pathways, including ROS, hormonal and photo-electrophysiological signalling (PEPS; Szechyńska-Hebda et al., 2010; Karpinski and Szechyńska-Hebda, 2012).

1.13 Hypothesis and project objectives

Plant performance and the predictability of crop yield are severely hampered by environmental factors that restrict plant vigour and create a "yield gap", which is the difference between the "yield potential" and the actual crop yield achieved by the farmer. Over the last 50 years, plant breeders have improved the yield potential by selecting improved genotypes, but the yield gap remains, due to environmental effects. Abiotic stresses alone reduce average yields by as much as 50% for most major crop plants (Boyer, 1982). Phloem-feeding insects such aphids can cause yield losses in wheat either directly (35-40%) by sucking the sap of the plants or indirectly (20-80%) by transmitting viral diseases (Aslam et al., 2005).

Improvements in yield production and stability in suboptimal and often stressful environmental conditions are needed in order to increase crop production in a sustainable manner to meet the demand of a growing human population in a changing climate, without adversely affecting our ecological footprint. The innovative approaches to enhancing tolerance to biotic and abiotic stresses and novel strategies for crop protection that are required are formidable challenges to current plant science research. The following studies are based on the concept that plants have co-evolved with an enormous variety of microbial pathogens and insect herbivores under conditions with very different types of abiotic stress (heat, cold, water-logging, drought, enhanced salinity, toxic compounds, etc.). Plants therefore harbour a largely untapped reservoir of natural adaptive mechanisms to simultaneously cope with different forms of stress and maximize growth and survival.

It is likely that plants respond differently to the simultaneous imposition of multiple stresses compared to individual stresses. The literature evidence discussed above suggests that plants rarely activate highly specific programmes of gene expression relating to the exact environmental conditions encountered. Moreover, rather than being additive, the presence of abiotic stress factors can either reduce or enhance susceptibility to a biotic pest or pathogen, and vice versa, depending on the hormone signalling pathways that are induced in the complex stress response network. The following studies therefore seek to resolve some of the outstanding issues by characterising plant responses to a biotic stress (aphid attack) and an abiotic (high light) stress alone, and then in combination.

The specific aim is to characterise the respective roles of redox signalling in pathways leading to resistance to the abiotic stress imposed by high light, the biotic stress imposed by aphid feeding and the combined stress imposed by high light and aphid feeding. This analysis will employ a range of mutants and transgenic plants that are modified in components of redox signalling pathways. These include:

1) Transgenic tobacco lines:

Transgenic tobacco (*Nicotiana tabacum* L.; T3 generation) expressing pumpkin (*Cucurbita maxima*) ascorbate oxidase (AO) in the sense orientation [AO sense lines (P221 and P372)] and partial tobacco AO in antisense orientation [AO antisense lines (P271 and P161)] as described by (Pignocchi et al., 2003).

2) *Arabidopsis thaliana pp2a* and *cat2* mutants:

1. *gamma*: *pp2a-b'γ* [SALK_039172 for At4g15415].
2. *zeta1*: *pp2a-b'ζ1-1* [SALK_107944C for At3g21650].
3. *zeta2*: *pp2a-b'ζ1-2* [SALK_150586 for At3g21650].
4. *gamma-zeta* double mutant: *pp2a-b'γζ* [cross between *pp2a-b'γ* and *pp2a-b'ζ1-1*] as described by (Trotta et al., 2011a).
5. Catalase-deficient mutant (*cat2*) and (*cat2 pp2a-b'γ*) double mutants as characterised by (Li et al., 2013).

3) *Arabidopsis thaliana* glutathione and catalase-glutathione deficient mutants:

1. *cad2*: defective in glutathione, as described by (Yi et al., 2013a,b).
2. *pad2*: defective in glutathione, as described by (Parisy et al., 2007).
3. *clt*: the export of γ-glutamylcysteine and glutathione from the chloroplast, as described by (Maughan et al., 2010).
4. *cat2 cad2*: defective in both catalase and glutathione, as described by (Yi et al., 2013a,b).

4) *Arabidopsis thaliana* ascorbate-deficient mutant lines:

1. *vtc2-1* (EMS)
2. *vtc2* (T-DNA): [SAIL_769_H05 for At4g26850].

5) *Arabidopsis thaliana* ascorbate oxidase (AO) transgenic lines:

1. *amiR-AO (3.6*)
2. *amiR-AO (8.5),* as characterised by (Lim, 2012).

The specific objectives of this project are:

1. To assess the importance of the redox state of the apoplast in plant responses to high light and aphid infestation

This analysis will use transgenic tobacco and Arabidopsis plants that are modified in the abundance of ascorbate oxidase. These plants have already been generated and extensively characterised in terms of growth and resistance to fungal pathogens (Pignocchi et al., 2003; 2006; Lim, 2012). The transgenic tobacco and Arabidopsis lines, which have altered activity of ascorbate oxidase in the apoplast will be used to investigate importance of the redox state of the apoplast in regulation of the signal transduction pathways involved in plant resistance to aphids and responses to high light. The effects of increased or decreased abundance of ascorbate oxidase in the apoplast on aphid fecundity will determined. The effects of increased or decreased abundance of ascorbate oxidase in the apoplast on the responses of photosynthetic CO_2 assimilation and chlorophyll *a* fluorescence quenching to a high light treatment (a 8h exposure to an irradiance of 1600 µmol m^{-2} s^{-1} for tobacco and 800 µmol m^{-2} s^{-1} for Arabidopsis) will then be determined. Thereafter, plants will first be subject to a 8h high light treatment and the high light exposed leaves will then tested for altered responses to aphid attack in terms of aphid fecundity. The tobacco lines that show the greatest differences in the aphid response relative to the wild type under optimal and/or high light conditions will be selected for further analysis. Samples will be harvested from infested leaves at time points early (12h) in aphid infestation process under optimal irradiance conditions and after the high light stress treatment. Aphid and light dependent gene expression and metabolite changes changes will be analysed by microarray analysis and metabolic profiling for the tobacco plants.

2. To assess the roles of protein phosphatase signalling cascades and catalase in plant responses to high light and aphid infestation

The host lab has already characterised the local and systemic responses of leaves of wild type *Arabidopsis thaliana* plants to aphid infestation using a combined transcriptomic and metabolomics approaches (Kerchev et al., 2013). The lab has also documented the altered responses of a range of *Arabidopsis thaliana* mutants that are defective either in ascorbate (*vtc1, vtc2*) or redox and associated signalling pathways (*abi4*, *rrft1*,*oxy1*, *aba1* and *abi4vtc2*). The following studies will extend this analysis to include other components that are downstream in the oxidative signalling cascades.

This analysis will include *Arabidopsis thaliana* mutants that are defective in protein phosphatase pathways, particularly mutants that are deficient in PP2A-B'γ and in a double mutant that is defective in both PP2A-B'γ and catalase.

Light and CO_2 response curves for photosynthesis will be measured together with chlorophyll *a* fluorescence quenching analysis in wild type and mutant lines before and after exposure to a high light treatment (8h at 800 µmol m^{-2} s^{-1}). Aphid fecundity will be compared in the wild type and mutant lines. The effects of a pre-treatment with high light on aphid fecundity will determined on wild type and mutant plants that have first been subjected to a 8h high light treatment. The high light exposed leaves will then tested for altered responses to aphid attack in terms of aphid fecundity. The effects of these treatments on transcripts that have been identified previously as important in the responses of *Arabidopsis thaliana* to aphids will be measured using qPCR.

3. To assess the roles of low ascorbate content in plant responses to aphid infestation

The host lab has already documented the responses of *Arabidopsis thaliana* mutants *vtc2-1* (EMS), which are defective in ascorbate, to aphid infestation compared to the wild type using combined transcriptomic and metabolomics approaches (Kerchev et al., 2013). The following analysis will therefore compare aphid responses in the *vtc2-1* (EMS) line and a T-DNA insertion mutant, *vtc2* (T-DNA).

4. To assess the roles of low glutathione (GSH) content in plant responses to aphid infestation under low and high light growth conditions

Although the host lab has already extensively characterised a range of *Arabidopsis thaliana* mutants that are defective in either GSH synthesis (*cad2-1, pad 2-1*) or GSH transport (*clt*). They have never however been tested in terms of responses to aphid infestation. The following analysis will therefore compare aphid responses in the wild type, *cad2-1, pad 2-1* and *clt* lines, as well as in a double mutant that is defective in both catalase and GSH synthesis (*cat2cad2-1*).

Chapter 2. Materials and Methods

2.1 Plant material and growth conditions

2.1.1 Tobacco lines

Seeds of wild type and transgenic tobacco (*Nicotiana tabacum* L.; T3 generation) lines expressing a pumpkin (*Cucurbita maxima*) ascorbate oxidase (AO) gene in the sense orientation [sense lines (cj102-*PAO* sense-P221 and P372)] GenBank accession number (X55779) or a partial tobacco AO sequence in the antisense orientation [antisense lines (cj102-*TAO* antisense (P271 and P161)] GenBank accession number (D43624) were obtained from Prof. Christine Foyer's laboratory seed stocks. These lines had been produced and characterised in previous studies in the lab by Pignocchi et al. (2003, 2006).

2.1.2 *Arabidopsis thaliana* lines

2.1.2.1 Protein phosphatase 2A (PP2A) mutant lines

Seeds of wild type *Arabidopsis thaliana* accession Columbia 0 (Col-0) and mutants lacking the gamma ('γ) and zeta ('*ζ)* subunits of regulatory subunit B of protein phosphatase 2A (PP2A) were provided by Dr. Saijaliisa Kangasjärvi, of the University of Turku, Finland. These lines had been characterised in previous studies (Trotta et al., 2011a, b).

Homozygous *pp2a-b'γ* (SALK_039172 for At4g15415), *pp2a-b'ζ1-1* and *pp2a-b'ζ1-2* (SALK_107944C and SALK_150586 for At3g21650, respectively) mutant lines were identified from the SALK institute's collection by PCR analysis according to the institute's protocols (Alonso et al., 2003). A *pp2a-b'γζ* double mutant was constructed by crossing the SALK_039172 and SALK_107944C single mutants and selecting homozygotes from the F2 generation using the same set of PCR primers that were used to screen for the single *pp2a-b'γ* and *pp2a-b'ζ1-1* mutants. Insertion mutant information was obtained from the SIGnAL website at http://signal.salk.edu.

2.1.2.2 Catalase-deficient (*cat2*) mutant and (*cat2 pp2a-b'γ*) double mutants

Seeds of catalase-deficient (*cat2*) mutant and (*cat2 pp2a-b'γ*) double mutants were provided by Prof. Graham Noctor of the University of South Paris, France. These lines had been characterised in previous studies (Li et al., 2013).

2.1.2.3 Glutathione-deficient mutants (*cad2, pad2, clt*) and (*cat2 cad2*) double mutants

Seeds of glutathione-deficient mutant [cadmium sensitive (*cad2*)] and catalase-glutathione deficient (*cat2 cad2*) double mutants were provided by Prof. Graham Noctor of the University of South Paris, France. These lines had been characterised in previous studies (Yi et al., 2013).

Seeds of phytoalexin-deficient (*pad2*) mutant were provided by the Arabidopsis Biological Resource Center (ABRC). This line had been characterised in previous studies (Parisy et al., 2007).

The CHLOROQUINERESISTANCE TRANSPORTER (PfCRT)-LIKE TRANSPORTER1 (*clt*) mutants were originally generated in the lab of Chris Cobbett (University of Melbourne) but have been grown and characterised in our lab since 2004 and also described by (Maughan et al., 2010).

2.1.2.4 Vitamin C defective 2 (*vtc2*) mutant lines

Ascorbate-deficient mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA) insertion line were used in the following study. The *vtc2-1* (EMS) seeds were obtained from the laboratory of Robert Last and grown in our lab since 2003. The *vtc2* (T-DNA) seeds, which is T-DNA insertion line from SAIL, were obtained from Nottingham Arabidopsis Stock Centre (NASC; SAIL_769_H05 for At4g26850).

2.1.2.5 Arabidopsis ascorbate oxidase (AO) transgenic lines

Seeds of Arabidopsis ascorbate oxidase (AO) transgenic lines *amiR-AO (3.6*) and *amiR-AO (8.5)* were provided by Prof. Nicholas Smirnoff of the University of Exeter, UK.

2.1.3 Growth conditions

Unless otherwise stated, *Arabidopsis thaliana* and tobacco plants were grown in compost (SHL professional potting compost) in controlled environment chambers under an 8h/16 h day/night regime, with an irradiance of 250 μmol m^{-2} s^{-1} (low light conditions). The relative humidity was 60% and day/night temperatures were 20˚C.

2.2 High light (HL) treatments

LED light was used for all HL treatments and it was provided by a PhytoLux LED Plant Growth Lighting, Surrey, UK. The spectral composition of the LED light is explained in figure (2.1).

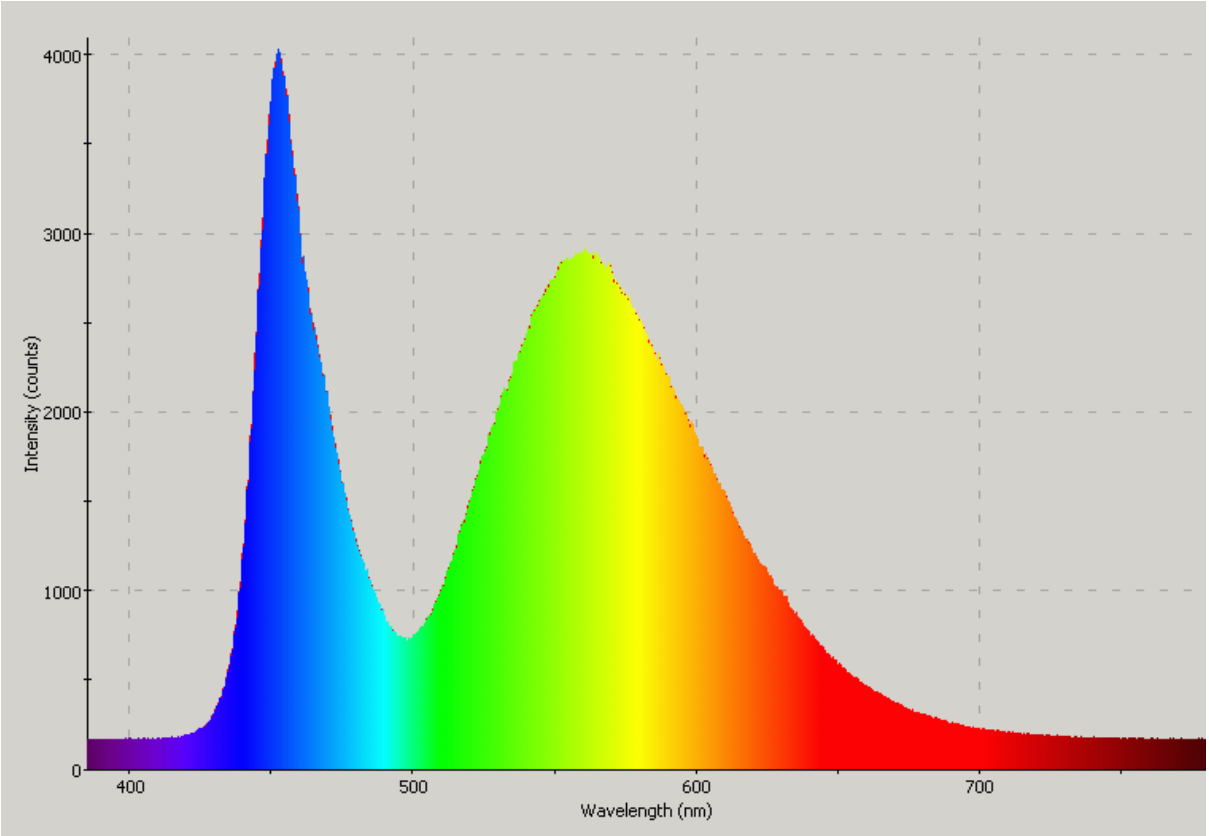


Figure 2.1 spectral composition of the LED light (adapted from PhytoLux LED Plant Growth Lighting).

2.2.1 HL-treatments in tobacco plants

Tobacco plants were grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) conditions. They were then either grown for a further seven days under LL conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) growth conditions for a further seven days before measurement of harvest (Fig. 2.2). The LED light that used for HL pre-treatment was provided by a PhytoLux LED Plant Growth Lighting, Surrey, UK.

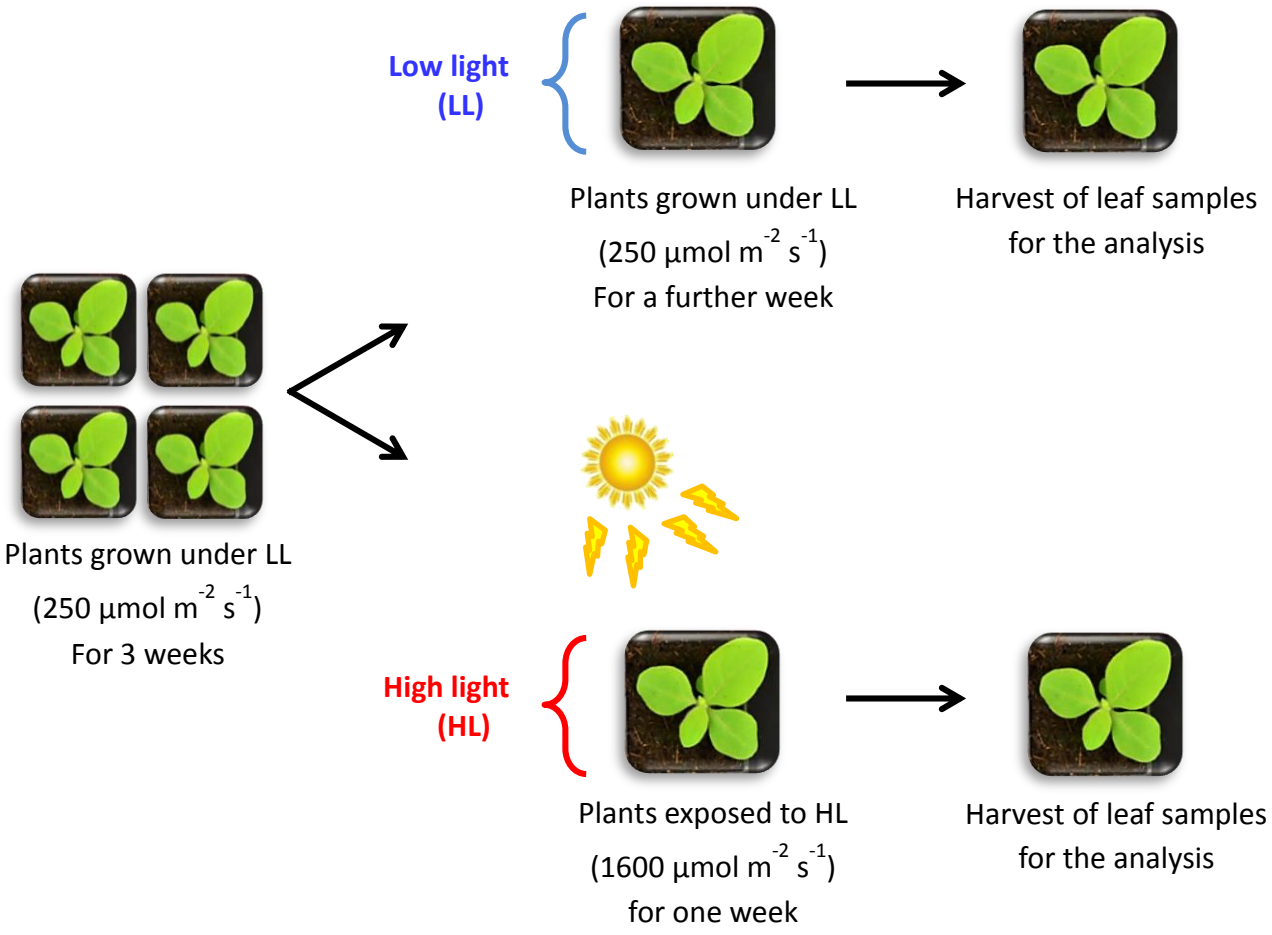


Figure 2.2 Illustration of high light (HL) treatment prior to measurement of harvest in tobacco plants. Plants were grown for three weeks under low light conditions (LL; 250 µmol m^{-2} s^{-1}). They were then either grown for a further seven days under LL conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) growth conditions for a further seven days before collection of leaf samples for analysis. The HL pre-treatment was provided by a PhytoLux LED Plant Growth light array.

2.2.2 HL-treatments in Arabidopsis plants

Arabidopsis plants were grown for two weeks under low light conditions (LL; 250 µmol m^{-2} s^{-1}). They were then either grown for a further seven days under LL conditions prior to measurement of harvest or transferred to high light (HL; 800 µmol m^{-2} s^{-1}) growth conditions for a further seven days before measurement of harvest (Fig. 2.3). The LED light that used for HL pre-treatment was provided by a PhytoLux LED Plant Growth Lighting.

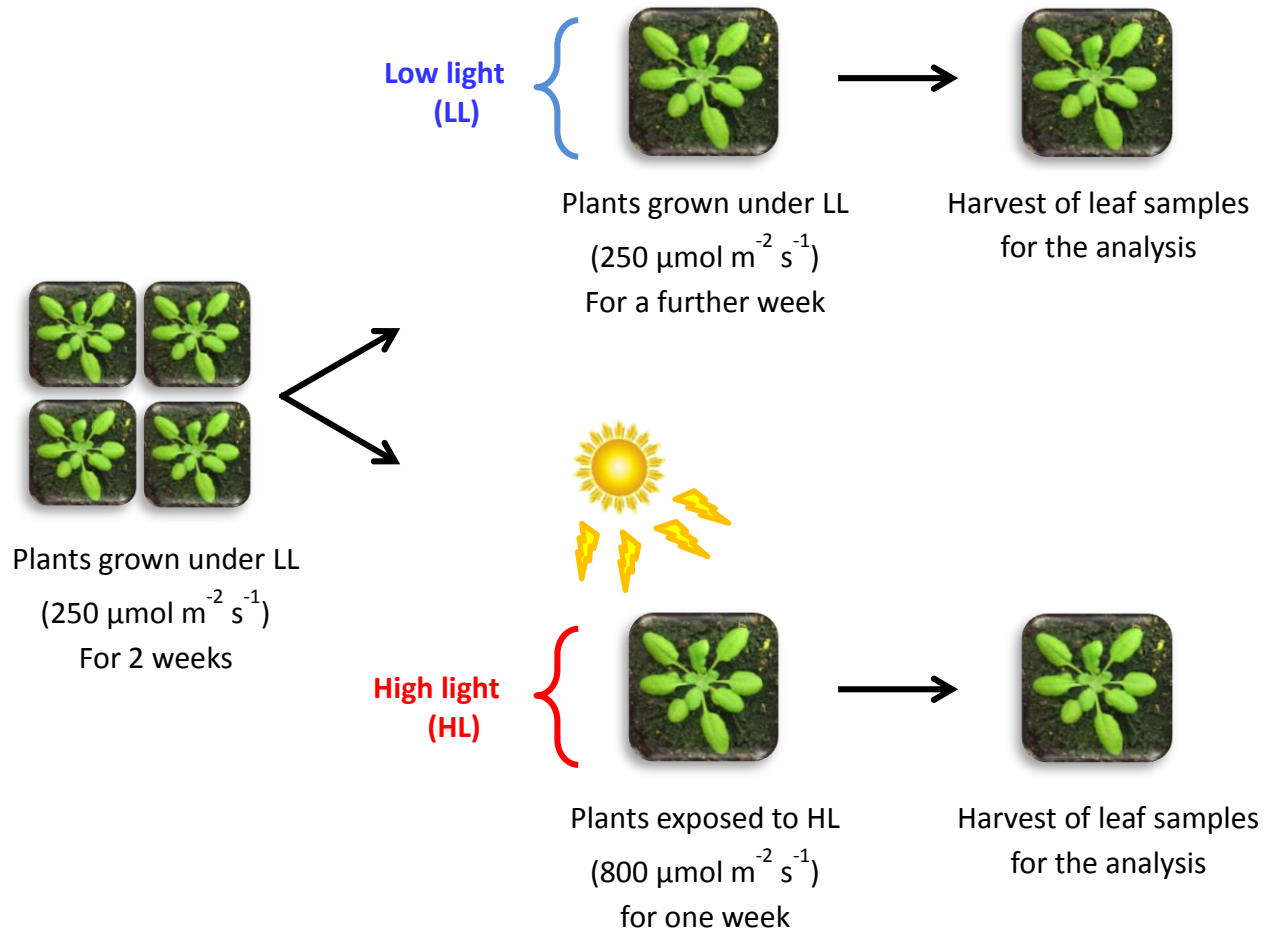


Figure 2.3 Illustration of the high light (HL) pre-treatment in Arabidopsis. Plants were grown for two weeks under low light conditions (LL; 250 µmol m^{-2} s^{-1}). They were then either grown for a further seven days under LL conditions or transferred to high light (HL; 800 µmol m^{-2} s^{-1}) growth conditions for a further seven days before collection of leaf samples for analysis. The high light pre-treatment was provided by a PhytoLux LED Plant Growth Lighting.

2.3 Phenotypic analysis

2.3.1 Leaf area

Leaf area was determined on 4-week-old tobacco plants and on 3-week-old Arabidopsis plants. Each measurement involved 10 plants per genotype per experiment. In all cases, photographs of shoot phenotypes were taken using a Canon EOS 450D digital camera. For scaling, a ruler was placed beside the plants. Images were analysed using Image J programme, version 1.41a.

2.3.2 Number of leaves

The total number of leaves was measured on 4-week-old tobacco plant and in different developmental stages of Arabidopsis plants. Each measurement involved 10 plants per genotype.

2.3.4 Rosette diameter

Rosette diameter in Arabidopsis was determined using a ruler, two measurements were recorded on the same rosette and the mean value was calculated.

2.3.5 Biomass (Fresh and dry weight)

Fresh weight of Arabidopsis plants was measured using microbalance. For dry weight, the rosette was put in oven at 70°C for 3 days to dry out then the dried rosette was weighed. Each measurement involved 10 plants per genotype.

2.3.6 Flowering time

Flowering time was determined depending on the appearance of the first flower bud, which indicates the transition from vegetative growth to reproductive stage.

2.3.7 Seed production

Seed of 10 plants per genotype of Arabidopsis plants was collected and weighed.

2.4 Ascorbate oxidase (AO) assay

The youngest fully expanded leaves were harvested from 4-week-old tobacco plants and the whole rosette of 3-week-old Arabidopsis plants were immediately frozen in liquid nitrogen. Leaf tissue was ground to a fine powder in liquid nitrogen. Extraction buffer (0.1 M sodium phosphate pH 6.5) was added (1 ml per 0.1 g fresh weight) and the mixture was ground again. The extract was centrifuged for 10 min at 15000g and 4°C. The supernatant was discarded and the pellet was resuspended in the extraction buffer (0.1 M sodium phosphate pH 6.5) and (1 M NaCl) was added then vortexed at 4°C for 10 min. The mixture was centrifuged for 10 min at 15000g and 4°C. The supernatant was used to assay AO activity.

The activity of ascorbate oxidase was measured as described by Pignocchi et al., (2003) following the decrease in absorbance at 265 nm (at 25°C) following the addition of 50 µl of extract to a reaction mixture containing 0.1 M sodium phosphate (pH 5.6), 0.5 mM EDTA and 100 µM ascorbate. One unit of ascorbate oxidase activity is defined as the amount of enzyme required to oxidise of 1 µmol ascorbate min^{-1} at 25°C. The extinction coefficient for ascorbate at 265 nm was used in these calculations was 14 mM^{-1} cm^{-1} (Nakano and Asada, 1981).

2.5 Ascorbate (AsA) measurements

2.5.1 Extraction and measurement of whole leaf AsA

Leaves of tobacco or Arabidopsis plants were harvested and frozen in liquid nitrogen. Leaf tissue was ground to a fine powder in liquid nitrogen. Perchloric acid (1M $HClO_4$) was added (1 ml per 0.1 g fresh weight) and the mixture was ground again. The homogenate was centrifuged for 10 min at 15000g and 4°C. Potassium carbonate (5M K_2CO_3) was added to 200 µl of supernatant until pH 5-6. The mixture was again centrifuged at 14000g for 2 min at 4°C. The supernatant was used for AsA assay as described by Foyer et al (1983).

Reduced ascorbate (AsA) was determined by decrease in absorbance at 265 nm (at 25°C) following the addition of 5 µl of ascorbate oxidase (AO) to a reaction mixture containing [20 µl neutralized extract, 100 µl 0.2 M NaH_2PO_4 (pH 5.6), 75 µl H_2O].

Total ascorbate was assayed in the same raction mixture of the reduced ascorbate after reducing dehydroascorbate (DHA) in the neutralized extract into AsA through the reaction mixture containing [100µl neutralized extract, 140 µl 0.12 M NaH_2PO_4 (pH7.5), 10 µl 25 mM DDT (Dithiothreitol)] and Incubated at room temperature for 30 min.

Oxidised ascorbate (DHA) was determined by subtraction of reduced ascorbate from total ascorbate.

2.5.2 Extraction and measurement of apoplastic AsA

The intracellular washing fluid (Apoplastic fluid) was extracted using the method described by Pignocchi et al., (2003). The youngest fully expanded leaves from 4-week-old tobacco plants and the whole rosette of 3-week-old Arabidopsis plants were harvested, weighed and vacuum infiltrated at -70 kPa with ice-cold citrate buffer [10 mM, (pH 3)] for 3 min using a vacuum pump. The infiltrated leaves or rosettes were dried with tissue paper, weighed, rolled and put in pre-cooled 10 ml syringe then put in pre-chilled 15 ml Falcon tube for centrifugation, 5 min at 2000 rpm and 4°C. The amount of extracted fluid was determined in (µl g FW^{-1}) then reduced, total and oxidised ascorbate was measured in the same way of whole leaf AsA measurement (Fig. 2.4). Total and oxidised ascorbate were also analysed after centrifugation of the intracellular washing fluid into a Falcon tube contained chilled 2% metaphosphoric acid (MPA). Extraction of intracellular washing fluid in MPA provided similar results to those obtained with the citric acid extraction procedure.

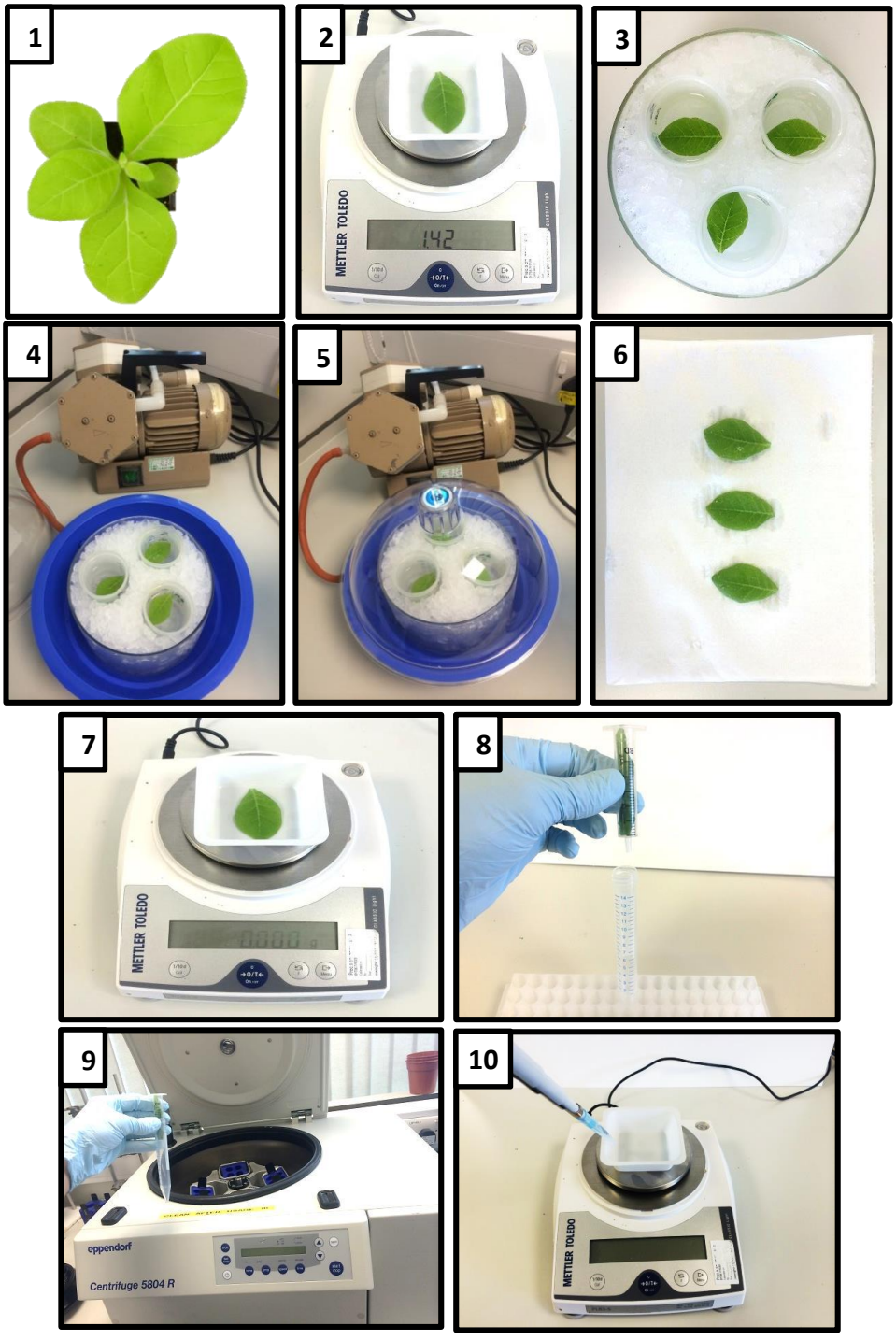


Figure 2.4 Extraction of intracellular washing fluid (Apoplastic fluid).

Fresh leaves were harvested **(1),** weighed **(2),** put in ice-cold extraction buffer **(3),** placed in a vacuum pump container **(4)**, vacuum infiltrated with the extraction buffer **(5).** Infiltrated leaves were dried out with tissue paper **(6)** and weighed again **(7)**. Leaves were then rolled and inserted into pre-cooled 10 ml syringe and put in a pre-chilled 15 ml Falcon tube **(8)** and centrifuged **(9)**. The amount of extracted fluid was determined in (µl g FW^{-1}) **(10)** then measurement of AsA was performed directly after collection of the fluid.

2.6 Pigment analysis

The youngest fully expanded leaves were harvested from 4-week-old plants grown in LL conditions. Leaves were weighed and samples (100 mg fresh weight) were ground in liquid nitrogen. 1 ml of ice-cold 95% ethanol was added to each sample and the mixture was ground again. The extracts were centrifuged for 10 min at 14,000g and 4°C. The supernatants were collected and used for pigment determination. The absorbance values of the supernatant samples were measured at 470, 664 and 649 nm using 95% ethanol as a blank. Values were recorded and used to calculate pigment concentrations using the equations of Lichtenthaler (1986) as follows:

Chlorophyll *a* = 13.36 $A_{664.2}$ - 5.19 $A_{648.6}$

Chlorophyll *b* = 27.43 $A_{648.6}$ - 8.12 $A_{664.2}$

Total Chlorophyll (*a*+*b*) = 5.24 $A_{664.2}$ + 22.24 $A_{648.6}$

Total Carotenoids = (1000 A_{470} - 2.13 C*a* - 97.64 C*b*)/209

2.7 Photosynthetic gas exchange measurements

Photosynthesis was measured in the leaves of tobacco plants and Arabidopsis whole rosettes that had been grown for three weeks under LL conditions (250 µmol m^{-2} s^{-1}) and then for a further 7 days either under LL conditions or under HL [(800 µmol m^{-2} s^{-1}) for Arabidopsis and (1600 µmol m^{-2} s^{-1}) for tobacco].

Photosynthetic CO_2 assimilation rates, transpiration rates, stomatal conductance values and intracellular CO_2 (C_i) concentrations were measured using a portable Infrared Gas Analyser (model LI-6400XT) LI-COR. These measurements were performed at 20°C in the leaf chamber with a light intensity of (250 µmol m^{-2} s^{-1}) photosynthetically active radiation (PAR) and an atmospheric CO_2 concentration of (400 µmol mol^{-1}). In all cases, leaves or rosettes were allowed to acclimatize to the chamber for 15 min prior to measurement to allow stabilization of parameters. Measurements were made on 3 plants per line per experiment.

2.7.1 Light response curves for photosynthesis

Light response curves for photosynthesis were measured on tobacco leaves and Arabidopsis whole rosettes using a portable Infrared Gas Analyser (model LI-6400XT) LI-COR. Plants had been grown for three weeks under LL conditions (250 µmol m^{-2} s^{-1}) and then for a further seven days either under LL conditions or under HL [(800 µmol m^{-2} s^{-1}) for Arabidopsis and (1600 µmol m^{-2} s^{-1}) for tobacco]. Measurements were performed at 20°C and a CO_2 concentration of (400 µmol mol^{-1}) in the leaf chamber. The leaves were exposed to each of the following light intensities: [(0, 20, 50, 200, 400, 800 up to 1600 µmol m^{-2} s^{-1}) photosynthetically active radiation (PAR)] allowing the leaves to acclimatize to each irradiance for at least 15 min prior to measurement to allow stabilization of parameters. Measurements were made on 3 plants per line per experiment.

2.7.2 CO_2 response curves for photosynthesis

The CO_2 response curves for photosynthesis were measured on tobacco leaves and Arabidopsis whole rosettes using a portable Infrared Gas Analyser (model LI-6400XT) LI-COR. Plants had been grown for three weeks under LL conditions (250 µmol m^{-2} s^{-1}) and then for a further seven days either under LL conditions or under HL [(800 µmol m^{-2} s^{-1}) for Arabidopsis and (1600 µmol m^{-2} s^{-1}) for tobacco plants]. Measurements were performed at 20°C, (250 µmol m^{-2} s-1) photosynthetically active radiation (PAR) and the photosynthetic values were taken at different CO_2 concentrations: (0, 200, 400, 600, 800 up to 1000 µmol mol^{-1}) allowing the leaves to acclimatize to each CO_2 concentrations for at least 15 min prior to measurement to allow stabilization of parameters. Measurements were made on 3 plants per line per experiment.

2.8 Measurement of chlorophyll *a* fluorescence quenching parameters

The ratio of dark adapted variable chlorophyll *a* fluorescence (Fv) to the maximal value of chlorophyll a fluorescence (Fm) in the dark adapted state was measured in the leaves of 4-week-old plants following the transfer from LL growth conditions to HL conditions using a Fluorometer (FP 100-SN-FP-680, Drasov, Czech Republic, http://www.psi.cz).

2.9 Aphid infestation and culture conditions

Populations of green peach aphids (*Myzus persicae* Sulzer) that had originally been collected in Scotland in the years 2002-2004 and propagated at the James Hutton Institute, Invergowrie, UK were obtained from Dr. Robert Hancock. Aphid stocks were maintained on mature potato plants in transparent cages in an insectary under controlled environment conditions (8h photoperiod and day/night temperatures of 20°C).

2.9.1 Aphid fecundity

2.9.1.1 Aphid fecundity in Arabidopsis plants

Arabidopsis plants had either been grown for two weeks under LL (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL conditions or transferred to HL (800 µmol m^{-2} s^{-1}) for seven days. Aphid fecundity was determined by the method of Fenton et al. (2010). A single one-day-old nymph was placed in the centre of a rosette and enclosed in transparent container (10 cm internal diameter and 15 cm height) capped with a thin mesh (mesh size 200 µm). The required light intensities were obtained under the mesh. Plants with containers were then returned to the LL controlled environment chamber conditions. After 15 days the total number of offspring was counted (Fig. 2.5). Each fecundity experiment involved 10 plants per genotype per experiment and repeated 3 times.

2.9.1.2 Aphid fecundity in tobacco plants

Aphid fecundity in tobacco plants that had received a HL pre-treatment

Tobacco plants had either been grown for three weeks under LL (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL conditions or transferred to different high light intensities (800, 1000 or 1600 µmol m^{-2} s^{-1}) for seven days. Aphid fecundity was determined by the method of Fenton et al. (2010). A single one-day-old nymph was placed in the centre of a rosette and enclosed in transparent container (10 cm internal diameter and 15 cm height) capped with a thin mesh (mesh size 200 µm). The required light intensities were obtained under the mesh. Plants with containers were then returned to the LL controlled environment chamber conditions. After 15 days the total number of offspring was counted (Fig. 2.6). Each fecundity experiment involved 10 plants per genotype per experiment and repeated 3 times.

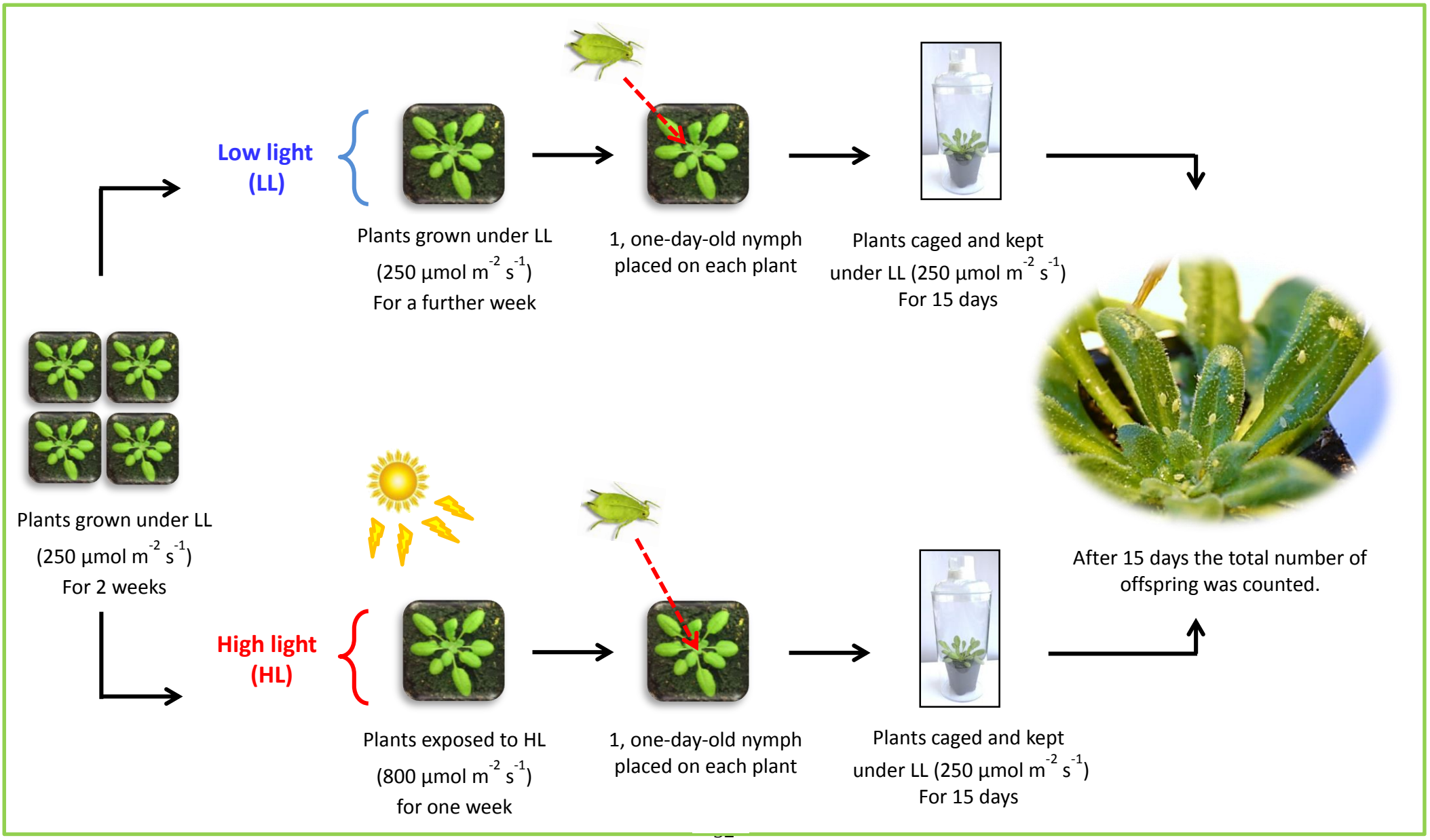


Figure 2.5 Experimental design of aphid fecundity measurements on Arabidopsis plants.

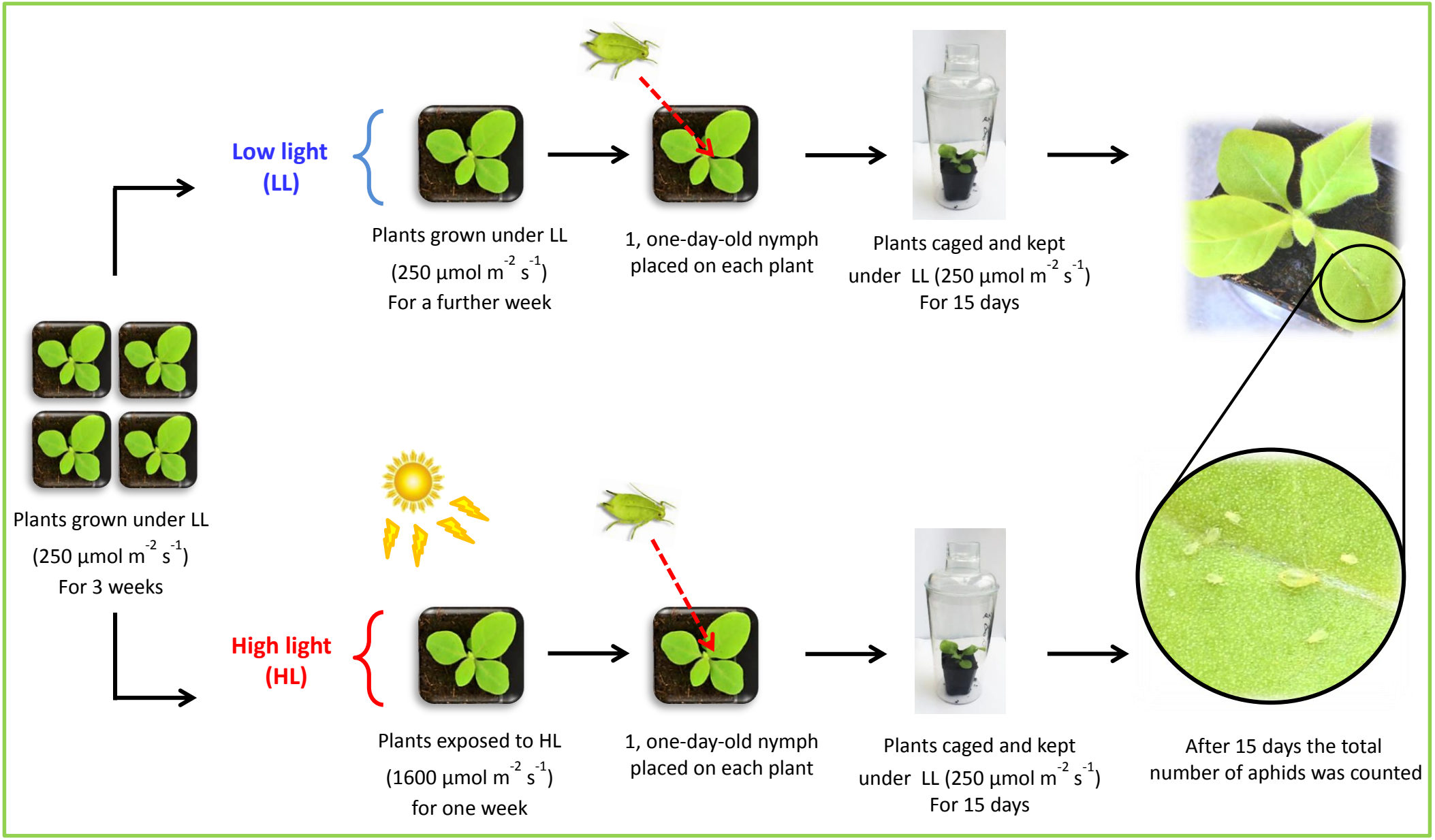


Figure 2.6 Experimental design of aphid fecundity measurements on tobacco plants.

Aphid fecundity in tobacco plants grown under LL or HL at the time of infestation

In the experiments above, the aphid fecundity analyses has been performed on plants that had received a HL pre-treatment. Aphid fecundity was measured after plants had been returned to the LL growth conditions. Aphid numbers were counted for 15 days after the return to LL growth conditions.

A further set of experiments were performed in which aphid fecundity was measured in tobacco plants that grown under LL (250 μmol m^{-2} s^{-1}) for 4 weeks. A single one-day-old nymph was placed in the centre of each rosette and enclosed in a transparent container (10 cm internal diameter and 15 cm height) capped with a thin mesh (mesh size 200 μm). Plants were then exposed to either LL or HL (1000 μmol m^{-2} s^{-1}) for 15 days. The total number of offspring was then counted (Fig. 2.7). Each fecundity experiment involved 10 plants per genotype per experiment and repeated 3 times. The LED light that used for HL treatment was provided by a PhytoLux LED Plant Growth Lighting.

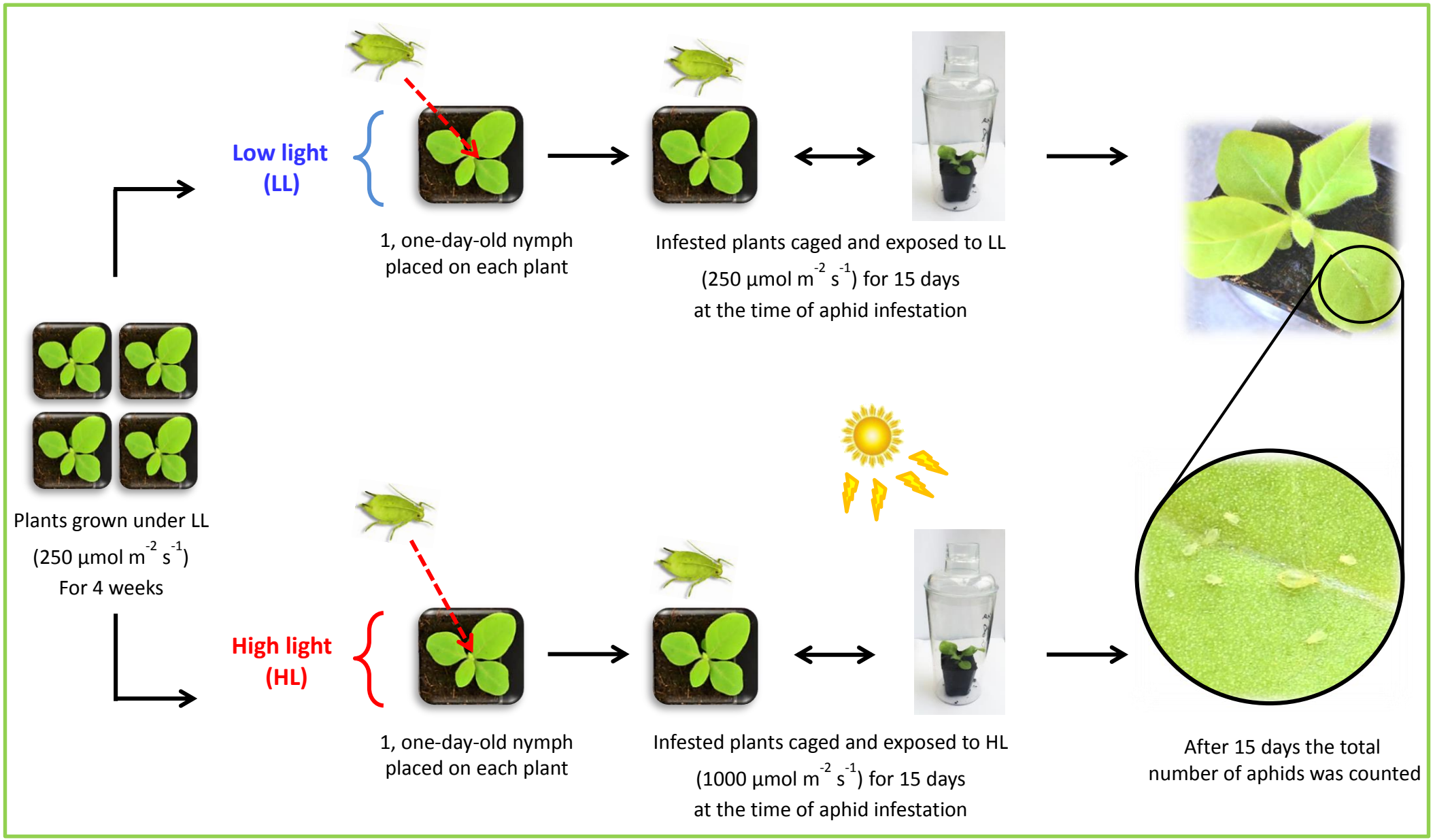


Figure 2.7 Experimental design for the comparison of aphid fecundity on tobacco plants grown under either LL or HL.

2.10 Collection of leaf samples for transcriptome profiling analysis in Arabidopsis

The following experiments were performed to assess the effects of light intensity and aphid infestation on the gene expression patterns in Arabidopsis rosettes. Leaf transcriptome profiling analysis was performed on fully expanded leaves of 4-week-old rosettes under LL or after a HL pre-treatment, in the absence or presence of aphids.

Arabidopsis plants had either been grown for three weeks under LL (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL conditions or transferred to HL (800 µmol m^{-2} s^{-1}) for seven days. Sixty adult wingless aphids were transferred to the leaf surface of LL and HL pre-treated plants with a small paint brush and enclosed in a mesh (mesh size 200 µm) covered clip cage (2.5 cm diameter) for 6h as illustrated in Figure (2.8). Cages without aphid were used as controls for LL and the HL-pre-treatment. Plants were kept under LL condition during infestation. Infested and non-infested leaves were harvested 6h following the onset of aphid infestation for the analysis of gene expression. Leaf samples were immediately frozen in liquid nitrogen and kept in -80˚C until analysis.

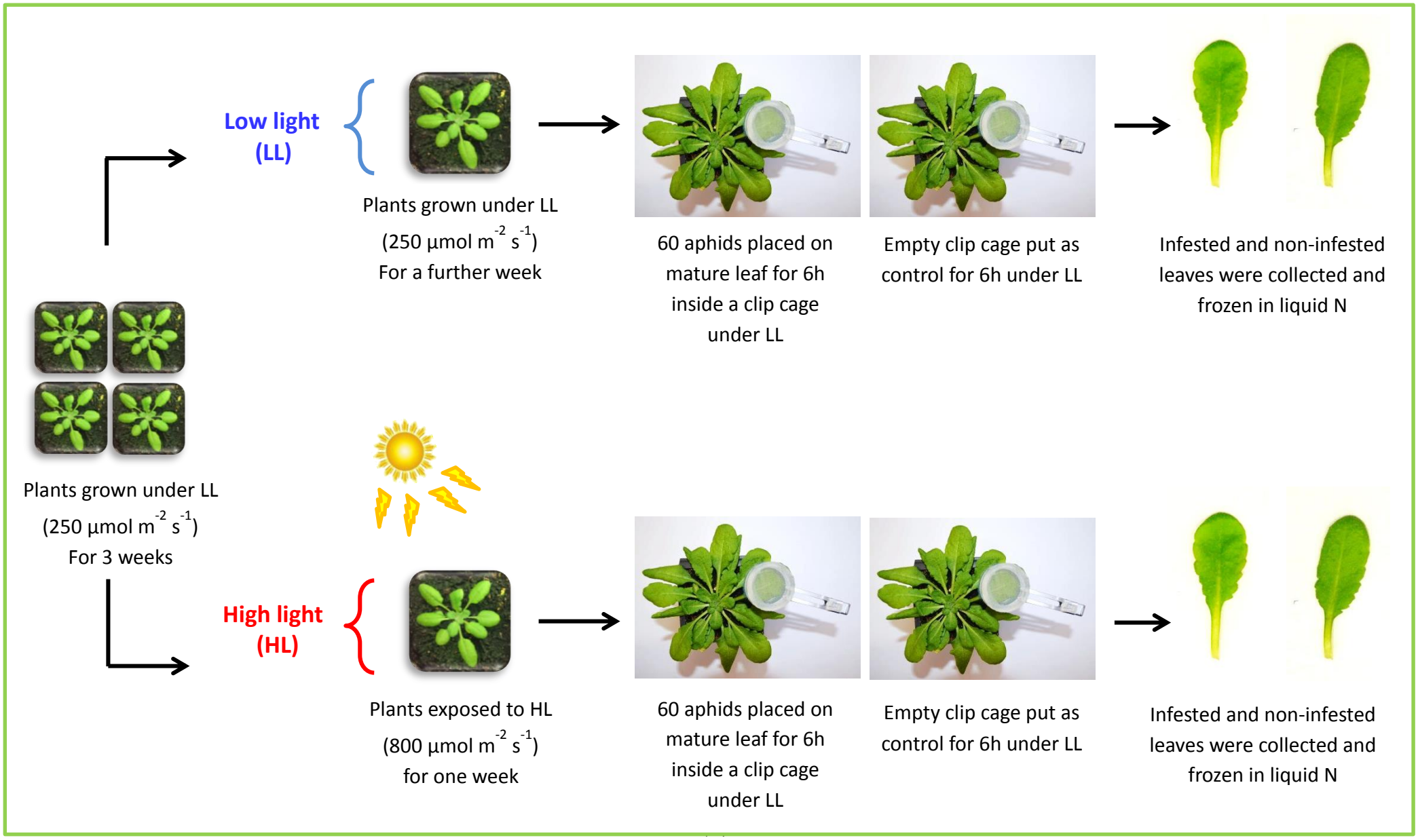


Figure 2.8 Experimental design for harvest of material for qPCR analysis. Leaves were either subjected to aphid infestation for 6h, or maintained in the absence of aphids (empty clip cages).

2.11 Microarray and metabolite processing and analysis

2.11.1 Collection of leaf samples for microarray and metabolite analysis in tobacco

The microarray processing procedures were carried out by Jenny Morris, data extraction, quality control analysis and initial statistical analysis were carried out by Pete Hedley at the James Hutton Institute, Dundee.

Four-week-old tobacco plants were used for the analysis of transcriptome and metabolite profiles under LL (250 μmol m^{-2} s^{-1}) and HL (1600 μmol m^{-2} s^{-1}) in the absence or presence of aphids.

Tobacco plants were grown for three weeks under LL and then either maintained for a further seven days under LL growth conditions or transferred to HL for seven days. Leaf samples were harvested from LL and HL-grown plants and frozen in liquid nitrogen until analysis (Fig. 2.9). Plants that had been pre-treated with HL were then transferred back to LL growth conditions. Sixty adult wingless aphids were transferred to upper surface of the youngest mature leaves of LL and HL pre-treated plants with a small paint brush and enclosed in a mesh (mesh size 200 μm) covered clip cage (2.5 cm diameter) for 12h. Plants with cages without aphids were used as controls for these experiments. Plants were then maintained under LL for the period of aphid infestation. The infested and non-infested leaves were collected 12h following the onset of aphid infestation and frozen in liquid nitrogen until the analysis (Fig. 2.9). In addition, other samples of LL and HL pre-treated leaves were harvested again at the 12h time point in order to determine the effects of the transition (12h) from HL to LL on the abundance and composition of metabolites.

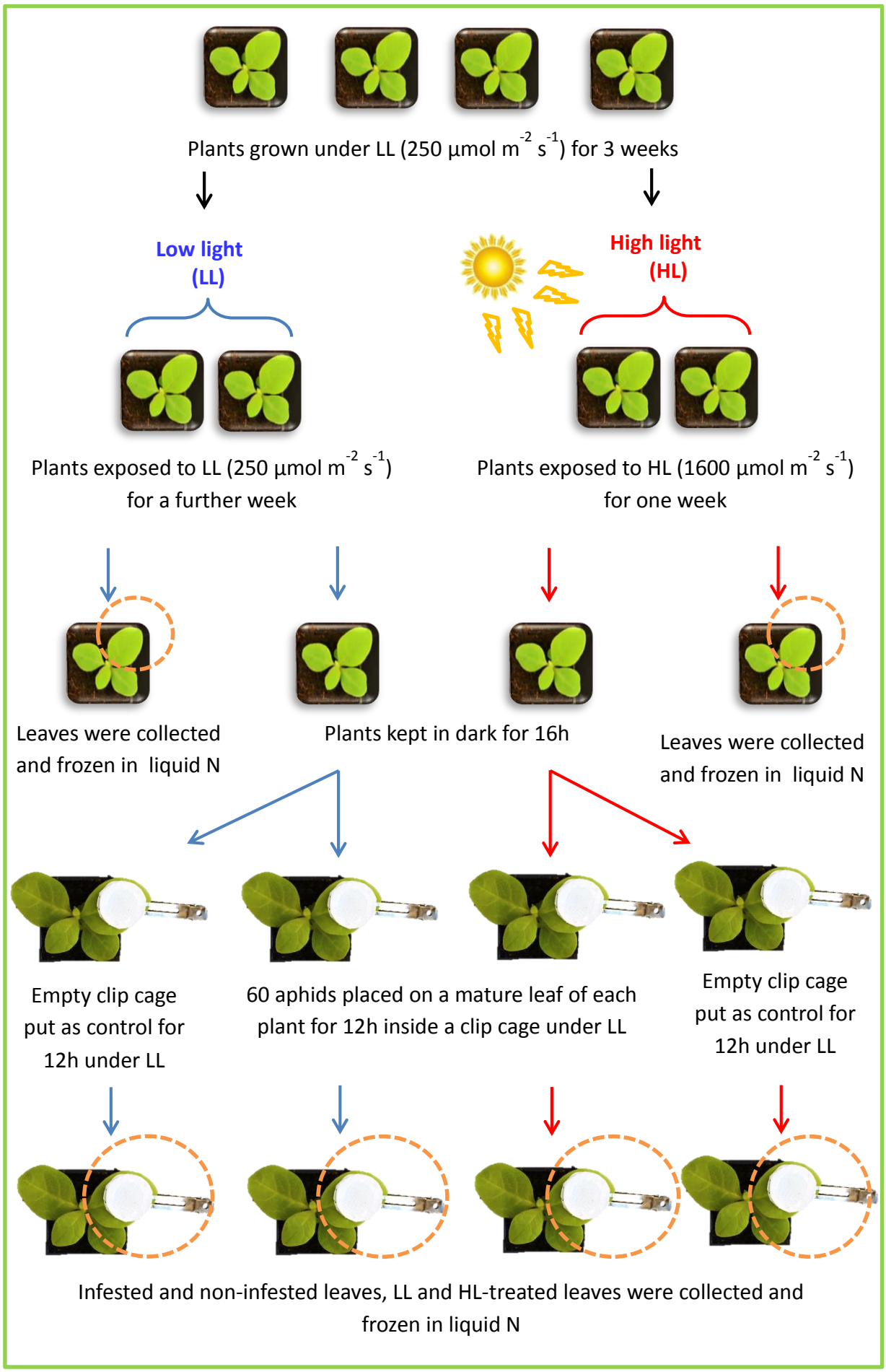


Figure 2.9 Experimental design for microarray and metabolite profiling analysis of tobacco plants under LL and after a HL pre-treatment in the absence or presence of aphids.

2.11.2 Microarray analysis

Agilent tobacco 44K microarray

Tobacco microarray design ID 021113 (Agilent Technologies) in 4x 44k format was used in the experiment. This is a catalogue gene expression microarray designed by Agilent to represent all publicly available expressed sequence tags (ESTs) at the time of design from UniGene (Build 11), TIGR (Release 3) and IGR Plant Transcript Assemblies (Release 5) databases. In total 43,803 transcripts are represented as 60mer probes on the array.

The Qiagen® RNeasy Plant Mini Kit was used according to the manufacturer's protocol for total RNA extraction from the harvested leaf materials described in section 2.11.1. Three biological replicates were used per treatment. One-Color Microarray-Based Gene Expression Analysis protocol (v. 6.5; Agilent Technologies) was used for microarray processing. Briefly, cRNA was synthesized from cDNA which was then linearly amplified and labelled with Cy3 prior to purification (Fig. 2.10). Labelled cRNA quality was evaluated using spectrophotometry. Throughout the experiment, tobacco microarray design ID 021113 (Agilent Technologies) was used. Labelled samples were hybridized to the microarrays overnight at 65ºC. Next day, the hybridized microarrays were disassembled, washed once for 1 min with GE Wash 1 buffer (Agilent Technologies) at room temperature and once for 1 min with GE Wash Buffer 2 (Agilent Technologies) at 37ºC, and then dried by centrifugation. The hybridized slides were scanned using the Agilent G2505B scanner at resolution of 5 μm at 532 nm (Fig. 2.10).

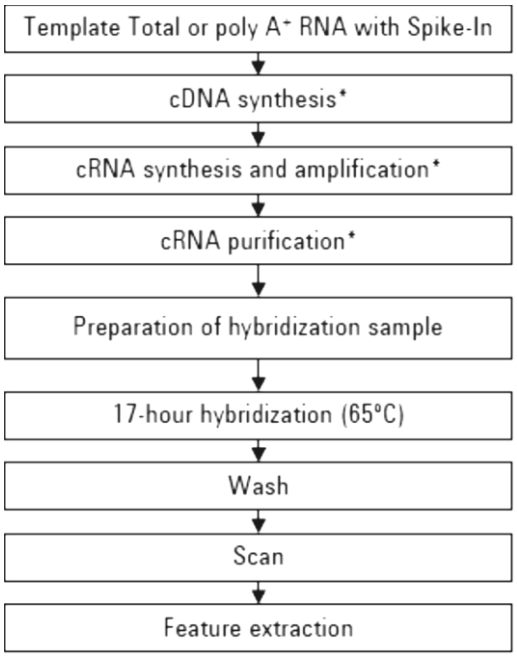


Figure 2.10 Preparation of samples for microarray processing

Feature Extraction (FE) software (v. 10.7.3.1; Agilent Technologies) with default settings was used for data extraction from the image files. Subsequent data quality control, pre-processing and analyses were performed using GeneSpring GX (v. 7.3; Agilent Technologies) software. Agilent FE one-colour settings in GeneSpring were used to normalise data and a filter used to remove inconsistent probe data flagged as absent in more than one replicate per sample. Statistically significant differentially expressed genes between LL and HL or infested and non-infested leaves were identified by using pairwise Student's *t*-test with a p-value of <0.05 and Bonferroni multiple-testing correction applied to adjust the false-discovery rate.

2.12 Metabolite analysis

The harvested leaf materials described in section 2.11.1 were weighed and freeze-dried for 48h. Three biological replicates were used per treatment. Gamma 1-16 LSC freeze drier (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) was used to lyophilise the samples at pressure of 0.7 mbar with a shelf temperature of 25°C and a condenser temperature of -50°C. Sequential extraction with methanol, chloroform and water in the presence of internal standards of ribitol and nonadecanoic acid methyl ester was used for extraction and derivatization of polar and non-polar metabolites from the freeze-dried samples. After phase separation of the extraction medium Polar (upper layer) and non-polar (lower layer) were transferred to amber vials using Pasteur pipette.

Extraction of polar and non-polar fractions

Accurately 100 mg of freeze-dried tobacco leaves were weighed out in to a culture tube (150 x 16 mm). To each tube 3 ml methanol was added and shaken at 1500 revolutions min^{-1} at 30°C for 30 min using a vortex-type shaker. 100 µl of polar (ribitol) and non-polar (nonadecanoic acid methyl ester) internal standards were added to samples with 0.75 ml water and the sample were subjected to shake for a further 30 min at 1500 revolutions min^{-1} at 30°C. The mixtures were shaken for 30 min at 2500 revolutions min^{-1} at 30°C after adding 6 ml chloroform. Finally, 1.5 ml water was added to the mixture, vigorously shaken by hand and the the polar and non-polar phases were separated by centrifugation at 1000 g for 10 min. Upper layer (Polar) and lower layer (non-polar) were transferred to amber vials using Pasteur pipette and kept in -20°C until next day.

Derivatization of polar fraction

The polar extracts were removed from freezer and warmed up to room temperature then 250 µl were pipetted into culture tubes and taken to dryness in a centrifugal evaporator. 80 µl of methoxylamine hydrochloride (20 mg methoxylamine hydrochloride/ml anhydrous pyridine) were added to to the dried fraction to oximate the carbonyl functional groups for 4h at 50°C. Simultaneously, 50 µl of a retention standard mixture (undecane, tridecane, hexadecane, eicosane, tetracosane, triacontane, tetratriacontane, and octatriacontane) which dissolved in isohexane (0.2mg/ml) were added to amber autosampler vials (300 µl fixed glass inserts with PTFE coated snap caps) and allowed to evaporate at the room temperature. After oximation, 80 µl of N-methyl, N-trimethylsilyl trifluroacetamide (MSTFA) were added to samples and incubated for 30 min at 37°C. Finally, 40 µl of the derivatized polar fractions and 40 µl of dry pyridine were added to the amber autosampler vials that contained the dried retention standards. The polar fraction was then ready for GC-MS analysis.

Derivatization of non-polar fraction

Non-polar fraction was taken to dryness in a centrifugal evaporator for 30 min, and then 1 ml chloroform with 2 ml 1% methanolic sulphuric acid was added. The mixture was incubated at 50°C for 16h then cooled down to room temperature. 3 ml chloroform and 5 ml of 5% (w/v) aqueous sodium chloride were added with vigorous shaking to allow the polar and non-polar layers to settle. The upper aqueous layer was discarded and 3 ml of 2% (w/v) aqueous potassium hydrogen carbonate was added to the lower layer then vigorously shaken. After settling, again the upper layer was discarded and the chloroform:methanol layer (lower layer) was pipetted through columns contained anhydrous sodium sulphate (3 cm columns prepared in cotton wool plugged Pasteur pipettes, and prewashed with 4 ml chloroform) in order to remove all residual water. The mixture was transferred to culture tube after washing the columns for a further time with 2 ml chloroform. The collected fraction was taken to dryness in a centrifugal evaporator for 60 min. Next, 10 µl anhydrous pyridine, 50 µl chloroform and 40 µl MSTFA were added to the dried sample and incubated at 37°C for 30 min.

Then, 40 µl of the derivatized non-polar fraction were transferred to autosampler vials which contained 40 µl anhydrous pyridine that previously been prepared with retention standards. The non-polar fraction was then ready for GC-MS analysis.

Sample analysis

DSQ II Single Quadrupole GC-MS system (Thermo) was used to analyse the samples. In a split ratio of 40:1, 1 µl of the sample was injected into a vaporising injector. The injection temperature was 132°C for 1 min, transfer rate was 14.5°C /s, transfer temperature was 320°C for 1 min, clean rate was 14.5°C /s and clean temperature was 400°C for 2 min. Analytes were chromatographed on a DB5-MSTM column (15 m x 0.25 mm x 0.25 µm; J&W, Folsom, USA) using helium at 1.5 ml/min in constant flow mode as mobile phase. The temperature gradient was 100°C for 2.1 min, 25°C /min to 320°C and isothermal for 3.5 min. The interface temperature was 250°C. Mass data were acquired at 70 eV electron impact ionization conditions over a 35 – 900 a.m.u mass range at 6 scans per sec with a source temperature 200°C and a solvent delay of 1.3 min. Acquisition rates were set to give approximately ten data points across a chromatographic peak. XcaliburTM v1.4 and XcaliburTM v2.0.7 software packages were used to acquire and analyze the data, respectively. A processing method developed at James Hutton Institute was used to assign identities to the peaks. It uses the retention times and masses of known standards and the Genesis algorithm (part of the XcaliburTM package) for peak integration.

The expected retention time for each peak was adjusted using the retention times of the retention standards (Appendix III, IV). The integrated area of the annotated peaks was normalized against the integrated area of the respective internal standards, ribitol and nonadecanoic acid for the polar and non-polar fractions, respectively. The peak area ratios were normalized on a dry weight basis.

Statistical analysis for metabolite data was performed with 2-way Analysis Of VAriance (ANOVA) with a p-value of <0.05.

2.13 Extraction of RNA and synthesis of cDNA

RNA for quantitative reverse transcriptase real time PCR (qRT-PCR) of tobacco leaf materials that were harvested and described in section (2.11.1), and for Arabidopsis leaf materials that were harvested and described in section (2.10), was extracted using Qiagen® RNeasy Plant Mini Kit according to the manufacturer's protocol.

The RNase-Free DNase Set (Qiagen) was used for DNA digestion during RNA isolation according to the manufacturer's protocol. The isolated RNA was quantified with Nanodrop ND-1000 (Thermo).

The QuantiTect Reverse Transcription Kit (Qiagen) was used for reverse transcription of 1 µg RNA following the manufacturer's protocol.

2.14 Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

The QuantiFast SYBR® Green PCR Kit (Qiagen) was used according to the manufacturer's protocol on a C1000™ Thermal Cycler (BIO-RAD) real-time PCR system.

A total 20 µl PCR reaction mixture consisted of: 3 µl cDNA (30 ng), 10 µl SYBR Green, 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM) and 5 µl H_2O. Three biological replicates of each sample were used in all experiments. The 96 well plates were used with three technical replicates for each sample.

The thermal Real-time cycler conditions were programmed as follows:

(1) Initial denaturation at 95°C for 5 min; (2) 40 cycles of denaturation and amplification comprised of 95°C for 10 s, 60°C for 30 s and (3) 72°C for 30 s.

2.14.1 Primer design

Primers for *Arabidopsis thaliana* genes were designed using sequence viewer at TAIR (http://www.arabidopsis.org). To design the primers based on the sequence of target genes the SDSC Biology WorkBench software (http://workbench.sdsc.edu/) was used.

Two housekeeping genes were used to normalize the expression level of target genes in Arabidopsis experiments (according to Czechowski et al., 2005): At3g18780 (*Actin-2*) and At1g13320 (*PDF2*). Arabidopsis genes and their primer sequences are listed in Table (2.1).

The microarray results of tobacco experiments were confirmed by qRT-PCR. Eight transcripts were selected for this analysis based on their transcriptional patterns. Two housekeeping genes were used to normalize the expression level of target genes in tobacco experiments according to (Levy et al., 2004; Pignocchi et al., 2006; Porta et al., 2011): AJ421413 ($\alpha-$ *tubulin A3*) and AJ236016 (*18S rRNA*). Tobacco genes and their primer sequences are listed in Table (2.2).

Table 2.1 List of primer sequences used for qRT-PCR in Arabidopsis. Primers were designed using sequence viewer at TAIR (http://www.arabidopsis.org) through the SDSC Biology WorkBench software (http://workbench.sdsc.edu/).

Primers were designed for two housekeeping genes (At3g18780 and At1g13320) and eight interest genes. (Fwd) forward and (Rev) reverse are directions of the primers.

Primer	Sequence 5'- 3'		Accession
ACTIN2	Fwd	GGCTCCTCTTAACCCAAAGG	At3g18780
	Rev	GAGAGAACAGCTTGGATGGC	
PDF2	Fwd	TAACGTGGCCAAAATGATGC	At1g13320
	Rev	GTTCTCCACAACCGCTTGGT	
VTC5	Fwd	AATGTGAGTCCGATTGAGTATGG	At5g55120
	Rev	TAAGCCTGAAAGTGAAGATGG	
RRTF1	Fwd	GGGCTAAACTCAACTTCCCC	At4g34410
	Rev	ATATTGCAATCCCCTCCTCC	
BAP2	Fwd	ATCAAATGTGGAGACCGAGG	At2g45760
	Rev	TGATACGCACACCAAACAGG	
WRKY62	Fwd	GTTTCTCAGATGCGCTCTCC	At5g01900
	Rev	GTGAAGTGGTTTCCTGGAGC	
NIM1	Fwd	GTGTCGTACGGGTTTGAAGC	At1g02450
	Rev	AAAGCCTTGTCTTCGTTTCG	
AOC3	Fwd	GACCGAAAACTCCAGACCAA	At3g25780
	Rev	TTTGTTTGTGAATGGGACGA	
AZF1	Fwd	CAGCAACAGCGTAGAACTCG	At5g67450
	Rev	CACTGAGACTTGATCAGCCG	
MAPKKK21	Fwd	ATTGGTTCGTATTGGTTCCG	At4g36950
	Rev	GATCCAAAAGCATCTCAGCC	

Table 2.2 List of primer sequences used for qRT-PCR in Tobacco. Primers were designed using the NCBI Primer-Blast tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Sol genomic network (http://solgenomics.net/) through the SDSC Biology WorkBench software (http://workbench.sdsc.edu/). Tobacco genes and their primer sequences are listed in Table (2.2). Primers were designed for two housekeeping genes (AJ421413 and AJ236016) and eight interest genes. (Fwd) forward and (Rev) reverse are directions of the primers.

Primer	Sequence 5'- 3'		Accession
α-tubulin A3	Fwd	TCCTCATATGCTCCTGTC	AJ421413
	Rev	AGCAGACAAGCATTCTAC	
18S rRNA	Fwd	GACGAACAACTGCGAAAG	AJ236016
	Rev	CATCTAAGGGCATCACAG	
Chlorophyll a-b binding protein 1	Fwd	GTCCATTGCGAGAACCCTTG	CV017417
	Rev	TCCCAACCAATTTACACCACA	
Disease resistance protein	Fwd	AAGGTTGTGGCAATGACAGG	EH617861
	Rev	CTTGCGCCTTCTTATGGTGA	
PDR-type ABC transporter 1	Fwd	AGGACCATGGGAGTTGCTAG	EH622485
	Rev	TCAGTTCCACCGGCTACAAT	
Thionin-like protein (D6)	Fwd	TCATGGCAGTGAAAGGTTTAGC	CV019616
	Rev	ACCTAGCTAAGCATACAGGCC	
Epi-arisotolchene synthase 110	Fwd	AGGCAGCATGGAAGGATCTT	EH623458
	Rev	GGAGTCCACAAGTAGGGCAT	
SAR8.2c protein	Fwd	GCCGGCAAAGCTTGTAAAAT	EH622851
	Rev	TGACCCAAAGACCTGTTCCA	
Stress. abiotic. heat	Fwd	CCGAAAGAGAACGCCAACAA	AY329066
	Rev	AGCACCACCTTCGTCCATAA	
Putative	Fwd	AGCTCACTCATGTAGCCATCT	FG634212
	Rev	TAGCAATGACCAAGCTGACG	

2.15 Statistical analysis

Statistical analysis was performed by One-way Analysis Of Variance (ANOVA) using IBM SPSS Statistics-version 20. Data represent the mean ± standard error of the mean (SEM).

Chapter 3. Role of ascorbate in plant responses to aphid attack

3.1 Introduction

Ascorbic acid vitamin C (L-ascorbic acid; AsA) is the most abundant low molecular weight antioxidant in plants. Together with the low molecular weight thiol and glutathione, ascorbate plays a central role in H_2O_2 detoxification (Zheng and Vanhuystee, 1992; Noctor and Foyer, 1998). The first step of the ascorbate-glutathione cycle is the oxidation of ascorbate by H_2O_2, which catalysed by the enzyme ascorbate peroxidase. The ascorbate peroxidase reaction produces monodehydroascorbate (MDHA), which can then either be reduced by MDHA reductase, or be further oxidised to dehydroascorbate (DHA). DHA is then reduced to ascorbate by the enzyme DHA reductase (DHAR).

In addition to its antioxidant role, ascorbate is also an important co-factor for many enzymes and it is essential for plant growth and development, as was demonstrated by knockout mutants defective in GDP- L-galactose phosphorylase and hence ascorbate synthesis, which were embryo lethal (Dowdle et al., 2007). GDP- L-galactose phosphorylase, which is required to convert (GDP- L-galactose) to (L-galactose-1-P) in the ascorbate biosynthesis pathway, is encoded by two genes; *VITAMIN C DEFECTIVE 2* (*VTC2*) and *VITAMIN C DEFECTIVE 5* (*VTC5*; Dowdle et al., 2007). Mutants that have less severe mutations in enzymes of the ascorbate synthesis pathway have been useful in characterizing ascorbate functions. For example, the *A. thaliana* vitamin C-defective *vtc2-1* (EMS) mutants, *vtc1 and vtc2,* which have only about 30% of the wild type ascorbate levels in their leaves have a slow growth phenotype and they show hypersensitivity to abiotic stresses such as ozone and UV-B radiation (Conklin et al., 1999; Pastori et al., 2003). These mutants show constitutive up-regulation of SA-mediated resistance to biotrophic pathogens that is linked to higher levels of ABA and SA (Pastori et al., 2003; Kerchev et al., 2013). In addition, while the *vtc1* (EMS) mutants showed a similarly level of aphid sensitivity to the wild type plants, the *vtc2-1* (EMS) mutants were more resistant to aphid infestation (Kerchev et al., 2011; 2013). Moreover, the higher aphid resistance observed in the *vtc2-1* (EMS) mutants was dependent on ABSCISIC ACID INSENSITIVE-4 (ABI4) transcription factor (Kerchev et al., 2013). The *abi4vtc2* double mutants had a similar level of sensitivity of aphids as the wild type, showing that low ascorbate levels activate ABA-dependent signaling pathways that exert an influence over aphid resistance (Kerchev et al., 2011; 2013).

The *vtc2-1* (EMS) mutation was first isolated in a screen for ozone-sensitive mutants (Conklin et al., 1996). An additional three alleles (*vtc2-2*, *vtc2-3*, and *vtc2-4*) were then isolated based on this phenotype. The first-pass map position (between CAPS markers WU95 (74 cM) and PRHA (78 cM) on chromosome 4) for the *vtc2* mutation was reported by Conklin et al., (2000). Sequencing comparisons of the *vtc2-1* (EMS) line with the wild type Col-0 sequence identified a mis-sense change, resulting in a Gly to Asp change in the predicted exon 5 (Jander et al., 2002).

Recently, an ascorbate-defective T-DNA insertion mutant in *VTC2*, which encodes GDP- L-galactose phosphorylase, was identified. The T-DNA insertion mutant is insertion of 620 bp downstream from start codon in the coding sequence (TAIR database).

Seeds of the *vtc2* (T-DNA) line were obtained and used in the following studies, which was designed to compare the effects of low ascorbate on aphid infestation in the *vtc2-1* (EMS) and the *vtc2* (T-DNA) lines. Furthermore, since the phenotype of the *vtc2* (T-DNA) line has not been characterised, studies on the growth and gene expression patterns in this line were performed relative to the wild type to determine whether the *vtc2-1* (EMS) and the *vtc2* (T-DNA) lines have similar characteristics, particularly in relation to shoot growth and development.

3.2 Results

3.2.1 Shoot phenotypes of Arabidopsis *vitamin C defective 2* mutant lines

Phenotypic comparisons of the wild type, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutant lines grown under a short day (8h) photoperiod (250 µmol m^{-2} s^{-2}) regime were made over a nine week period. At most stages of vegetative development the *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants had a similar shoot phenotype, the rosettes being visibly smaller than the wild type. While the 9-week-old *vtc2* (T-DNA) mutants showed a bigger rosette than *vtc2-1* (EMS) mutants (Fig. 3.1), the *vtc2* (T-DNA) mutants were still smaller than the wild type (Figs. 3.1).

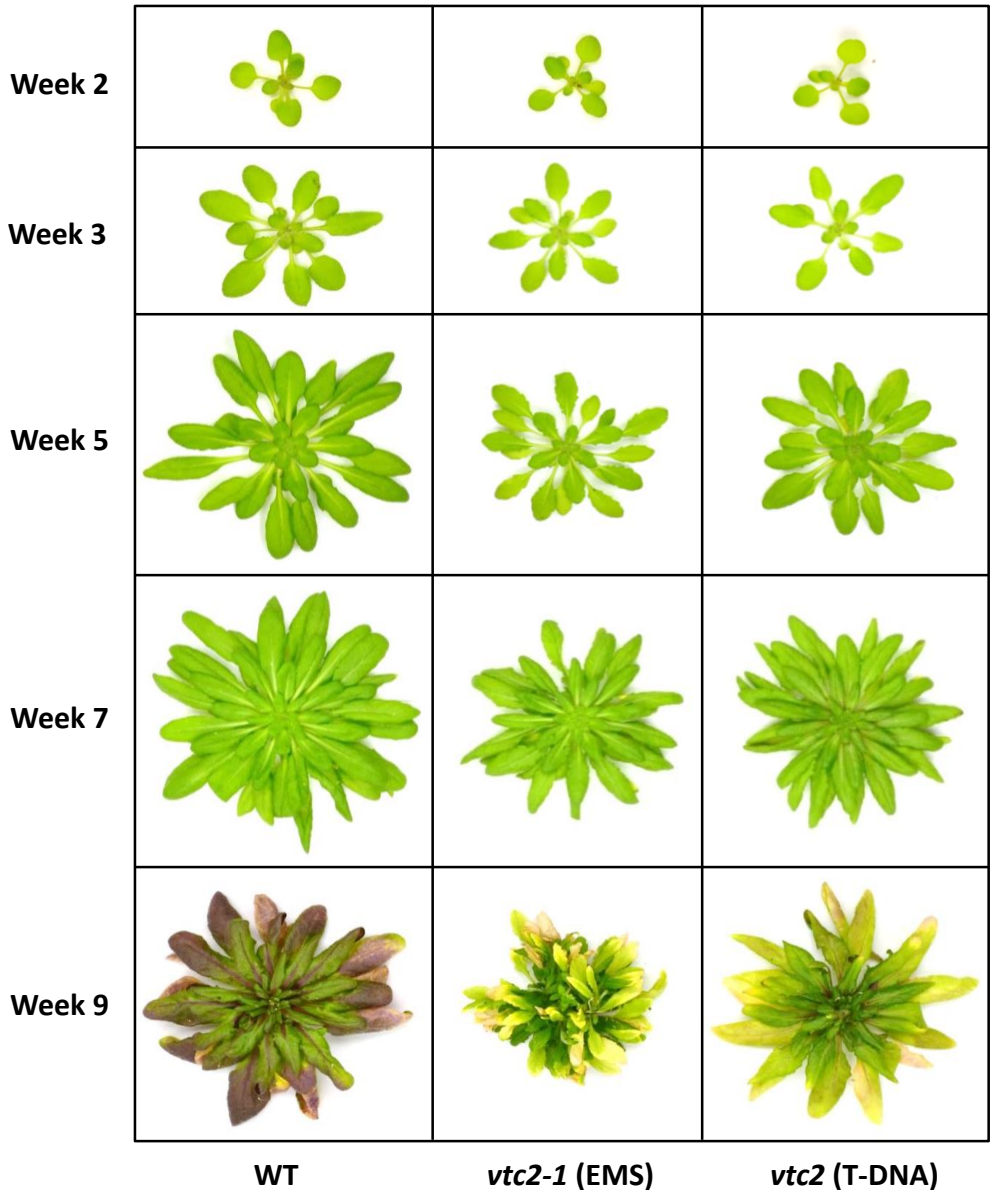


Figure 3.1 A comparison of rosette growth phenotypes in wild type (WT) Arabidopsis and *vitamin C defective 2* mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA) at 2-, 3-, 5-, 7-, and 9-week-old plants.

Leaf area, rosette diameter and number of leaves measurements were performed at different stages of vegetative development in wild type and *vtc2* mutant lines. Apart from the earliest stages of rosette growth (week-2), the *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants had a significantly smaller leaf area than the wild type (Fig. 3.2A). Even in 9-week-old plants. The *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants had a smaller leaf area than the wild type (Fig. 3.2A). Similarly, except of week-2 where the rosette size was similar in all genotypes, the diameter of the *vtc2-1* (EMS) and *vtc2* (T-DNA) rosettes was significantly smaller than that of wild type plants throughout development (Fig 3.2B). Regardless of the differences in leaf area (Fig. 3.2A) and rosette diameter (Fig. 3.2B) between wild type and ascorbate-defective *vtc2* mutant lines, all the genotypes had the same number of leaves (Fig. 3.2C).

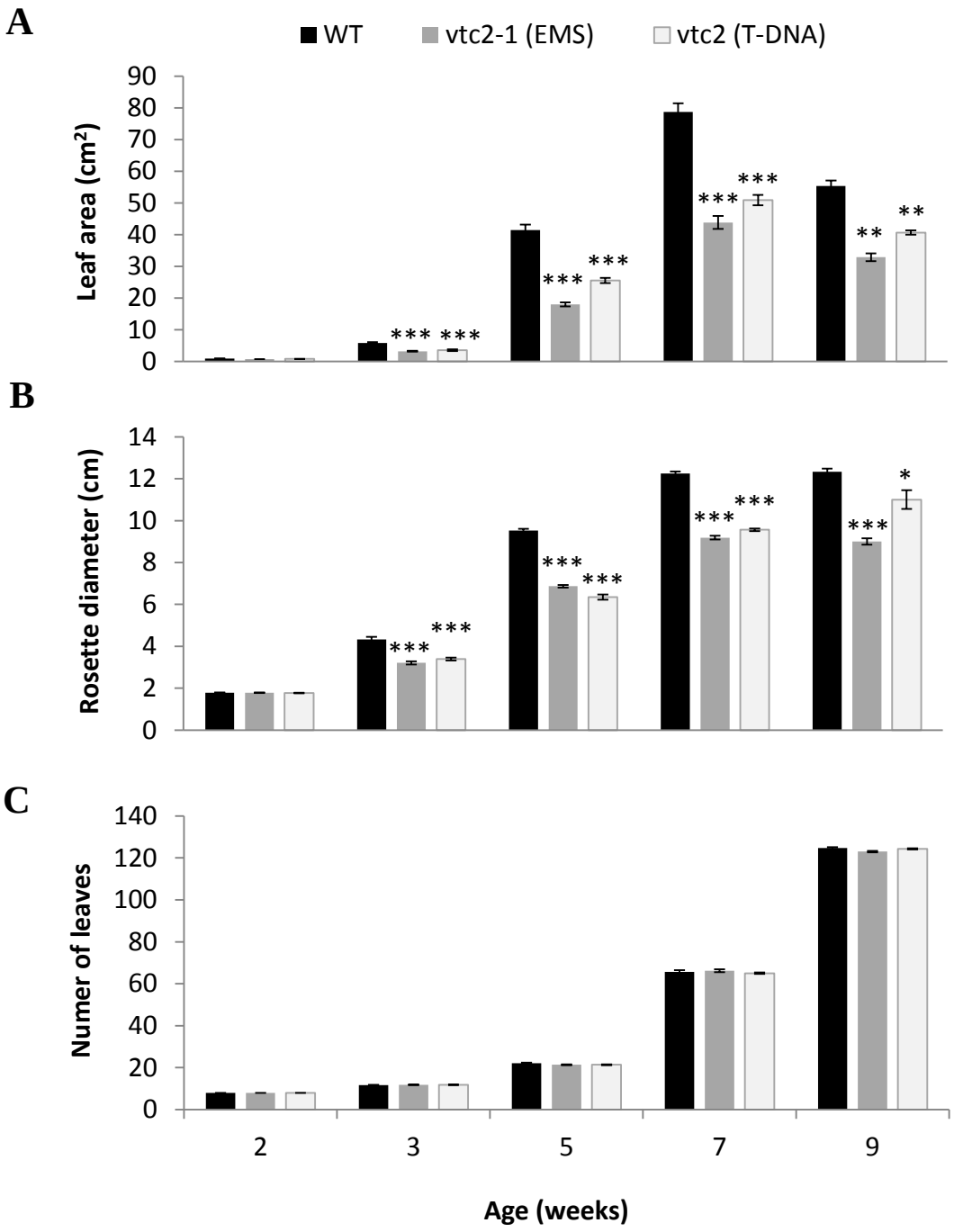


Figure 3.2 A comparison of rosette leaf areas (A), rosette diameter (B) and leaf numbers (C) in the Arabidopsis *vitamin C defective 2* mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA) to the wild type (WT) in 2-, 3-, 5-, 7-, and 9-week-old plants. Data are the mean values ± SE (n = 10). (*p < 0.05); (**p < 0.01); (***p < 0.001) in Significance given from analysis by One-Way ANOVA comparisons between the mutant lines and wild type.

The shoot of *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants significantly accumulated lower biomass, expressed on fresh and dry weights, than the wild type at all stages of development (Fig. 3.3A, B).

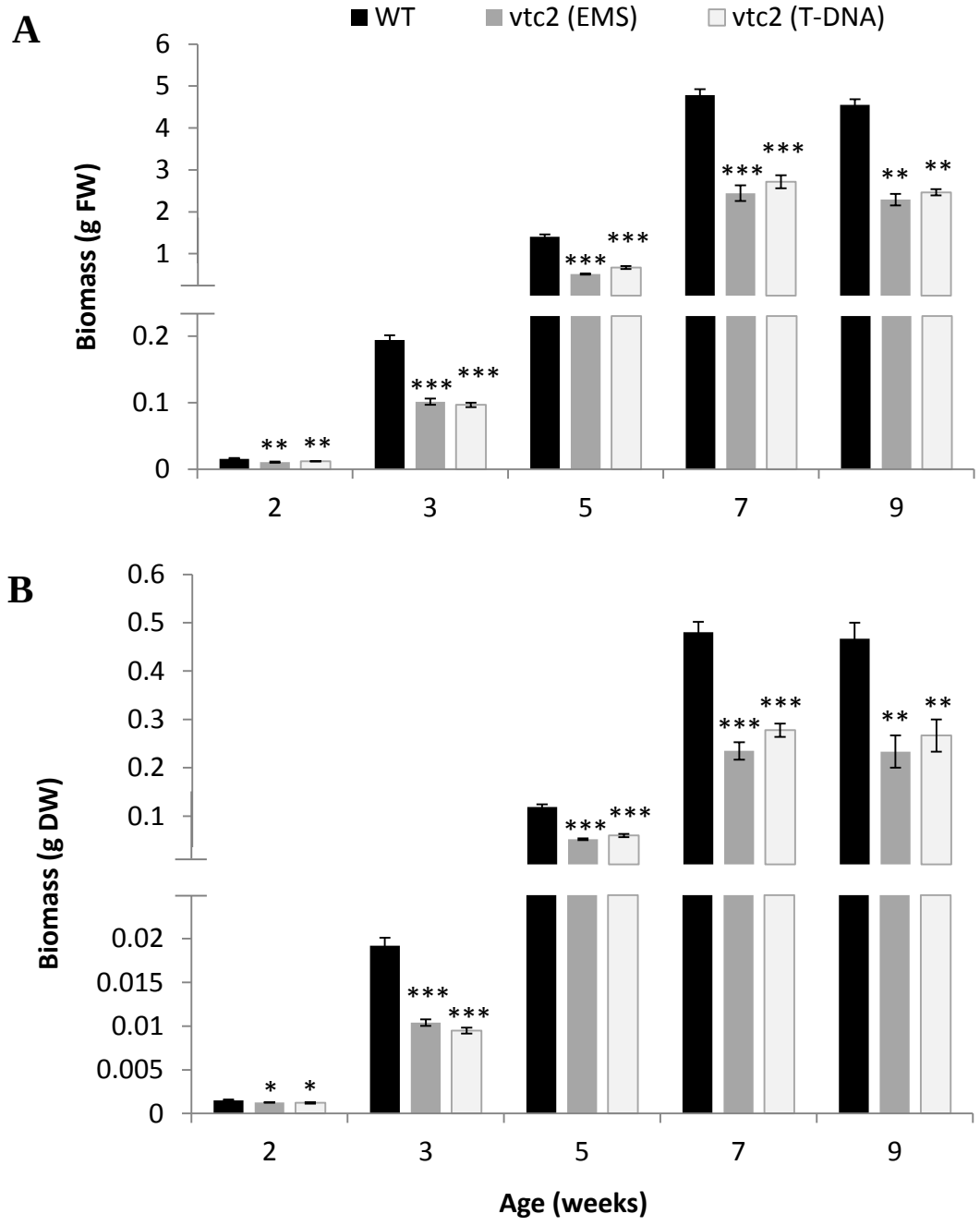


Figure 3.3 A comparison of shoot biomass expressed on a fresh weight (A) and dry weight (B) basis in 2-, 3-, 5-, 7-, and 9-week-old wild type (WT) Arabidopsis and *vitamin C defective 2* mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA). Data are the mean values ± SE (n = 10). (*p < 0.05); (**p < 0.01); (***p < 0.001) in Significance given from analysis by One-Way ANOVA comparisons between the mutant lines and wild type.

3.2.2 Whole leaf ascorbate content

To determine the whole leaf ascorbate content and the ratio of reduced to oxidised ascorbate, the total ascorbate was extracted from the wild type and *vitamin C defective 2* mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA) at different stages of development.

Both ascorbate-defective *vtc2* mutants contain about 25-30% of the wild types level of total ascorbate throughout development (Fig. 3.4). More than 80% of the total pool of ascorbate in all genotypes was in the reduced form (AsA) and about 20% was in the oxidised form (dehydroacorbate; DHA). Moreover, the total pool of ascorbate was increased during development in the wild type plants (Fig. 3.4). This increase was also showed by the *vtc2* mutants but in smaller amount (Fig. 3.4).

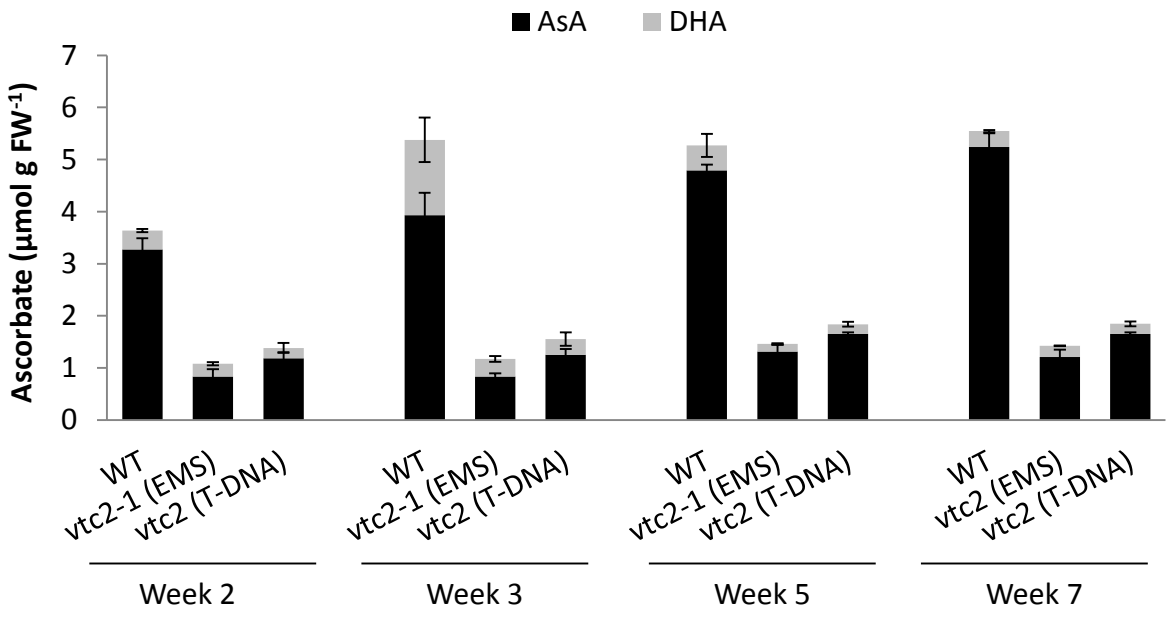


Figure 3.4 A comparison of whole leaf ascorbate content in 2-, 3-, 5-, and 7-week-old wild type (WT) Arabidopsis and *vitamin C defective 2* mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA). Black columns (AsA) represent the reduced form of ascorbate, silver columns (DHA) represent the oxidised (Dehydroascorbate) form of ascorbate and both columns together represent the total pools of ascorbate. Data are the mean values ± SE (n = 3).

3.2.3 Expression of *VITAMIN C DEFECTIVE 5* (*VTC5*)

The GDP-L-galactose phosphorylase, which is required enzyme to convert (GDP-L-galactose) to (L-galactose-1-P) in ascorbate biosynthesis, is encoded by two genes; *VITAMIN C DEFECTIVE 2 (VTC2) and VITAMIN C DEFECTIVE 5* (*VTC5*; Dowdle et al., 2007). In *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants the vitamin C defective 2 (*VTC2*) gene was knocked out, therefore to analyse how (*VTC5*) is altered, its expression level was determined by qRT-PCR in the wild type and both *vtc2* mutants.

The expression of vitamin C defective 5 (*VTC5*) gene was up-regulated in both *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants relative to wild type in 3-week-old plants (Fig. 3.5).

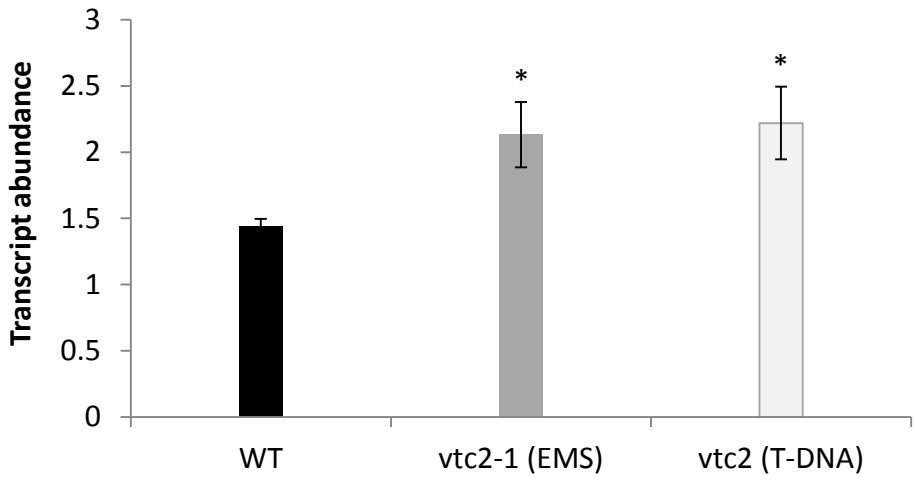


Figure 3.5 Expression level of vitamin C defective 5 (*VTC5*) gene encoding (GDP-L-**galactose phosphorylase) in 3-week-old wild type (WT) Arabidopsis and *vitamin C defective 2* mutant lines *vtc2-1* (EMS) and *vtc2* (T-DNA).** Data are the mean values ± SE (n = 3). (*p < 0.05) in Significance given from analysis by One-Way ANOVA comparisons between the mutant lines and wild type.

3.2.4 Apoplastic ascorbate content and ascorbate oxidase activity

To investigate the effect of mutation in the (*VTC2*) gene in *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants, on the apoplastic ascorbate content, the apoplastic ascorbate concentration was determined in the wild type and both *vtc2* mutant lines. The apoplastic or intracellular washing fluid was extracted by vacuum infiltration for the whole rosette at week-3.

No ascorbate was detected in the extracted apoplastic fluid of *vtc2-1* (EMS) and *vtc2* (T-DNA) (Fig. 3.6A). The majority of wild types apoplastic ascorbate was in the oxidised (dehydroacorbate; DHA) form (Fig. 3.6A). Furthermore, no significant differences were observed in the extracted amount of intracellular washing fluid in all genotypes (Fig. 3.6B).

The activity of apoplastic ascorbate oxidase (AO) enzyme was assayed, which is apoplastic enzyme that oxidises ascorbate (AsA) to dehydroacorbate (DHA). AO activity was similar in both *vtc2* mutants and wild type plants in spite of different abundance of total ascorbate (Fig. 3.6C).

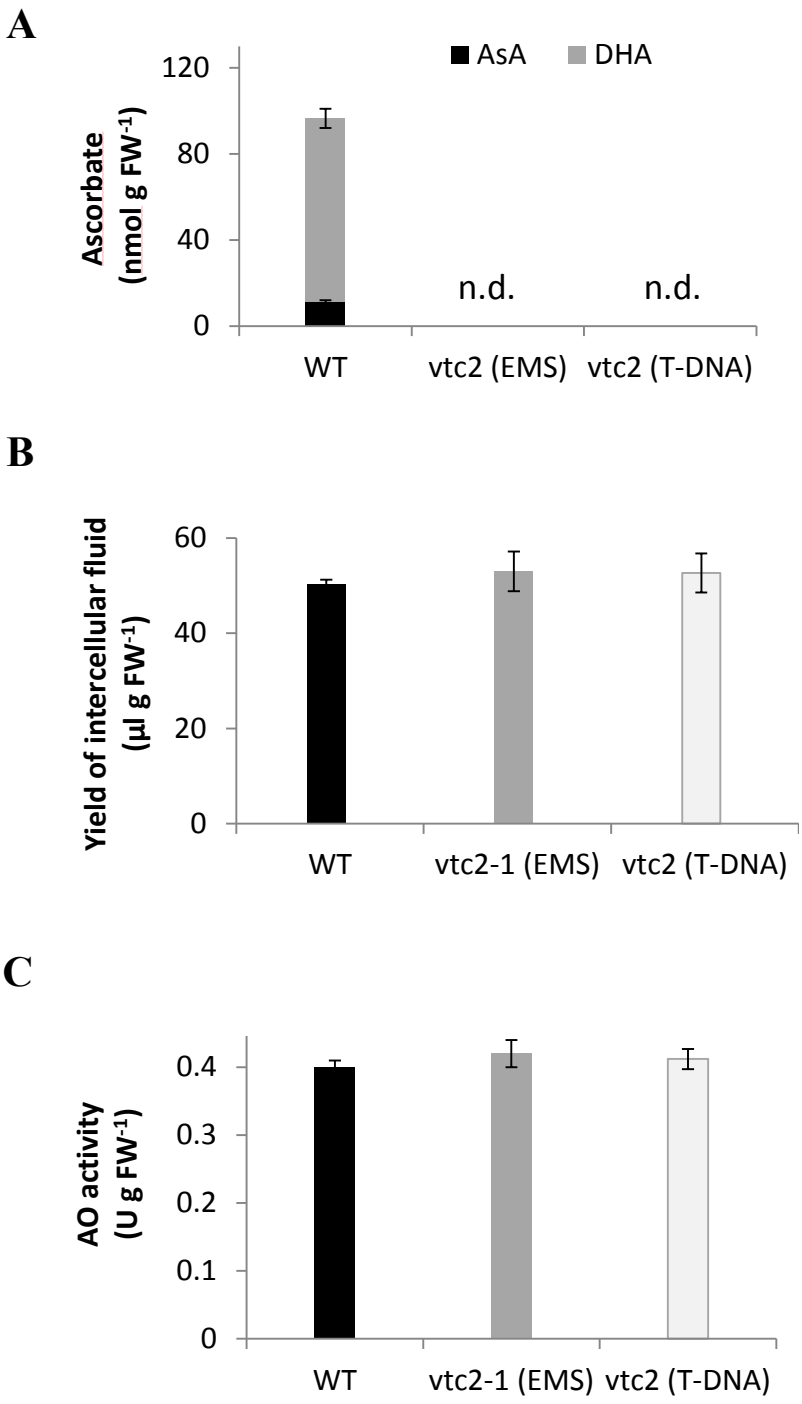


Figure 3.6 A comparison of apoplastic ascorbate content (A), yield of intracellular (apoplastic) washing fluid after vacuum infiltration of the leaves (B) and ascorbate oxidase (AO) activity (C) in 3-week-old wild type (WT) Arabidopsis, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutant lines. Black column (AsA) represents the reduced form of ascorbate, silver column (DHA) represents the oxidised (Dehydroascorbate) form of ascorbate and both columns together represent the total pools of ascorbate (A). One unit of AO activity equal the amount of enzyme required to oxidise (1µmol ascorbate min^{-1}) at 25°C. Data are the mean values ± SE (n = 3). N.d., not detected.

3.2.5 Leaf pigment content

The chlorophyll and carotenoid contents of the rosette leaves were similar in all genotypes at all stages of development (Fig. 3.7A, B, C, D).

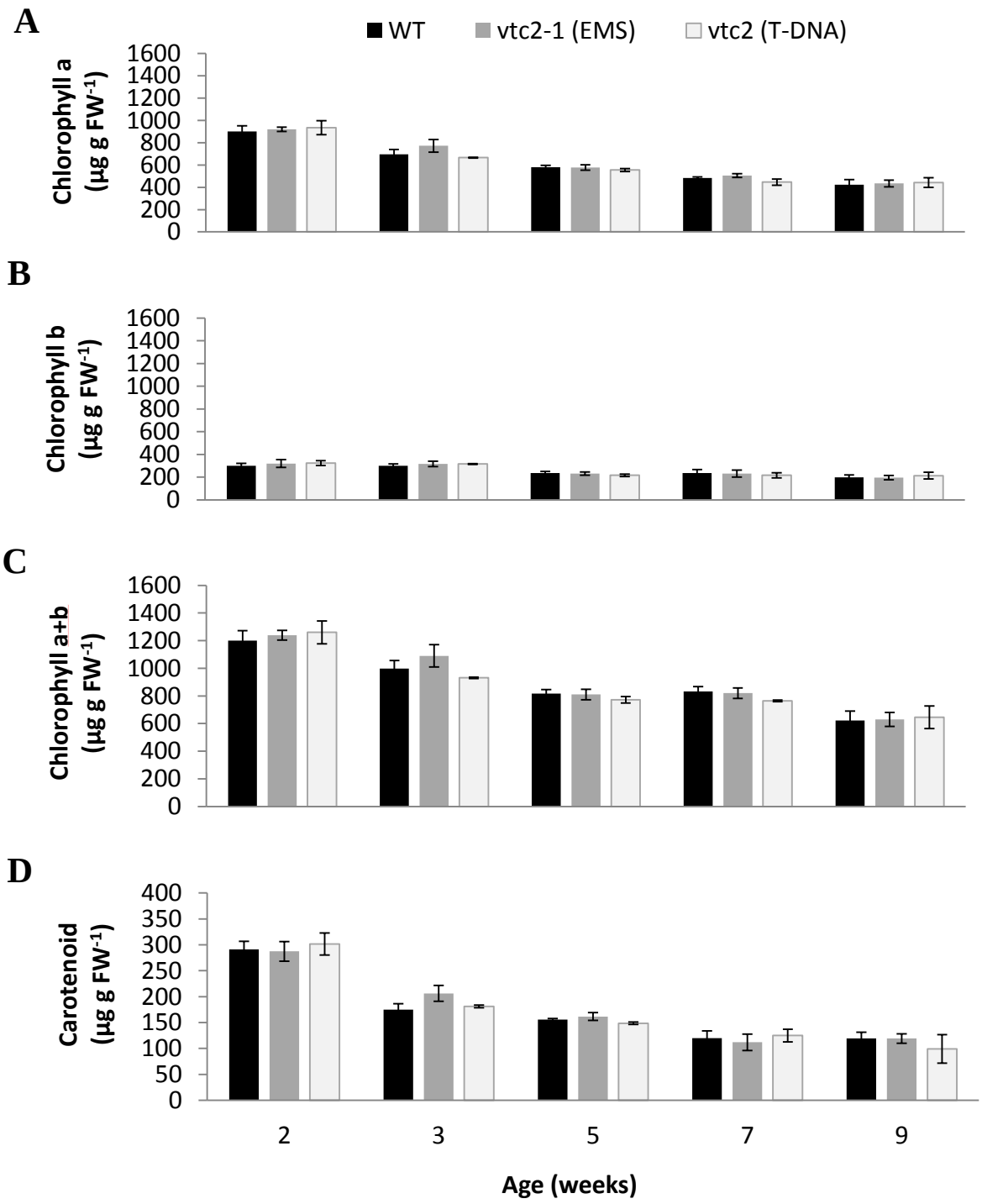


Figure 3.7 A comparison of leaf pigment contents in 2-, 3-, 5-, 7-, and 9-week-old wild type (WT) Arabidopsis, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutant lines. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and total carotenoid pigments (carotene; D). Data are the mean values ± SE (n = 3).

3.2.6 Flowering time

There were no significant differences in the flowering time between the wild type and both *vtc2* mutant lines. Similarly, all genotypes had the same number of leaves when the first flower bud appeared (Fig. 3.8A, B) and (Fig. 3.9). In contrast, the seed production capacity was highly significantly reduced in both ascorbate-defective *vtc2* mutant lines. This reduction in seed production capacity was more marked in *vtc2* (T-DNA) mutants (Fig. 3.8C) and (Fig. 3.9).

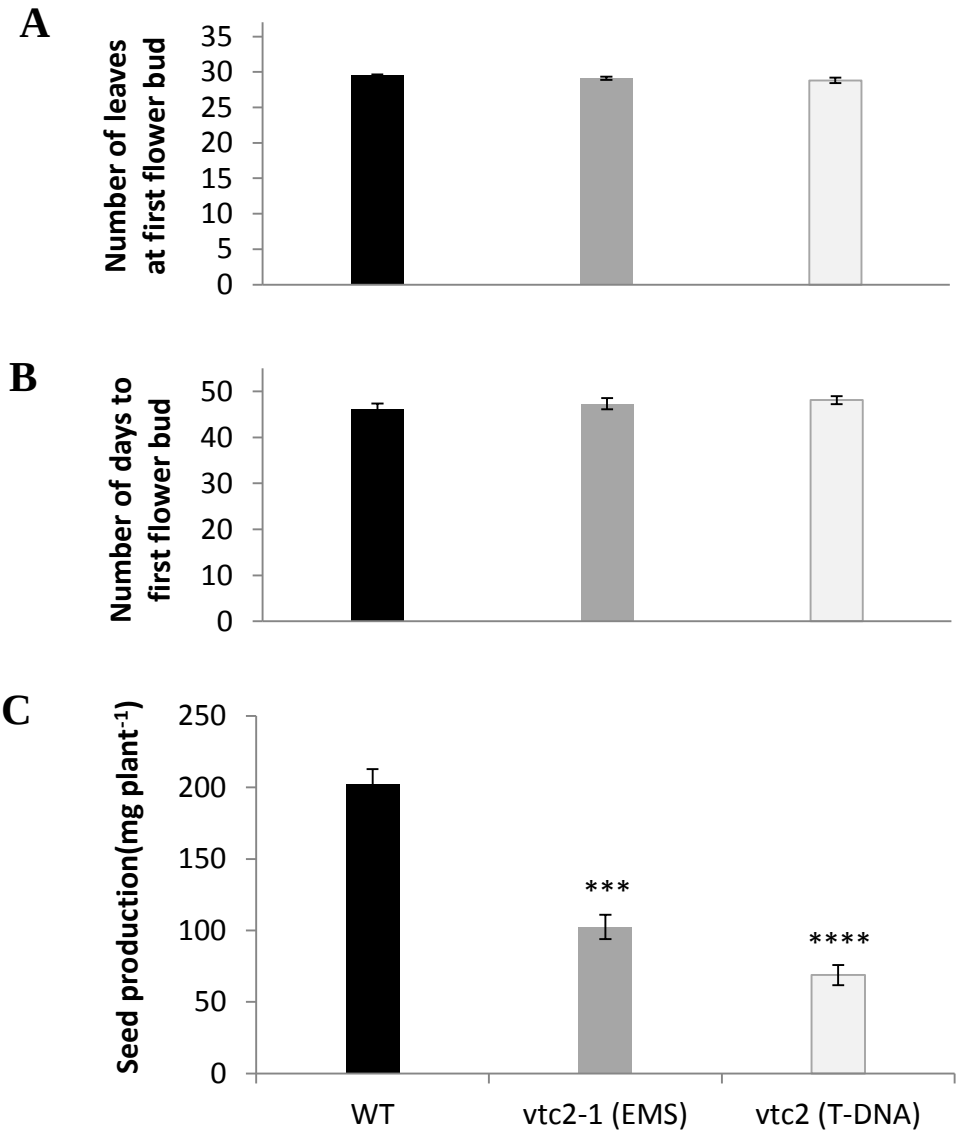


Figure 3.8 A comparison of flowering time and seed production capacity in wild type (WT) Arabidopsis, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutant lines. Number of leaves at first flower bud (A), number of days to first flower bud (B) and seed production capacity (C). Data are the mean values ± SE (n = 10). (***p < 0.001); (****p < 0.0001) in Significance given from analysis by One-Way ANOVA comparisons between the mutant lines and wild type.

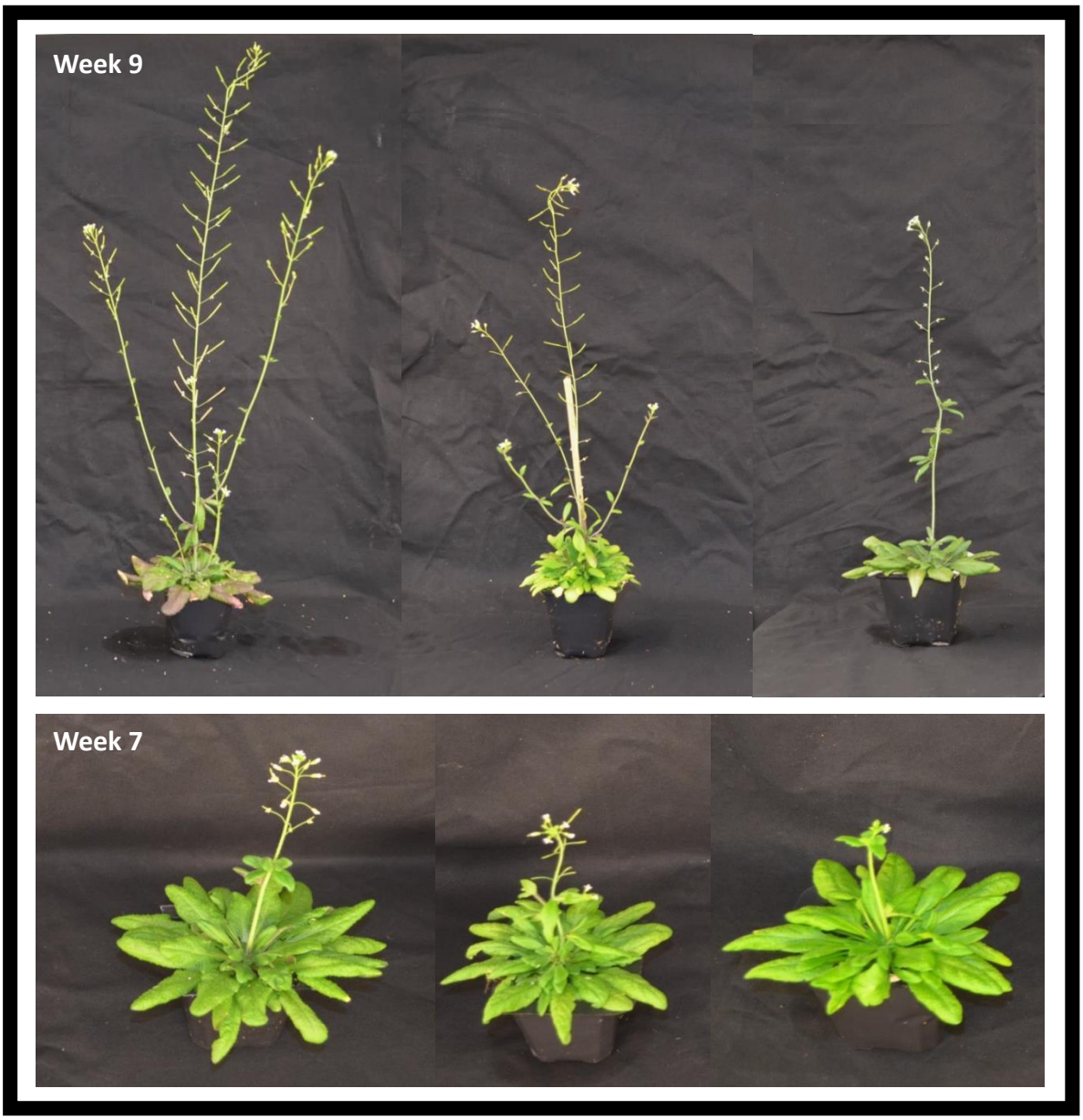


Figure 3.9 A comparison of rosette phenotype at flowering point in wild type (WT) Arabidopsis, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants at weeks 7 and 9.

3.2.7 Aphid fecundity

Aphid fecundity was measured in wild type and both *vtc2* mutant lines. A single one day-old nymph was placed on each plant and after 15 days the total number of offspring was counted (Fig. 3.10).

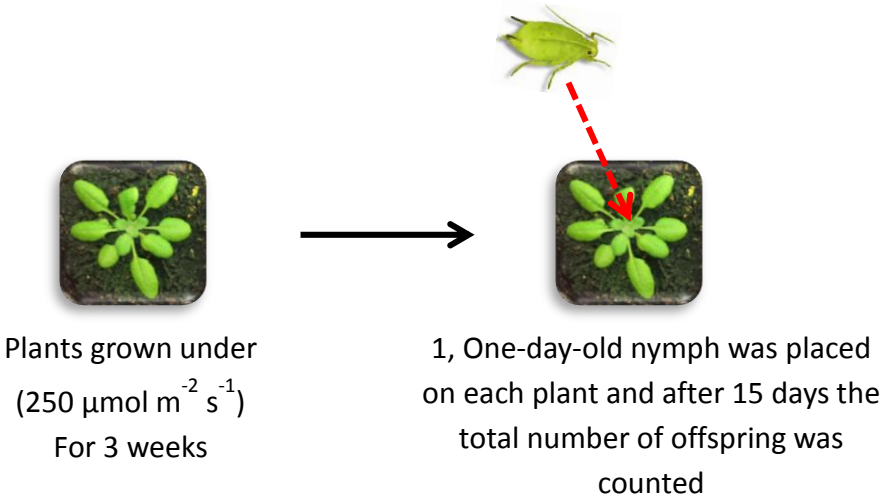


Figure 3.10 Experimental designs illustrate aphid fecundity on 3-week-old wild type and *vtc2* mutant lines. Wild type and mutant lines were grown under (250 µmol m^{-2} s^{-1}) for three weeks and then 1, one-day-old nymph was placed on the rosette centre of each plant. After 15 days the total number of aphids was counted.

The number of aphids were highly significantly lower on the leaves of the *vtc2-1* (EMS) mutants compared to the wild type plants. Conversely, there was no significant difference in aphid fecundity between *vtc2* (T-DNA) mutant and wild type plants (Fig. 3.11A, B).

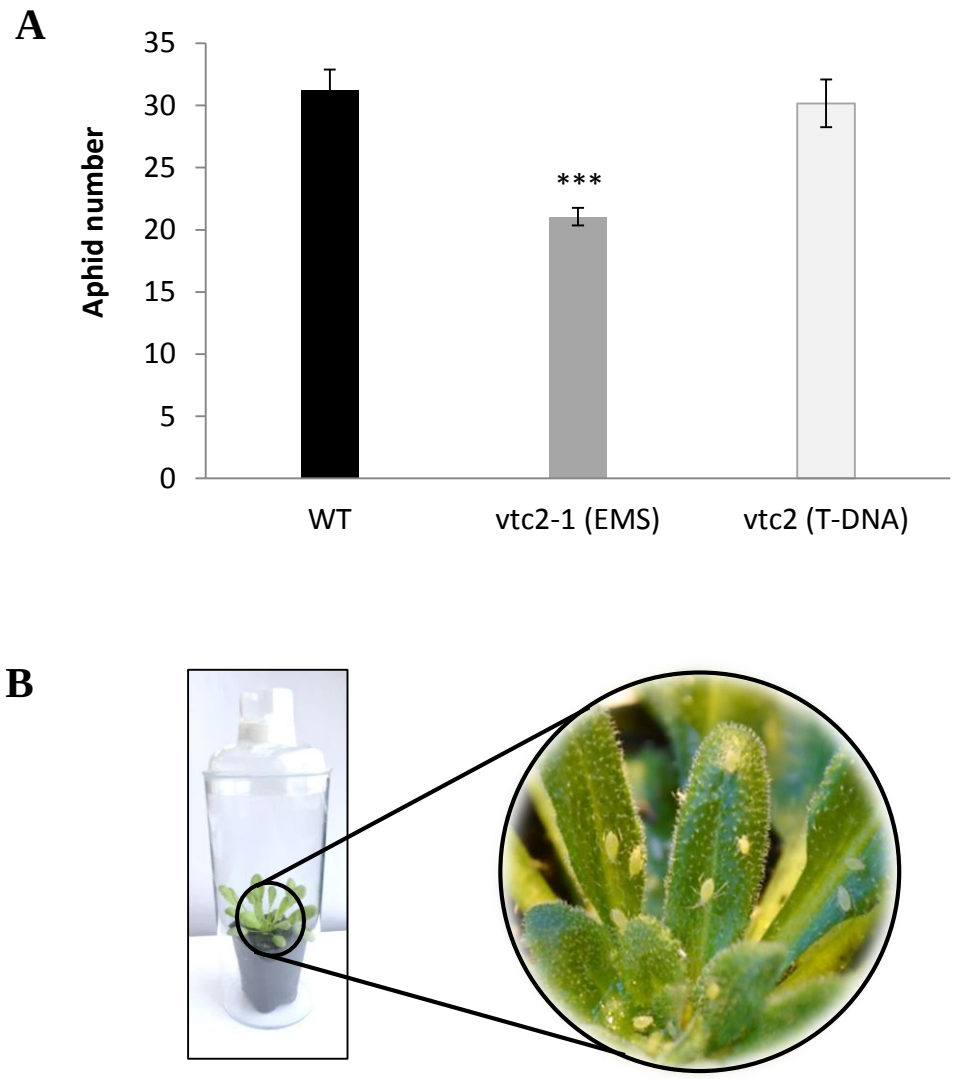


Figure 3.11 A comparison of aphid fecundity in wild type (WT) Arabidopsis, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants. (A) Aphid fecundity. (B) Representative images of adult aphids and new nymphs at 15-days on wild type leaves. The numbers of aphids present on leaves two weeks after the onset of infestation were measured on plants that had been grown for 3 weeks under (250 μmol m^{-2} s^{-1}) growth conditions. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times. (***$p < 0.001$) in Significance given from analysis by One-Way ANOVA comparisons between the mutant lines and wild type.

3.2.8 Transcript changes upon aphid infestation

To assess plant responses to aphid infestation, the quantitative real-time PCR was used to analyse the induction of transcripts that related to various defence pathways.

A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four-week-old wild type and mutants that grown for four weeks under (250 μmol m^{-2} s^{-1}) growth conditions. An empty cage was attached as aphid free control plants. The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. To assess the induction of different defence pathways, the RNA was isolated and quantitative real-time PCR (qRT-PCR) was performed to analyse the expression level of seven genes that they each involve in different pathways.

Aphid infestations quickly change the expression of a range of genes that involve in the maintenance of redox homeostasis (Kerchev et al., 2013). The REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1), which involves in maintenance of redox homeostasis and considered as an essential key regulator of cellular redox networks (Khandelwal et al., 2008), was significantly induced in the infested leaves of wild type and *vtc2-1* (EMS) mutants compared to non-infested corresponding genotypes (Fig. 3.12). In contrast, this induction was not shown by *vtc2* (T-DNA) mutants (Fig. 3.12).

The expression of BON1-associated protein 2 (BAP2), which encodes inhibitors of hydrogen peroxide-induced cell death, was up-regulated in the infested leaves of both *vtc2* mutant lines compared to non-infested corresponding genotypes (Fig. 3.12). While, the abundance of (BAP2) transcript was not altered by aphid feeding in the wild type plants (Fig. 3.12).

Another pathway that is induced quickly due to aphid attack in the plant cells is alteration in the hormone signalling pathways including salicylic acid (SA). A large numbers of SA signal transduction-related genes were altered up to aphid infestation (Kerchev et al., 2013).

The SA-associated transcript, WRKY DNA-binding protein 62 transcription factor (WRKY62), was induced in the infested leaves of all genotypes compared to non-infested corresponding genotypes (Fig. 3.12). This induction in the expression of (WRKY62) was much stronger in the infested leaves of *vtc2-1* (EMS) mutants (Fig. 3.12).

The aphid feeding significantly enhanced the expression of NIM1-INTERACTING 1; protein binding (NIM1), a salicylic acid- mediated signal transduction, in all genotypes relative to non-infested corresponding genotypes (Fig. 3.12).

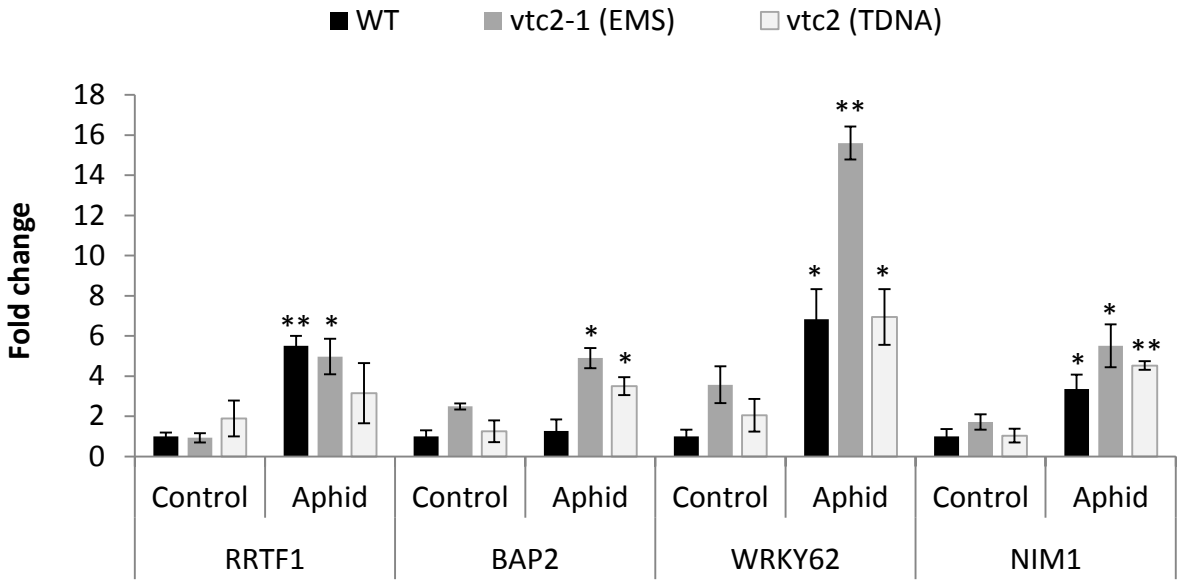


Figure 3.12 Effect of aphid infestation on the transcript abundance of REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1), BON1-associated protein 2 (BAP2), WRKY DNA-binding protein 62 transcription factor (WRKY62) and INTERACTING 1- protein binding (NIM1) in the infested leaves of wild type (WT) Arabidopsis, *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants relative to the aphid-free controls of the corresponding genotype. Sixty wingless adult aphids were placed inside a clip cage attached to a mature rosette leaf of four weeks old plants. The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$); (***$p < 0.001$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) for each genotype.

Transcripts related to jasmonic acid (JA) biosynthesis and signalling pathways were induced due to aphid attack such as ALLENE OXIDE CYCLASE 3 (AOC3) and lipoxygenase 5 (LOX5; Kerchev et al., 2013). The expression of ALLENE OXIDE CYCLASE 3 (AOC3), which is associated to JA synthesis (Schaller et al., 2008), was only enhanced in the infested leaves of *vtc2-1* (EMS) mutants (Fig. 3. 13). In contrast, the AOC3 gene was not altered upon aphid attack in the infested leaves of wild type and *vtc2* (T-DNA) mutants relative to non-infested corresponding genotypes (Fig. 3. 13). Transcripts encoding abscisic acid (ABA)-mediated transcription factors or ABA signalling pathways were altered in the local leaves of the infested plants with aphid (Kerchev et al., 2013).

The abundance of ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) transcript, which is ABA-related transcription factor, was significantly increased in the infested leaves of all genotypes compared to non-infested corresponding genotypes (Fig. 3.13). This induction of (AZF1) transcript due to aphid feeding was much stronger in *vtc2-1* (EMS) mutants (Fig. 3.13).

Mitogen activated protein kinases (MAPKs) play important role in the signal transduction pathways such as reactive oxygen species (ROS) and calcium signalling (Takahashi et al., 2011). MAPKs involve or regulate intracellular and extracellular signal transductions through protein phosphorylation on their serine and threonine residues (Rodriguez et al., 2010).

The transcript encoding ATP binding / protein kinase (MAPKKK21), which implicated in mitogen activated protein kinase (MAPK) pathway, was highly significantly enhanced in the infested leaves of *vtc2-1* (EMS) mutants compared to non-infested corresponding genotypes (Fig. 3.13). However, the (MAPKKK21) was not induced upon aphid infestation in wild type and *vtc2* (T-DNA) mutants relative to non-infested corresponding genotypes (Fig. 3.13).

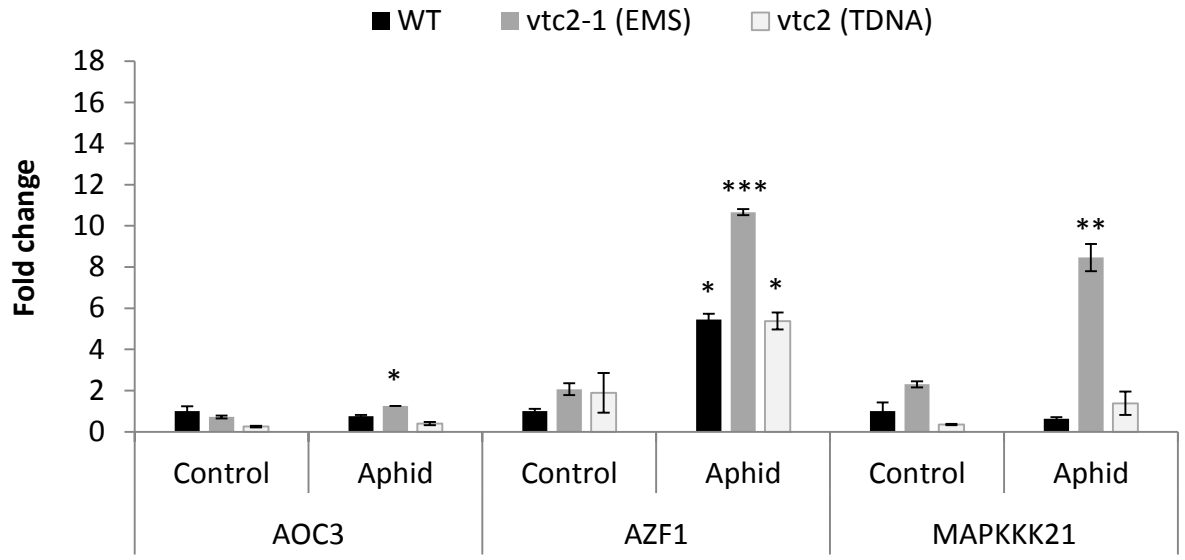


Figure 3.13 Effect of aphid feeding on the expression level of ALLENE OXIDE CYCLASE 3 (AOC3), ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) and ATP binding / protein kinase (MAPKKK21) on the leaves of wild type (WT), *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants relative to the non-infested corresponding genotype. Sixty wingless adult aphids were placed inside a clip cage attached to a mature rosette leaf of four weeks old plants. The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$); (***$p < 0.001$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) for each genotype.

3.3 Discussion

The *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants had lower amounts of total ascorbate than the wild type. Both mutant genotypes had about 25-30% of the total ascorbate levels measured in the wild type throughout development (Fig. 3.4). These values are consistent with previously published values for *vtc2* (EMS; Pavet et al., 2005; Olmos et al., 2006). The maximum extractable ascorbate oxidase activities were similar in all genotypes. However, in contrast to the wild type the levels of ascorbate (AsA) and dehydroascorbate DHA were below the levels of detection in the apoplastic fluid extracted from the *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants. This finding is in agreement with previously published observations in the *vtc1* (EMS) mutant, which had no detectable AsA or DHA in the apoplast (Veljovic-Jovanovic et al., 2001). The ascorbate present in the *vtc2-1* (EMS) and *vtc2* (T-DNA) leaves was likely to be the result of GDP-L-galactose phosphorylase activity encoded by the *VTC5* gene. The levels of *VTC5* transcripts were higher in the leaves of the *vtc2-1* (EMS) and *vtc2* (T-DNA) mutants than the wild type plants (Fig. 3.5).

Although all genotypes had the same number of leaves and amounts of leaf pigments, the growth of the *vtc2-1* (EMS) and *vtc2* (T-DNA) rosettes, measured as either rosette diameter, leaf area, or fresh and dry weight, was slower than that the wild type. Thus, the ascorbate-deficient mutants had smaller leaves than the wild type. This finding is consistent with previous observations of the leaves of the *vtc2-1* (EMS) mutants, which had the same numbers of cells in the leaves as the wild type, but the cell size was smaller (Pavet et al., 2005). All lines had the same numbers of leaves at flowering. However, the *vtc2-1* (EMS) and *vtc2* (T-DNA) produced significantly fewer seeds than the wild type, suggesting that low ascorbate impairs seed production.

Aphid fecundity was compared in the wild type, *vtc2-1* (EMS) and *vtc2* (T-DNA) lines by placing a single one-day-old nymph on each plant and then counting the numbers of offspring per plant after 15 days (Fig. 3.10). Aphid fecundity was significantly lower on the *vtc2-1* (EMS) plants than the wild type as observed in a previous study (Kerchev et al., 2013). In contrast, the *vtc2* (T-DNA) mutants had similar numbers of aphids to the wild type plants (Fig. 3.11A). The variation observed between the *vtc2-1* (EMS) and *vtc2* (T-DNA) lines in terms of aphid resistance is surprising, and suggests that the lower aphid fecundity observed in the *vtc2-1* (EMS) mutants is not caused by low ascorbate. Previous studies in a large number of different low ascorbate mutants has shown that they are more resistant to

biotrophic pathogens, a trait that is associated with high levels of salicylic acid (SA), together with the constitutive expression of PR genes (Pavet et al., 2005; Colville and Smirnoff, 2008). It has been suggested that aphids have ability to manipulate the hormonal cross talk through suppression of jasmonic acid (JA)-mediated defence pathways via stimulation of SA-mediated defence signalling pathways (Zarate et al., 2007). A previous study on the *vtc2-1* (EMS) mutants implicated abscisic acid (ABA)-dependent pathways in the higher resistance to aphids in *vtc2-1* (EMS) mutants relative to the wild type (Kerchev et al., 2013).

An analysis of the abundance of transcripts that can be used as markers for the redox, SA, JA and ABA-dependent signalling pathways that are triggered by aphid infestation (Kerchev et al., 2013), revealed differences in the transcriptome profiles of the *vtc2-1* (EMS) and *vtc2* (T-DNA) lines compared to the wild type in the absence of aphid infestation (Figs. 3.12 and 3.13). However, large differences in the responses of the selected transcripts were observed between the *vtc2-1* (EMS) and *vtc2* (T-DNA) lines, 6h after the onset of addition of aphids to the leaves.

The analysis of the aphid-dependent changes in abundance of transcripts related to defence pathways performed in these studies revealed that levels of mRNAs encoding REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1) were significantly increased following aphid infestation in the leaves of wild type and *vtc2-1* (EMS) mutants but not in *vtc2* (T-DNA) leaves. The levels of BON1-associated protein 2 (BAP2) transcripts were increased to a similar extent by aphid infestation in both mutant genotypes but not in the wild type (Fig. 3.12). In contrast to RRTF1 and BAP2 transcripts, the levels of several marker transcripts were increased to a greater extent in the *vtc2-1* (EMS) mutants as a result of aphid infestation than the wild type and *vtc2* (T-DNA) lines. For example, WRKY DNA-binding protein 62 transcription factor (WRKY62) transcripts were significantly higher in the infested leaves of *vtc2-1* (EMS) mutants than the wild type or *vtc2* (T-DNA) lines. The WRKY62 transcription factor functions downstream of NPR1 and negatively regulates JA signalling (Mao et al., 2007). Moreover, overexpression of WRKY62 decreases basal resistance to *Pseudomonas syringae* (Kim et al., 2008). However, it is unlikely that the higher aphid resistance observed in the *vtc2-1* (EMS) mutants is related to the increased levels WRKY62 transcripts because the SA-mediated defence pathway does not appear to be effective in protecting plants against aphid attack (Pegadaraju et al., 2005).

Other transcripts may provide information concerning the nature of the greater aphid resistance observed in the *vtc2-1* (EMS) mutants compared to the wild type and *vtc2* (T-DNA) lines. For example, ALLENE OXIDE CYCLASE 3 (AOC3) and ATP binding / protein kinase (MAPKKK21) transcripts were increased to a greater extent in the infested leaves of *vtc2-1* (EMS) mutants than the wild type or *vtc2* (T-DNA) lines as a result of aphid infestation (Fig. 3.13). The allene oxide cyclase family are involved in JA synthesis and related defence signalling pathways. While little is known about the precise functions of MAPKKK21, MAP Kinase signalling pathways are known to be important regulators of the plant immune response. The levels of ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) transcripts, which is ABA-associated transcript, were also increased to a greater extent by aphid feeding in the leaves of *vtc2-1* (EMS) mutants than the wild type and *vtc2* (T-DNA) lines. Taken together, these transcripts may indicate that JA, ABA and related defence signalling pathways were differentially expressed in the *vtc2-1* (EMS) mutants compared to the wild type and *vtc2* (T-DNA) lines.

Chapter 4. Oxidative stress signalling linked to PP2A-B'γ, catalase and glutathione in plant responses to aphids under low and high light growth conditions

4.1 Introduction

Protein phosphorylation and de-phosphorylation, which are catalysed by protein kinases and phosphatases respectively, serve as an "on-off" switch that regulates many biological processes. In particular, protein phosphorylation and de-phosphorylation are used in the transduction of oxidative signals, a process that is considered to be mediated by mitogen-activated protein (MAP) kinase (MAPK) cascades (Nakagami et al., 2005; Xia et al., 2015). MAPK cascades comprise of MAPK, MAPK kinase (MAPKK/MKK) and MAPKK kinase (MAPKKK/MEKK) and are highly conserved central regulators of diverse cellular processes, such as differentiation, proliferation, growth, death and stress responses (Xia et al., 2015). Moreover, a previous study concerning the responses of *A. thaliana* leaves to aphid infestation identified a large number of transcripts encoding protein kinases and protein phosphatases that were changed in abundance within the first 24h of the aphids being placed on the leaves (Kerchev et al., 2013).

Protein phosphatases function in conjunction with protein kinases to regulate cell signalling, particularly in plant stress responses (Luan, 2003). Protein phosphatases 2A (PP2A), which is a serine/threonine (Ser/Thr) phosphatase, is a major phosphatase that accounts for about 25% of the total protein phosphatase activity in crude homogenates from several plants (MacKintosh and Cohen, 1989). The trimeric form of the PP2A holoenzyme consists of a scaffold subunit A (65 kDa) that binds the catalytic subunit C (36 kDa) and a regulatory subunit B (Mayer-Jaekel and Hemmings, 1994). The B subunits, which influence the structural conformation and determine the substrate specificity and subcellular localization of PP2As, are highly variable. The B subunits are classified as either B (or B55; 55 kDa), B' (54-74 kDa), or B" (72-130 kDa) subfamilies (Janssens and Goris, 2001). The B proteins are encoded by four related genes (Bα, Bβ, Bγ, Bδ). The B' family are encoded by the B'α, B'β, B'γ, B'ζ, B'δ and B'ε genes while the B" family are encoded by three related genes, PR48, PR59, and PR72/130 (Janssens and Goris, 2001).

While relatively little information is available on the roles of the different subunits, the functions of the PP2A-B'γ subunit have been studied in detail (Trotta et al., 2011a, b). Loss of function mutants in PP2A-B'γ were shown to have constitutive activation of pathogenesis responses, an effect that was linked to enhanced ROS signalling (Trotta et al., 2011a).

The lack of a functional regulatory B'γ subunit in the *pp2a-b'γ* mutant resulted in increased resistance against the virulent biotrophic pathogen *Pseudomonas syringae* pv *tomato* DC3000 and to the necrotic pathogen *Botrytis cinerea* compared to the wild type. Further analysis showed that a number of salicylic acid (SA)-related genes were more highly expressed in the *pp2a-b'γ* mutant compared with the wild type plants (Hirai et al., 2007; Fan et al., 2011).

Aphid fecundity was examined on the mutants with defects in various PP2A-B' subunits. This work was undertaken because the *pp2a-b'γ* mutant showed constitutive expression of defence related genes (Trotta et al., 2011a) and therefore it was possible that plant responses to aphids were also changed relative to the wild type. Moreover, a synergistic interaction has been demonstrated between the Arabidopsis *pp2a-b'γ* mutants and mutants deficient in leaf catalase (*cat2*) that have impaired antioxidant defences (Li et al., 2013). Analysis of *cat2 pp2a-b'γ* double mutants demonstrated that PP2A-B'γ is a crucial regulator of intracellular oxidative stress signalling (Li et al., 2013).

Catalase is a key antioxidant enzyme, which decomposes hydrogen peroxide (H_2O_2) $_{to}$ water and oxygen in leaf peroxisomes (Zamocky et al., 2008). H_2O_2 is generated in leaf peroxisomes by glycolate oxidase in the photorespiratory pathway (Corpas et al., 2008). The three catalase genes in Arabidopsis (CAT1, CAT2 and CAT3) have a high degree of sequence similarity and they each encode a protein of 492 amino acids (Frugoli et al., 1996; McClung, 1997). Arabidopsis *cat2* mutants lack the major leaf form of catalase (CAT2), having only about 10% of the catalase activity of the wild type plants (Mhamdi et al., 2010). When the *cat2* mutants were grown air (i.e. photorespiratory conditions) they have decreased rosette biomass, but only show lesion development on leaves under long day conditions (Queval et al., 2007; Chaouch et al., 2010). The day length-dependent effects on oxidative signalling leading to lesion formation on the leaves were linked to an accumulation of SA and constitutive activation of pathogenesis-related (PR) genes (Li et al., 2013). Moreover, the *cat2* mutants showed altered responses to pathogens and constitutive resistance to bacterial attack (Chaouch et al., 2010). In contrast, when the mutants were grown under short day conditions, they did not develop lesions and the SA-related responses were absent (Queval et al., 2007; Chaouch et al., 2010).

In the following studies, the responses of the *cat2 pp2a-b'γ* double mutants to aphid infestation were examined to determine whether aphid responses were modified. The absence of a functional PP2A-B'γ protein in the *cat2* mutant background resulted in the formation of lesions, SA and phytoalexin accumulation and PR gene expression under short days growth conditions.

These pathogenesis-related responses were not activated in the *cat2* mutant alone under short day conditions suggesting that the *cat2* and *pp2a-b'γ* mutations interact synergistically in oxidative signalling and in control of day length-dependent responses to intracellular oxidative stress. Analysis of the *cat2 pp2a-b'γ* double mutants might therefore provide new information on the mechanisms of plant resistance to aphid infestation.

In the following studies, aphid fecundity was compared in wild type Arabidopsis plants and PP2A mutants lacking either the gamma (γ) or zeta (ζ) subunits, *cat2* mutants and in *cat2 pp2a-b'γ* double mutants. Moreover, the effects of light intensity on aphid fecundity in these different mutants was assessed by first growing the plants for two weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then either maintaining them for a further seven days under low light conditions or transferring them to high light (HL; 800 μmol m^{-2} s^{-1}) for seven days, prior to the onset of aphid infestation. Photosynthetic CO_2 assimilation rates were determined, together with chlorophyll *a* fluorescence quenching in all mutants to determine the effects of the light treatments on photosynthesis. In addition, samples were harvested from leaves infested with aphids at an early time-point (6h) following the onset of aphid feeding under the different conditions in order to characterise effects on the transcripts that related with various defence pathways by the quantitative real-time PCR.

4.2 Results

4.2.1 Shoot phenotypes under low and high light conditions

When wild type and mutant lines were grown for three weeks under short day conditions (8h photoperiod) with LL, all of the mutant genotypes except for *pp2a-b'ζ1-1* had visibly smaller rosettes than the wild type (Fig. 4.1). However, when the plants were grown for two weeks under LL and then exposed for seven days to HL, the rosette phenotypes were more similar in wild type, *pp2a-b'γ, pp2a-b'ζ1-1* mutants than under LL, although the *pp2a-b'ζ1-2*, *cat2*, *pp2a-b'γζ* and *cat2 pp2a-b'γ* double mutants were visibly smaller than the other lines under HL conditions (Fig. 4.1).

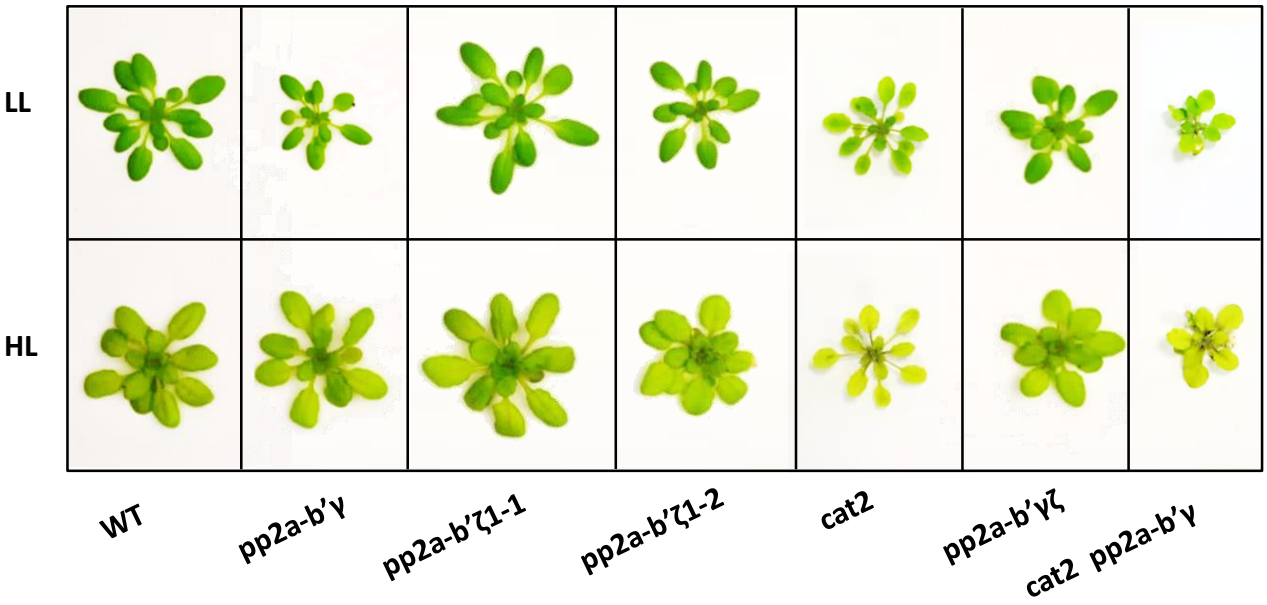


Figure 4.1 A comparison of the rosette phenotypes at week 3 in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL) and high light (HL) conditions. Plants grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions (top row) or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days (bottom row).

Leaf area and rosette diameter measurements were performed on plants that had either been grown for three weeks under LL or for two weeks under LL followed by seven days HL. The wild type plants had similar leaf areas under both light conditions (Fig. 4.2A). In contrast, the leaf area was significantly increased in all of the mutant genotypes, except for *cat2*, under HL relative to LL conditions (Fig. 4.2A). The HL-dependent increase in leaf area was most marked in the *pp2a-b'γ* mutants (Fig. 4.2A).

All of the mutants that grown for three weeks under LL, except for *pp2a-b'ζ1-1*, had significantly smaller rosette diameter relative to wild type plants (Fig. 4.2B). This decrease in rosette diameter was most noticeable in the *pp2a-b'γ* and *cat2 pp2a-b'γ* double mutants (Fig. 4.2B). Moreover, the rosette diameter was significantly increased in the *pp2a-b'γ* mutants under HL relative to LL conditions (Fig. 4.2B).

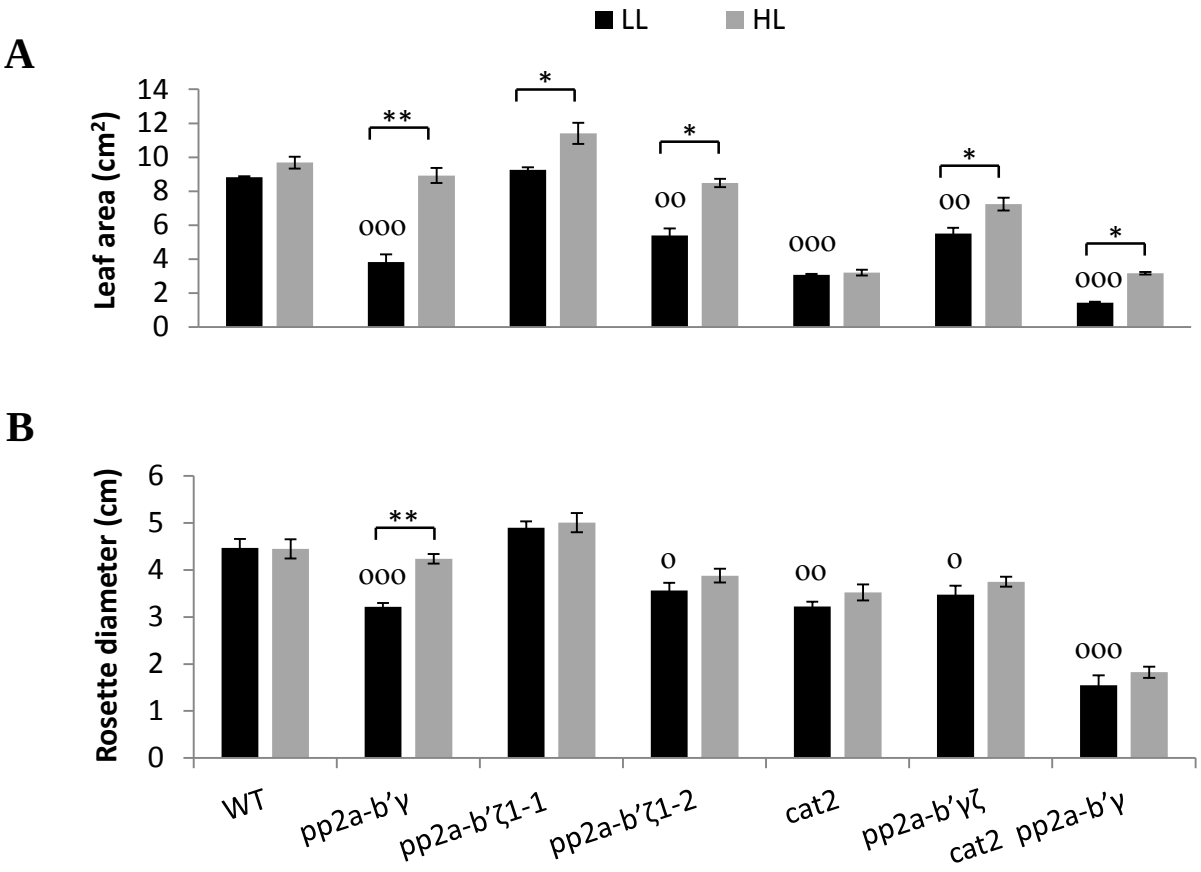


Figure 4.2 A comparison of the rosette phenotypes in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL) and high light (HL) conditions. (A) Rosette leaf areas. (B) Rosette diameter. Plants grown for two weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 800 μmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 10). (*p < 0.05); (**p < 0.01) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype, as follows (◦p < 0.05); (◦◦p < 0.01); (◦◦◦p < 0.001); (◦◦◦◦p < 0.0001) in One-Way ANOVA comparisons between the mutant lines and wild type under LL conditions.

4.2.2 Leaf pigment content

The chlorophyll and carotenoid contents of the rosette leaves were similar in all genotypes under LL conditions (Fig. 4.3A, B). Growth under HL for seven days decreased leaf chlorophyll by about 30% in all genotypes relative to the leaves grown under LL conditions. The light-dependent decreases in leaf chlorophyll were similar in all genotypes (Fig. 4.3 A, B)

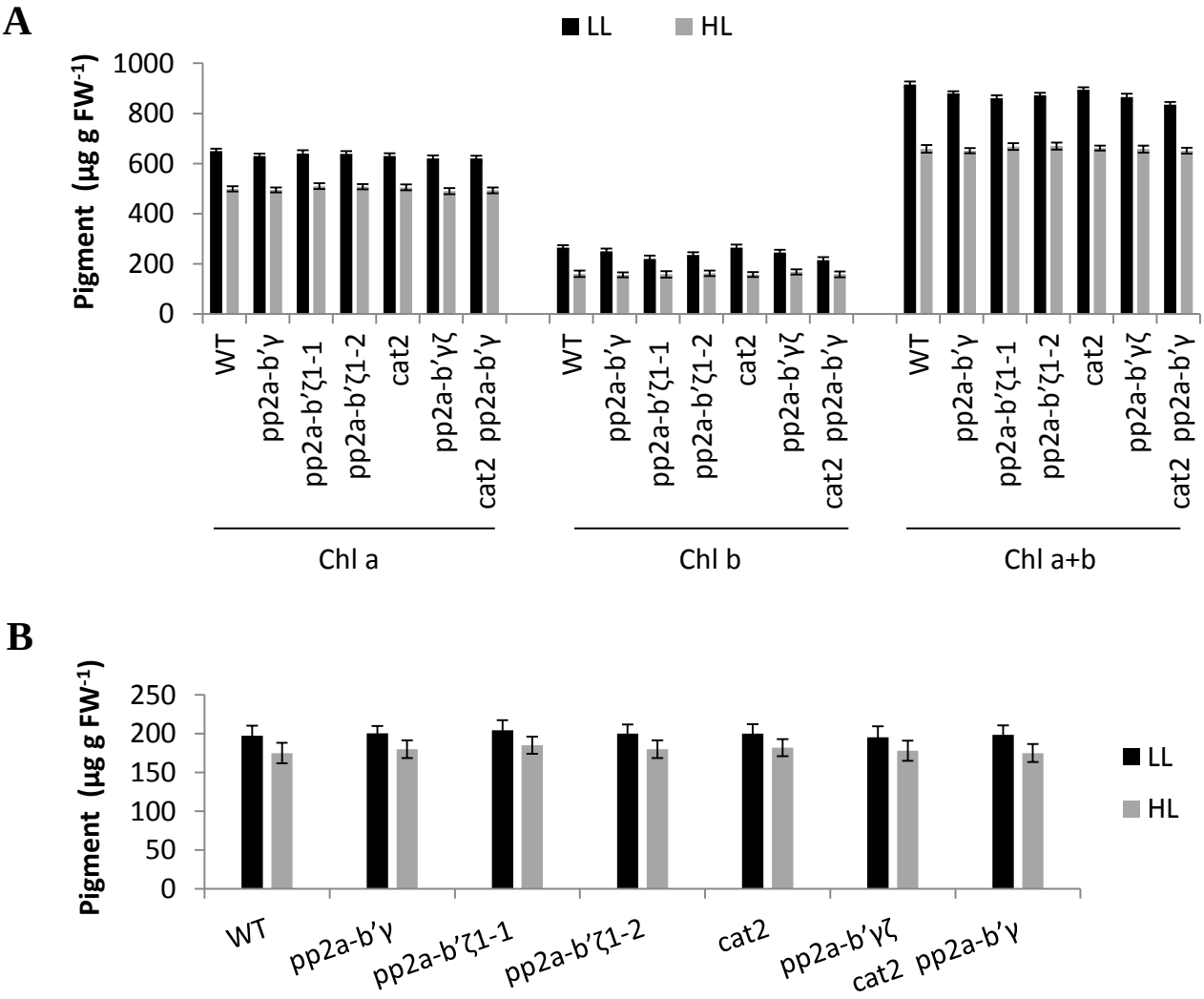


Figure 4.3 A comparison of leaf chlorophyll (A) and carotenoid (B) contents in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL) and high light (HL) conditions. Chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b) and total carotenoid pigments (carotene) were performed on the whole rosettes of plant grown for two weeks under low light (250 µmol m^{-2} s^{-1}) or for two weeks under low light followed by seven days high light (800 µmol m^{-2} s^{-1}). Data are the mean values ± SE (n = 3).

4.2.3 Chlorophyll *a* fluorescence

The ratio of dark adapted variable chlorophyll *a* fluorescence (Fv) to the maximal value of chlorophyll *a* fluorescence (Fm) was measured in the leaves of plants following the transfer from LL to HL conditions (Fig. 4.4). The Fv/Fm ratios and hence the PSII maximum efficiency were decreased in the leaves of all lines during the first 24h of exposure to HL. The HL-induced decreases in the Fv/Fm ratios were similar in all genotypes (Fig. 4.4).

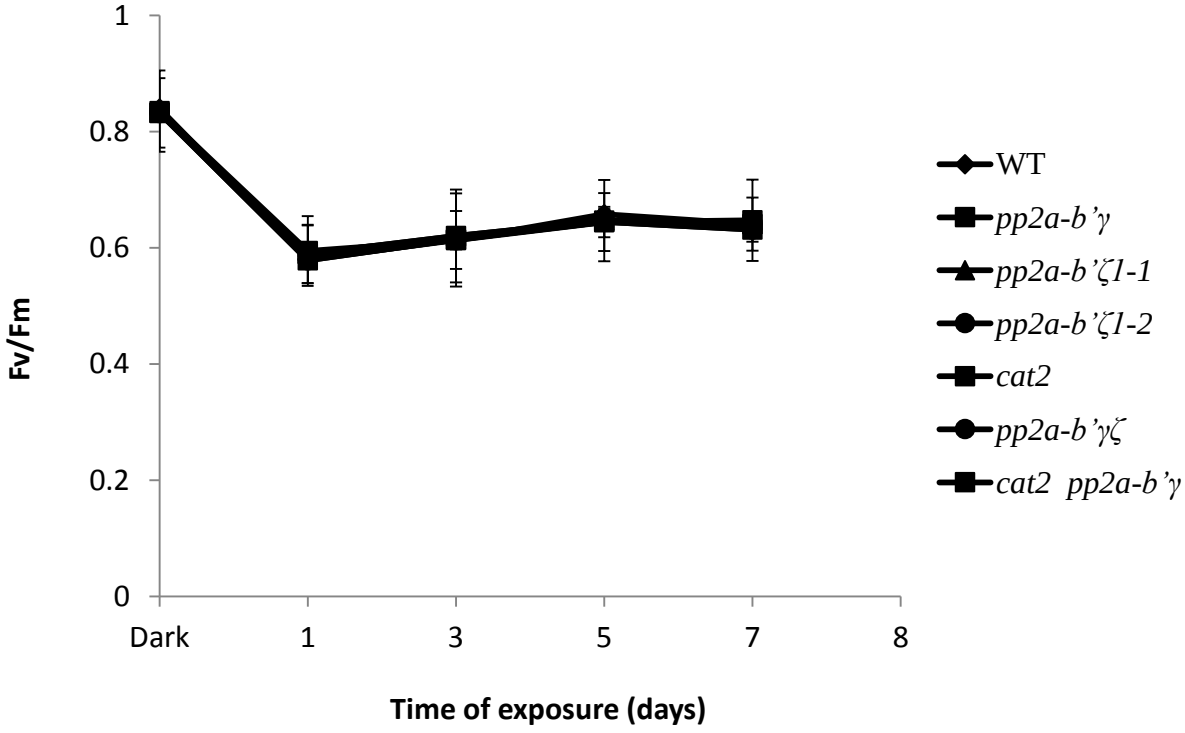


Figure 4.4 A comparison of the ratio of dark-adapted variable chlorophyll *a* fluorescence (Fv) to maximal chlorophyll a fluorescence (Fm) in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL) and high light (HL) conditions. Plants grown for two weeks under low light (250 μmol m^{-2} s^{-1}) and then transferred to high light (800 μmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 10).

4.2.4 Photosynthesis, light and CO_2 response curves

Photosynthetic CO_2 assimilation rates were similar in the leaves of all genotypes under LL conditions. Growth under HL for seven days decreased maximal rates of photosynthesis by about 40% relative to the leaves of plants that had been grown only under LL (Fig. 4.5).

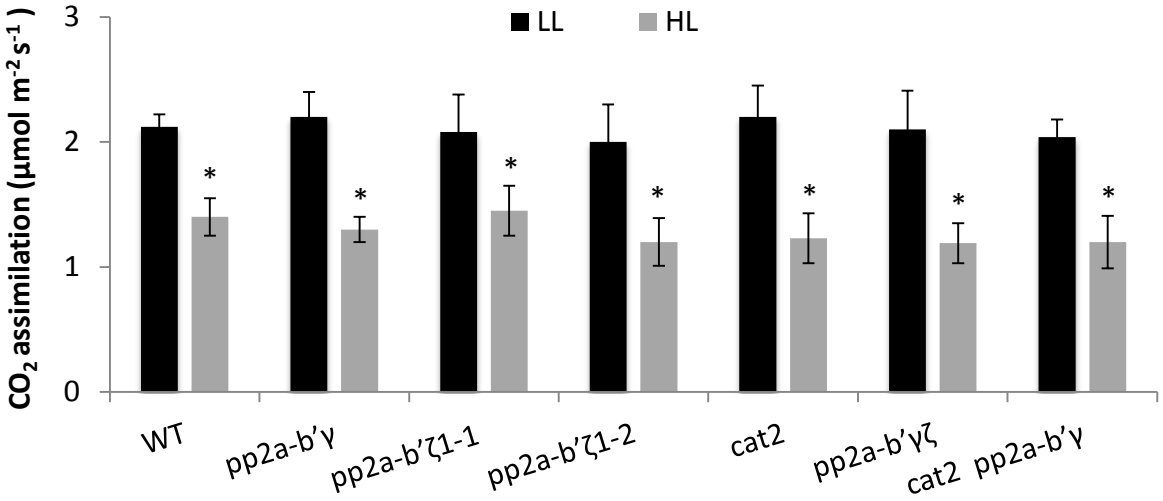


Figure 4.5 A comparison of photosynthetic CO_2 assimilation rates in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL) and high light (HL) conditions. The photosynthetic CO_2 assimilation was measured on the whole rosette leaves of plants that had either been grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days. Photosynthesis was measured at 20°C with an irradiance of 250 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) and an atmospheric CO_2 concentration of 400 µmol mol^{-1}. Data are the mean values ± SE (n = 3). (*$p < 0.05$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype.

Analysis of the light response curves (Fig. 4.6A, B) and the CO_2 response curves for photosynthesis (Fig. 4.7A, B) showed that the initial slopes of both curves were decreased in the leaves of all genotypes that had been grown under HL for seven days compared to those that has been maintained under LL conditions. Moreover, there was a significant HL-dependent decrease in the CO_2 saturated rates of photosynthesis measured in the CO_2 response curve analysis in all genotypes (Fig. 4.7A, B). The HL-dependent decrease in the light-saturated rates of photosynthesis was less marked in light response curve analysis (Fig. 4.6A, B). No significant differences in these parameters were observed between the wild type and mutant lines.

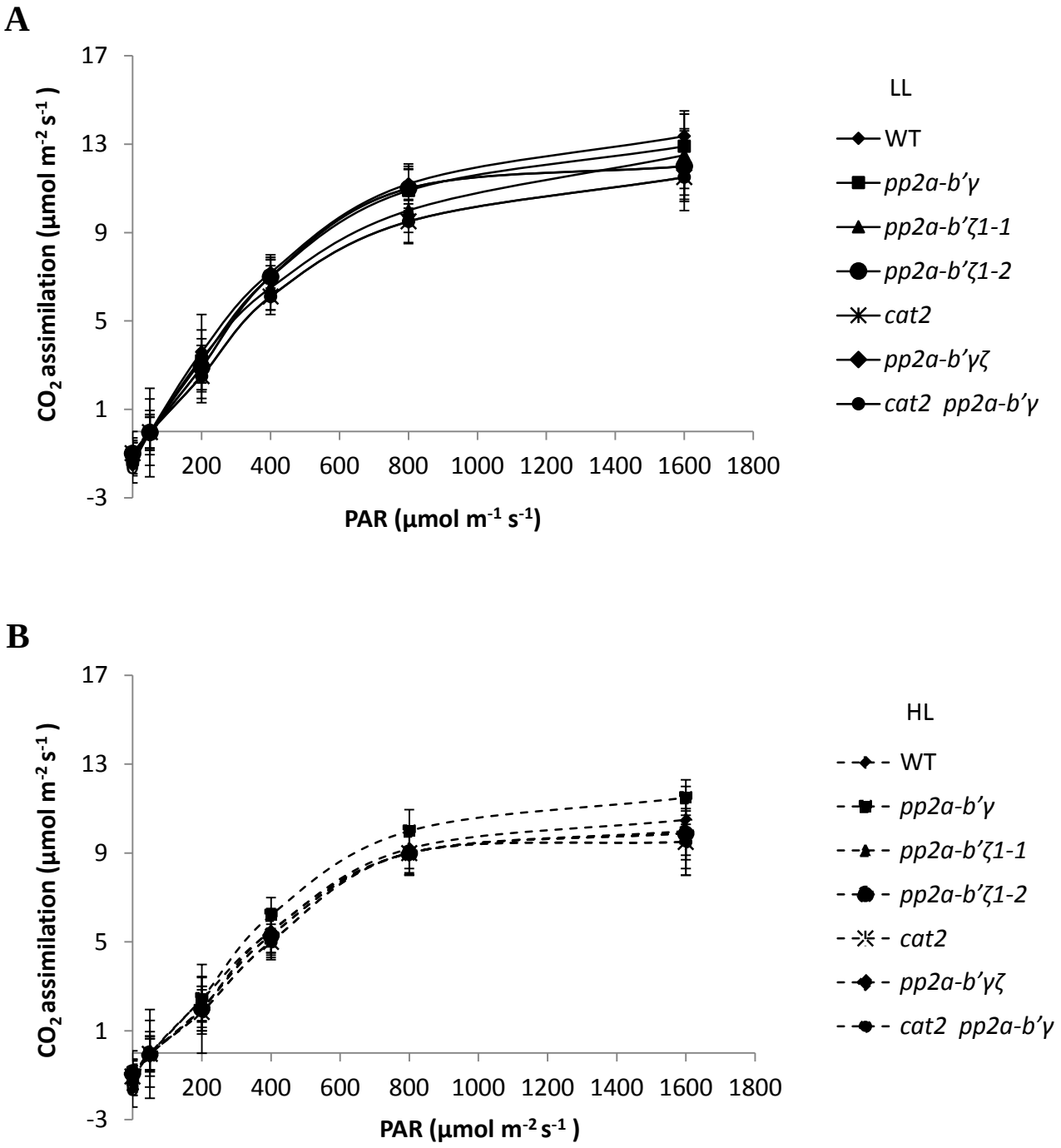
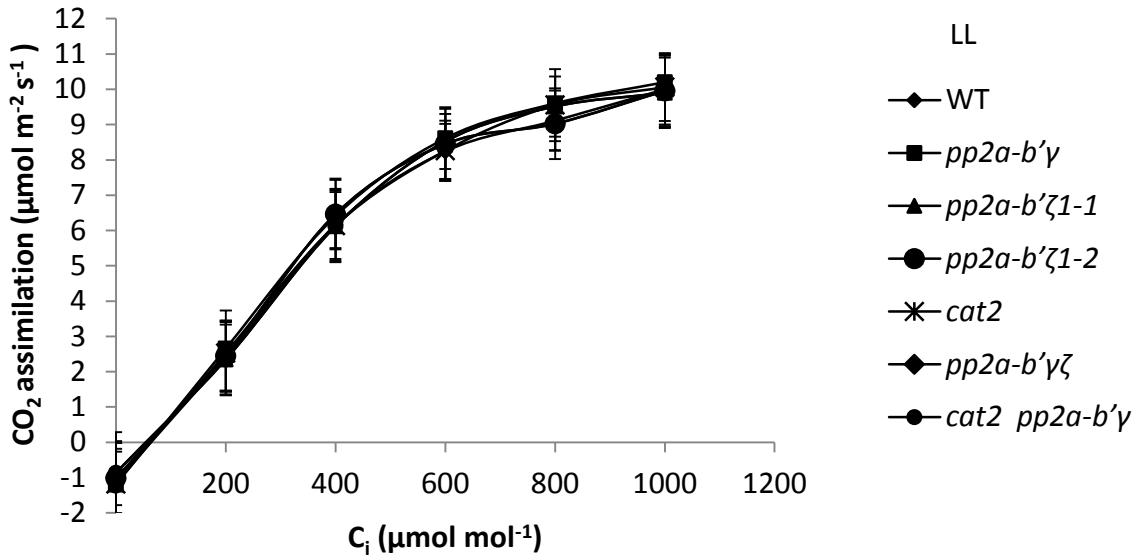


Figure 4.6 A comparison of the light saturation curves for photosynthesis in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL; A) and high light (HL; B) conditions. The light saturation curves for photosynthesis were measured on the whole rosette leaves of plants that had either been grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions **(A)** or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days **(B)**. Data are the mean values ± SE (n = 3).

A



B

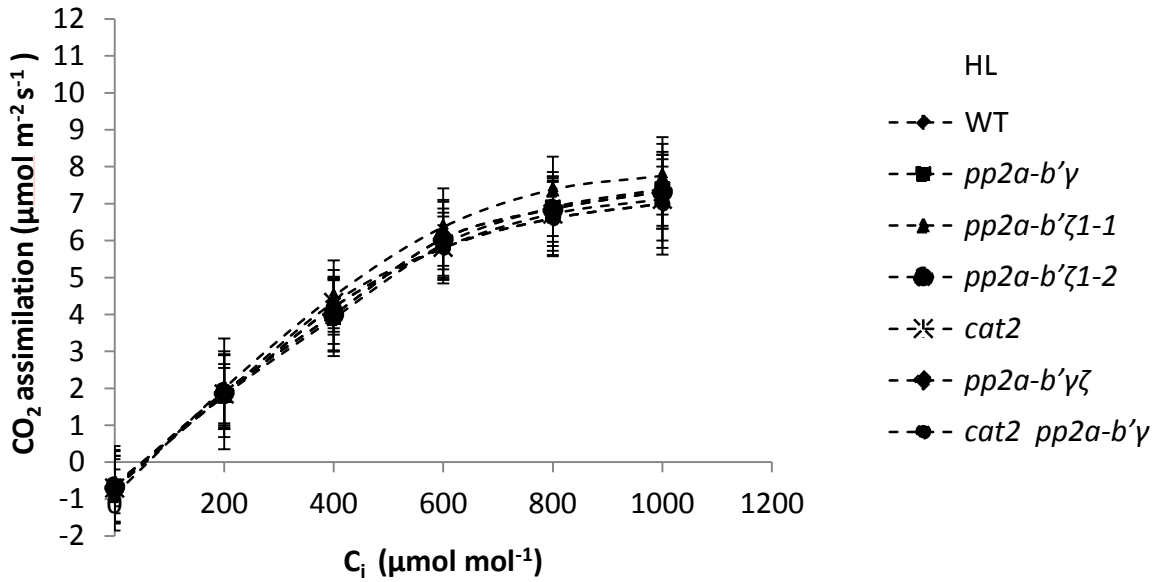


Figure 4.7 A comparison of the CO_2 response curves for photosynthesis in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL; A) and high light (HL: B) conditions. The CO_2 response curves for photosynthesis were measured on the whole rosette leaves of plants that had either been grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions **(A)** or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days **(B)**. For CO_2 response curves measurements were performed at (250 µmol m^{-2} $s^{-}1$) photosynthetically active radiation (PAR). Data are the mean values ± SE (n = 3).

4.2.5 Aphid fecundity

Aphid fecundity was measured in plants that had been grown for two weeks under LL and then either maintained for a further seven days under LL or transferred to HL for seven days (Fig. 4.8). A single one-day-old nymph was placed on each plant and then the total number of offspring was counted after 15 days.

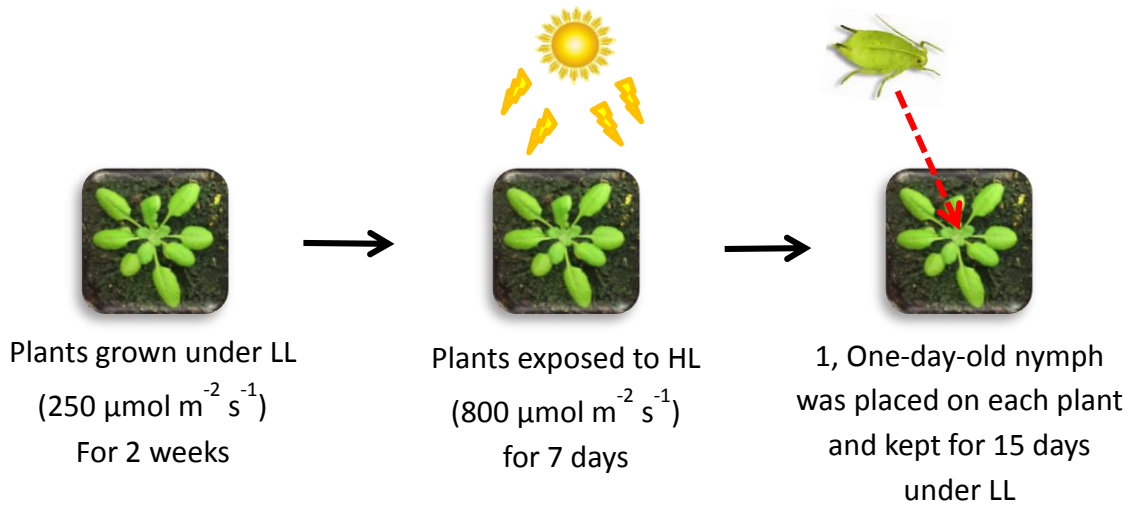


Figure 4.8 Experimental design illustrates aphid fecundity on plants that grown for two weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then transferred to high light (HL; 800 µmol m^{-2} s^{-1}) for seven days.

Aphid fecundity in plants that had been grown only under LL was similar in all genotypes, except for the mutants that lack the γ (*pp2a-b'γ*) subunit of PP2A, catalase-deficient mutant (*cat2*) and the *cat2 pp2a-b'γ* double mutant (Fig. 4.9A, B). The number of aphids was significantly lower on the leaves of the *pp2a-b'γ* mutant and highly significantly lower on the *cat2* and *cat2 pp2a-b'γ* double mutant compared to the wild type under LL conditions (Fig. 4.9). Interestingly, the decrease in aphid fecundity observed in the *pp2a-b'γ* mutant under LL was not observed in the *pp2a-b'γζ* double mutant (Fig. 4.9).

Growth under HL for seven days prior to the analysis of aphid fecundity led to a significant light-dependent decrease in aphid numbers on all genotypes, except for the *cat2* mutant (Fig. 4.9).

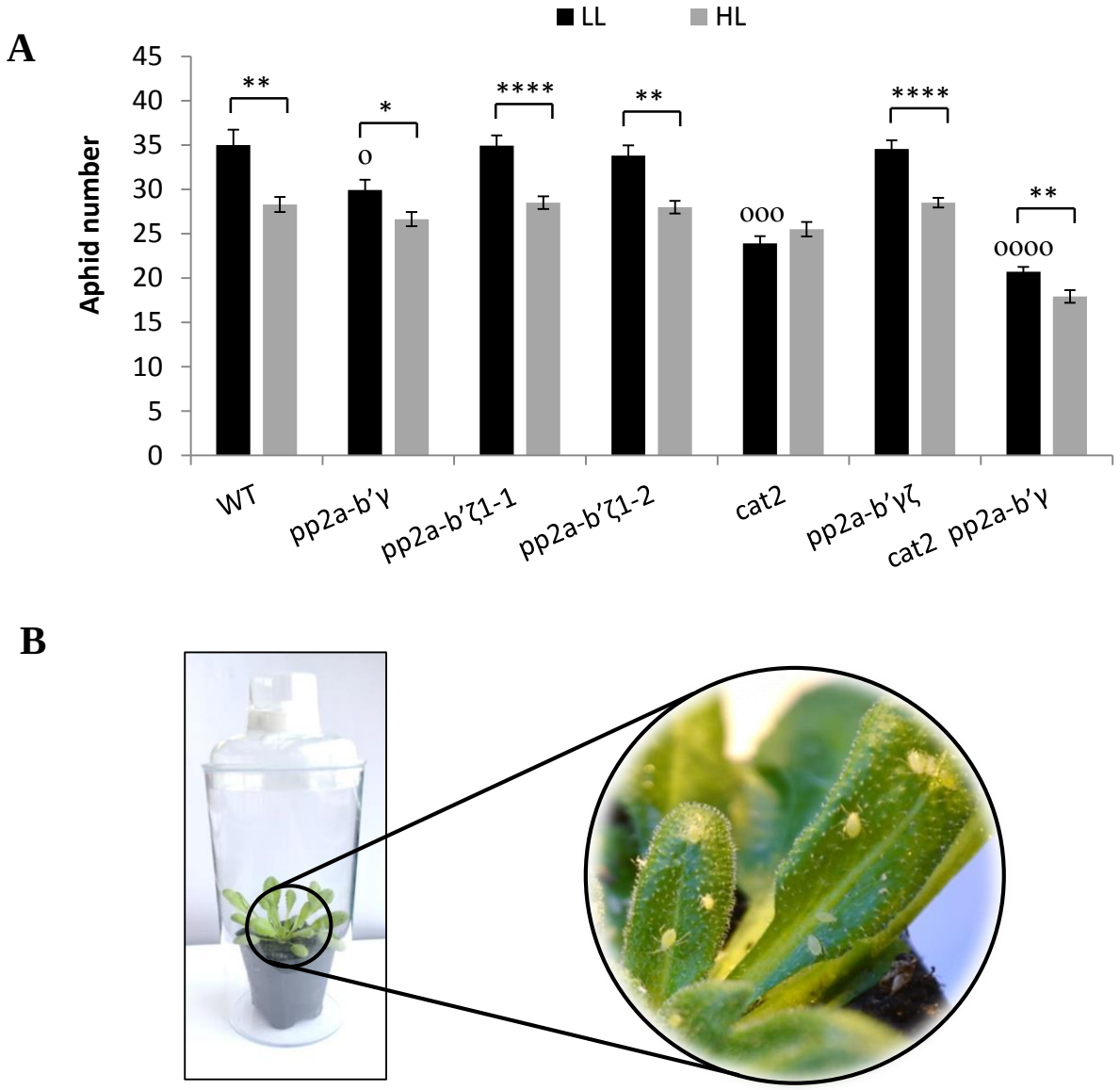


Figure 4.9 A comparison of aphid fecundity in wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*pp2a-b'ζ1-2*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants grown under low light (LL) and high light (HL) conditions. (A) Aphid fecundity. (B) Representative images of adult aphids and new nymphs at 15-days on wild type leaves. The numbers of aphids present on leaves two weeks after the onset of infestation were measured on plants that had either been grown for five weeks under low light (250 μmol m^{-2} s^{-1}) or that had been grown for two weeks under low light followed by seven days under high light (800 μmol m^{-2} s^{-1}) and were then returned low light growth conditions for infestation and subsequent aphid growth for two weeks. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times. (*$p < 0.05$); (**$p < 0.01$); (****$p < 0.0001$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype, as follows (◦$p < 0.05$); (◦◦$p < 0.01$); (◦◦◦$p < 0.001$); (◦◦◦◦$p < 0.0001$) in One-Way ANOVA comparisons between the mutant lines and wild type under LL conditions.

4.2.6 Transcript changes upon aphid infestation

To assess plant responses to aphid infestation, the quantitative real-time PCR was used to analyse the induction of transcripts that related to various defence pathways.

A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four-week-old wild type and mutants that had either been grown for four weeks under low light (250 μmol m^{-2} s^{-1}) or that had been growth for three weeks under low light followed by seven days high light (800 μmol m^{-2} s^{-1}). An empty cage was attached as aphid free control plants. The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid feeding. To assess the induction of different defence pathways, the RNA was isolated and quantitative real-time PCR (qRT-PCR) was performed to analyse the expression level of five genes that they each involve in different defence pathways.

Aphid infestations quickly change the expression of a range of genes that involve in the maintenance of redox homeostasis (Kerchev et al., 2013). The REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1), which is involved in maintenance of redox homeostasis and considered as regulator of cellular redox networks (Khandelwal et al., 2008), was significantly induced in the infested leaves of wild type, *pp2a-b'γ, cat2* and *cat2 pp2a-b'γ* mutants that grown under LL compared to non-infested corresponding genotype under the same conditions (Fig. 4.10A). This induction was more marked in wild type plants (Fig. 4.10A). The induction present in LL grown plants were maintained in HL-treated plants and the magnitude of induction was higher in HL growth conditions (Fig. 4.10B). Furthermore, *RRTF1* was also up-regulated in the infested leaves of the *pp2a-b'γζ* double mutants grown under HL for seven days compared to non-infested leaves under the same conditions. Interestingly, a HL pre-treatment for seven days increased transcript abundance of (RRTF1) in all non-infested genotypes relative to non-infested corresponding genotypes under LL (Fig. 4.10A, B)

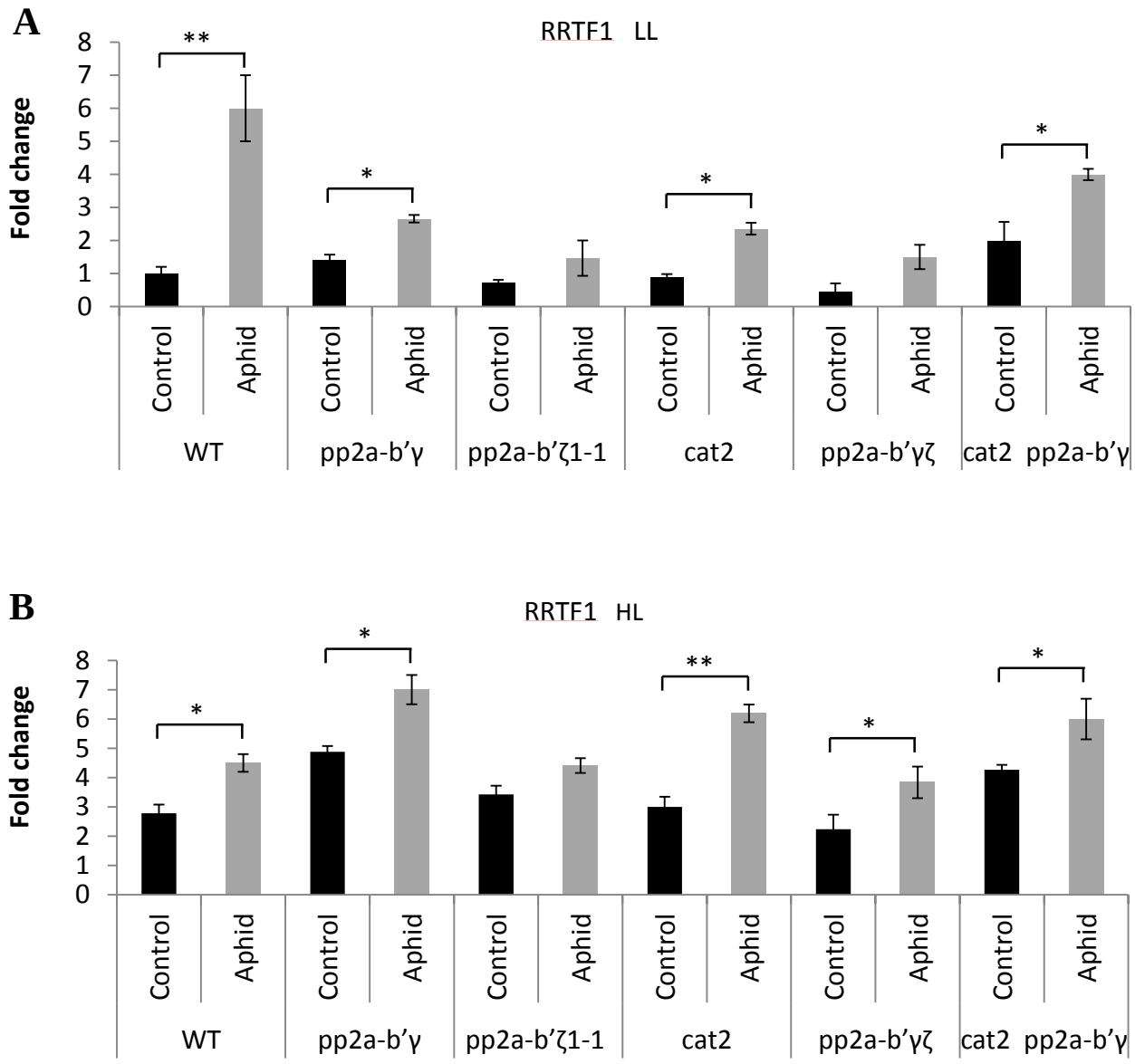


Figure 4.10 Effect of aphid infestation on the abundance of REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1) transcripts in the infested leaves of wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants relative to the aphid-free controls of the corresponding genotype grown under low light (A) and high light (B) conditions. A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four weeks old plants that had either been grown for four weeks under low light (250 μmol m^{-2} s^{-1}) or had been grown for three weeks under low light followed by seven days high light (800 μmol m^{-2} s^{-1}). The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) in LL or HL for each genotype.

Another pathway that is induced quickly due to aphid attack in the plant cells is hormone signalling pathways including salicylic acid (SA). A large numbers of SA signal transduction-related genes were regulated upon aphid infestation (Kerchev et al., 2013).

The SA-associated transcript, WRKY DNA-binding protein 62 transcription factor (WRKY62), was strongly up-regulated upon aphid infestation in the wild type, *pp2a-b'γ, cat2* and *cat2 pp2a-b'γ* mutants grown under LL compared to non-infested corresponding genotypes under the same conditions (Fig. 4.11A). A HL pre-treatment for seven days led to a significant increase in the transcript abundance of (WRKY62) in all genotypes grown under HL relative to non-infested corresponding genotypes under the same conditions (Fig. 4.11B). Interestingly, the transcript of (WRKY62) was present in greater abundance in the leaves of all non-infested genotypes grown under HL relative to non-infested corresponding genotype in LL (Fig. 4.11A, B).

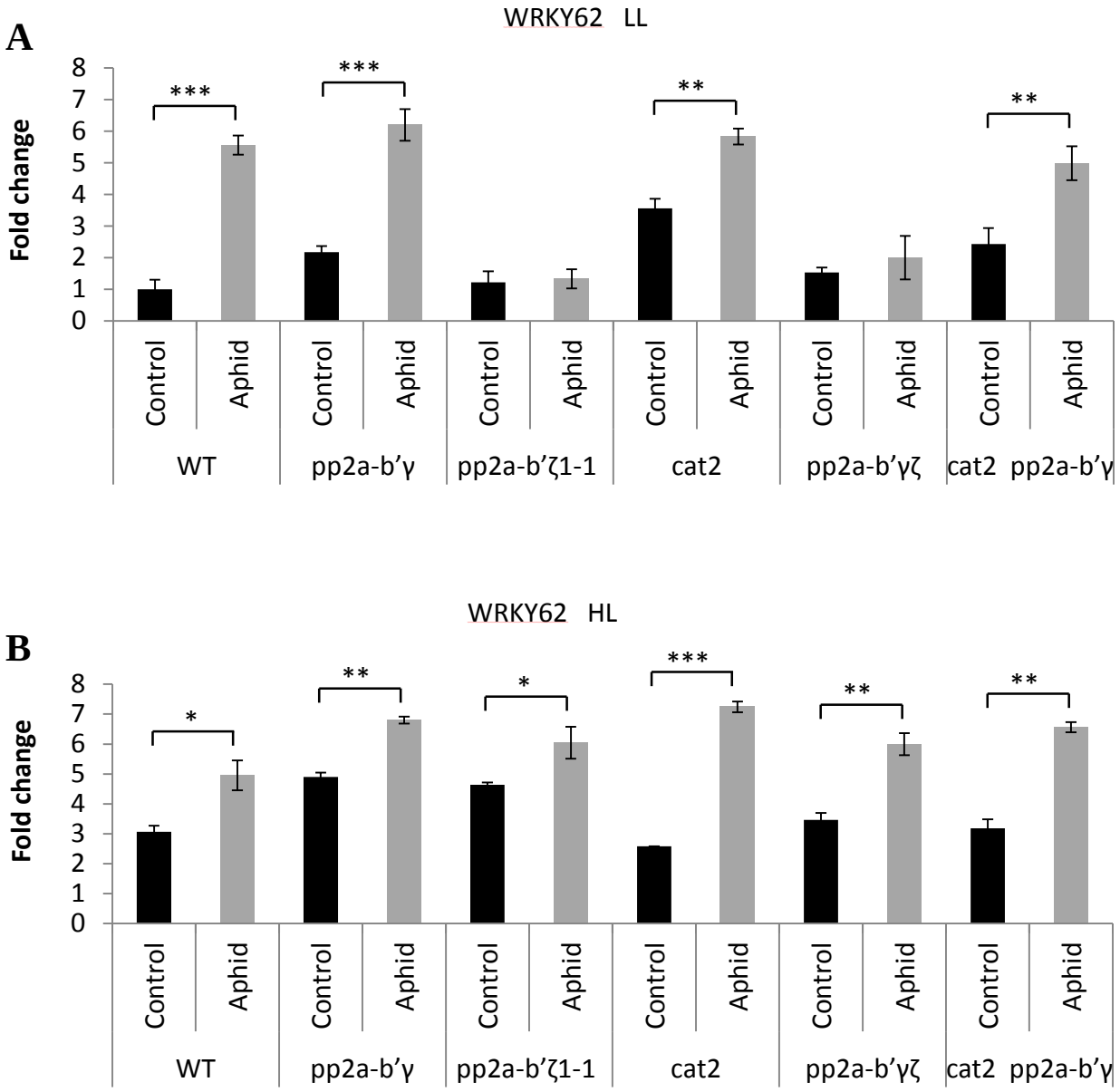


Figure 4.11 Effect of aphid feeding on the expression level of WRKY DNA-binding protein 62 transcription factor (WRKY62) in the infested leaves of wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants relative to the aphid-free controls of the corresponding genotype grown under low light (A) and high light (B) conditions. A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four weeks old plants that had either been grown for 4 weeks under low light (250 μmol m^{-2} s^{-1}) or had been grown for three weeks under low light followed by seven days high light (800 μmol m^{-2} s^{-1}). The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$); (***$p < 0.001$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) in LL or HL for each genotype.

Transcripts related to jasmonic acid (JA) biosynthesis and signalling pathways were induced due to aphid attack such as ALLENE OXIDE CYCLASE 3 (AOC3) and lipoxygenase 5 (LOX5; Kerchev et al., 2013).

The expression of ALLENE OXIDE CYCLASE 3 (AOC3), which is associated to JA synthesis and signalling (Schaller et al., 2008), was only enhanced due to aphid infestation in *cat2 pp2a-b'γ* double mutants grown under LL conditions (Fig. 4.12A). In contrast, the (AOC3) gene was significantly induced in all aphid-infested genotypes that exposed to HL compared to aphid-free plants of the corresponding genotypes grown under the same conditions (Fig. 4.12B). In addition, the transcript of (AOC3) was present in higher abundance in all aphid-free plants exposed to HL for seven days compared to aphid-free plants of the corresponding genotypes grown only under LL (Fig. 4.12A, B).

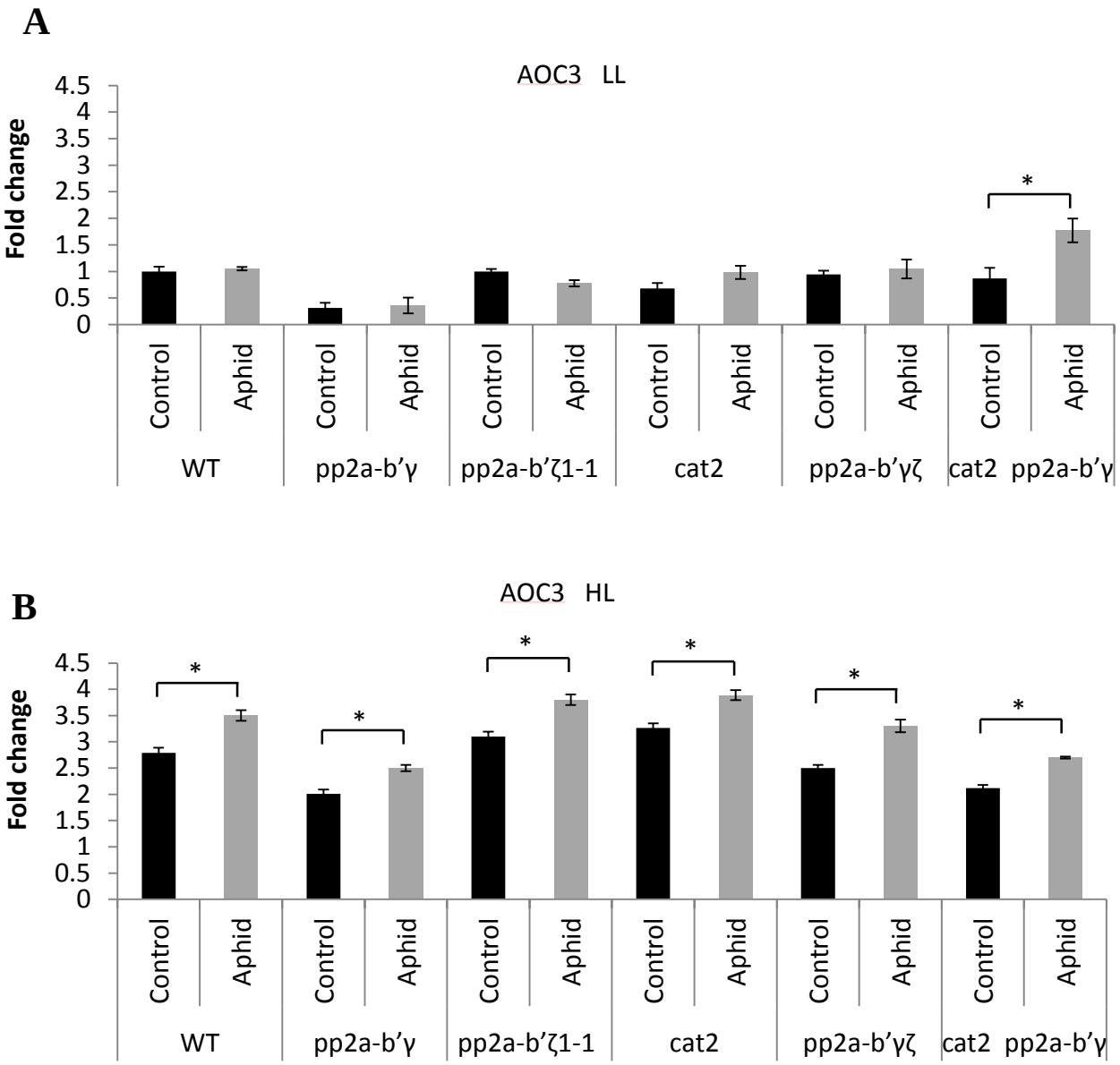


Figure 4.12 Effect of aphid infestation on the expression of ALLENE OXIDE CYCLASE 3 (AOC3) gene in the infested leaves of wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants relative to the aphid-free controls of the corresponding genotype grown under low light (A) and high light (B) conditions. A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four weeks old plants that had either been grown for four weeks under low light (250 µmol m^{-2} s^{-1}) or had been grown for three weeks under low light followed by seven days high light (800 µmol m^{-2} s^{-1}). The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) in LL or HL for each genotype.

Transcripts encoding abscisic acid (ABA)-mediated transcription factors or ABA signalling pathways were altered in the local leaves of the infested Arabidopsis with aphids (Kerchev et al., 2013).

The abundance of ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) transcript, which is ABA-related transcription factor, was significantly increased by aphid feeding on the leaves of wild type, *pp2a-b'γ* and *cat2* mutants grown under LL compared to non-infested leaves of the corresponding genotypes under the same condition (Fig. 4.13A). The expression of (AZF1) was up-regulated in the infested leaves of HL-treated *pp2a-b'γ*, *cat2*, *pp2a-b'γζ* and *cat2 pp2a-b'γ* mutants relative to non-infested leaves of the corresponding genotypes under the same conditions (Fig. 4.13B).

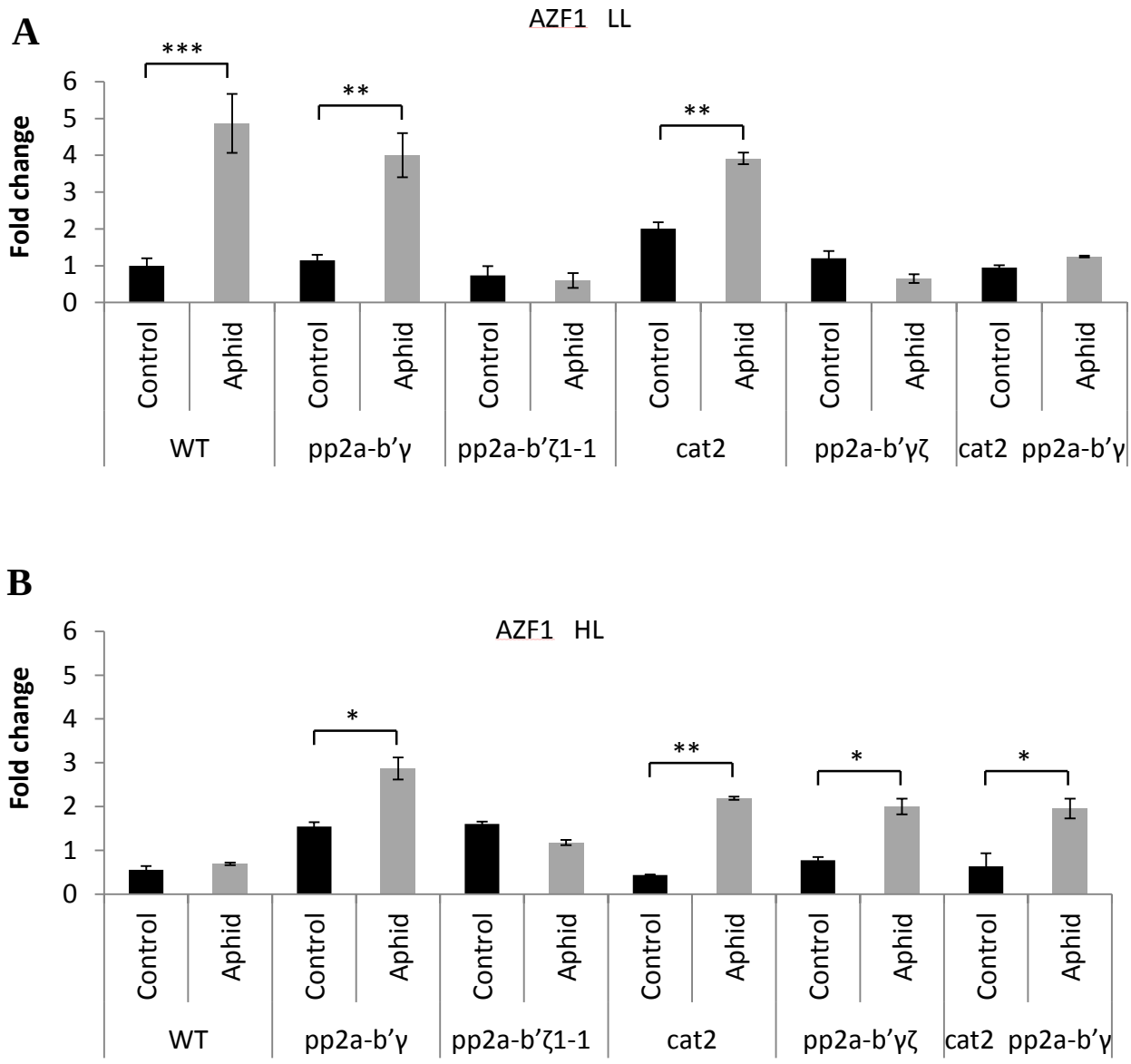


Figure 4.13 Effect of aphid infestation on ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) gene in the infested leaves of wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants relative to the aphid-free controls of the corresponding genotype grown under low light (A) and high light (B) conditions. A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four weeks old plants that had either been grown for 4 weeks under low light (250 µmol m^{-2} s^{-1}) or had been grown for three weeks under low light followed by seven days high light (800 µmol m^{-2} s^{-1}). The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$); (***$p < 0.001$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) in LL or HL for each genotype.

Mitogen activated protein kinases (MAPKs) play important role in the signal transduction pathways such as reactive oxygen species (ROS) and calcium signalling (Takahashi et al., 2011). MAPKs involve or regulate intracellular and extracellular signal transductions through protein phosphorylation on their serine and threonine residues (Rodriguez et al., 2010).

The transcript encoding ATP binding / protein kinase (MAPKKK21), which implicated in mitogen activated protein kinase (MAPK) pathway, was highly significantly enhanced in the infested leaves of *pp2a-b'γ* and *cat2 pp2a-b'γ* mutants relative to non-infested leaves of the corresponding genotypes grown under LL conditions (Fig. 4.14A). Moreover, the expression level of (MAPKKK21) gene was induced by aphid feeding in all genotypes treated with HL for seven days (Fig. 4.14B). Similarly, a HL pre-treatment for seven days increased transcript abundance of (MAPKKK21) in all non-infested genotypes grown under HL relative to non-infested corresponding genotype under LL conditions (Fig. 4.14A, B).

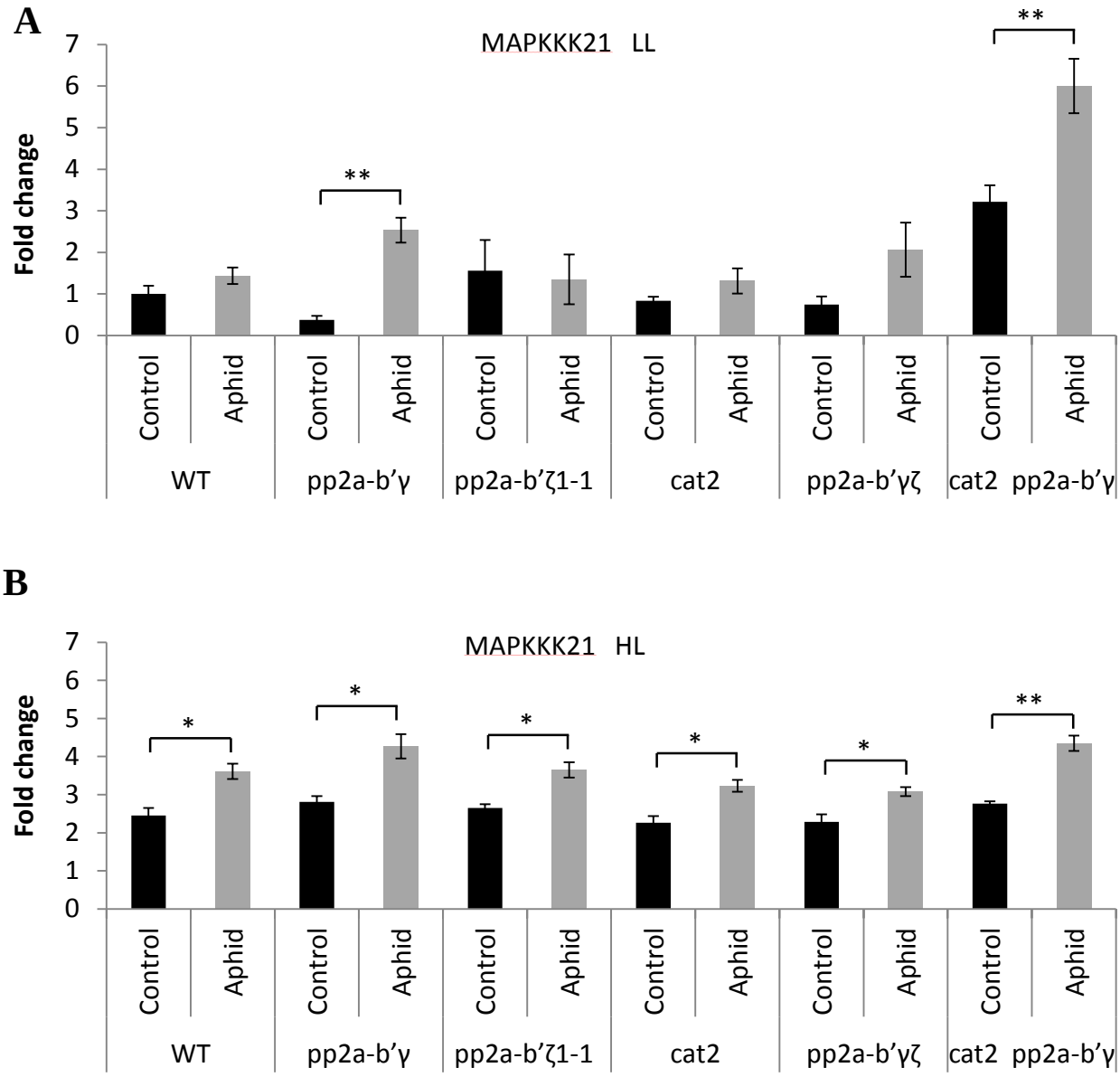


Figure 4.14 Effect of aphid infestation on the expression of ATP binding / protein kinase (MAPKKK21) gene in the infested leaves of wild type (WT) Arabidopsis, (*pp2a-b'γ*), (*pp2a-b'ζ1-1*), (*cat2*), (*pp2a-b'γζ*) and (*cat2 pp2a-b'γ*) mutants relative to the aphid-free controls of the corresponding genotype grown under low light (A) and high light (B) conditions. A clip cage that contained sixty wingless adult aphids was attached to a mature rosette leaf of four weeks old plants that had either been grown for four weeks under low light (250 µmol m^{-2} s^{-1}) or had been grown for three weeks under low light followed by seven days high light (800 µmol m^{-2} s^{-1}). The infested and non-infested caged leaves were collected at an early time-point (6h) following the onset of aphid infestation. RNA was isolated and the expression level estimated as explained in Material and Methods section. Data are the mean values ± SE (n = 3). (*$p < 0.05$); (**$p < 0.01$) in Significance given from analysis by One-Way ANOVA analysis of infested leaves (Aphid) and aphid-free controls (Control) in LL or HL for each genotype.

4.2.7 Aphid fecundity in Arabidopsis mutants altered in glutathione (GSH) synthesis or intracellular partitioning

In the above experiments the *cat2* mutants showed greater resistance to aphid infestation than the wild type plants under LL conditions (Fig. 4.9A). The *cat2* mutants accumulate high levels of camalexin (Chaouch et al., 2010; Han et al., 2013), which are a toxic secondary metabolites to aphids (Kettles et al., 2013; Prince et al., 2014).

The following experiments were performed in order to investigate the role of camalexin in the enhanced aphid resistance in the *cat2* mutants. Aphid fecundity was compared in the wild type Arabidopsis plants and in mutants that are either defective in GSH synthesis [phytoalexin-deficient (*pad2*); cadmium sensitive (*cad2*)] or deficient in both catalase and GSH synthesis (*cat2cad2*), or they are impaired in the partitioning between the chloroplasts and cytosol (*clt*). GSH synthesis mutants such as *pad2* and *cad2* are also camalexin-defective mutants (Parisy et al., 2006). The *pad2* mutants have only about 20% of the GSH levels found in the wild type plants (Parisy et al., 2006). Although *pad2* mutants are more susceptible to pathogens such as *Pseudomonas syringae* and *Pieris brassicae*, it appears that the camalexin deficiency does not have a role in this enhanced susceptibility (Glazebrook and Ausubel, 1994; Roetschi et al., 2001). The *cad2* mutants have about 20-30% of the GSH levels found in the wild type plants (Cobbett et al., 1998). The *cad2* mutants also have low camalexin contents and are more susceptible to the pathogen *Pseudomonas syringae* (Ball et al., 2004). The CHLOROQUINERESISTANCE TRANSPORTER (PfCRT)-LIKE TRANSPORTER1 (CLT1) are GSH transporters on the plastid envelope membranes, transporting GSH and γ-EC from the chloroplasts to the cytosol (Maughan et al., 2010). The *clt* mutants have a similar amount of GSH in the chloroplasts to the wild type plants but they have low cytosolic GSH levels and are consequently more sensitive to the pathogen *Phytophthora brassicae* relative to the wild type plants (Maughan et al., 2010). Plants from all genotypes were grown for two weeks under LL (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL conditions or transferred to HL (800 μmol m^{-2} s^{-1}) for seven days, prior to the onset of aphid infestation.

4.2.7.1 Shoot phenotypes of GSH-defective mutants under LL and HL conditions

When wild type and mutant lines were grown for three weeks under short day conditions (8h photoperiod) with LL, the *cat2* mutants and *cat2 cad2* double mutants had visibly smaller rosettes than the wild type (Fig. 4.15).

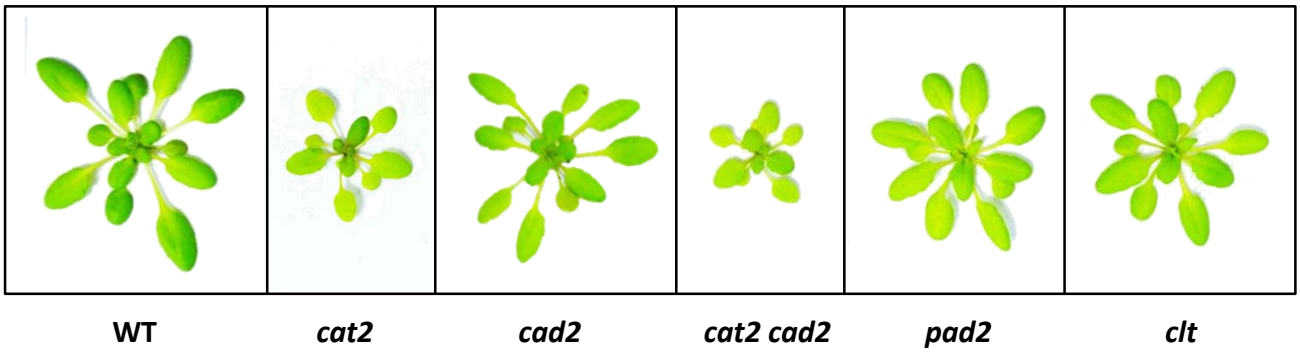


Figure 4.15 A comparison of the rosette phenotypes at week 3 in wild type (WT) Arabidopsis, *cat2*, *cad2*, *cat2 cad2*, *pad2* and *clt* mutants grown under low light (LL) conditions. Plants were grown for three weeks under low light (250 μmol m^{-2} s^{-1}) conditions.

4.2.7.2 Aphid fecundity on GSH-deficient mutants

Aphid fecundity was significantly lower in all the mutant genotypes, except for *clt*, compared to wild type plants when the experiment was performed under LL (Fig. 4.16). Growth under HL for seven days prior to the analysis of aphid fecundity led to a significant light-dependent decrease in aphid numbers only on wild type plants (Fig. 4.16). In all other genotypes aphid fecundity was similar under LL and HL conditions. Moreover, aphid numbers were similar on the *cat2*, *cad2* mutants and *cat2cad2* double mutants under both LL and HL conditions.

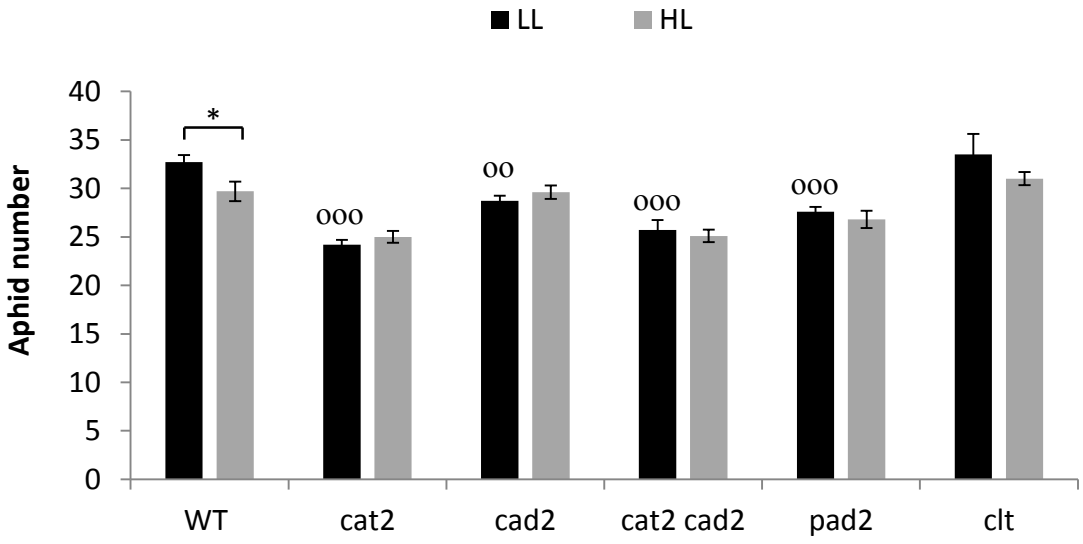


Figure 4.16 A comparison of aphid fecundity in wild type (WT) Arabidopsis, *cat2*, *cad2*, *cat2 cad2*, *pad2* and *clt* mutants grown under low light (LL) and high light (HL) conditions. The numbers of aphids present on leaves two weeks after the onset of infestation were measured on plants that had either been grown for five weeks under low light (250 μmol m^{-2} s^{-1}) or that had been growth for two weeks under low light followed by seven days under high light (800 μmol m^{-2} s^{-1}) and were then returned low light growth conditions for infestation and subsequent aphid growth for two weeks. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times. (*$p < 0.05$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype, as follows (◦◦$p < 0.01$); (◦◦◦$p < 0.001$) in One-Way ANOVA comparisons between the mutant lines and wild type under LL conditions.

4.3 Discussion

The role of protein phosphatase 2A (PP2A) and increased intracellular oxidation, caused by loss of a functional respiratory catalase, in plant responses to growth light intensity and to aphid infestation were studied in the mutants lacking either the γ or ζ of subunits of PP2A, or CATALASE-2 (*cat2*) and in the *cat2 pp2a-b'γ* double mutants. All mutant genotypes except for *pp2a-b'ζ1-1* had visibly smaller rosettes than the wild type when grown under short days with LL (250 μmol m^{-2} s^{-1}; Fig. 4.1) conditions. These findings are consistent with previous reports concerning the shoot phenotypes of these mutants grown under similar growth conditions (Trotta et al., 2011a, b; Li et al., 2013).

A pre-exposure to HL (800 μmol m^{-2} s^{-1}) under short day conditions for seven days significantly increased the rosette size in all of the mutant genotypes except for *cat2*, relative to LL conditions (Fig. 4.2B). The HL-dependent increase in growth in the mutants lacking the B'γ (gamma; *pp2a-b'γ*) or B'ζ (zeta; *pp2a-b'ζ1-1* and *pp2a-b'ζ1-2*) subunits or lacking both subunits suggests that the restriction on growth imposed by the loss of the regulation afforded by these subunits is overcome by HL-dependent pathways. This was also the case for the *cat2 pp2a-b'γ* double mutants but not the *cat2* mutants, suggesting that the limitation on growth observed in *cat2*, could be partially overcome in HL manner by loss of a function PP2A-B'γ protein. The decrease in shoot growth resulting from increased oxidation in the *cat2* mutants, which might be related to altered auxin metabolism and signalling, is therefore at least in part regulated in a PP2A-dependent manner.

Leaves of all genotypes that has been given a HL pre-treatment for seven days had lower chlorophyll contents, with decreased Fv/Fm ratios compared to the plants that been grown under LL conditions alone (Figs. 4.3 and 4.4). Moreover, the rates of photosynthetic CO_2 assimilation measured at 250 μmol m^{-2} s^{-1} in the leaves of HL-treated plants were lower than those of plants that had only experienced LL (Fig. 4.5). The light-dependent decreases in photosynthetic CO_2 assimilation were similar in all lines. These finding suggest that the PP2A subunit composition and the presence of catalase does not influence the acclimation of photosynthesis to HL observed in the wild type plants.

Aphid fecundity under LL conditions was similar on all genotypes, except for the *pp2a-b'γ*, *cat2* and the *cat2 pp2a-b'γ* double mutants (Fig. 4.9). Aphid fecundity was significantly lower on the *pp2a-b'γ*, *cat2* and the *cat2 pp2a-b'γ* double mutants relative to the wild type under LL conditions, being most decreased in the *cat2 pp2a-b'γ* double mutants. These results show that PP2A-B'γ and catalase negatively control plant resistance to aphids under LL. While knock-down *pp2a-b'γ* mutants show decreased aphid fecundity, the *pp2a-b'γζ* double mutant supports aphid propagation in a similar manner to wild type plants (Fig. 4.9) suggesting that the pathway influencing aphid resistance is functionally connected with PP2A-B'ζ, which in turn seems to have a positive impact on defence signalling. In contrast, the *cat2 pp2a-b'γ* double mutants, which were reported to show constitutive induction of pathogenesis responses, were more resistant to aphids than either of the single mutants alone. The observed decrease in aphid fecundity suggests that control of cellular redox state and PP2A-B'γ have roles in the control of aphid resistance.

Growth under HL for seven days prior to the analysis of aphid fecundity led to a significant light-dependent decrease in aphid numbers on all genotypes, except for the *cat2* mutant (Fig. 4.9). These findings suggest that the HL pre-treatment activated the innate immune defences that limit aphid infestation in all genotypes, except for the *cat2* mutants. Like the HL-dependent effects on shoot biomass accumulation, a HL-dependent inhibition of aphid fecundity was observed in the *cat2 pp2a-b'γ* double mutants but not in the *cat2* mutants. This finding suggests that the PP2A-B'γ protein mediates the HL-dependent regulation of aphid fecundity. The analysis of the aphid-dependent changes in abundance of transcripts related to defence pathways performed in these studies revealed that levels of mRNAs encoding REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1), WRKY DNA-binding protein 62 transcription factor (WRKY62) and ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) were significantly increased following aphid infestation in all genotypes under LL conditions except for the *pp2a-b'ζ1-1* mutant and *pp2a-b'γζ* double mutant. The PP2A-B*'ζ* subunit may therefore positively influence the pathways that trigger *AZF1* and *RRTF1* expression in response to aphids. In contrast, after the HL pre-treatment WRKY62, ALLENE OXIDE CYCLASE 3 (AOC3) and ATP binding / protein kinase (MAPKKK21) were increased in all genotypes following aphid infestation.

The PP2A-B'γ regulates organellar ROS signalling and plays a key role in the negative control of SA-linked responses and associated metabolic alterations in *A. thaliana* (Trotta et al., 2011; Li et al., 2014). Metabolite profiling of the *pp2a-b'γ* mutants showed that PP2A-B'γ modulates amino acid metabolism and the biosynthesis of camalexin under conditions of intracellular oxidative stress (Li et al., 2014). Moreover, an analysis of previously published microarray data revealed up-regulation of SA signalling and cell death pathways for *pp2a-b'γ* mutants (Trotta et al., 2011). While no transcripts related to callose synthesis are differentially increased in the gamma (*pp2a-b'γ*) mutants, mRNAs encoding the beta-glucanase pathogenesis-related protein (PR2), which negatively regulates the deposition of the callose, were increased relative to the wild type, together with other PR transcripts. The observed decreases in aphid fecundity on the *pp2a-b'γ*, *cat2* and the *cat2 pp2a-b'γ* double mutants relative to the wild type under LL conditions might therefore be related to the differential accumulation of amino acids and secondary metabolites in these mutants relative to the wild type. However, future work is required to identify the precise nature of the the roles of secondary metabolites in the regulation of aphid fecundity in these mutants because the aphid numbers were similar on the *pp2a-b'γζ* double mutants to the wild type controls under LL conditions.

Aphid fecundity was similar on the *cat2, cad2* mutants and *cat2cad2* double mutants under both LL and HL conditions. While the *cat2* mutants accumulate camalexins, the *cad2* mutants are deficient in this secondary metabolite, as are as the *cat2cad2* mutants. These findings would suggest that that lower aphid numbers on these mutants compared to the wild type plants under both LL and HL conditions, was not caused by effects on camalexin production or accumulation.

Chapter 5. Role of apoplastic redox state on Arabidopsis responses to aphid infestation

5.1 Introduction

The apoplast/cell wall compartment of the plant cell has crucial roles in the transport of water and nutrients, preserving cell shape and regulating growth, as well as being the first line of defence against environmental changes and biotic stresses (Sakurai, 1998). For example, it is the site of the pathogen-triggered oxidative burst that has a key role in plant immune responses. Unlike many of the intercellular compartments, apoplast/cell wall has very few low molecular weight antioxidants. However, unlike most other low molecular weight antioxidants, L-ascorbic acid (vitamin C) is abundant in the apoplast/cell wall, where it fulfils important roles in the control of redox reactions (Foyer and Noctor, 2000).

Ascorbic acid is the most abundant low molecular weight antioxidant in plants, with a central role in H_2O_2 detoxification (Zheng and Vanhuystee, 1992; Noctor and Foyer, 1998). The levels of ascorbate in the apoplast are tightly controlled in relation to cell growth and expansion (Horemans et al., 2000). Although some ascorbate may be degraded within the symplasm, the major pathway of ascorbate degradation may occur within the apoplast, following oxidation of ascorbate to DHA (Green and Fry 2005). The oxidation of ascorbate in the apoplast is catalysed by the enzyme ascorbate oxidase (AO), which is encoded by three genes (*AO1*, *AO2*, and *AO3*) in Arabidopsis (Lim, 2012). AO is a copper-containing protein catalysing the reduction of molecular oxygen to water using ascorbate as the electron donor (Suzuki and Ogiso, 1973; Kato and Esaka, 1999). The activity of AO is considered to be important in limiting the accumulation of ascorbate in the apoplast (Pignocchi and Foyer, 2003). The Arabidopsis *ao1* and *ao3* mutants and *ao1ao3* double mutants were used to assess the functions of AO on tolerance to either high light or drought (Lim, 2012). While the *ao1* had a similar level of AO activity to that of wild type, the *ao3* and *ao1ao3* double mutant contained only about 10-20% of wild type AO levels (Lim, 2012). Crucially, no phenotypic differences were observed in any of the AO mutant lines, and they showed similar responses to high light or drought stress conditions to the wild type plants (Lim, 2012).

In addition, the *AO1*, *AO2*, and *AO3* genes were silenced using microRNA technology in transgenic Arabidopsis lines (*amiR-AO*; Lim, 2012). AO activities were below the levels of detection in the transgenic amiR-*AO* lines, which had larger rosettes than wild type plants under either optimal or stress conditions (Lim, 2012).

The following studies were performed on the transgenic amiR-*AO* lines, to assess the role of AO activity on plant responses to high light and to aphid infestation. To test the effects of high light, wild type plants and transgenic lines were grown for two weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light or transferred to high light (HL; 800 μmol m^{-2} s^{1}) for seven days. Ascorbate and photosynthesis measurements were then performed on leaves of plants grown under either low or high light conditions. Similarly, aphid fecundity measurements were performed on wild type plants and transgenic lines grown under low or high light conditions.

5.2 Results

5.2.1 Shoot phenotypes under low and high light conditions

The Arabidopsis *amiR-AO* transgenic lines, *amiR-AO (3.6*) and *amiR-AO (8.5)*, that were grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) had visibly bigger rosette phenotypes than the wild type plants (Fig. 5.1). This difference in growth phenotype was maintained in the plants that were grown for two weeks under LL and then transferred for seven days to high light (HL; 800 µmol m^{-2} s^{-1}) growth conditions (Fig. 5.1).

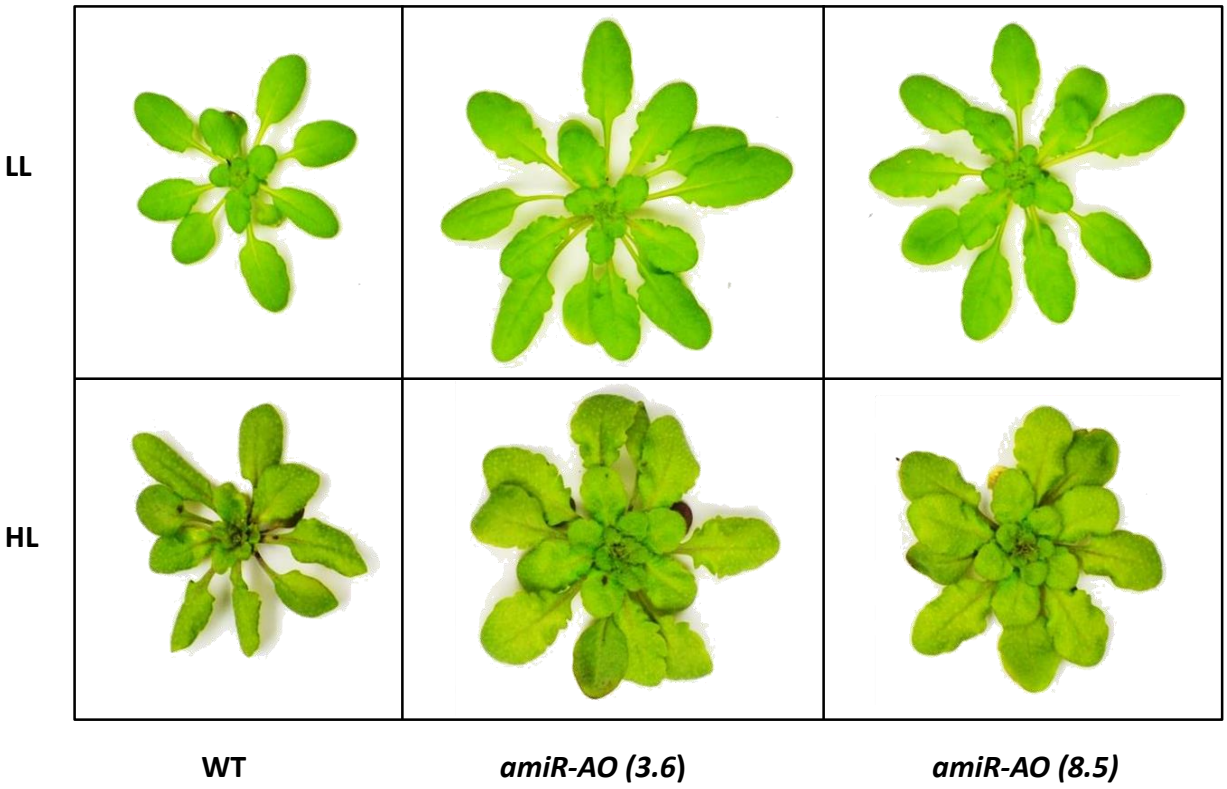


Figure 5.1 A comparison of the rosette phenotypes in 3-week-old wild type (WT) Arabidopsis, *amiR-AO (3.6*) and *amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. Plants grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions (top row) or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days (bottom row).

Leaf area and rosette diameter measurements were performed on plants that had either been grown for three weeks under LL or for two weeks under LL followed by seven days HL.
Both *amiR-AO* transgenic lines, *amiR-AO (3.6*) and *amiR-AO (8.5)*, had significantly bigger leaf areas and rosette diameter under both LL and HL conditions compared to the wild type plants (Fig. 5.2A, B). Furthermore, a HL pre-treatment for seven days did not lead to significant changes in the leaf areas and rosette diameters in any genotypes compared to the corresponding genotypes that grown only under LL conditions (Fig. 5.2A, B).

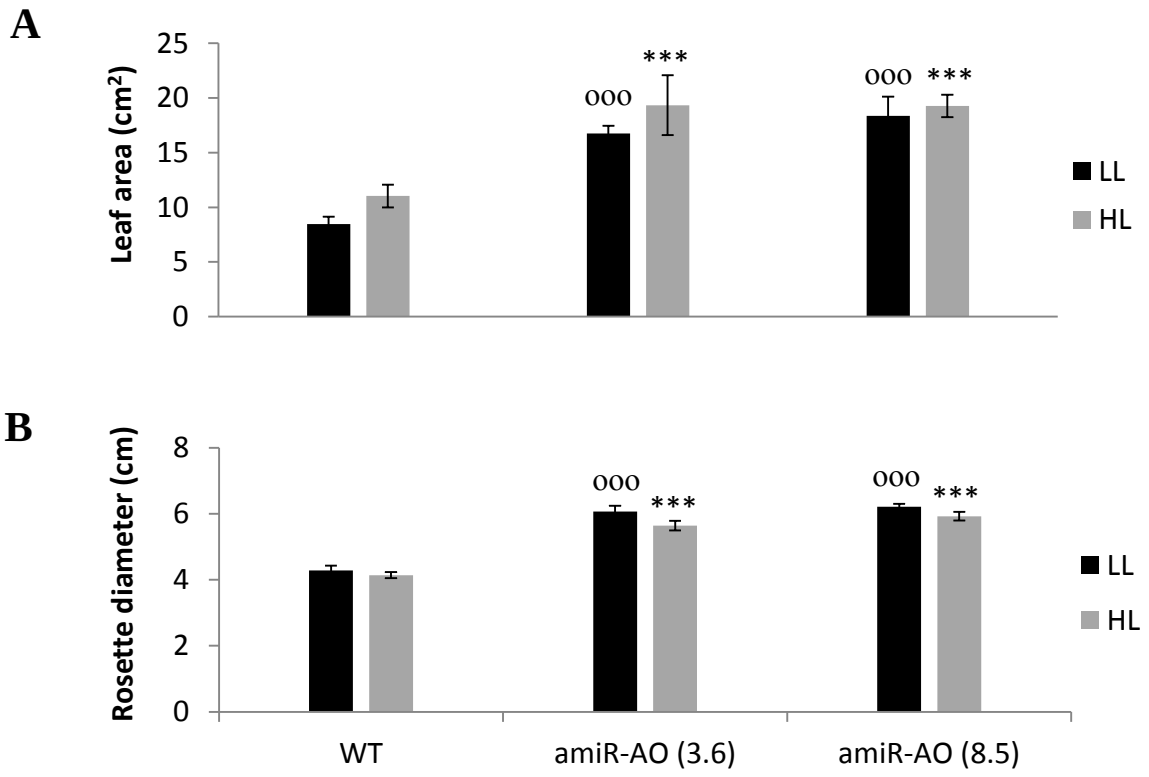


Figure 5.2 A comparison of rosette leaf areas (A) and rosette diameter (B) in the Arabidopsis *amiR-AO (3.6)* and *amiR-AO (8.5)* transgenic plants to the wild type (WT) plants grown under low light (LL) and high light (HL) conditions. Plants either grown for three weeks under low light (250 µmol m^{-2} s^{-1}) or for two weeks under low light and then transferred to high light (800 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 10). (◦◦◦$p < 0.001$) in Significance given from analysis by One-Way ANOVA comparisons between the transgenic lines and wild type under LL conditions, as follows (***$p < 0.001$) in One-Way ANOVA comparisons between the mutant lines and wild type under HL conditions.

5.2.2 Ascorbate oxidase (AO) activity

The activity of apoplastic ascorbate oxidase (AO) enzyme was assayed in the wild type and the *amiR-AO* transgenic lines. AO is apoplastic enzyme that oxidises ascorbate (AsA) to dehydroacorbate (DHA).

The AO activities were below the levels of detection in the leaves of *amiR-AO* transgenic lines grown under LL or HL conditions. This result confirms that the AO activity was fully abolished in these lines due to the transgene (Fig. 5.3). Moreover, the leaves of wild type plants had similar AO activity under both LL and HL conditions (Fig. 5.3).

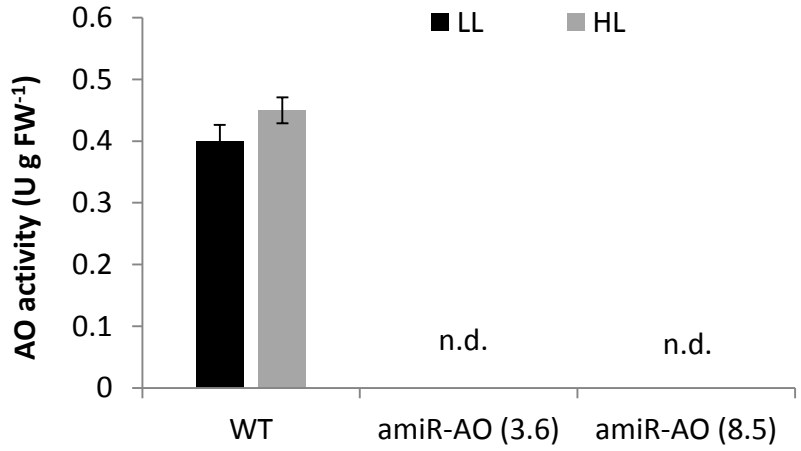


Figure 5.3 Assay of apoplastic ascorbate oxidase (AO) activity in 3-week-old wild type (WT) Arabidopsis and *amiR-AO (3.6), amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. Plants grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days. One unit of AO activity equal the amount of enzyme required to oxidise (1µmol ascorbate min^{-1}) at 25°C. Data are the mean values ± SE (n = 3). N.d., not detected**.**

5.2.3 Whole leaf and apoplastic ascorbate content

To investigate the effect of the *amiR-AO* transgene in both (3.6) and (8.5) transgenic lines on the redox state of apoplastic ascorbate, the ascorbate concentration was determined in the apoplastic of wild type and both *amiR-AO* transgenic lines. The apoplastic or intercellular washing fluid was extracted by vacuum infiltration for the whole rosette at week-3.

The reduced ascorbate (AsA) content in both *amiR-AO* transgenic lines was higher than that of wild type under both LL and HL conditions (Fig. 5.4A). The highest level of (AsA) was in the *amiR-AO (8.5)* line followed by the *amiR-AO (3.6)* line and then wild type plants (Fig. 5.4A).

There was no significant difference in the total apoplastic ascorbate content in all genotype plants grown under both LL and HL conditions (Fig. 5.4A). Furthermore, no significant difference was found in the extracted amount of intercellular washing fluid in all genotypes grown under both LL and HL conditions (Fig. 5.4B).

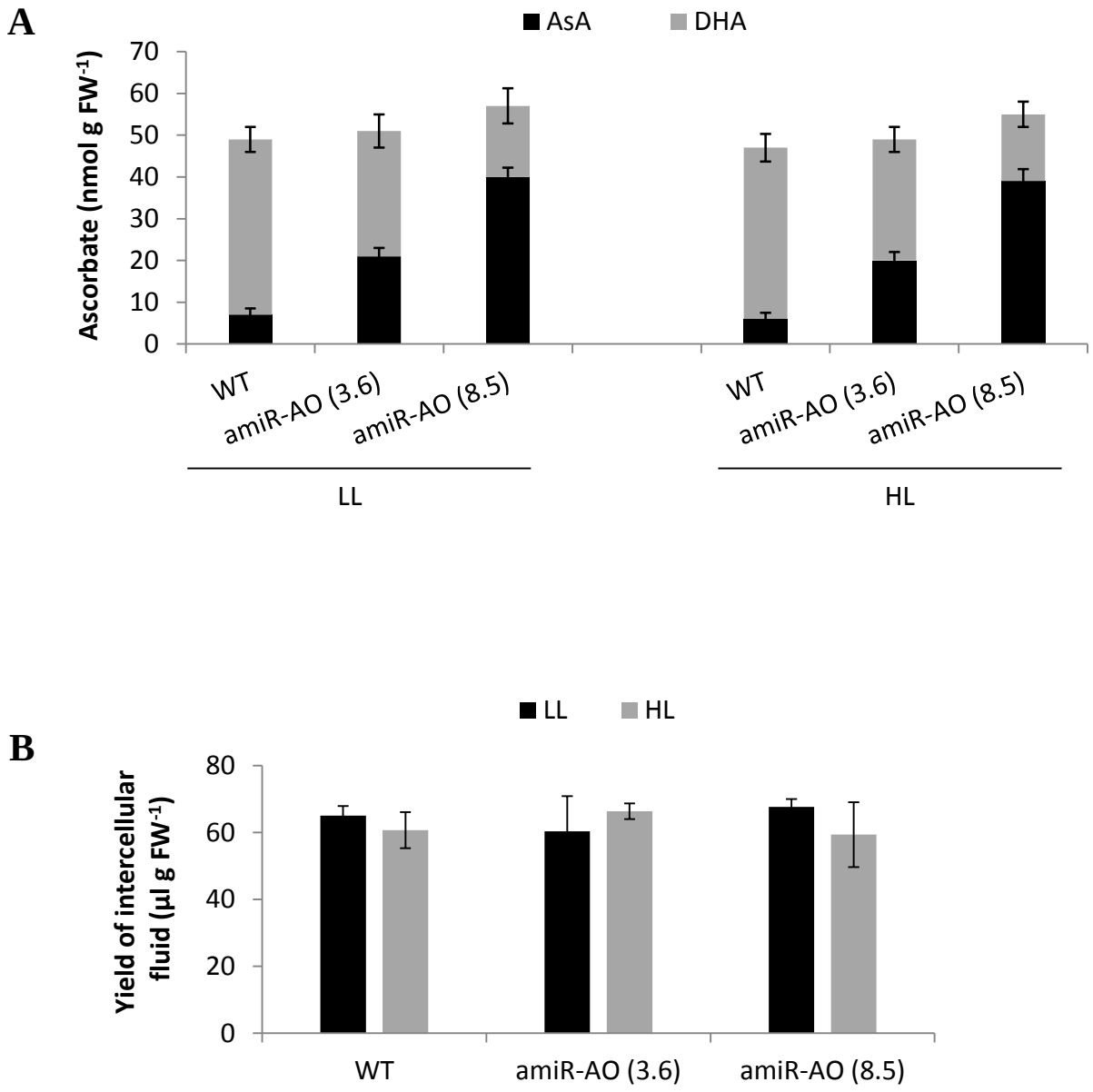


Figure 5.4 A comparison of apoplastic ascorbate content (A), and yield of intercellular (apoplastic) washing fluid after vacuum infiltration of the leaves (B) in 3-week-old wild type (WT) Arabidopsis and *amiR-AO (3.6)*, *amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. Black columns (AsA) represent the reduced form of ascorbate, silver columns (DHA) represent the oxidised (dehydroascorbate) form of ascorbate and both columns together represent the total pools of ascorbate (A). Plants grown for two weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 800 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

Total leaf ascorbate was measured in the leaves of the wild type and *amiR-AO* transgenic plants at week-3. More than 80% of the total pool of ascorbate in all genotypes was in the reduced form (AsA) under both LL and HL growth conditions (Fig. 5.5). The amount of total ascorbate pool was similar in the wild type and *amiR-AO* transgenic lines grown under LL conditions (Fig. 5.5). Likewise, there was no difference in the total ascorbate content of the different genotypes that had been subjected to HL growth conditions for seven days (Fig. 5.5). However, growth under HL for seven days resulted in a significant increase (about 100%) in the total pool of ascorbate in the leaves of all genotypes compared to plants grown only under LL conditions (Fig. 5.5).

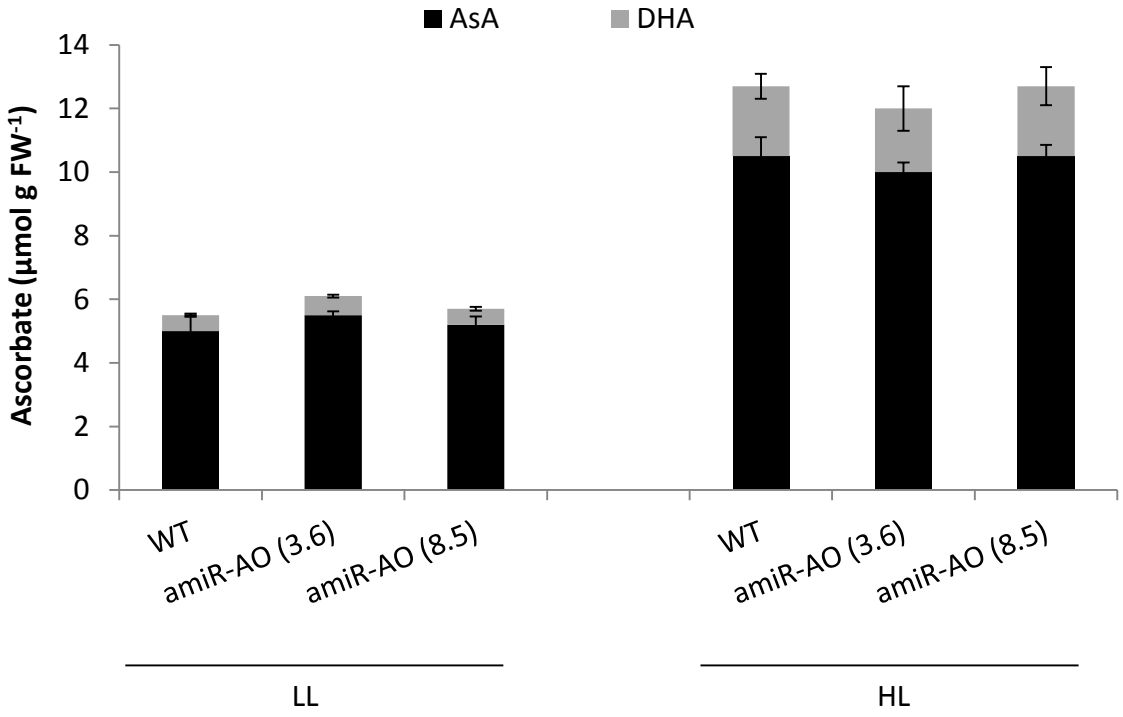


Figure 5.5 A comparison of whole leaf ascorbate content in 3-week-old wild type (WT) Arabidopsis and *amiR-AO (3.6), amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. Black columns (AsA) represent the reduced form of ascorbate, silver columns (DHA) represent the oxidised (dehydroascorbate) form of ascorbate and both columns together represent the total pools of ascorbate. Plants grown for two weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 800 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

5.2.4 Leaf pigment content

Chlorophyll and carotenoid contents of the rosette leaves were similar in all genotypes under LL conditions (Fig. 5.6A, B). Growth under HL for seven days significantly decreased leaf chlorophyll in all genotypes relative to the leaves of LL grown plants (Fig. 5.6A). Similarly, The HL pre-treatment for seven days decreased leaf carotenoid in all genotypes relative to the leaves of LL grown plants (Fig. 5.6B).

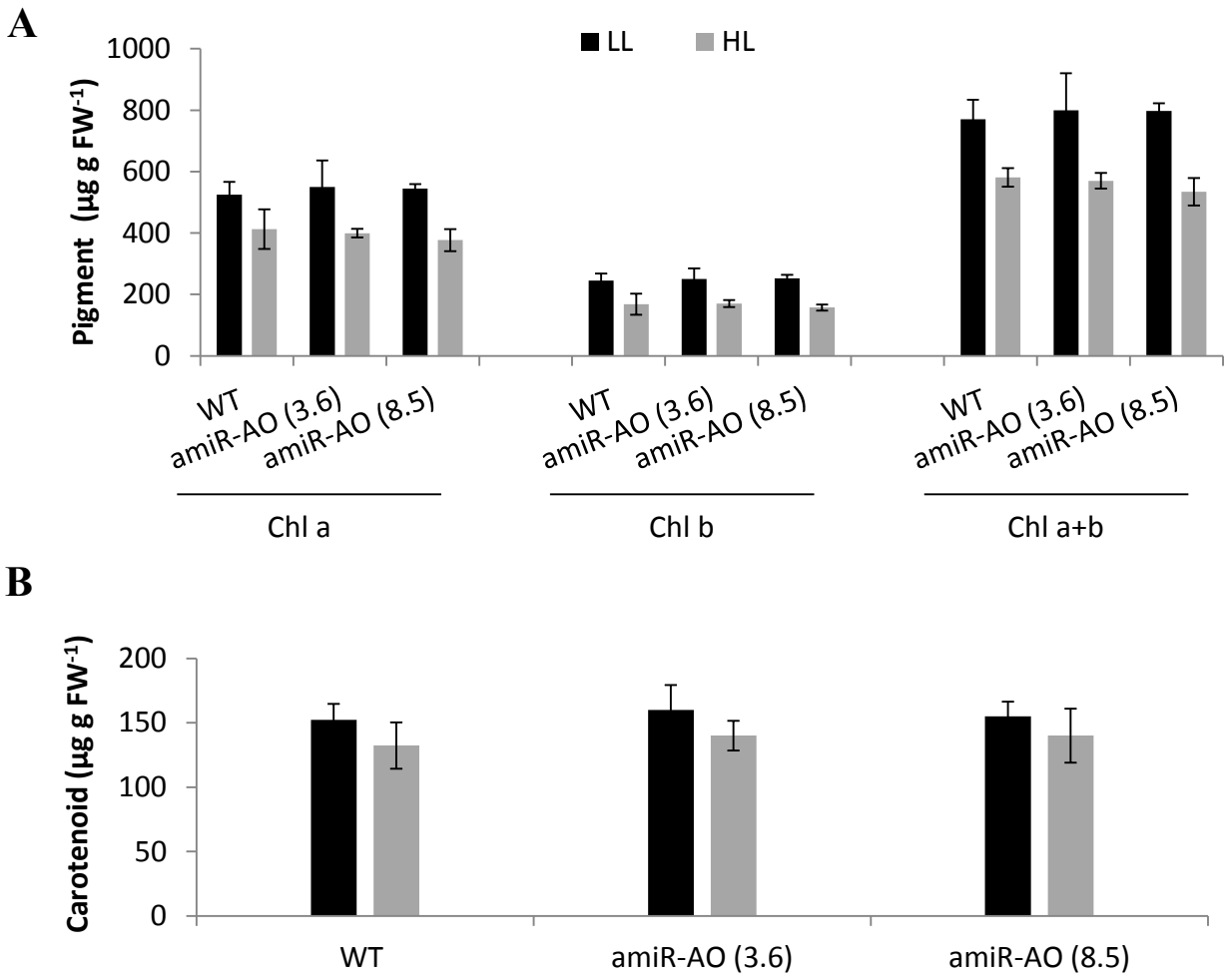


Figure 5.6 A comparison of leaf pigment contents in wild type (WT) Arabidopsis and *amiR-AO (3.6), amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. (A) Leaf chlorophyll content. (B) Leaf carotenoid contents. Chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b) and total carotenoid pigments (carotene) were performed on the whole rosettes of plants that had either been grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

5.2.5 Chlorophyll *a* fluorescence

The ratio of dark adapted variable chlorophyll *a* fluorescence (Fv) to the maximal value of chlorophyll *a* fluorescence (Fm) was measured in the leaves of plants following the transfer from LL (250 μmol m^{-2} s^{-1}) growth conditions to HL (800 μmol m^{-2} s^{-1}) conditions. The Fv/Fm ratios, and hence the PSII maximum efficiencies, were decreased in the leaves of all genotypes over the first 24h of exposure to HL conditions. The HL-induced decreases in the Fv/Fm ratios were similar in all genotypes (Fig. 5.7).

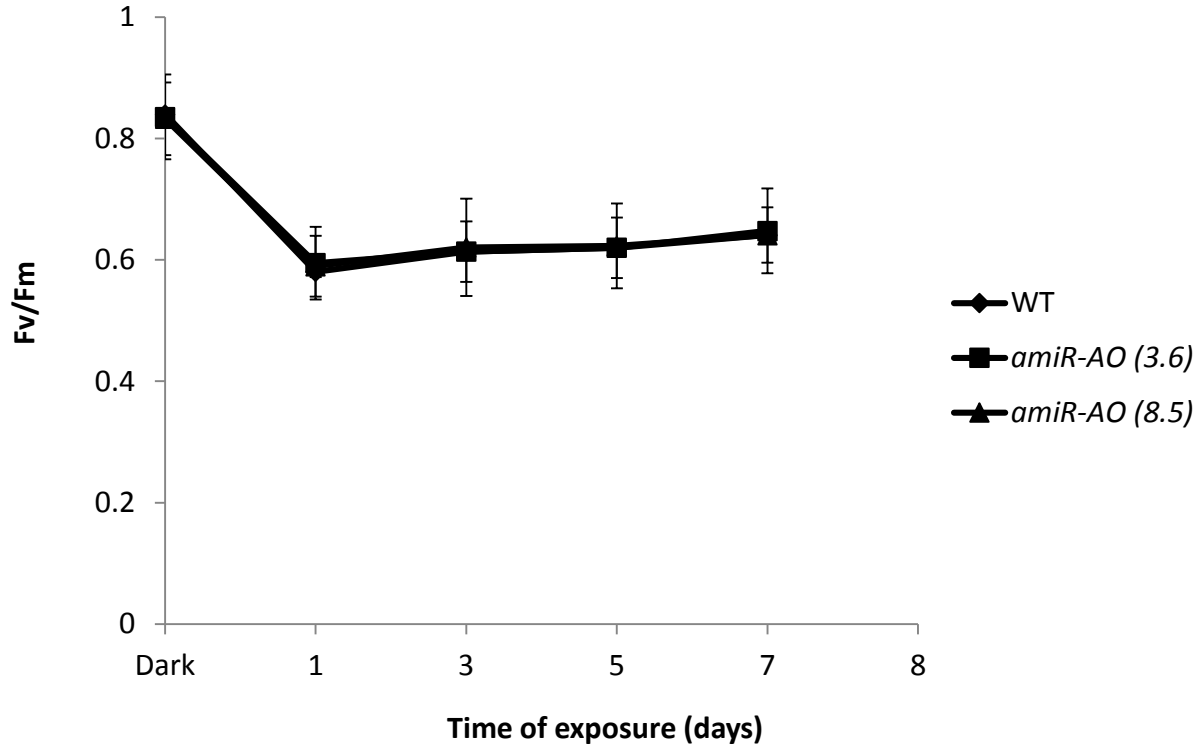


Figure 5.7 A comparison of the ratio of dark-adapted variable chlorophyll *a* fluorescence (Fv) to maximal chlorophyll a fluorescence (Fm) in wild type (WT) Arabidopsis, *amiR-AO (3.6)* and *amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. Plants were grown for two weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then transferred to high light (HL; 800 μmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 10).

5.2.6 Photosynthesis, light and CO_2 response curves

Photosynthetic CO_2 assimilation rates were similar in the leaves of all genotypes under LL conditions. A HL pre-treatment for 7 days decreased maximal rates of photosynthesis by about 40% relative to the leaves of plants that had been grown under LL (Fig. 5.8).

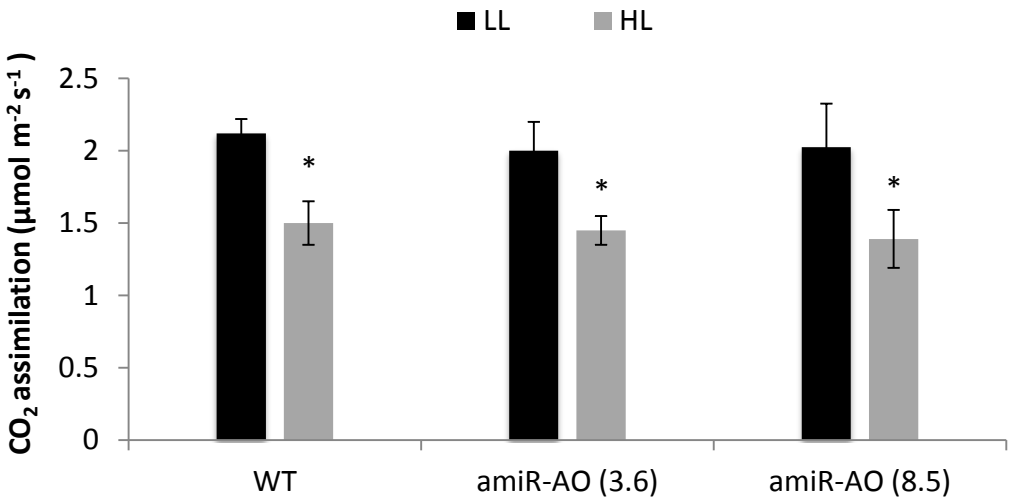


Figure 5.8 A comparison of photosynthetic CO_2 assimilation rates in wild type (WT) Arabidopsis, *amiR-AO (3.6)* and *amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. The photosynthetic CO_2 assimilation was measured on the whole rosette leaves of plants that had either been grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days. Photosynthesis was measured at 20°C with an irradiance of 250 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) and an atmospheric CO_2 concentration of 400 µmol mol^{-1}. Data are the mean values ± SE (n = 3). (*$p < 0.05$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype.

Analysis of the light response curves (Fig. 5.9A) and the CO_2 response curves for photosynthesis (Fig. 5.9B) showed that the initial slopes of both curves were decreased in the leaves of all genotypes that had been grown under HL for 7 days compared to those that has been maintained under LL conditions. Moreover, there was a significant HL dependent decrease in the CO_2 saturated rates of photosynthesis measured in the CO_2 response curve analysis in all genotypes (Fig. 5.9B). The HL-dependent decrease in the light saturated rates of photosynthesis was less marked in light response curve analysis (Fig. 5.9A). No significant differences in these parameters were observed between the wild type and the transgenic lines (Fig. 5.9A, B).

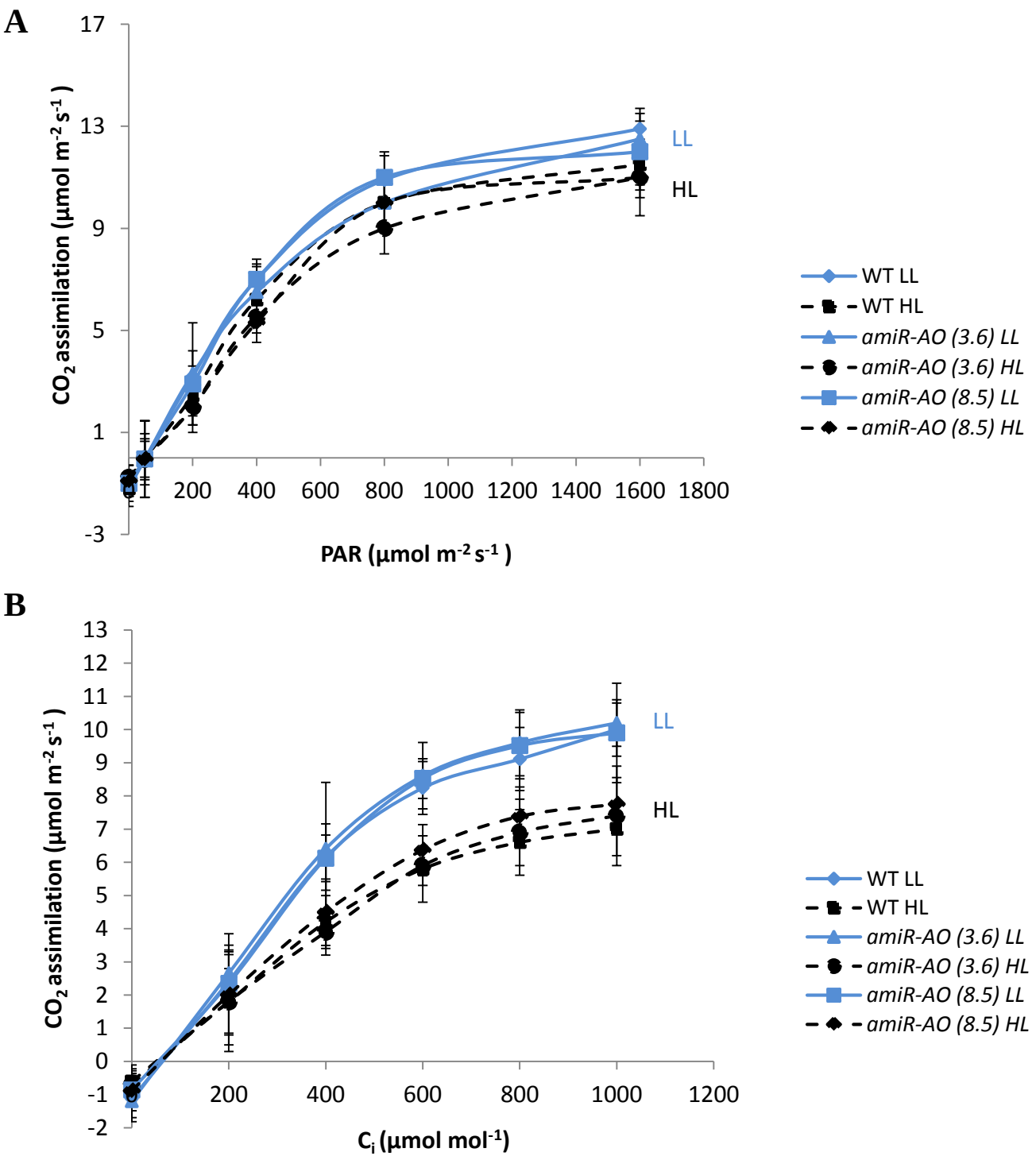


Figure 5.9 A comparison of the light saturation curves for photosynthesis (A) and the CO_2 response curves for photosynthesis (B) in wild type (WT) Arabidopsis and *amiR-AO (3.6)*, *amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. The light saturation curves and CO_2 response curves for photosynthesis were measured on the whole rosette leaves of plants that had either been grown for two weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions **(A)** or transferred to high light (800 µmol m^{-2} s^{-1}) for seven days **(B)**. Data are the mean values ± SE (n = 3).

5.2.7 Aphid fecundity

Aphid fecundity was measured in plants that had either been grown for two weeks under LL (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL conditions or transferred to HL (800 µmol m^{-2} s^{-1}) for seven days (Fig. 5.10). A single one-day-old nymph was placed on each plant and then the total number of offspring was counted after 15 days.

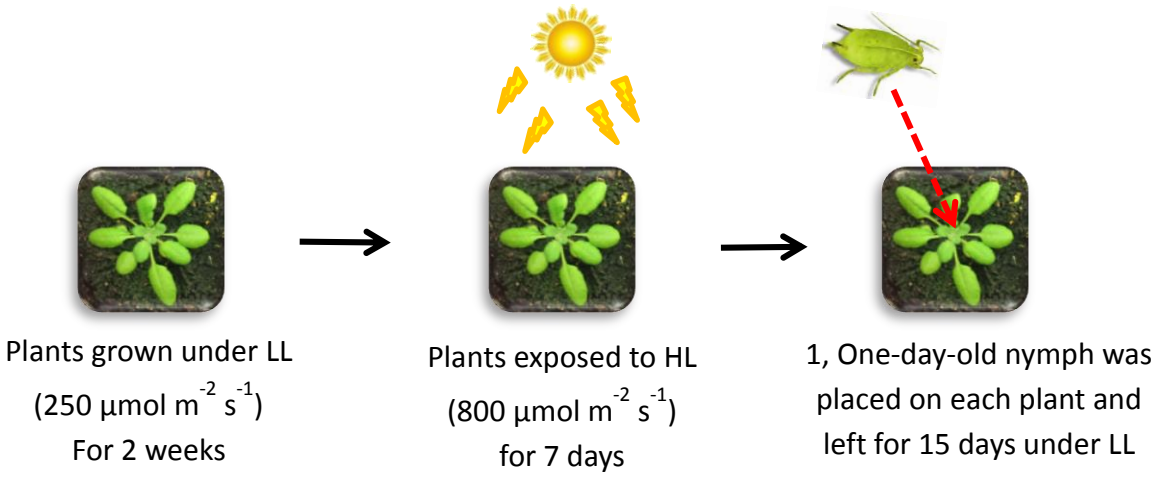


Figure 5.10 Experimental design illustrates aphid fecundity on plants that grown for two weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then transferred to high light (HL; 800 µmol m^{-2} s^{-1}) for seven days.

The numbers of aphids was similar on the leaves of all lines that had been grown under LL (Fig. 5.11A, B). However, aphid fecundity was decreased on the leaves of wild type plants that had been grown under HL for seven days, relative to wild type plants that been grown under LL alone (Fig. 5.10A). In contrast, aphid fecundity was similar in the *amiR-AO (8.5)* and *amiR-AO (3.6)* leaves, regardless of the light pre-treatment.

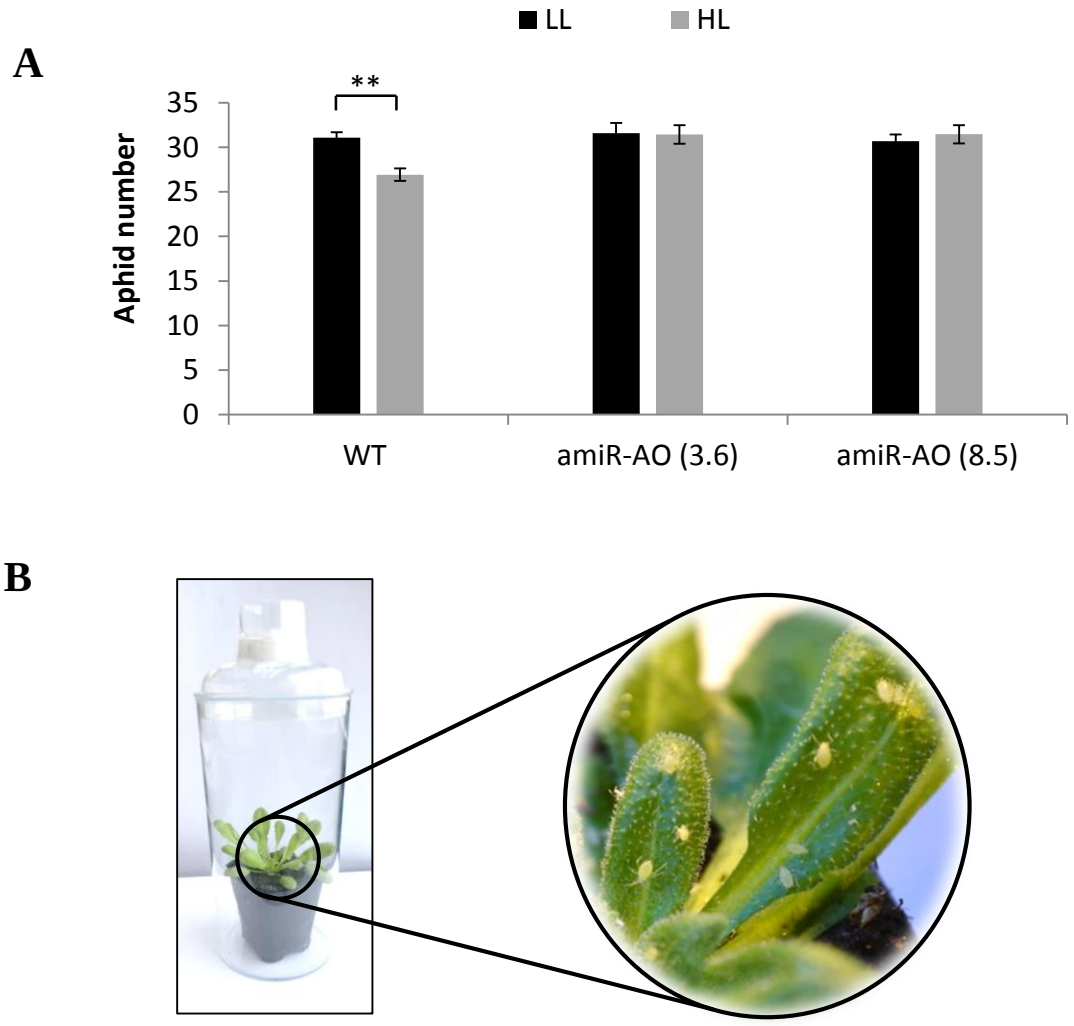


Figure 5.11 A comparison of aphid fecundity in wild type (WT) Arabidopsis and *amiR-AO (3.6)*, *amiR-AO (8.5)* transgenic plants grown under low light (LL) and high light (HL) conditions. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times. (*p < 0.05); (**p < 0.01); (****p < 0.0001) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype, as follows (◦p < 0.05); (◦◦p < 0.01); (◦◦◦p < 0.001); (◦◦◦◦p < 0.0001) in One-Way ANOVA comparisons between the mutant lines and wild type under LL conditions.

5.3 Discussion

The data presented here demonstrate that the Arabidopsis ascorbate oxidase (AO) transgenic lines, *amiR-AO (3.6*) and *amiR-AO (8.5)* had no detectable ascorbate oxidase activity in their leaves (Fig. 5.3), in agreement with previous observations (Lim, 2012). Moreover, the AsA/DHA ratios of the apoplastic fluid extracted from the *amiR-AO (3.6*) and *amiR-AO (8.5)* leaves was much high than that obtained from wild type leaves (Fig. 5.4A). The absence of apoplastic AO activities and the higher apoplastic AsA/DHA ratios of the *amiR-AO (3.6*) and *amiR-AO (8.5)* lines were associated with larger rosette phenotypes under both LL and HL growth conditions (Fig. 5.1). The rosette growth of both *amiR-AO* lines measured as either rosette diameter or leaf area was faster than that observed in the wild type (Fig. 5.2A, B). Although little is known about the precise functions of AO in the control of growth, AO activity has been linked to effects on cell division and expansion. The larger phenotype of the *amiR-AO* rosettes might be resulted from an altered hormone balance or related signalling that controls growth. For example, it was previously been shown that high AO activity in maize roots decreased auxin concentrations (Kerk et al., 2000). Furthermore, the growth of transgenic tobacco plants overexpressing pumpkin *AO* in sense orientation, which had 97% oxidised apoplastic ascorbate, was not stimulated by auxin treatment to the same extent as was observed in the wild type plants (Pignocchi et al., 2006). Taken together, these findings suggest that the enhanced growth of the *amiR-AO* shoots might be linked to direct or indirect effects on auxin concentration or auxin signalling.

The leaves of all genotypes had similar photosynthetic CO_2 assimilation under LL conditions (Fig. 5.8). The HL pre-treatment resulted in decreased photosynthetic CO_2 assimilation and lower chlorophyll contents in all genotypes, together with decreased Fv/Fm ratios compared to the plants that been grown under LL conditions alone (Fig. 5.6A, B). The rates of photosynthetic CO_2 assimilation measured at 250 μmol m^{-2} s^{-1} in the leaves of HL-treated plants were lower than those of plants that had only experienced LL (Fig. 5.9). However, the HL-dependent decreases in photosynthetic CO_2 assimilation were similar in all lines.

Aphid fecundity under LL conditions was similar on all genotypes (Fig. 5.11). However, while a pre-exposure to HL led to a significant decrease in aphid fecundity in the wild type plants relative to LL conditions, aphid fecundity was similar in the *amiR-AO* lines under both LL and HL conditions. This finding suggests that the absence of ascorbate oxidase activity and the resultant higher apoplastic AsA/DHA ratios prevent the HL-induced increase in aphid resistance that was observed in the wild type leaves.

Chapter 6. Role of apoplastic redox state on tobacco responses to aphid infestation

6.1 Introduction

The apoplast/cell wall compartment of the plant cell has crucial roles in the transport of water and nutrients, preserving cell shape and regulating growth, as well as being the first line of defence against environmental changes and biotic stresses (Sakurai, 1998). For example, it is the site of the pathogen-triggered oxidative burst that has a key role in plant immune responses. Unlike many of the intracellular compartments, apoplast/cell wall has very few low molecular weight antioxidants. However, unlike most other low molecular weight antioxidants, L-ascorbic acid (vitamin C) is abundant in the apoplast/cell wall, where it fulfils important roles in the control of redox reactions (Foyer and Noctor, 2000). Ascorbic acid is the most abundant low molecular weight antioxidant in plants, with a central role in H_2O_2 detoxification (Zheng and Vanhuystee, 1992; Noctor and Foyer, 1998). The levels of ascorbate in the apoplast are tightly controlled in relation to cell growth and expansion (Horemans et al., 2000). In particular, the enzyme ascorbate oxidase (AO), which catalyses the first step in the pathway of ascorbate degradation, is localized in the apoplast. AO is a copper-containing protein catalysing the reduction of molecular oxygen to water using ascorbate as the electron donor. In this reaction ascorbate is oxidised to monodehydroacorbate (MDHA) and then to dehydroacorbate (DHA). AO activities are high in rapidly growing tissues such as fruits and germinating seeds (Suzuki and Ogiso, 1973; Kato and Esaka, 1999). AO is considered to be important in limiting the accumulation of ascorbate in the apoplast (Pignocchi and Foyer, 2003). However, little is known about the regulation of AO activity and its functions, which have largely been characterized by the analysis of transgenic plants with altered levels of AO expression (Pignocchi et al., 2003). Constitutive expression of a melon AO gene in transgenic tobacco plants led to a decrease in PR1a transcripts. In addition, the AO-overexpressing plants were more susceptible to infection by the necrotrophic fungus *Botrytis cinerea* than the wild type (Fotopoulos et al., 2006). In contrast, AO overexpression in antisense orientation resulted in the expression of defence genes and enhanced resistance to biotrophic pathogens (Pignocchi et al., 2006). These studies suggest that AO regulates the innate immune response of the plant and may therefore also be important in plant resistance to aphid infestation. The following experiments were therefore performed to test this hypothesis.

In the following studies, aphid fecundity was compared in wild type tobacco plants and transgenic tobacco plants that either over express a pumpkin (*Cucurbita maxima*) ascorbate oxidase (AO) i.e. lines with high AO activity (PAO), or a partial tobacco AO sequence in the antisense orientation i.e. lines with low AO activity (TAO). Moreover, the effects of light intensity on aphid fecundity in these different lines was assessed by first growing the plants for three weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then either maintaining them for a further seven days under low light growth conditions or transferring them to high light (HL; 800, 1000 or 1600 μmol m^{-2} s^{-1}) for seven days, prior to the onset of aphid infestation. Photosynthetic CO_2 assimilation rates were determined together with chlorophyll *a* fluorescence quenching in all lines to determine the effects of the light treatments on photosynthesis. In addition, samples were harvested from leaves infested with aphids at an early time-point (12h) following the onset of aphid feeding under the different conditions in order to characterise effects on the transcript and metabolite profiles.

6.2 Results

6.2.1 Shoot phenotypes under low and high light conditions

No visible differences were observed in the shoot phenotypes of the wild type and the transgenic lines with either low (TAO) or high (PAO) ascorbate oxidase activity under low (LL) and high light (HL) growth conditions (Fig. 6.1). However, the appearance of the youngest leaves on the plants of all genotypes that had been grown under HL conditions for seven days was faster than that observed in LL conditions (Fig. 6.1).

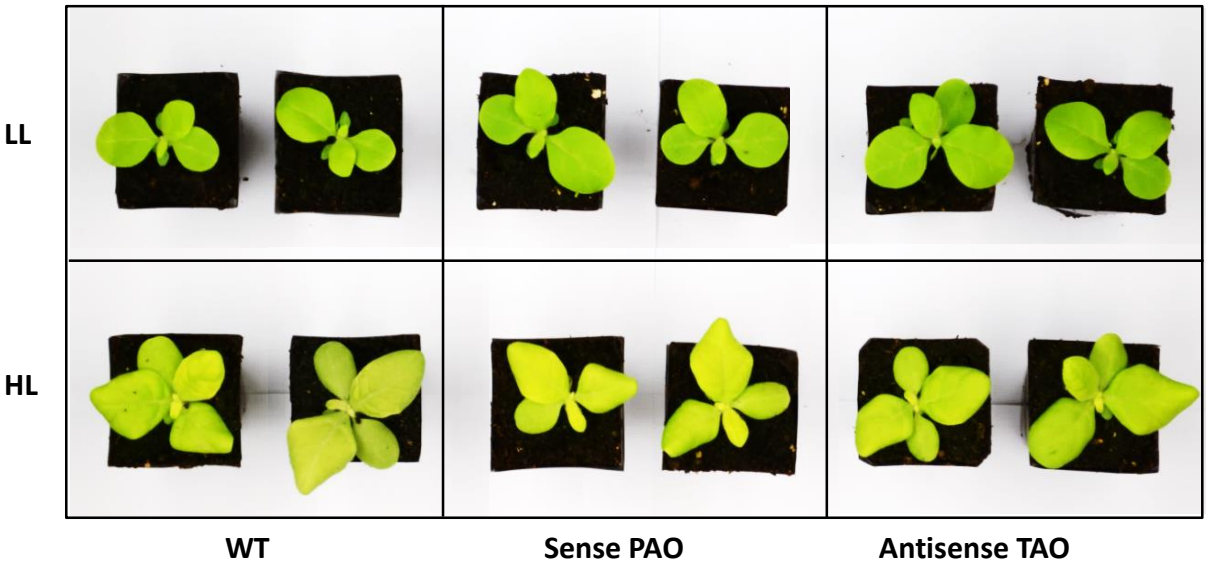


Figure 6.1 A comparison of shoot phenotypes in wild type (WT) tobacco plants and in transgenic lines in which ascorbate oxidase was expressed in either the sense (PAO) or antisense (TAO) orientations grown under low light (LL) and high light (HL) conditions. Plants grown for three weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions (top row) or transferred to high light (HL; 1600 μmol m^{-2} s^{-1}) for seven days (bottom row).

Leaf area and leaf number measurements were performed on plants that had either been grown for four weeks under LL or for three weeks under LL followed by seven days HL. There were no statistically significant differences in total leaf area in any of the lines grown under LL (Fig. 6.2A). Similarly, the shoot of all plants had similar leaf area after seven days of HL treatment (Fig. 6.2A). In contrast, a HL pre-treatment for seven days resulted in grow of one more leaf in all genotypes compared to the corresponding genotypes under LL (Fig. 6.2B). However, all lines had the same number of leaves under LL conditions (Fig. 6.2B).

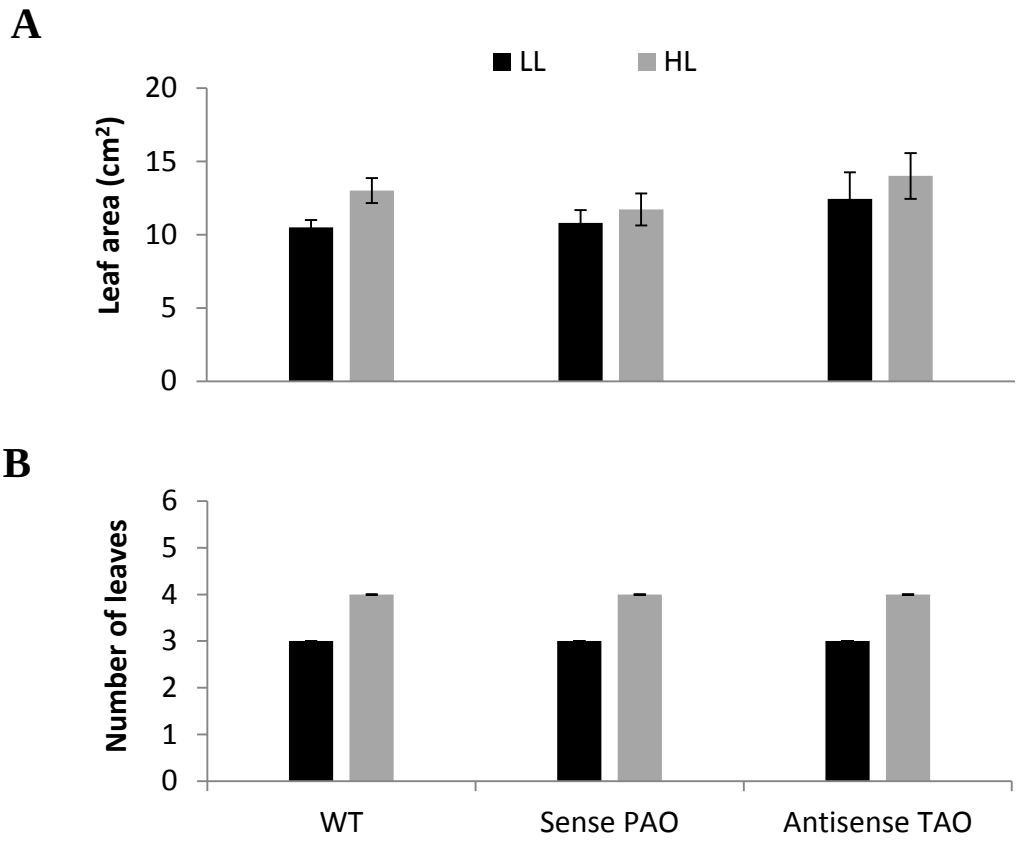


Figure 6.2 A comparison of leaf area (A) and leaf number (B) of wild type (WT) and in transformed tobacco plants that had high (PAO) or low (TAO) AO activity grown under low light (LL) and high light (HL) conditions. Plants grown for three weeks under low light (LL; 250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 1600 μmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 10).

6.2.2 Ascorbate oxidase (AO) activity

To confirm the over-expression of ascorbate oxidase (AO) in sense (PAO) and antisense (TAO) orientations in the transgenic lines, the maximal extractable AO activity was therefore measured in sense, antisense and in wild type plants.

The data shown in figure (6.3) confirm that ectopic over-expression of the pumpkin AO in sense orientation resulted in a higher (ca.15-fold) increase in leaf AO activity relative to the wild type under both LL and HL conditions. In contrast, over-expression of partial tobacco AO sequence in antisense orientation in the leaves of the TAO lines resulted in a (ca. 0.4-fold) reduction in extractable leaf AO activity under both light conditions (Fig. 6.3). Moreover, no significant increase in AO activity was shown by any genotype after HL treatment (Fig. 6.3).

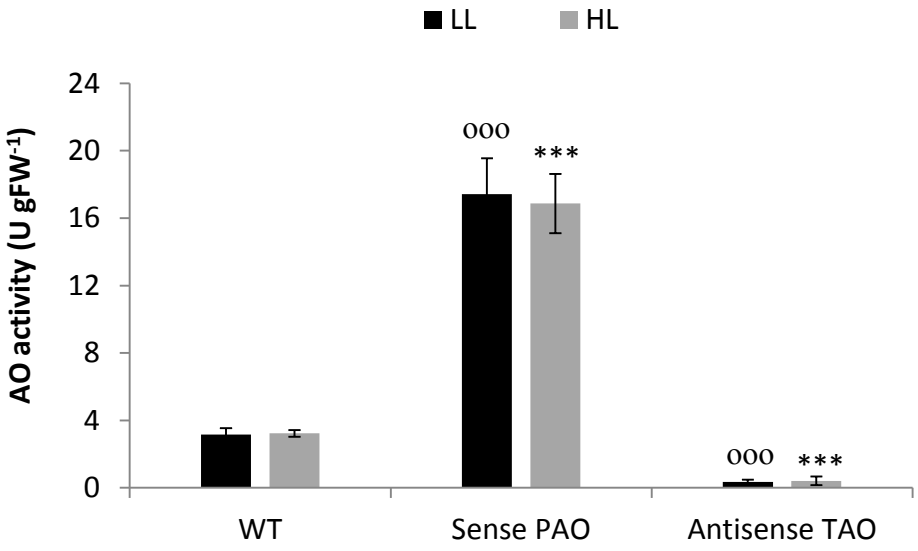


Figure 6.3 A comparison of the maximum extractable ascorbate oxidase (AO) activities in the leaves of sense PAO, antisense TAO and the wild type tobacco (WT) plants under low (LL) and high light (HL) growth conditions. Plants grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) for seven days. One unit of AO activity equal the amount of enzyme required to oxidise (1µmol ascorbate min^{-1}) at 25°C. Data are the mean values ± SE (n = 3). (◦◦◦$p < 0.001$) in Significance given from analysis by One-Way ANOVA comparisons between the transgenic lines and wild type under LL conditions, as follows (***$p < 0.001$) in One-Way ANOVA comparisons between the transgenic lines and wild type under HL conditions.

6.2.3 Whole leaf and apoplastic ascorbate content

To investigate the effect of over-expression of ascorbate oxidase (AO) in sense (PAO) and antisense (TAO) orientations on the apoplastic AsA/DHA ratios, the ascorbate concentration was determined in the apoplast of wild type and both transgenic lines. The apoplastic or intracellular washing fluid was extracted by vacuum infiltration for the fully matured leaves of 4-week-old plants.

In transgenic tobacco plants with low ascorbate oxidase (TAO) activity, approximately 70% of the apoplastic ascorbate was present in the reduced form (AsA). This value compared with only 3% of the ascorbate pool in the reduced form in the transgenic sense (PAO) or the wild type plants, in which about 40% of the apoplastic ascorbate pool was in the reduced form under both LL and HL growth conditions (Fig. 6.4A). A HL pre-treatment for seven days did not lead to a significant change in the redox state and total ascorbate contents of apoplast (Fig. 6.4A).

Furthermore, no significant differences were found in the extracted amount of intracellular washing fluid in all genotypes grown under LL or HL conditions (Fig. 6.4B).

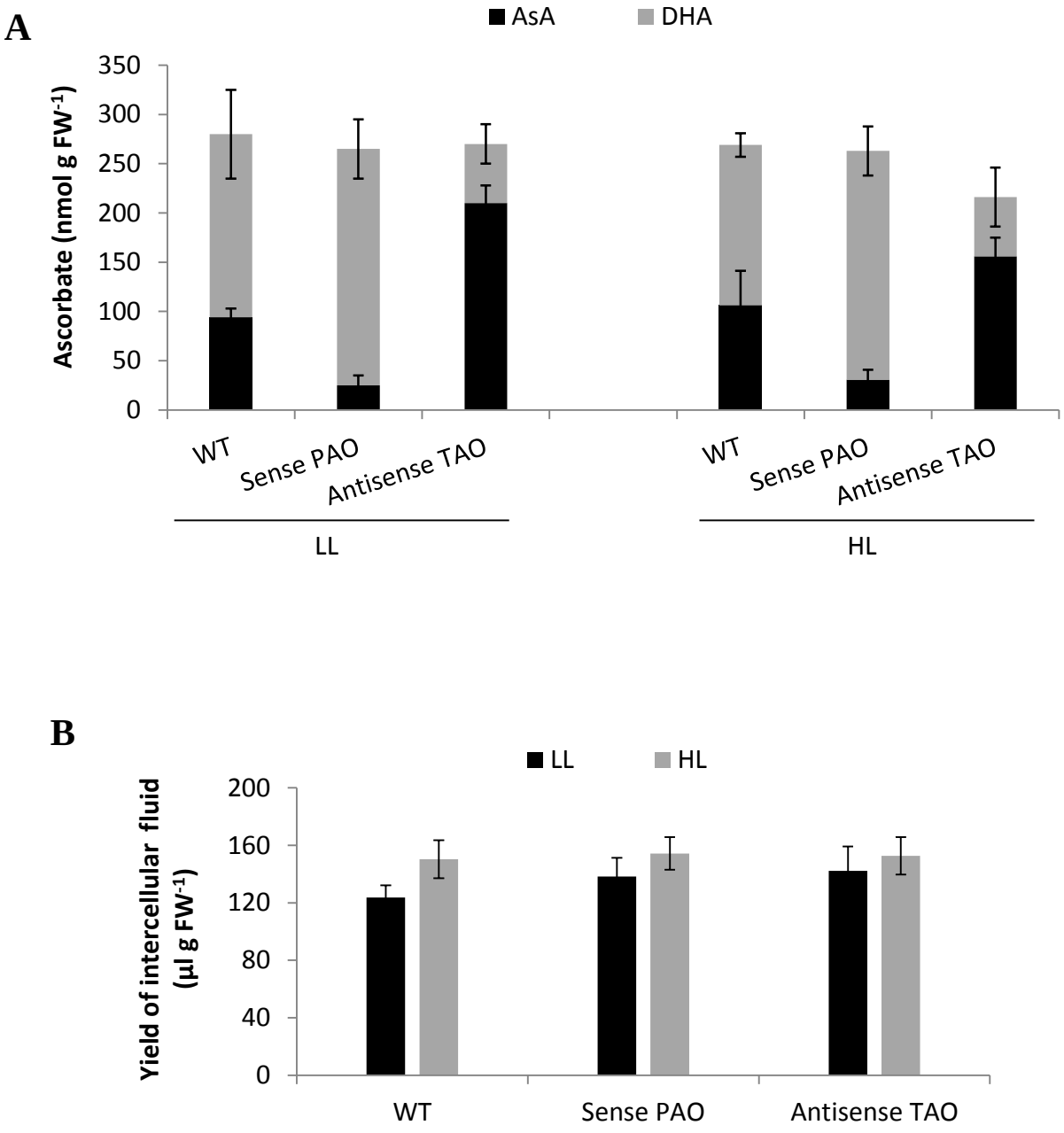


Figure 6.4 A comparison of apoplastic ascorbate content (A) and yield of intracellular (apoplastic) washing fluid after vacuum infiltration of the leaves (B) in wild type (WT) and in transformed tobacco plants that had high (PAO) or low (TAO) AO activity grown under low light (LL) and high light (HL) conditions. Black columns (AsA) represent the reduced form of ascorbate, silver columns (DHA) represent the oxidised (dehydroascorbate) form of ascorbate and both columns together represent the total pools of ascorbate (A). Plants grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

To determine the whole leaf total ascorbate content and AsA/DHA ratios, the total leaf ascorbate was extracted from the leaves of wild type and both AO transgenic lines.

About 80% of the total pool of ascorbate in all genotypes was in the reduced form (AsA) under both LL and HL conditions (Fig. 6.5). The amount of total ascorbate was similar in the wild type and both AO transgenic lines grown under LL or HL conditions (Fig. 6.5). Likewise, there were no differences in the total ascorbate content in all genotypes grown under HL conditions (Fig. 6.5).

However, a HL pre-treatment for seven days led to a significant increase in the total pool of ascorbate content in all genotypes compared to corresponding genotypes in LL conditions (Fig. 6.5).

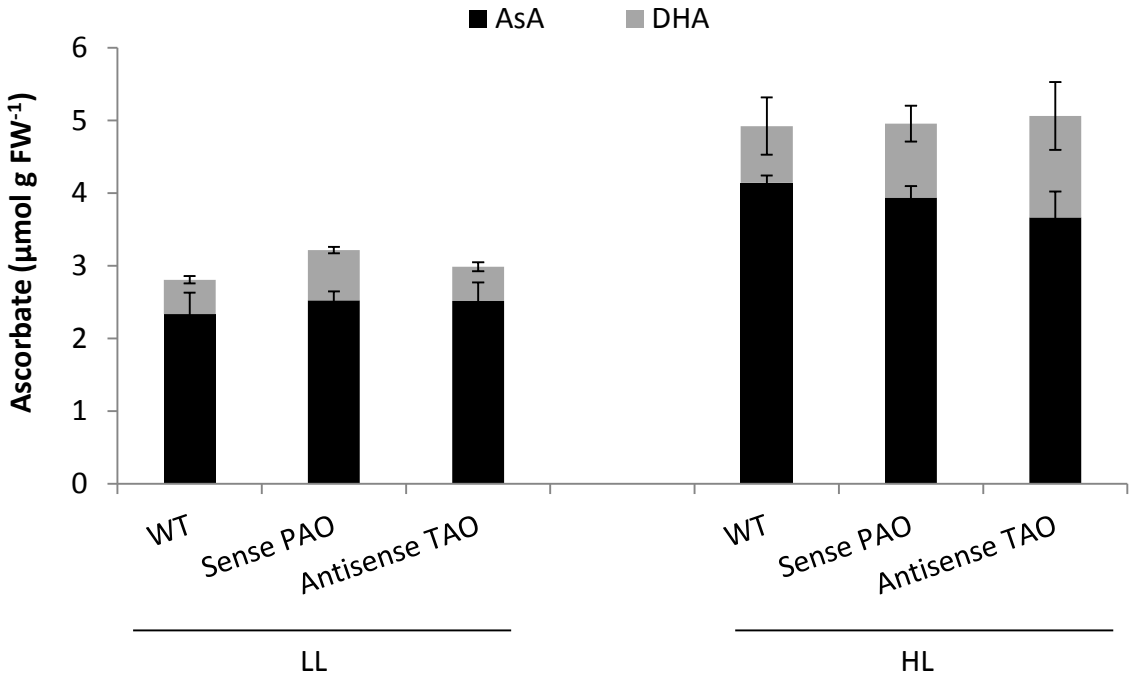


Figure 6.5 A comparison of whole leaf ascorbate content in sense PAO, antisense TAO and the wild type tobacco (WT) plants under low (LL) and high light (HL) growth conditions. Black columns (AsA) represent the reduced form of ascorbate, silver columns (DHA) represent the oxidised (dehydroascorbate) form of ascorbate and both columns together represent the total pools of ascorbate. Plants grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

6.2.4 Leaf pigment content

The leaves of all genotypes that grown under LL had similar amounts of chlorophyll and carotenoids irrespective of the level of AO activity in the lines (Fig. 6.6A, B). However, growth under HL for seven days decreased leaf chlorophyll by about 30% in all genotypes relative to the leaves grown under LL (Fig. 6.6A, B). The light-dependent decreases in leaf chlorophyll were similar in all genotypes (Fig. 6.6A, B).

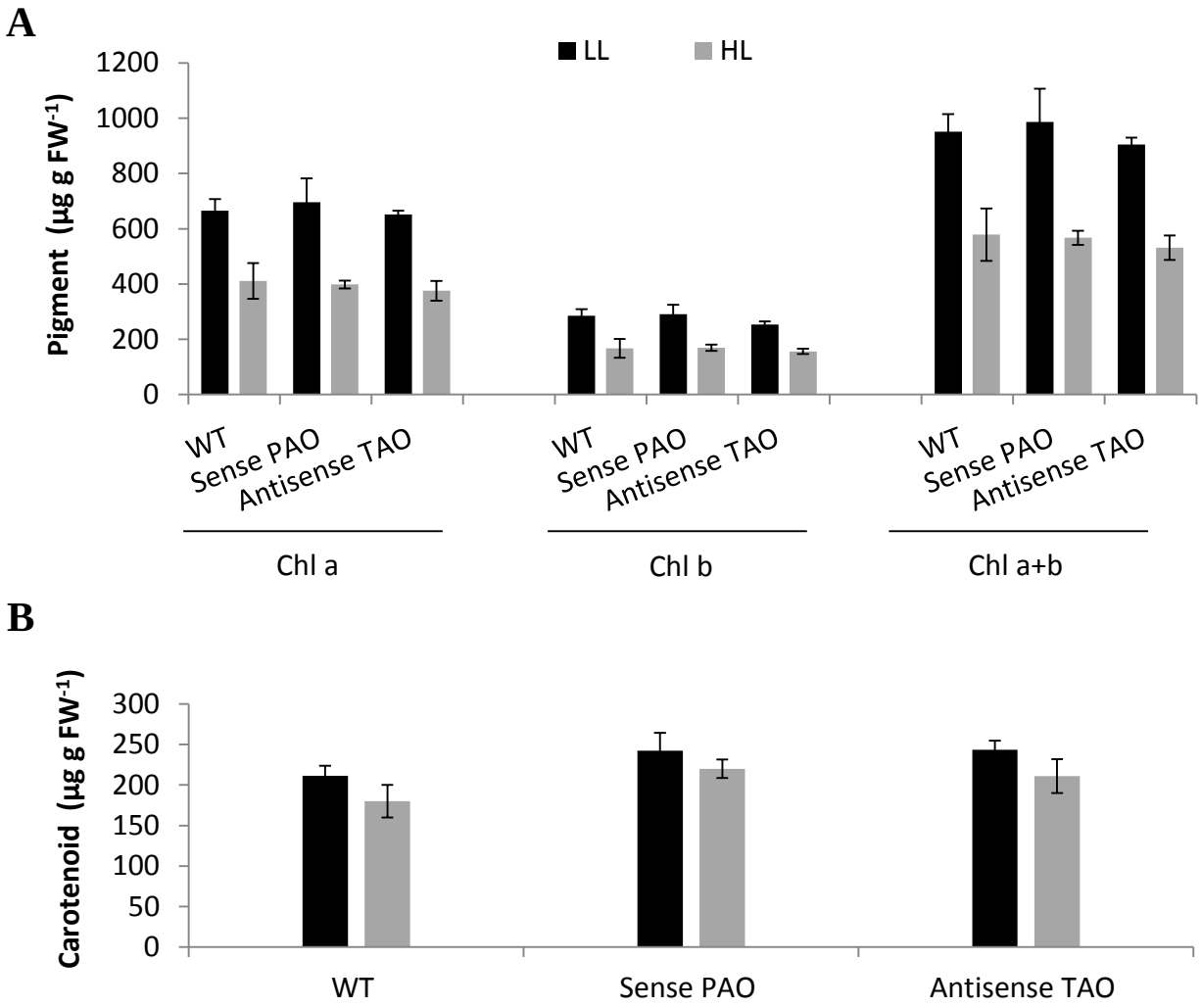


Figure 6.6 A comparison of leaf pigment contents in wild type (WT) tobacco plants and transgenic lines sense (PAO) or antisense (TAO) grown under low light (LL) and high light (HL) conditions. (A) Leaf chlorophyll content. (B) Leaf carotenoid contents. Chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b) and total carotenoid pigments (carotene) were performed on the youngest fully expanded leaves of plants that had either been grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

6.2.5 Chlorophyll *a* fluorescence

The ratio of dark adapted variable chlorophyll *a* fluorescence (Fv) to the maximal value of chlorophyll a fluorescence (Fm) in the dark adapted state is a measure of the maximum efficiency at which light absorbed by photosystem (PSII) is used for photochemistry. This parameter was measured in the leaves of 4-week-old plants following the transfer from LL growth conditions to HL (Fig. 6.7). Fv/Fm values and hence the PSII maximum efficiency were decreased in the leaves of all lines during the first 24h of exposure to HL conditions. The HL-induced decrease in this parameter was similar in wild-type, PAO and TAO leaves (Fig. 6.7).

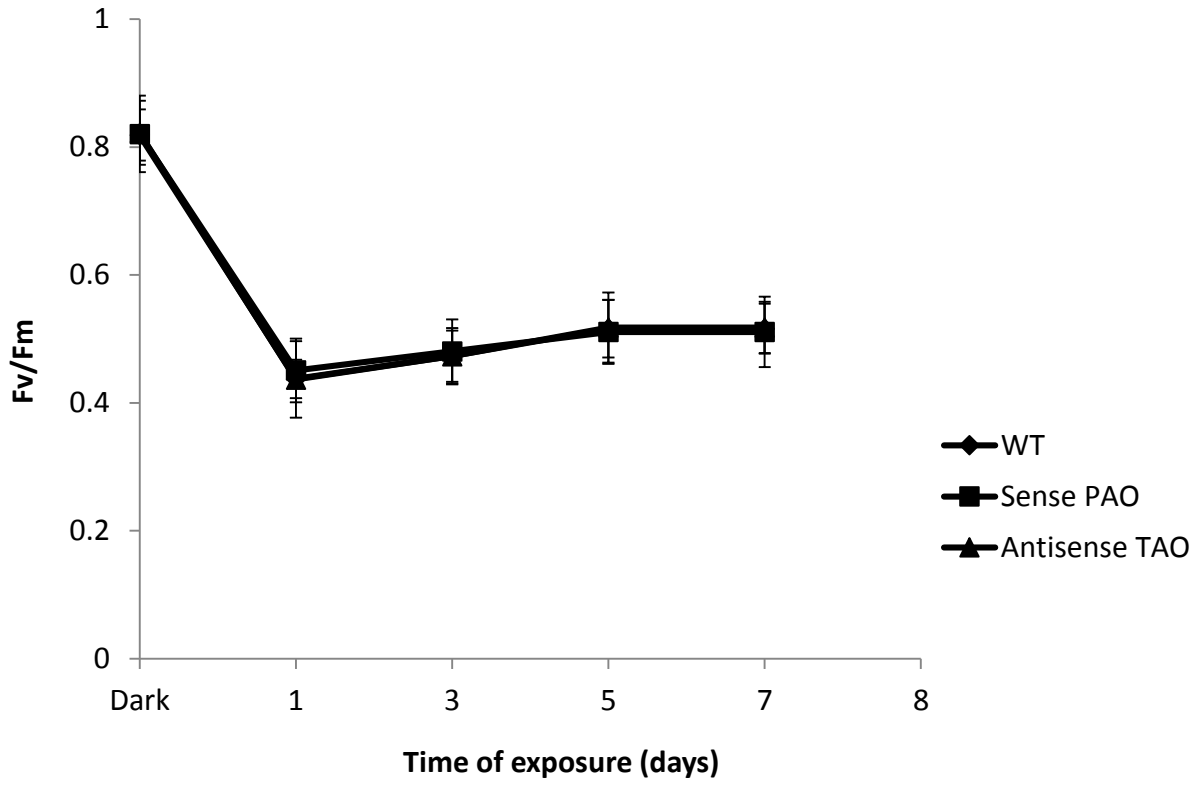


Figure 6.7 A comparison of the ratio of dark-adapted variable chlorophyll *a* fluorescence (Fv) to maximal chlorophyll a fluorescence (Fm) in wild type (WT) and in transformed tobacco plants that had high (PAO) or low (TAO) AO activity grown under low light (LL) and high light (HL) conditions. Plants grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then transferred to high light (1600 μmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 10).

6.2.6 Photosynthesis, light and CO_2 response curves

The leaves of all lines grown under LL conditions had similar rates of photosynthetic CO_2 assimilation (Fig. 6.8A), similar intracellular CO_2 concentration values (Fig. 6.8B), similar stomatal conductance values (Fig. 6.8C) and similar leaf transpiration rates (Fig. 6.8D).

The plants grown under HL for seven days had lower rates of photosynthetic CO_2 assimilation (Fig. 6.8A) and slightly higher intracellular CO_2 concentrations (Fig. 6.8B) when measured under low irradiance (250 µmol m^{-2} s^{-1}) than the leaves of plants grown and maintained under LL (Fig. 6.8B). Stomatal conductance values were almost doubled in the leaves of plants grown under HL growth conditions (Fig. 6.8C) compared to those grown under LL when this parameter was measured under low irradiance (250 µmol m^{-2} s^{-1}). Similarly, leaf transpiration rates were increased under HL growth conditions (Fig. 6.8D).

The data shown in figure (6.9A) suggests that photosynthetic CO_2 assimilation rates were decreased in light response curves for photosynthesis in all genotypes grown under HL compared to LL conditions but the HL-induced decrease in photosynthesis was marked only under irradiance (200-400 µmol m^{-2} s^{-1}). This trend was also observed in CO_2 response curves for photosynthesis (Fig. 6.9B).

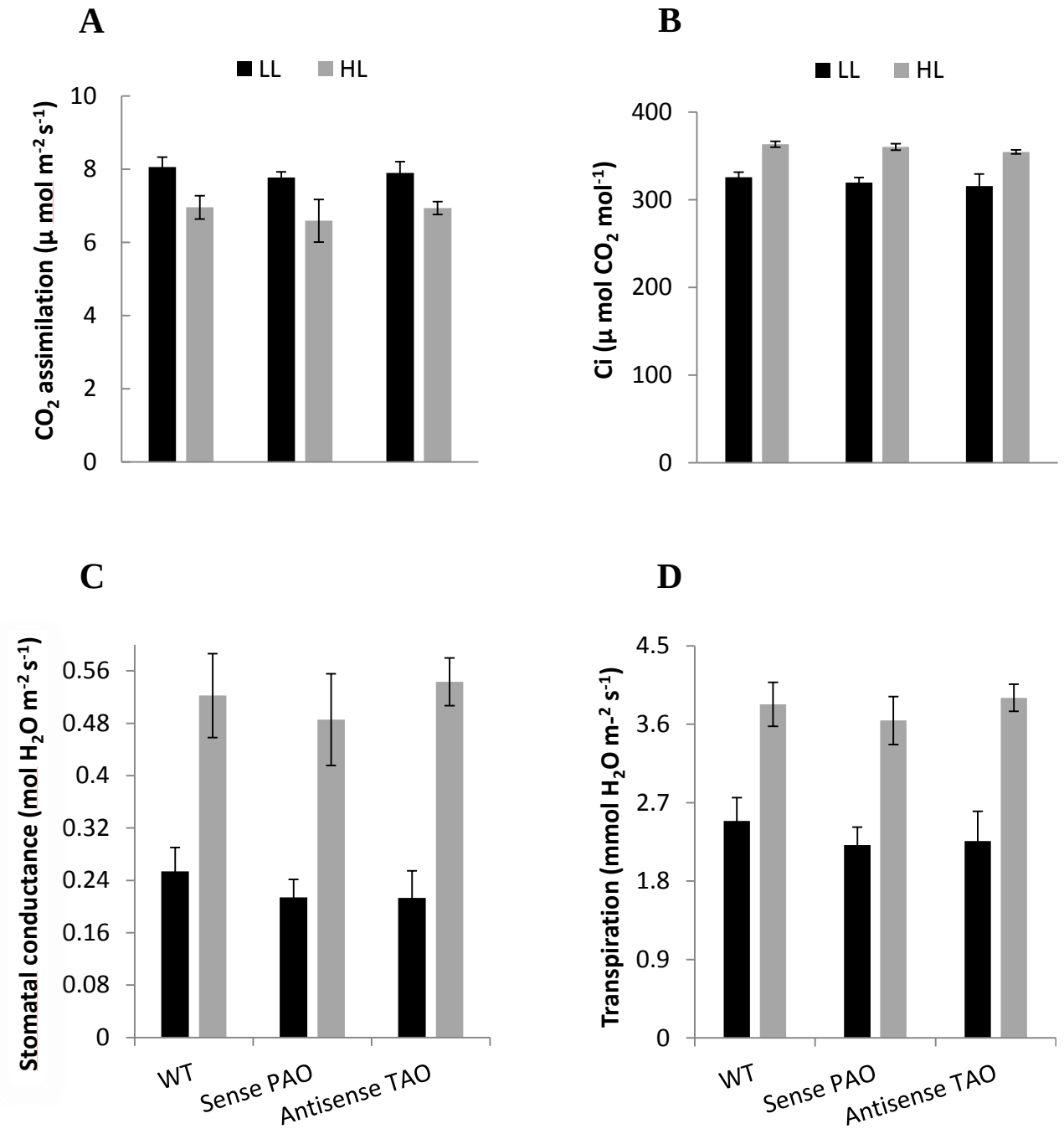


Figure 6.8 A comparison of photosynthetic CO_2 assimilation rates in sense PAO, antisense TAO and the wild type tobacco (WT) plants under low and high light growth conditions. (A): CO_2 assimilation (µmol m^{-2} s^{-1}); (B): intracellular CO_2 concentration (Ci; µmol CO_2 mol^{-1}); (C): Stomatal conductance (mol H_2O m^{-2} s^{-1}); (D): Transpiration (mmol H_2O m^{-2} s^{-1}). Photosynthesis was measured at 20°C with an irradiance of 250 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) and an atmospheric CO_2 concentration of 400 µmol mol^{-1}. Plants grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to high light (HL; 1600 µmol m^{-2} s^{-1}) for seven days. Data are the mean values ± SE (n = 3).

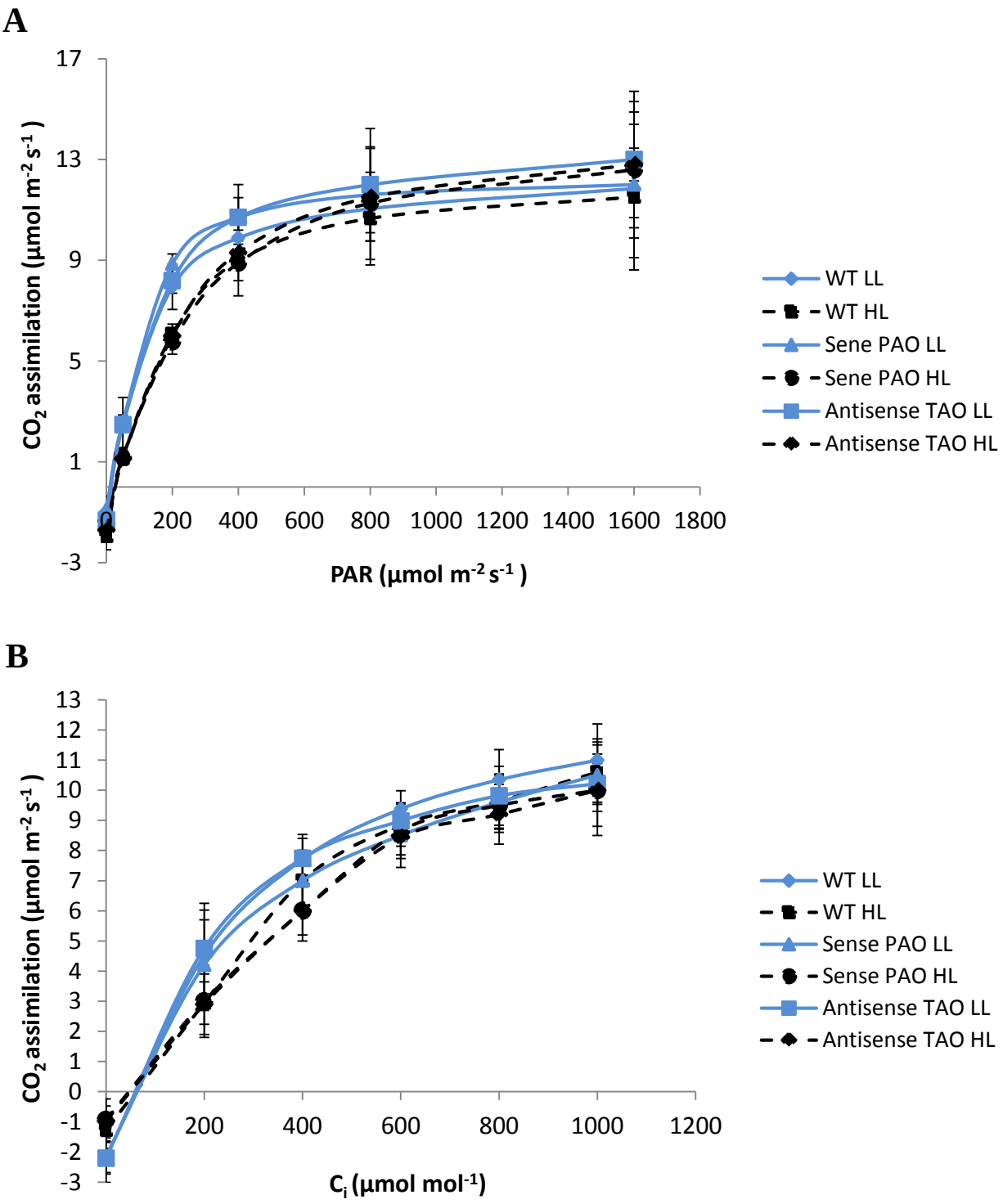


Figure 6.9 A comparison of the light saturation curves for photosynthesis (A) and the CO_2 response curves for photosynthesis (B) in sense PAO, antisense TAO and the wild type (WT) tobacco plants under low and high light growth conditions. The light saturation curves and CO_2 response curves for photosynthesis were measured on the whole rosette leaves of plants that had either been grown for three weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions **(A)** or transferred to high light (1600 µmol m^{-2} s^{-1}) for seven days **(B)**. Data are the mean values ± SE (n = 3).

6.2.7 Aphid fecundity

Aphid fecundity or reproductive performance on a given host plant is determined by the endogenous constitutive physical defenses to aphid attack and also by the ability of the plant to elicit rapid and effective inducible defense systems.

In these experiments the aphid fecundity analyses has been performed on plants that had received a HL pre-treatment with different irradiances prior to the analysis of aphid fecundity, which was measured in plants returned to the LL growth conditions for 15 days.

Aphid fecundity measured in plants that had either been grown for three weeks under LL (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL or exposed to different HL intensities (800, 1000 or 1600 µmol m^{-2} s^{-1}) for seven days (Fig. 6.10). A single one-day-old nymph was placed on each plant and then the total number of offspring was counted after 15 days.

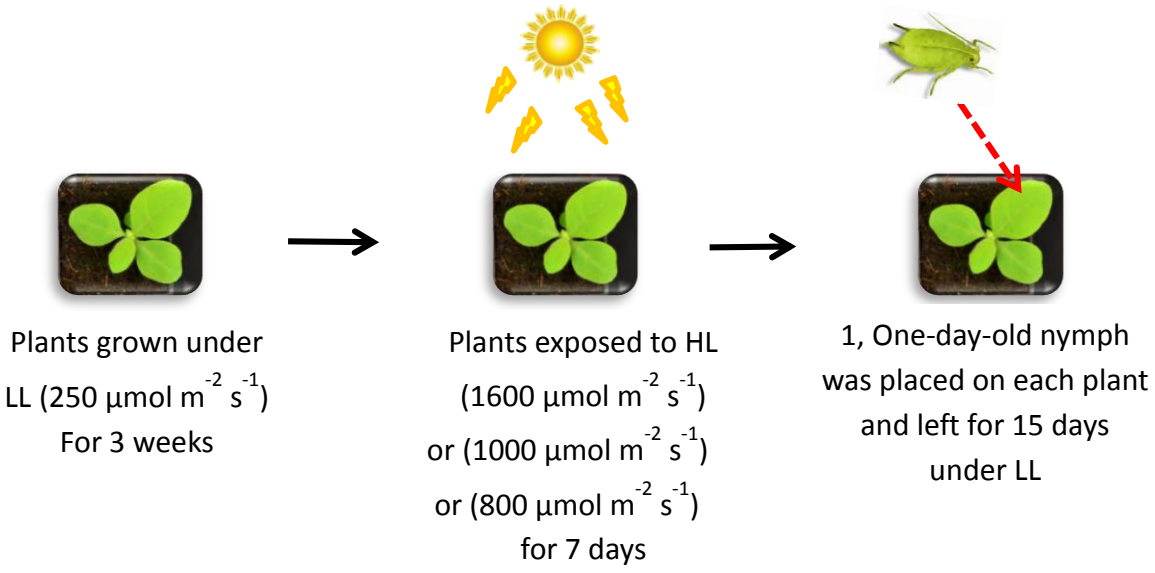


Figure 6.10 Experimental design illustrates aphid fecundity in sense PAO, antisense TAO and the wild type (WT) tobacco plants that grown for three weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then transferred to high light (HL; 800, 1000 or 1600 µmol m^{-2} s^{-1}) for seven days.

Aphid fecundity in plants that grown only under LL was similar in all genotypes (Fig. 6.11). Similarly, growth under HL (800 μmol m^{-2} s^{-1}) for seven days did not have a significant effect on aphid fecundity in any of genotypes compared to LL-grown plants (Fig. 6.11).

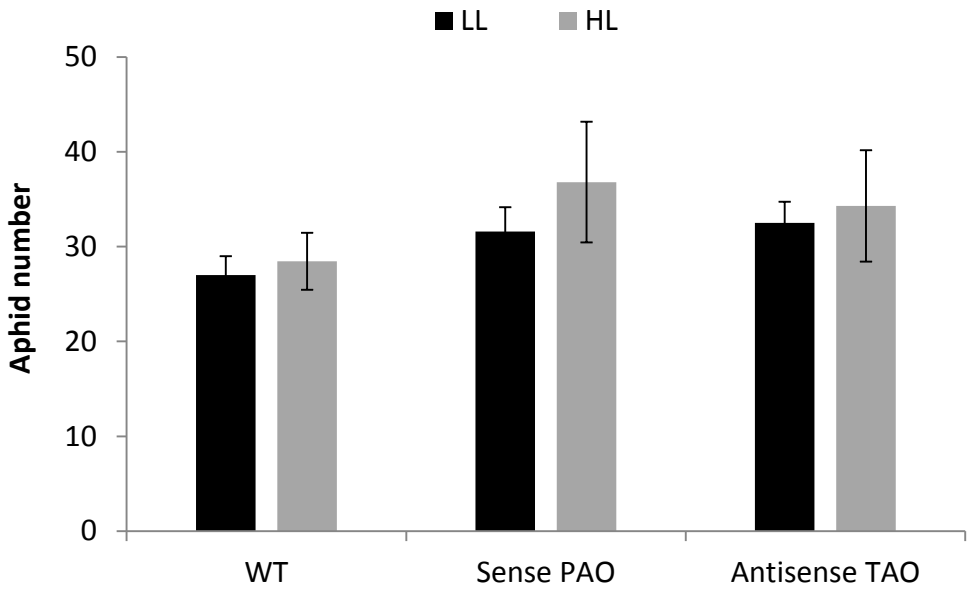


Figure 6.11 A comparison of aphid fecundity in sense PAO, antisense TAO and the wild type tobacco (WT) plants grown under low (LL) and high light (HL) conditions. The numbers of aphids present on leaves two weeks after the onset of infestation were measured on plants that had either been grown for four weeks under low light (250 μmol m^{-2} s^{-1}) or that had been growth for three weeks under low light followed by seven days under high light (800 μmol m^{-2} s^{-1}) and were then returned low light growth conditions for infestation and subsequent aphid growth for two weeks. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times.

Growth under HL (1000 μmol m^{-2} s^{-1}) conditions for seven days prior to analysis of aphid fecundity significantly decreased the number of aphids only on the leaves of antisense (TAO) plants compared to the corresponding genotype grown only under LL conditions (Fig. 6.12).

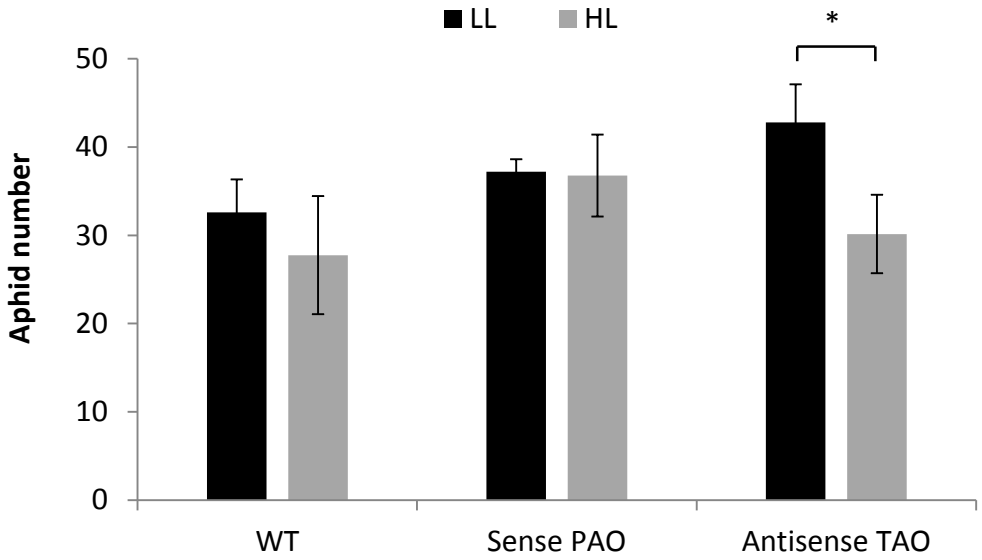


Figure 6.12 A comparison of aphid fecundity in wild type (WT) and in transformed tobacco plants that had high (PAO) or low (TAO) AO activity grown under low light (LL) and high light (HL) conditions. The numbers of aphids present on leaves two weeks after the onset of infestation were measured on plants that had either been grown for four weeks under low light (250 μmol m^{-2} s^{-1}) or that had been growth for three weeks under low light followed by seven days under high light (1000 μmol m^{-2} s^{-1}) and were then returned low light growth conditions for infestation and subsequent aphid growth for two weeks. (*$p < 0.05$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times.

Plant exposure to HL (1600 µmol m^{-2} s^{-1}) for seven days prior to analysis of aphid fecundity led to a significant light-dependent decrease in aphid numbers only on antisense (TAO) leaves compared to the corresponding genotype grown only under LL condition (Fig. 6.13A, B).

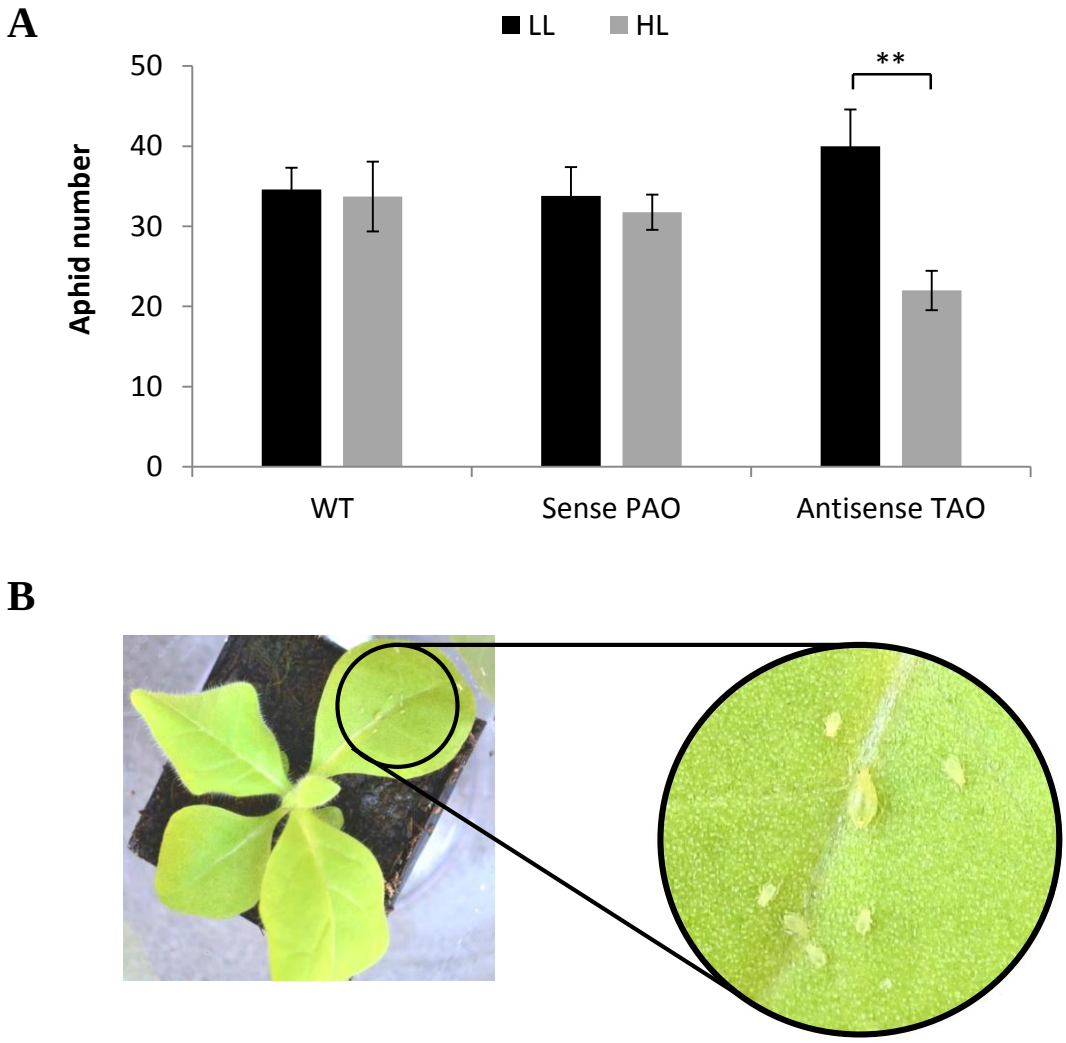


Figure 6.13 A comparison of aphid fecundity in sense PAO, antisense TAO and the wild type tobacco (WT) plants grown under low (LL) and high light (HL) conditions.
(A) Aphid fecundity. (B) Representative images of new-born nymphs at 15-days on antisense TAO leaves. The numbers of aphids present on leaves three weeks after the onset of infestation were measured on plants that had either been grown for four weeks under low light (250 µmol m^{-2} s^{-1}) or that had been growth for three weeks under low light followed by seven days under high light (1600 µmol m^{-2} s^{-1}) and were then returned low light growth conditions for infestation and subsequent aphid growth for two weeks. Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times. (**$p < 0.01$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype.

In the above experiments the aphid fecundity analysis has been performed on plants that had received a HL pre-treatment with different irradiances prior to the analysis of aphid fecundity, which was measured in plants returned to the LL growth conditions for 15 days.

A further set of experiments were performed in which aphid fecundity was measured in plants that grown under LL (250 µmol m^{-2} s^{-1}) for four weeks then a single, one-day-old nymph was placed on each plant and the infested plants were exposed to either LL or HL (1000 µmol m^{-2} s^{-1}) at the time of aphid infestation for 15 days (Fig. 6.14).

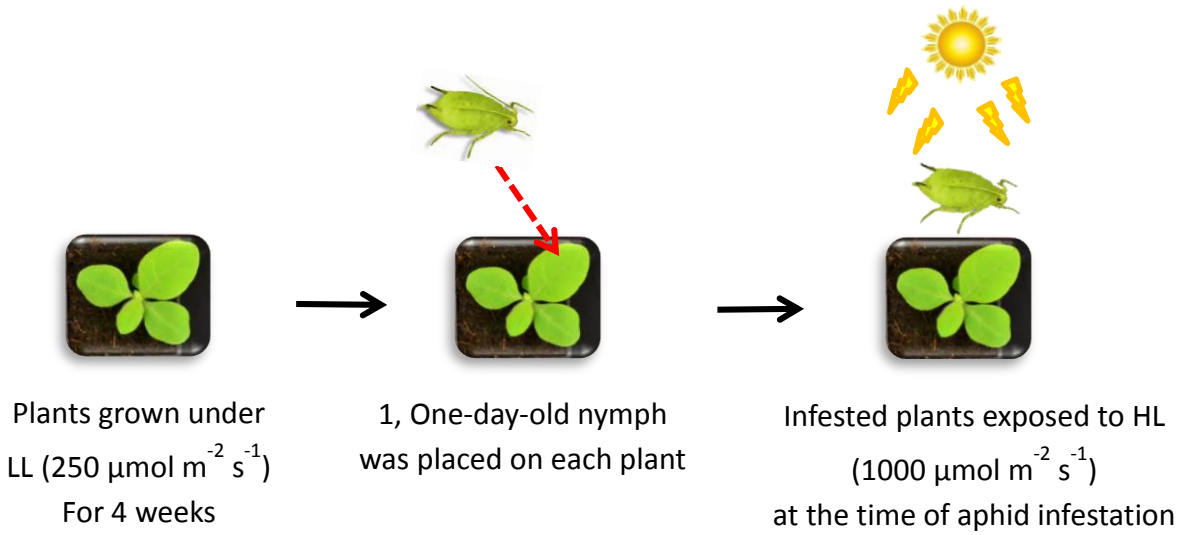


Figure 6.14 Experimental design illustrates aphid fecundity in sense PAO, antisense TAO and the wild type tobacco (WT) plants that grown for 4 weeks under low light (LL; 250 µmol m^{-2} s^{-1}) and then exposed to either LL or HL (1000 µmol m^{-2} s^{-1}) at the time of aphid infestation (15 days).

Growth under HL condition at the time of aphid infestation (15 days) led to a significant increase in all genotypes susceptibility to aphid infestation compared to the corresponding genotype that grown under LL condition at the time of aphid infestation (Fig. 6.15).

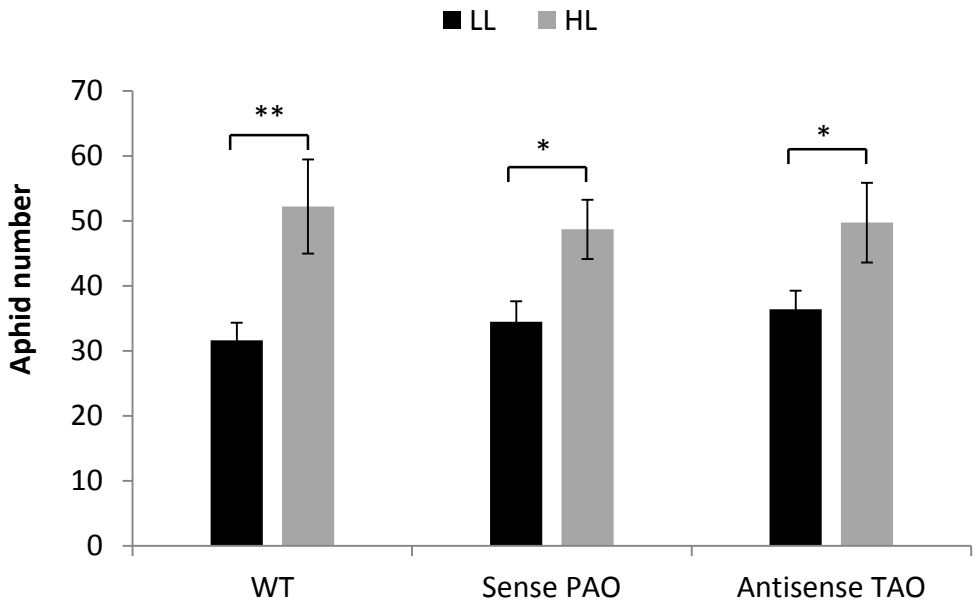


Figure 6.15 A comparison of aphid fecundity in sense PAO, antisense TAO and the wild type tobacco (WT) plants grown under low (LL) or high light (HL) conditions at the time of aphid infestation. The numbers of aphids present on leaves two weeks after the onset of infestation were measured on plants that had been grown for four weeks under low light (250 μmol m^{-2} s^{-1}), a single, one-day-old nymph was placed on each plant then the infested plants were either exposed to LL or HL (1000 μmol m^{-2} s^{-1}) at the time of aphid infestation (15 days). Data are the mean values ± SE (n = 10). Each experiment was repeated 3 times. (*$p < 0.05$); (**$p < 0.01$) in Significance given from analysis by One-Way ANOVA analysis of LL and HL values for each genotype.

6.3 Discussion

The role of ascorbate oxidase (AO) in plant responses to growth light intensity and to aphid infestation were studied in transformed tobacco plants that had high or low AO activity (Fig. 6.3). Although the leaves of the transformed lines had similar levels of leaf ascorbate content, the TAO with low AO activity had increased amounts of AsA in the extracted apoplastic fluid and less DHA than the wild type (Fig. 6.4). Conversely, the PAO with high AO activity had lower amounts of AsA in the extracted apoplastic fluid and more DHA than the wild type (Fig. 6.4). These findings are consistent with previous observation on these transgenic lines (Pignocchi et al., 2003). The earlier study reported a relationship between AO activity and plant height and biomass (Pignocchi et al., 2003) but in the present study the shoots of all the lines had a similar growth phenotype.

Earlier studies on plants with different AO activities were made only in the transgenic tobacco lines grown under LL intensities (250 μmol m^{-2} s^{-1} at with a (16h) photoperiod; Pignocchi et al., 2003). In the present experiments, plants were grown for three weeks under similar LL (250 μmol m^{-2} s^{-1}) conditions and then either maintained for a further seven days under LL growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. The plants that had been grown under HL for seven days had significantly more leaves than those grown under the LL conditions alone, but no significant light-dependent differences in leaf area were observed (Fig. 6.2A). The HL-induced acceleration in the development of the leaves is consistent with previously published observations in barley plants (Humbeck and Krupinska, 2003). Growth under HL resulted in higher levels of leaf ascorbate content but no change in the AsA/DHA ratios. In contrast, leaves grown for seven days under HL had lower chlorophyll contents with decreased Fv/Fm ratios (Figs. 6.6 and 6.7).

The rates of photosynthetic CO_2 assimilation measured at (250 μmol m^{-2} s^{-1}) in the leaves of HL grown plants were lower than that of the leaves that had only been grown under LL (Fig. 6.8). The light-dependent changes in leaf number (Fig. 6.2), ascorbate (Fig. 6.5), pigments (Fig. 6.6) and photosynthesis (Fig. 6.8) were similar in all lines.

The effects of light on aphid fecundity were studied in tobacco plants that had been exposed to HL pre-treatments, consisting of a seven days exposure (8h photoperiod) to either 800, 1000 or 1600 μmol m^{-2} s^{-1}. Aphid fecundity was similar in the leaves of the wild type and PAO plants, regardless of the light pre-treatment, demonstrating that the light history of the tobacco plants had no effect on the ability of aphids to infest and reproduce on the leaves. In contrast, aphid fecundity was significantly lower on the TAO lines that had been pre-treated with HL at intensities of 1000 or 1600 but not 800 μmol m^{-2} s^{-1} (Figs. 6.12 and 6.13), the numbers of aphids on the TAO leaves that had been exposed to the highest irradiance for seven days were about 50% lower than the TAO leaves grown under LL or the wild type under similar growth conditions. These data suggest that the lower AO activities and/or the higher AsA/DHA ratios in the TAO leaves conferred resistance to aphid infestation in leaves that had previously been exposed to HL stress. Given that the light-dependent changes in leaf number, ascorbate, pigments and photosynthesis were similar in all lines, the HL stress protection conferred by lower AO activities is likely to be largely independent of these parameters. Further experiments were therefore performed to investigate the role of AO in aphid resistance by comparing the transcript and metabolite profiles of the leaves of all lines grown under either LL (250 μmol m^{-2} s^{-1}), or given a HL pre-treatment at (1600 μmol m^{-2} s^{-1}) and then infested with 60 adult aphids for 12h (Chapters 7 and 8).

In these experiments discussed above, the aphid fecundity analyses has been performed on plants that had received a HL pre-treatment with different irradiances prior to the analysis of aphid fecundity, which was measured in plants returned to the LL growth conditions for 15 days. These experiments were designed to determine whether a “memory”, of the abiotic HL stress persisted in plants returned to LL conditions in such a way as to influence aphid fecundity.

A further set of experiments were performed in which aphid fecundity was measured in plants that were exposed to either LL or HL at the time of aphid infestation (Fig. 6.15). In this situation, aphid fecundity was significantly higher in the leaves under HL (1000 μmol m^{-2} s^{-1}) conditions than LL (250 μmol m^{-2} s^{-1}) conditions (Fig. 6.15).

These results shown in figure 6.15 demonstrate that HL growth conditions enhance aphid fecundity if both the abiotic and biotic stresses are imposed simultaneously. The light and CO_2 response curves for photosynthesis (Fig. 6.9) show that the maximal rates of photosynthesis were similar in all plants irrespective of the growth irradiance. It is likely that the leaves of plants grown under HL had higher levels of sucrose and nutrients than those grown under LL. The transcript and metabolite profiles of the leaves grown under LL and HL support this hypothesis (Chapters 7 and 8).

Chapter 7. Transcript profile of ascorbate oxidase transgenic tobacco plants grown either under low or high light in the absence or presence of aphids

7.1 Introduction

Literature evidence shows that leaf transcriptome responses to HL include effects on plant responses to biotic stresses (Hihara et al., 2001; Rossel et al., 2002; Kimura et al., 2003; Galvez-Valdivieso et al., 2009; Rasmussen et al., 2013). However, while the abiotic stresses in the environment are known to influence aphid infestation, the relationships between growth light intensity and aphid infestation are relatively poorly documented. Aphid infestation leads to substantial transcriptome re-programming (Thompson and Goggin, 2007; Kerchev et al., 2013; Coppola et al., 2013). Moreover, plants show systemic responses to aphids such that the abundance of transcripts is altered in systemic leaves that are far removed from the site of aphid infestation (Kerchev et al., 2013). Aphid infestation leads to increased levels of transcripts involved in redox signalling, as well as salicylic acid (SA), jasmonic acid (JA) and ethylene-related signalling pathways (Kerchev et al., 2013; Coppola et al., 2013). An analysis of the transcriptional responses to the generalist phloem feeders (*Myzus persicae* and *Bemisia tabaci*) and the specialist *Brevicoryne brassicae* were compared in Arabidopsis, highlighted the importance of calcium, WRKY transcription factors, receptor kinase signalling, and wall associated kinases (Foyer et al., 2015). Another common feature of plant response to phloem feeding insects was the decrease in the levels of transcripts encoding components of secondary metabolism. For example, transcripts associated with glucosinolate metabolism were decreased in the plant response to *M. persicae* but not *B. brassicae* (Foyer et al., 2015).
The studies reported in Chapter (6) show that aphid fecundity in tobacco is influenced by environmental (light intensity) and metabolic (ascorbate oxidase, AO) factors. AO catalyses the oxidation of ascorbate in the apoplast/cell wall compartment of the cell and plays an important role in responses to external stimuli (Pignocchi and Foyer, 2003). Aphid fecundity was similar on the leaves of wild type tobacco plants and transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations grown under low light (LL) conditions. However, unlike the wild type and PAO lines, aphid fecundity was significantly decreased by a HL pre-treatment in the TAO plants.

The following studies were performed in order to determine the leaf transcriptome responses to growth light intensity and aphid infestation in the wild type, PAO and TAO tobacco lines. Plants were grown under LL (250 μmol m^{-2} s^{-1}) for three weeks and then either maintained for a further seven days under LL conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Leaf transcriptome profile were first compared in wild type tobacco plants and in PAO and TAO lines that had been grown under LL or HL in the absence of aphids. Thereafter, plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions. Sixty adult wingless aphids were transferred to upper surface of the youngest mature leaves of LL and HL pre-treated plants with a small paint brush and enclosed in a mesh (mesh size 200 μm) covered clip cage (2.5 cm diameter) for 12h. Plants with cages without aphids were used as controls for these experiments. Plants were then maintained under LL for the period of aphid infestation. Leaf samples were harvested from LL and HL-grown plants, as well as infested and non-infested leaves were collected 12h following the onset of aphid infestation and frozen in liquid nitrogen until the analysis.

7.2 Results

7.2.1 Transcript changes in response to light availability in all genotypes

Number of differentially expressed transcripts in response to high light (HL) treatment

In total, 4465 transcripts were differentially expressed in the tobacco leaves in response to HL regardless of genotype in these experiments (Fig. 7.1A, B, C, Appendix I). Of these, 2665 were increased in abundance and 1800 were lower than in the leaves grown under LL (Fig. 7.1B, C).

Of the 4465 transcripts were differentially expressed in response to changes in growth irradiance, 977 transcripts were more abundant in the leaves of all genotypes under HL conditions relative to LL-grown plants. Similarly, the levels of 356 transcripts were lower in the leaves of all genotypes under HL conditions relative to LL-grown plants.

Of the transcripts that were increased in response to the growth light conditions, 317 were unique to wild type plants, 273 were unique to sense PAO plants and 508 were unique to antisense TAO plants (Fig. 7.1B, C). In addition, of the transcripts that were lower under HL, 192 were changed only in the leaves of wild type plants, 284 were lower only in PAO plants and 629 transcripts were lower only in TAO plants (Fig. 7.1B). In addition to the transcripts that were changed in a genotype-specific manner in response to the growth light level, others were altered in a similar manner in more than one genotype. For example, the levels of 154 transcripts were higher in abundance in both the wild type and PAO lines (Fig. 7.1B). Similarly, the levels of 123 mRNAs were lower in abundance in both the wild type and PAO lines. In addition, 281 transcripts were increased and 102 transcripts were decreased in a similar manner in both wild type and TAO plants. Moreover, HL treatment led to an increase in the abundance of 155 transcripts in both the PAO and TAO leaves while 114 transcripts were lower than in plants grown under LL (Fig. 7.1B, C).

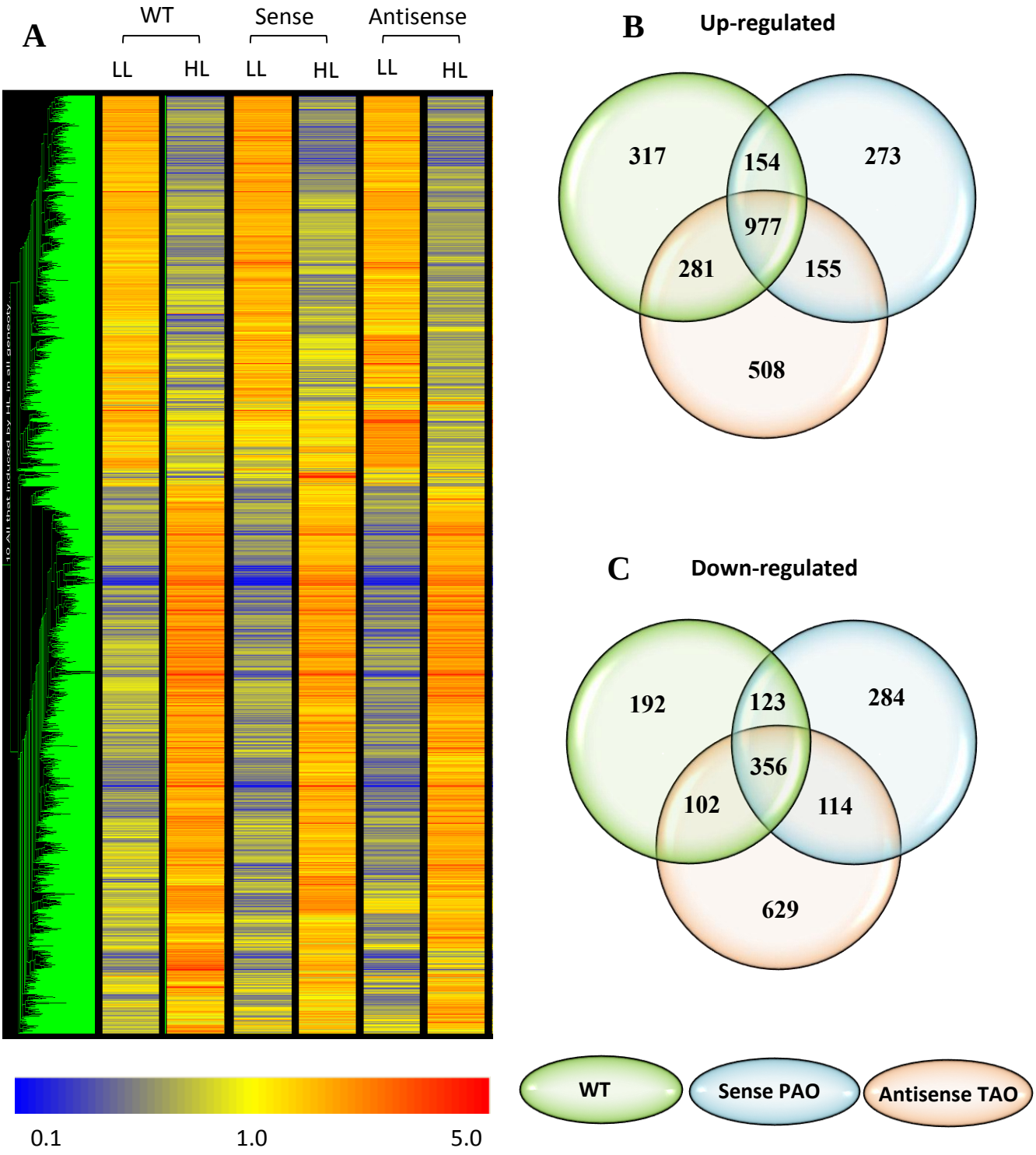


Figure 7.1 Hierarchical clustering and venn diagrams of differentially expressed transcripts under high light (HL) conditions relative to low light (LL) in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. (A): Hierarchical clustering. **(B)**: Common and unique up-regulated genes. **(C)**: Common and unique down-regulated genes. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg).

Of the 977 transcripts that were increased in abundance in all genotypes under HL conditions relative to LL-grown plants (Fig. 7.1B), transcripts encoding proteins associated with photosynthesis (128 transcripts) or otherwise with chloroplasts (90 transcripts) were the largest functional group (Fig. 7.2A). Other important functional categories were transcripts encoding proteins associated with stress responses (106 transcripts), transcription (99 transcripts), transport (94 transcripts), signal transduction (80 transcripts) and metabolism (20 transcripts; Fig. 7.2A).

Of the 356 common transcripts that were decreased in abundance under HL conditions in all genotypes compared to LL plants (Fig. 7.1C), 98 were associated with transporter, 89 with transcription, 35 with signalling, 18 with chloroplast functions, 14 with stress and 13 with metabolism (Fig. 7.2B).

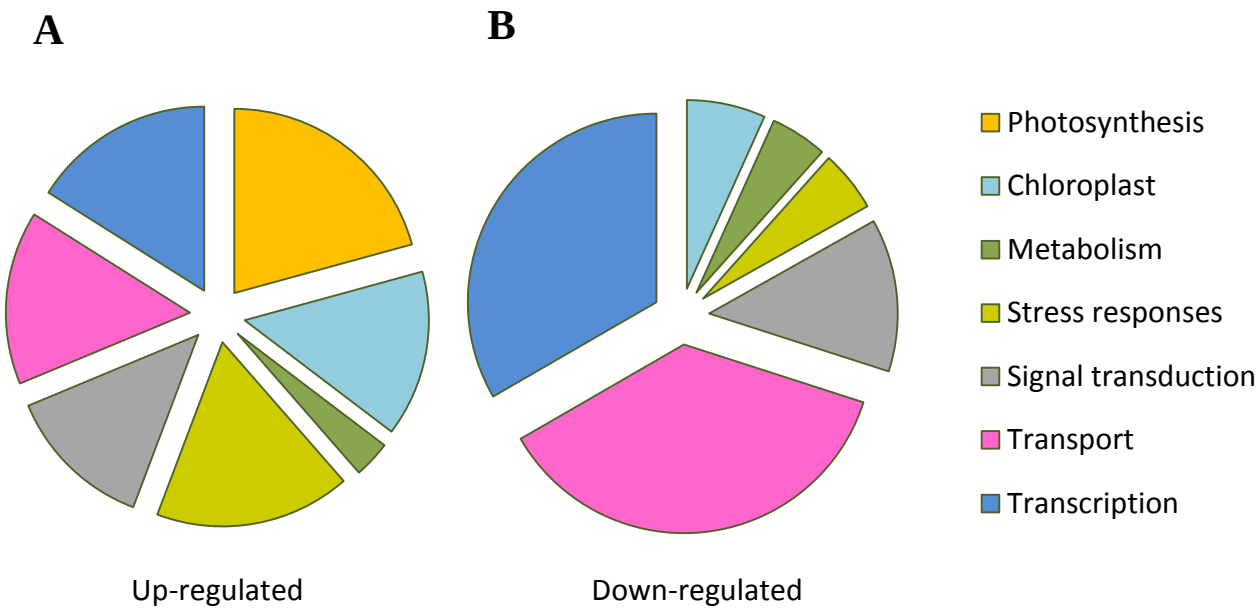


Figure 7.2 Functional groups of common genes that induced (A) and repressed (B) by high light treatment in the leaves of wild type tobacco plants and in transgenic lines sense (PAO) or antisense (TAO). To assign the genes to functional groups gene ontology (GO) annotation was carried out using agriGO software (http://bioinfo.cau.edu.cn/agriGO/).

Transcripts that were changed in response to growth irradiance are described in detail below:

a) Photosynthesis associated transcripts

The abundance of many transcripts that encode components associated with photosynthesis, such as the light-harvesting chlorophyll a-b binding proteins (LHCP) of photosystem I (PSI) and photosystem II (PSII) was increased in plants grown under HL compared to LL (Fig. 7.2A). The levels of LHCP transcripts were significantly increased in response to the HL treatment in all genotypes compared to LL grown plants (Table 7.1).

Table 7.1 Photosynthesis-related transcripts that their expression was altered in response to high light treatment commonly in the leaves of wild type tobacco plants and in transgenic lines sense PAO and antisense TAO. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
photosystem I				
A_95_P105332	CV017417	7.57	Chlorophyll a-b binding protein 1	PS.lightreaction.photosystem I.LHC-I
A_95_P125507	EB102904	6.52	Chlorophyll a-b binding protein 13	PS.lightreaction.photosystem I.LHC-I
A_95_P108792	CV019003	4.22	Chlorophyll a-b binding protein 6A	PS.lightreaction.photosystem I.LHC-I
A_95_P179347	DV998829	3.46	Chlorophyll a-b binding protein 1	PS.lightreaction.photosystem I.LHC-I
A_95_P111737	CV020355	3.38	Chlorophyll a-b binding protein 6A	PS.lightreaction.photosystem I.LHC-I
A_95_P012501	CV018784	3.22	Chlorophyll a-b binding protein 6A	PS.lightreaction.photosystem I.LHC-I
A_95_P105692	CV017571	1.89	Chlorophyll a-b binding protein 8	PS.lightreaction.photosystem I.LHC-I
A_95_P105282	CV017393	1.76	Photosystem I subunit O	PS.lightreaction.photosystem I.PSI polypeptide subunits
A_95_P110717	CV019869	1.61	Photosystem I subunit L	PS.lightreaction.photosystem I.PSI polypeptide subunits

A_95_P109852	EB681709	1.60	Photosystem I reaction center subunit VI	PS.lightreaction.photosystem I.PSI polypeptide subunits
A_95_P111617	CV020277	1.58	Photosystem I subunit L	PS.lightreaction.photosystem I.PSI polypeptide subunits
A_95_P110772	CV019890	1.56	Photosystem I subunit O	PS.lightreaction.photosystem I.PSI polypeptide subunits
A_95_P181257	EB679822	1.03	PGR5-like A	PS.lightreaction.cyclic electron flow-chlororespiration
photosystem II				
A_95_P002906	TA14592_4097	7.88	Chlorophyll a-b binding protein 7	PS.lightreaction.photosystem II.PSII
A_95_P003266	CV021520	7.63	Chlorophyll a/b binding protein	PS.lightreaction.photosystem II.LHC-II
A_95_P176552	TA11638_4097	7.59	Chlorophyll a-b binding protein 40	PS.lightreaction.photosystem II.LHC-II
A_95_P107827	CV018547	7.50	Chlorophyll a-b binding protein 40	PS.lightreaction.photosystem II.LHC-II
A_95_P003231	TA11623_4097	7.45	Chlorophyll a-b binding protein 40	PS.lightreaction.photosystem II.LHC-II
A_95_P110677	TA11624_4097	6.31	Chlorophyll a-b binding protein E	PS.lightreaction.photosystem II.LHC-II
A_95_P105757	CV018172	6.30	Chlorophyll a-b binding protein 1	PS.lightreaction.photosystem II.LHC-II
A_95_P002906	TC123590	7.88	Chlorophyll a-b binding protein 7	PS.lightreaction.photosystem II.PSII
A_95_P110677	TA11624	6.31	Chlorophyll a-b binding protein E	PS.lightreaction.photosystem II.LHC-II
A_95_P106947	CV018140	5.22	Chlorophyll binding protein	PS.lightreaction.photosystem II.LHC-II
A_95_P002941	FG134885	5.13	Chlorophyll a-b binding protein 16	PS.lightreaction.photosystem II.LHC-II
A_95_P105557	CV017513	5.09	Chlorophyll a-b binding protein 3A	PS.lightreaction.photosystem II.LHC-II
A_95_P247017	EB424655	3.73	Chlorophyll a-b binding protein CP24	PS.lightreaction.photosystem II.LHC-II

b) Transcripts associated with redox processes

The abundance of several transcripts encoding glutathione S-transferases (GSTs), which catalyse the oxidation of glutathione during the detoxification of reactive substrates, particularly during responses to pathogen attack or oxidative stress (Marrs, 1996), were increased in the leaves of HL-grown plants relative to LL plants (Table 7.2). Similarly, the leaves of HL grown plants accumulated transcripts encoding flavin mono-oxygenase (FMO)-like proteins, which catalyse the oxidation of low molecular weight substrates and play a role in pathogen defence and in the biosynthesis of auxin and glucosinolates (Schlaich, 2007).

Table 7.2 Redox processes-associated genes that altered in the leaves of wild type tobacco plants and in transgenic lines sense PAO and antisense TAO grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P010131	X64399	2.71	Glutathione S-transferase	misc.glutathione S transferases
A_95_P163312	EH622305	2.68	Glutathione S-transferase	misc.glutathione S transferases
A_95_P152072	EB682364	2.63	Gigantea protein (GI)	Circadian clock coupling factor ZGT
A_95_P292563	EB427707	2.26	FMO-like monooxygenase/ oxidoreductase	misc.oxidases - copper, flavone
A_95_P247207	EH622095	1.94	response to oxidative stress	stress.abiotic
A_95_P113872	CV021352	1.86	Senescence associated gene 21	development.late embryogenesis abundant
A_95_P159237	EH617190	1.81	Late embryogenesis abundant protein Lea5	development embryo related
A_95_P019621	DW004086	1.67	Superoxide dismutase	redox.dismutases and catalases
A_95_P007796	Y14972	1.35	Annexin	cell.organisation
A_95_P010971	DV162525	1.28	Glutathione S-transferase	misc.glutathione S transferases
A_95_P258281	EB431441	1.10	Glutathione S-transferase	misc.glutathione S transferases

c) Protein kinase associated transcripts

The levels of transcripts encoding a mitogen associate protein kinase 3 (MPK3), which is related to induced resistance to biotic and abiotic stresses (Beckers et al., 2009), were higher in leaves under HL than LL conditions (Table 7.3). Similarly, the abundance of transcripts encoding a serine/threonine protein kinase SAPK8-like protein was increased under HL relative to LL conditions (Table 7.3). The serine/threonine protein kinase SAPK8-like protein is a member of the sucrose nonfermenting1-related protein kinase 2 (SnRK2) proteins family that involve in the transduction of abscisic acid (ABA) signalling (Wang et al., 2013).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P290014	DV161729	1.95	Protein kinase	postranslational modification
A_95_P024461	AB020590	1.58	Mitogen associate protein kinase 3	signalling.MAP kinases
A_95_P311288	FG141368	1.27	Serine/threonine-protein kinase	signalling.receptor kinases.protein.postranslational modification
A_95_P157827	EH615857	1.08	Serine/threonine protein kinase SAPK8-like protein	postranslational modification
A_95_P155857	EG650296	1.02	Receptor-like kinase 4	stress.biotic
A_95_P267611	DV160845	-1.43	Receptor-like kinase	signalling.receptor kinases.leucine rich repeat III
A_95_P150902	TC134920	-1.46	Serine/threonine protein kinase 1	
A_95_P272651	EB451799	-1.57	High leaf temperature 1 kinase	postranslational modification

Table 7.3 Protein kinase-associated genes that altered in the leaves of wild type tobacco plants and in transgenic lines sense PAO and antisense TAO grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

d) Stress responses-associated transcripts

Large numbers of transcripts involved in plant stress responses were higher in abundance in the leaves pre-treated with HL compared to LL (Fig. 7.2A, Table 7.4). For example, transcripts encoding proteinase inhibitor I-B were higher under HL than LL (Table 7.4). Proteinase inhibitors regulate the activity of proteases during plant development and in response to stress (Samac and Smigocki, 2003). Furthermore, the abundance of transcripts encoding dirigent-like protein (pDIR12) was higher under HL than LL (Table 7.4). The dirigent-like proteins are localized in the cell wall and play important role in plant defences against pathogen and insect attack, through participation in lignin biosynthesis (Davin and Lewis, 2000; Ralph et al., 2006). Transcripts encoding Flavin-dependent monooxygenase 1 were also higher in leaves grown under HL than LL. This FMO enzyme is involved in pathogen defence responses and also in the biosynthesis of auxin and glucosinolates (Schlaich, 2007). Transcripts encoding an osmotin precursor, which is a pathogenesis-related (PR) protein that is accumulated under stress conditions (Singh et al., 1987; Venkatachalam et al., 2007), were also higher in leaves under HL than LL (Table 7.4).

Table 7.4 Stress responses-related transcripts that altered in the leaves of wild type tobacco plants and in transgenic lines sense PAO and antisense TAO grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P000771	X67076	3.04	Proteinase inhibitor I-B	protease inhibitor related
A_95_P220112	TC142248	3.01	Dirigent-like protein pDIR12	Wound and insect induced genes
A_95_P273666	EH617861	2.97	Disease resistance protein	verticillium wilt /Hcr2, stress.biotic.PR-proteins
A_95_P292563	EB427707	2.26	Flavin dependent mono oxygenase 1	Systemic Acquired Resistance
A_95_P004201	X65700	2.20	Osmotin precursor	stress.biotic
A_95_P298383	EH620366	2.03	NADPH oxidase	stress.biotic.respiratory burst
A_95_P121357	DW001395	1.90	Plant cadmium resistance 2	stress related
A_95_P091943	TC152875	1.90	Polygalacturonase-inhibiting protein -	inhibit pathogen polygalacturonase
A_95_P247062	EB428015	1.85	Ethylene-responsive element binding factor	stress biotic related
A_95_P247062	EB428015	1.85	Ethylene-responsive element binding factor	stress biotic related
A_95_P283429	AY639146	1.79	Proline oxidase	Osmotic stress-responsive proline dehydrogenase
A_95_P140917	EB444726	1.74	Ubiquitin-conjugating	protein.degradation.ubiquitin

A_95_P102522	CV016057	1.57	Stress-responsive protein	putative, stress.abiotic.cold
A_95_P000776	X67075	1.56	Microbial serine proteinases inhibitor	protease inhibitor related
A_95_P049626	BP132210	1.52	Disease resistance protein (TIR-NBS-LRR class)	putative, stress.biotic.PR-proteins
A_95_P002681	CV018508	1.49	Chitinase	stress.biotic
A_95_P105032	EB428527	1.46	Cold acclimation protein COR413	stress.abiotic.cold
A_95_P235119	EH615107	1.42	ERD15 protein	stress.abiotic.drought/salt
A_95_P106277	CV017834	1.39	Wound-induced protein	putative, Defense related
A_95_P119912	DV999542	-1.35	Auxin-binding protein ABP19a precursor	stress.abiotic.unspecified

e) Expression profile of transporters associated transcripts

The levels of many transcripts encoding transporters were higher in the tobacco leaves under HL (Fig 2A, Table 7.5). These included transcripts encoding an ATP-binding cassette (ABC) pleiotropic drug resistance transporter (PDR) transporter, which were increased in the leaves under HL (Table 7.5). This transporter is localized to the plasma membrane and is involved in the transport of ABA (Kang et al., 2010). Similarly, transcripts encoding a copper transporter 1, which is involved in copper transport and important in metal tolerance (Salt et al., 1998; Sancenon et al., 2003), were increased under HL (Table 7.5). Purine permease 9, iron transport protein 2 and sugar transporter transcripts, were also enhanced in the leaves under HL compared to LL (Table 7.5).

Table 7.5 Transcripts encoding transporters that were altered in abundance in the leaves of wild type, PAO and TAO tobacco plants under HL. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P004526	EH622485	2.72	PDR-type ABC transporter 1	putative, transport.ABC transporters and multidrug resistance systems
A_95_P196332	EB428726	2.61	Copper transporter 1	transport.metal
A_95_P291733	EB424741	2.13	Purine permease 9	putative, transport.nucleotides
A_95_P111707	CV020338	1.50	Iron transport protein 2	transporter related
A_95_P125897	EB425028	1.44	Sugar transporter	transport.sugars
A_95_P255849	AM817974	1.39	Sugar transporter st3 protein (st3)	transport.sugars
A_95_P268381	DW001009	1.27	Bile acid:sodium symporter	transport.unspecified cations
A_95_P253589	EH622381	1.24	SNAP25 homologous protein SNAP33	cell.vesicle transport
A_95_P004381	AF112863	1.23	Syntaxin 125	cell.vesicle transport
A_95_P092308	BP531742	1.14	Polyol transporter related cluster	transporter related
A_95_P122677	DW002655	1.12	Polyol transporter	transport.sugars
A_95_P308648	FG643148	1.10	Na+ symporter	transporter related
A_95_P226494	EB680189	-3.82	Vacuolar citrate/H+ symporter	transport.unspecified cations
A_95_P041366	BP130073	-4.66	ABC transporter family	transport.ABC transporters and multidrug resistance

f) Transcripts encoding transcription factors

Large numbers of transcripts encoding transcription factors were altered in abundance in response to HL (Fig. 7.2A, Table 7.6). Growth under HL for seven days significantly increased the abundance of WRKY33 transcripts, which encode a pathogen-inducible transcription factor (Table 7.6; Mao et al., 2011). Similarly, the levels of transcripts encoding ethylene-responsive transcription factors (ERFs) 1 and 4 were higher under HL (Table 7.6). ERFs either induce or repress the transcription of genes that encode proteins involved in ethylene signalling (Ohta et al., 2000). Of the four tobacco ERFs, ERF2 and ERF4 induce the transcription of ethylene-related genes, while ERF3 is a repressor of the expression of ethylene-related genes (Ohta et al., 2000). Moreover, transcripts encoding cycling dof factor 3 (CDF3) were higher in the leaves under HL (Table 7.6). The CDF transcription factors family plays an important role in plant defence responses against biotic and abiotic stresses. For example, overexpression of CDF1 and CDF3 enhanced drought and salt tolerance in Arabidopsis (Corrales et al., 2014). In contrast, a pre-exposure to HL led to a significant decrease in the abundance of transcripts encoding a basic helix-loop-helix (BHLH) transcription factor, which belongs to a large family of transcription factors that regulate many biological processes (Table 7.6; Bailey et al., 2003).

Table 7.6 Transcripts encoding transcription factors that were altered in abundance in the leaves of wild type, PAO and TAO tobacco plants under HL. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P101338	AB063573	2.17	WRKY33	RNA.regulation of transcription.WRKY domain transcription factor family
A_95_P237924	AB020023	1.84	WRKY 3	RNA.regulation of transcription.WRKY domain transcription factor
A_95_P034768	AY627865	1.83	Transcription factor	hormone metabolism.ethylene.signal transduction
A_95_P148192	EB677428	1.81	Zinc finger CCCH domain-containing protein 2-like	predicted, RNA.regulation of transcription.unclassified
A_95_P105832	DV999396	1.52	Ethylene-responsive transcription factor 4	hormone metabolism.ethylene.signal transduction
A_95_P271051	EB439258	1.39	Cycling dof factor 3 (CDF3)	RNA.regulation of transcription.C2C2(Zn) DOF zinc finger family
A_95_P235964	EB450575	1.36	NAC1-like	pathogen-induced transcription factor
A_95_P195572	DV999109	1.34	Ethylene-responsive transcription factor 1	hormone metabolism.ethylene.signal transduction
A_95_P004626	D61377	1.03	WRKY-type transcription factor	RNA.regulation of transcription.WRKY domain transcription factor family

A_95_P119577	DV998968	-2.33	BHLH transcription factor	putative, RNA.regulation of transcription.bHLH,Basic Helix-Loop-Helix family
A_95_P026211	TA14910_4097	-3.79	Sigma-like factor precursor	RNA.transcription
A_95_P011106	EB680133	-4.17	RNA polymerase sigma factor rpoD	putative, RNA.transcription
A_95_P196562	TA16366_4097	-4.72	Myb transcription factor	RNA.regulation of transcription.MYB-related transcription factor family

g) Metabolism-associated transcripts

Many metabolism-related transcripts were altered in abundance in tobacco leaves grown under HL compared to LL (Fig. 7.2A, Table 7.7). Transcripts encoding a fructokinase-like protein were higher under HL (Table 7.7). This fructokinase is a member of the chloroplast phosphofructokinase B-type (pfkB-type kinase) carbohydrate kinase family (Ogawa et al., 2009; Gilkerson et al., 2012). Similarly, the expression of gene encoding alpha-glucan, a starch-phosphorylating enzyme that catalyses the phosphorylation of starch (Mikkelsen et al., 2004), was up-regulated in the leaves under HL (Table 7.7). Moreover, the HL-grown leaves accumulated transcripts encoding 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase, which plays a role in ethylene biosynthesis (Table 7.7; Chung et al., 2002). Furthermore, the transcripts encoding zeaxanthin epoxidase, which participates in ABA biosynthesis as well as being an important enzyme in the xanthophyll cycle that protects PSII from the adverse effects of HL by participating in thermal energy dissipation (Part et al., 2008), were increased in the HL-grown leaves (Table 7.7).

Table 7.7 Transcripts encoding proteins associated with metabolism that were altered in abundance in the leaves of wild type, PAO and TAO tobacco plants under HL. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
Major CHO metabolism				
A_95_P249137	FG160796	2.80	Fructokinase-like	major CHO metabolism.synthesis.sucrose.FBPase
A_95_P050261	BP132352	2.19	Alpha-glucan	water dikinase, major CHO metabolism.degradation.starch.glucan water dikinase
A_95_P229449	FG146265	2.11	Starch associated protein R1	major CHO metabolism.synthesis.starch.starch branching
A_95_P231759	FG136072	1.37	Alpha-glucan phosphorylase type H isozyme	major CHO metabolism.degradation.starch.starch phosphorylase
A_95_P184752	TA13765_4097	-3.67	1,4-alpha-glucan-maltohydrolase	major CHO metabolism.degradation.starch.starch cleavage.beta amylase
Secondary metabolism				
A_95_P200827	EB448410	2.07	Isochorismatase hydrolase	secondary metabolism.phenylpropanoids
A_95_P291748	EB424773	1.49	Scenescence related gene 1	secondary metabolism.flavonoids
A_95_P031441	EB442855	1.37	Aldehyde dehydrogenase	fermentation.aldehyde dehydrogenase

A_95_P249212	AJ582651	-1.87	Hydroxycinnamoyl CoA quinate transferase	secondary metabolism.phenylpropanoids
A_95_P195322	AM845626	-4.13	UDP-glucosyl transferase 72E1	secondary metabolism.phenylpropanoids.lignin biosynthesis
Hormone metabolism				
A_95_P157492	EH615541	2.96	1-aminocyclopropane-1-carboxylic acid (ACC) oxidase	hormone metabolism.ethylene.synthesis-degradation
A_95_P016511	DV999707	2.47	Zeaxanthin epoxidase	hormone metabolism.abscisic acid.synthesis-degradation.synthesis.zeaxanthin epoxidase
A_95_P254344	AF190634	2.39	UDP-glucose	salicylic acid glucosyltransferase (SA-GTase)
A_95_P029796	EB444740	1.86	Ethylene forming enzyme	hormone metabolism.ethylene.synthesis-degradation
A_95_P011252	AB125233	1.59	Gibberellin 2-oxidase 2	hormone metabolism.gibberelin.synthesis-degradation.GA2 oxidase
A_95_P210807	DQ129870	1.18	Hydroperoxide lyase	hormone metabolism.jasmonate.synthesis-degradation.allene oxidase synthase

7.2.2 Unique transcripts that were changed in abundance in TAO plants under HL

Of the 508 unique transcripts that were increased in abundance in the leaves of TAO plants under HL conditions relative to LL-grown plants (Fig. 7.1B), 78 transcripts encoding transporter, 76 encoding transcription, 70 encoding signalling, 54 were stress-related, 32 encoding terpenoids, 23 were kinase-related, 21 were plasmalemma-related and 13 were cell wall-related (Fig. 7.3A).

Of the 629 unique transcripts that were decreased in abundance in the leaves of TAO plants under HL conditions compared to LL plants (Fig. 7.1C), 62 transcripts were transcription-related, 58 were signalling-related, 46 were membrane transporter-related, 42 were kinase-related, 38 were stress-related, 35 were cell wall-related and 34 were plasmalemma-related (Fig. 7.3B).

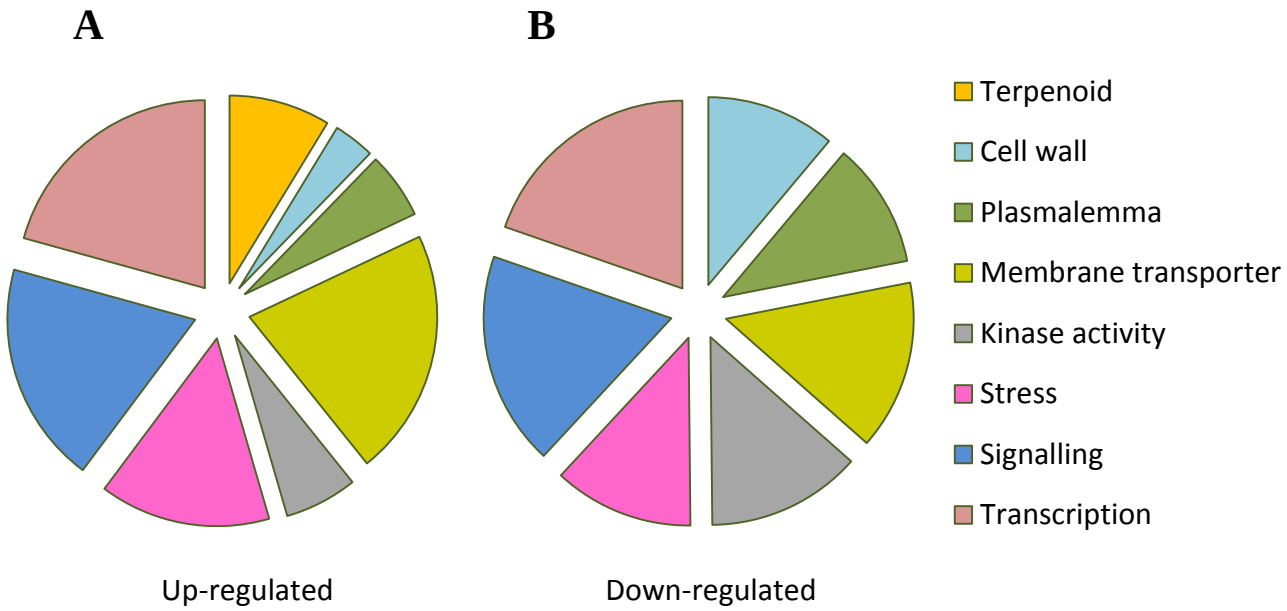


Figure 7.3 Functional groups of unique genes to antisense TAO plants that induced (A) and repressed (B) by high light treatment. To assign the genes to functional groups gene ontology (GO) annotation was carried out using agriGO software (http://bioinfo.cau.edu.cn/agriGO/).

a) Cell wall associated transcripts

The levels of cell wall-associated transcripts were changed in TAO leaves grown under HL relative to those of the wild type and PAO plants (Fig. 7.3A, Table 7.8). For example, transcripts encoding a thionin-like protein (D6) were increased in abundance in the HL-grown TAO leaves (Table 7.8). Thionins are a cysteine-rich family of proteins that play roles in defence mechanisms against fungi and insects, by inhibiting the activities of α-amylase and proteinases (Bloch and Richardson, 1991; Melo et al., 2002). This group of proteins, which accumulate following pathogen infestation, are localized in the cell wall (Kang and Buchenauer, 2003). Similarly, HL-grown leaves accumulated transcripts encoding a hydrolase. The function of these enzymes, which are synthesized in the cell wall in response to pathogen attack, is to hydrolyse polysaccharides in the pathogen cell wall (Rose et al., 2000). Transcripts encoding an endo-1,3-β-glucosidase were also increased in the leaves of HL-grown TAO (Table 7.8). The endo-1,3- β -glucosidases not only degrade β-1,3/1,6 glucans that are localized in pathogen cell walls (Rose et al., 2000) but they also function in oligosaccharide production in plant cell walls, which plays an important role in the induction of plant defence responses during pathogen attack (Ham et al., 1997). Moreover, transcripts encoding aldehyde dehydrogenase 7 (ALDH7) were increased in abundance in the HL-grown TAO leaves (Table 7.8). ALDH7 plays a central role in the detoxification of reactive aldehydes in the cell wall (Sunkar et al., 2003; Coppola et al., 2013).

The abundance of transcripts encoding xyloglucan endotransglucosylase/hydrolase 15 was also increased in the TAO leaves under HL (Table 7.8). Xyloglucan endotransglucosylases/hydrolases are involved in the remodelling of the cell wall during herbivore/insect attack in a way that increases the function of the wall as a physical barrier to attack, making insect settling and feeding more difficult (Divol et al., 2007). Furthermore, the HL-treated leaves accumulated transcripts encoding a cationic peroxidase, which is localized in the apoplast/cell wall compartment (Table 7.8; Young et al., 1995). Peroxidases have a number of important functions such as the regulation of cell elongation (Goldberg et al., 1986), polysaccharide cross-linking (Fry, 1986) and wound healing (Espelie et al., 1986), particularly during pathogen or insect attack.

Table 7.8 Cell wall associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P110192	CV019616	2.35	Thionin-like protein (D6)	cell wall related. Stress .biotic
A_95_P270056	EB451396	1.70	Hydrolase	cell wall.modification
A_95_P305833	FG172156	1.65	Phosphate-induced protein	putative
A_95_P164712	EH624023	1.64	Endo-1,3-beta-glucosidase	cell wall related
A_95_P260196	DV160279	1.35	LEXYL2 protein Xylan 1,4-beta-xylosidase	cell wall.degradation.mannan-xylose-arabinose-fucose
A_95_P115427	DV157630	1.24	Cationic peroxidase	putative, misc.peroxidases
A_95_P148597	EB677958	1.16	Arabinoxylan arabinofuranohydrolase isoenzyme AXAH-II	putative, Intersting and miscellaneous
A_95_P150987	EB449923	1.05	Carboxyesterase 18	Biodegradation of Xenobiotics
A_95_P010321	EB448853	1.04	xyloglucan endotransglucosylase/hydrolase 15	xyloglucan endotransglucosylase/hydrolase
A_95_P159537	TA17140_4097	1.00	Aldehyde dehydrogenase family 7	fermentation.aldehyde dehydrogenase
A_95_P032706	AF049355	-1.29	Alpha-expansion precursor (Nt-EXPA6)	cell wall.modification
A_95_P001171	FG640267	-1.47	Extensin protein	cell wall related, 1.9
A_95_P100938	X71602	-1.77	Extensin protein	cell wall related

b) Terpenoid biosynthesis associated transcripts

A large number of terpenoid biosynthesis-related transcripts were increased in the leaves of the TAO plants grown under HL (Fig. 7.3A, Table 7.9). For example, the HL-treatment led to a significant increase in the abundance of transcripts encoding epi-arisotolchene synthase 110 (Table 7.9). The epi-arisotolchene synthase family undertakes the catalysis the conversion of farnesyl diphosphate to 5-epiaristolochene, which is a precursor of phytoalexin capsidiol (Back et al., 1994). Phytoalexin capsidiol is a low molecular weight terpenoid that accumulates in tobacco plants in response to biotic stresses (Back et al., 1994; Maldonado-Bonilla et al., 2008). Aphid reproduction and performance was reduced on *Vicia fabae* plants that accumulated high levels of terpenes (Sadek et al., 2013).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P160707	EH623458	2.83	Epi-arisotolchene synthase 110	Secondary metabolism.isoprenoids. terpenoids
A_95_P007511	AF272244	2.81	Aristolochene synthase	Secondary metabolism.isoprenoids. terpenoids
A_95_P007511	AF272244	2.81	5-epi-aristolochene synthase	Secondary metabolism.isoprenoids. terpenoids
A_95_P229054	DV160959	1.03	Carotinoid cleavage dioxygenase 1	Secondary metabolism.isoprenoids.carotenoids.carotenoid cleavage dioxygenase

Table 7.9 Terpenoid associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

c) SAR proteins associated transcripts

Growth under HL increased the levels of *SAR8.2* transcripts, which are known to be involved in systemic acquired resistance (SAR; Alexander et al., 1992; Moraes and Goodman, 2002). Of the 12 SAR-proteins in tobacco leaves, transcripts encoding SAR2, 2a, 2c, 2d and 2e were more abundant under HL in the TAO plants (Table 7.10).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P004671	EH622851	2.49	SAR8.2c protein	stress.biotic
A_95_P006336	FG125386	1.83	SAR8.2e protein	stress.biotic.inducible by salicylic acid
A_95_P119112	EH621322	1.64	SAR8.2 protein	stress.biotic
A_95_P004421	TA11690_4097	1.59	SAR8.2d protein	stress.biotic
A_95_P004306	M97194	1.24	SAR8.2a protein	stress.biotic

Table 7.10 SAR proteins associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

d) Protein kinase-associated transcripts

Differential expression of a large number of protein kinase-related genes was observed in the TAO leaves grown under HL (Fig. 7.3A, Table 7.11). For example, transcripts encoding a NAK protein kinase were increased in abundance in the TAO leaves (Table 7.11). Although the functions of the NAK family have not been fully described, some members of this group are involved in plant hormone signalling (Shen et al., 2001). The leaves of the TAO plants accumulated transcripts encoding ATP/GTP binding protein under HL conditions (Table 7.11). This ATP/GTP binding protein is a member of the nucleotide binding site (NBS) proteins, which are encoded by disease resistance (R) genes and they are involved in plant resistance to *Fusarium oxysporum* and aphid infestation (Tameling et al., 2002). In contrast, the abundance of transcripts encoding two leucine-rich receptor-like kinases (LRR-RKs), which are involved in plant development and defence responses (Torii, 2004) was decreased in response to HL in the TAO leaves (Table 7.11).

Table 7.11 Protein kinase associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P254394	FG171627	1.36	MAPKK mRNA for mitogen-activated protein kinase	Kinase related
A_95_P254624	EB439565	1.23	Protein kinase / NAK, protein	protein.postranslational modification.kinase.receptor like cytoplasmatic kinase VII
A_95_P229404	DV160146	1.22	ATP/GTP-binding protein	putative, Intersting and miscellaneous
A_95_P027016	EB437209	1.12	ABC1 family protein kinase-like protein	kinase related
A_95_P015591	TA15397_4097	1.10	Serine/threonine protein kinase B-raf	protein.postranslational modification
A_95_P083590	AF435451	1.10	calcium-dependent protein kinase 16	calcium-dependent protein kinase
A_95_P234939	EB439485	1.06	Inositol 1,3,4-trisphosphate 5/6-kinase family protein	kinase family protein
A_95_P116567	DV159321	1.01	Receptor-like serine-threonine protein kinase	putative, signalling.receptor kinases
A_95_P040536	BP129857	-1.30	Leucine-rich receptor-like kinase, 2.9	Protein phosphorylation
A_95_P173657	EH665660	-1.46	Leucine-rich receptor-like kinase, 3.16	Protein phosphorylation

e) Stress responses associated transcripts

Stress-associated transcripts were another group that were differentially regulated in the TAO leaves under HL (Fig. 7.3A, Table 7.12). Transcripts encoding a tumor-related protein were increased in abundance in the TAO leaves under HL (Table 7.12). Tumor-related proteins and their homologues play a role in the induction of the hypersensitive response (HR; Karrer et al., 1998). Growth under HL also led to a significant increase in the abundance of transcripts encoding methanol inducible protein 21 (MIG-21; Table 7.12). MIG proteins are induced by methanol emitted from wounded plant cells in order to enhance pathogen resistance (Dorokhov et al., 2012). More than 300 *MIG* transcripts were up-regulated in the leaves of *Nicotiana benthamianain* in response to methanol treatment (Dorokhov et al., 2012). Transcripts encoding osmotin precursor 34 were also increased in TAO leaves under HL (Table 7.12). Osmotin precursors are pathogenesis-related (PR) proteins, which accumulate under stress conditions (Singh et al., 1987; Venkatachalam et al., 2007). The levels of many heat shock-related transcripts were also increased under HL compared to LL conditions (Table 7.12).

Table 7.12 Stress responses-associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P005501	TA12601_4097	2.20	Tumor-related protein	stress biotic
A_95_P009846	EB440040	1.78	Methanol inducible protein (MIG-21)	defence related
A_95_P180587	M64081	1.70	Osmotin 34 precursor	stress.abiotic
A_95_P030076	TA18497_4097	1.52	17.5 kDa class I heat shock protein	stress.abiotic.heat
A_95_P233289	CV016412	1.52	26.5 kDa class P-related heat shock protein (HSP26.5-P)	stress.abiotic.heat
A_95_P233289	TA18497_4097	1.52	Heat shock protein - like	stress.abiotic.heat
A_95_P215127	TA20416_4097	1.32	Chaperone protein dnaJ 20	stress.abiotic.heat
A_95_P162662	EH621663	1.17	Hypersensitive-induced reaction protein	stress biotic
A_95_P157317	FG640154	1.14	FG640154,	stress.biotic
A_95_P193372	TA15655_4097	1.12	Thaumatin-like protein	stress.biotic.Pathogenesis-related
A_95_P295468	FG637012	1.11	Thioredoxin-like protein	ROS related
A_95_P006256	AF154644	1.10	Glycine-rich RNA-binding protein (GRP1)	RNA binding related

A_95_P000576	EB446879	1.08	Dehydration-induced protein ERD15	stress.abiotic.drought/salt
A_95_P107322	CV018315	1.04	Thiol-disulfide interchange like protein	redox.thioredoxin
A_95_P102472	TA11752_4097	1.02	Elicitor resposible protein (TCIP)	stress.biotic
A_95_P184147	EB679701	1.00	Cold acclimation protein WCOR413-like	stress.abiotic.cold
A_95_P216542	EB684025	-1.96	Peroxidase superfamily protein	Defence and stress responses
A_95_P019246	FG638725	-2.80	Snakin-1 (SN1) gene	defense related
A_95_P122637	DW002621	-2.98	Heavy metal transport	detoxification domain-containing protein, stress abiotic

f) Expression profiles of transporter-associated transcripts

The levels of many transcripts encoding transporters were higher in the TAO leaves under HL compared to LL (Fig. 7.3A, Table 7.13). Transcripts encoding a sodium/calcium exchanger, which is a plasma membrane protein that regulates the concentration of Ca^{2+} ions in plant cells (Nicoll et al., 1990), were increased in abundance in the leaves of TAO plants under HL (Table 7.13). Moreover, transcripts encoding sugar, peptide, amino acid and metal transporters were increased in TAO plants grown under HL (Table 7.13). In contrast, transcripts encoding transporters that are associated with cell elongation were decreased in abundance under HL compared to LL conditions. For example, the levels of transcripts encoding myosin-9 were lower under HL than LL (Table 7.13). Myosin transporters play roles in intercellular communication, cell elongation and cytokinesis (Yokota and Shimmen, 2011). Similarly, the expression of *Delta-VM23* was down-regulated in TAO leaves under HL (Table 7. 13). Delta-VM23 is a membrane-intrinsic protein that functions in cell elongation. *VM23* expression was found to be inhibited by light (Higuchi et al., 1998).

Table 7.13 Transporters associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P220887	TA21705_4097	1.94	Sodium/calcium exchanger	transport.calcium
A_95_P223472	TA22258_4097	1.29	Sugar transporter	transport.sugars
A_95_P304348	FG636422	1.27	peptide transporter 3	peptide transporter 3
A_95_P163827	EH622876	1.25	Histidine amino acid transporter	transport.amino acids
A_95_P145447	EB449196	1.24	Metal transport related protein	metal handling.binding, chelation and storage
A_95_P267791	DV161961	1.24	ABC1 family protein, transport	transport.ABC transporters and multidrug resistance systems
A_95_P116932	DV159705	1.20	Zinc finger protein, RNA.regulation of transcription.C2C2(Zn) CO-like	Constans-like zinc finger family
A_95_P309468	FG167388	1.19	Ammonium transporter (PtrAMT1-1)	transport.ammonium
A_95_P223897	EB677784	1.14	nucleotide transporter 1	nucleotide transporter 1
A_95_P219747	EH618831	1.10	lysine histidine transporter 1	lysine histidine transporter 1
A_95_P133067	EB434138	1.09	ammonium transporter 1;1	ammonium transporter

A_95_P284423	DW000973	1.06	Amino acids transmembrane transporte	transport.ABC transporters and multidrug resistance systems
A_95_P096583	FG147325	1.04	GCN5-related N-acetyltransferase (GNAT) family protein	misc.GCN5-related N-acetyltransferase
A_95_P148292	EB677558	1.03	Integral membrane family protein	membrane related
A_95_P233344	FG168825	1.03	membrane-associated mannitol-induced	membrane related
A_95_P278333	FG643137	1.02	Plastid-lipid-associated protein 12	cell organisation related
A_95_P278708	AM815593	1.01	EamA-like transporter family	
A_95_P137987	EB441696	1.00	RING-H2 finger protein ATL1O/zinc finger (C3HC4-type RING finger) family protein	protein.degradation. ubiquitin
A_95_P138682	EB442402	-1.06	Proton-dependent oligopeptide transport (POT) family protein	ransport.peptides and oligopeptides
A_95_P031686	DW003455	-1.22	elongation factor 1-alpha	protein.synthesis.elongation
A_95_P147087	EB451334	-1.43	Sulfate transmembrane transporter	transport.sulphate
A_95_P135297	EB438685	-1.63	Plasmodesmata callose-binding protein 3	misc.beta 1,3 glucan hydrolases
A_95_P296198	EB450777	-1.75	Myosin-9	cell transport
A_95_P129297	EB429007	-2.03	Delta-VM23	transport.Major Intrinsic Proteins
A_95_P023711	TA14702_4097	-2.31	Auxin efflux carrier family protein	transport.misc
A_95_P223582	TA22284_4097	-2.38	Myb-like DNA-binding protein	putative, transcription related

g) Transcripts encoding transcription factors

Large numbers of transcripts encoding transcription factors were altered in abundance in response to HL only in the TAO plants (Fig. 7.3A, Table 7.14). For example, growth under HL significantly increased the abundance of transcripts encoding response regulator 9, which plays a role in the regulation of the circadian clock (Table 7.14; Nakamichi et al., 2010). Transcripts encoding the nam-no apical meristem (NAM) protein were higher in the TAO leaves under HL than LL (Table 7.14). NAM proteins are involved in the formation of the shoot apical meristem, as well as in defense responses and programmed cell death (Souer et al., 1996). Similarly, transcripts encoding a bell-like homeodomain protein 3, which plays a role in the regulation of the shoot apical meristem (Kumar et al., 2007), were higher under HL than LL in the TAO leaves (Table 7.14). The abundance of transcripts encoding a number of different WRKY transcription factors was also increased under HL (Table 7.14). In contrast, a pre-exposure to HL resulted in a down-regulation of the expression of a gene encoding a B-box type zinc finger-containing protein in the TAO leaves (Table 7.14). B-box type zinc finger-containing proteins are involved in protein-protein interactions during transcriptional processes (Khanna et al., 2009).

Table 7.14 Transcription factors associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P200697	EH623399	2.00	Response regulator 9	RNA.regulation of transcription.ARR
A_95_P159827	EH618297	1.85	Nam - No apical meristem (NAM) protein	development.unspecified
A_95_P037771	BP129089	1.76	Bell-like homeodomain protein 3	RNA.regulation of transcription.HB,Homeobox transcription factor family
A_95_P052011	BP132803	1.66	WRKY DNA binding protein, 3.69	Response to biotic and abiotic stress
A_95_P085905	BP529408	1.57	Homeobox transcription factor Hox7-like protein	transcription related
A_95_P161277	EH620028	1.42	Zinc finger, C2H2-type	RNA.regulation of transcription.unclassified
A_95_P150297	EB680396	1.35	MYB124 transcription factor, 2.67	Plant growth, development and stress response
A_95_P154892	EG649803	1.24	homeobox protein 5	homeobox protein 5
A_95_P006856	NP916818	1.22	Transcription factor	RNA.regulation of transcription.bZIP transcription factor family

A_95_P116932	DV159705	1.20	Zinc finger protein	RNA.regulation of transcription.C2C2(Zn) CO-like, Constans-like zinc finger family
A_95_P311638	FG153856	1.19	transcript elongation factor IIS	transcript elongation factor IIS
A_95_P024146	EB681090	1.12	BEL1-related homeotic protein 11	transcription factor related
A_95_P011571	DV160063	1.06	Zinc finger (AN1-like) family protein	RNA.regulation of transcription.unclassified
A_95_P074720	BP526539	1.05	SET domain-containing protein	RNA.regulation of transcription.NAC domain transcription factor family
A_95_P302878	TC62955	1.03	Zinc finger (B-box type) family protein	RNA.regulation of transcription.unclassified
A_95_P019061	TA12425_4097	1.02	Multiple stress-responsive zinc-finger protein ISAP1	putative, RNA.regulation of transcription.unclassified
A_95_P131357	EB431539	-1.02	C2H2-like zinc finger protein	transcription factor related
A_95_P307658	FG639045	-1.67	B-box type zinc finger-containing protein	putative, transcription factor related

h) Transcripts encoding proteins associated with metabolism, protein turnover and hormone signalling

A pre-exposure to HL significantly increased the abundance of major transcripts associated with carbohydrate metabolism. For example, transcripts encoding β-amylase, which is involved in starch breakdown (Scheidig et al., 2002), were higher in TAO plants grown under HL. Similarly, the abundance of many hormone-related transcripts was changed in response to HL. For example, transcripts encoding an auxin-responsive protein were enhanced in the TAO plants under HL (Table 7.15). This auxin-responsive protein plays a key role in light responses as well as in auxin signal transduction (Reed, 2001). Transcripts encoding an E_3 ubiquitin-ligase were increased in the TAO leaves under HL (Table 7.15). This E_3 ubiquitin-ligase has important functions in the signal transduction during biotic and abiotic stresses (Lee and Kim, 2011). For example, jasmonate-zim-domain (JAZ) proteins, which are repressors of JA-related genes, are degraded by this E_3 ubiquitin-ligase during JA signalling (Thines et al., 2007; Chung et al., 2008).

Table 7.15 Metabolism-associated transcripts that altered in the leaves of antisense TAO plants grown under high light for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions for 12h. The LL and HL-treated leaves were collected and frozen in liquid nitrogen until the analysis. The differentially expressed genes between LL and HL conditions were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P025341	TA12601_4097	2.20	E_3 ubiquitin-ligase	protein.degradation.ubiquitin
A_95_P006866	NP916855	1.90	Osmotic stress-responsive proline dehydrogenase	amino acid metabolism.degradation.glutamate family.proline
A_95_P110827	CV019927	1.76	Glutamate decarboxylase 4a related	amino acid metabolism.synthesis.central amino acid metabolism
A_95_P031291	EB433445	1.47	RNA polymerase sigma subunit SigD	protein.synthesis.ribosomal protein.unknown
A_95_P028741	EB451539	1.44	Bidirectional sugar transporter SWEET1	development.unspecified
A_95_P008316	EB449250	1.13	NAD-dependent epimerase/dehydratase	oxidoreductase
A_95_P228709	EB443398	-2.15	Lactoylglutathione lyase family protein	glyoxalase I family protein, Biodegradation of Xenobiotics.lactoylglutathione lyase
A_95_P160027	EH618659	-2.40	Acyl-protein thioesterase 2	putative, lipid metabolism.lipid degradation.lysophospholipases.carboxylesterase
A_95_P137507	EB441239	-2.44	Lipase/hydrolase family protein	misc.GDSL-motif lipase

Major CHO metabolism				
A_95_P267841	DV162243	1.29	Beta-amylase PCT-BMYI	major CHO metabolism.degradation.starch.starch cleavage.beta amylase
A_95_P207642	EB680934	1.22	Tyrosine phosphatase-like	major CHO metabolism.degradation.starch.laforin like phosphoglucan phosphatase (SEX4)
A_95_P273391	EB102906	1.20	Plastid alpha-amylase	major CHO metabolism.degradation.starch.starch cleavage
A_95_P251817	AY553218	1.09	Hexokinase 4a, major CHO metabolism	major CHO metabolism.degradation.sucrose.hexokinase
A_95_P176682	TA11720_4097	-2.12	Alpha-amylase	major CHO metabolism.degradation.starch.starch cleavage
Hormone metabolism				
A_95_P205037	TA18195_4097	2.05	Auxin-responsive family protein	hormone metabolism.auxin.induced-regulated-responsive-activated
A_95_P021861	AB433897	1.79	jasmonate-zim-domain protein 3	defence responses
A_95_P205537	EB425528	1.10	Short-chain dehydrogenase/reductase (SDR) family protein	absicic acid metabolism related
A_95_P201362	TA17407_4097	1.10	9-cis-epoxycarotenoid dioxygenase	hormone metabolism.abscisic
A_95_P258851	D29976	1.05	TFHP-1 protein	Ethylene-mediated signalling pathway related
A_95_P146282	EB450279	-1.05	Basic helix-loop-helix (bHLH) family protein	hormone metabolism.ethylene.induced-regulated-responsive-activated

7.2.3 Transcripts that show differential responses to aphid infestation under HL

In total, 503 transcripts were differentially expressed in the tobacco leaves in response to aphid infestation in all genotypes that had been pre-treated with HL (Fig. 7.4A, B, C, Appendix II). Of these, 255 were increased in abundance and 248 were lower than in non-infested leaves (Fig. 7.4B, C). Of the 255 transcripts that were differentially expressed in response to aphid infestation, 2 transcripts were more abundant in the leaves of all genotypes under HL conditions relative to LL-grown plants. Similarly, the level of only 1 transcript was lower in the aphid-infested leaves of all genotypes relative to non-infested leaves (Fig. 7.4B, C). Of the transcripts that were increased in response to aphid infestation, 94 were unique to wild type plants, 66 were unique to PAO plants and 60 were unique to TAO plants (Fig. 7.4B, C).

Of the transcripts that were lower in the infested leaves, 14 were changed only in the leaves of wild type plants, 67 were lower only in PAO plants and 140 transcripts were lower only in TAO plants (Fig. 7.4B).

In addition to the transcripts that were changed in a genotype-specific manner in response to aphid infestation, others were altered in a similar manner in more than one genotype. For example, the levels of 16 transcripts were higher in abundance in both the wild type and PAO lines (Fig. 7.4B). Similarly, the levels of 6 mRNAs were lower in abundance in both the wild type and PAO lines. Only 3 transcripts were increased and 3 transcripts were decreased in a similar manner in both the wild type and TAO plants. Moreover, aphid feeding led to an increase in the abundance of 14 transcripts in both the PAO and TAO leaves, while 17 transcripts were lower than non-infested leaves (Fig. 7.4B, C).

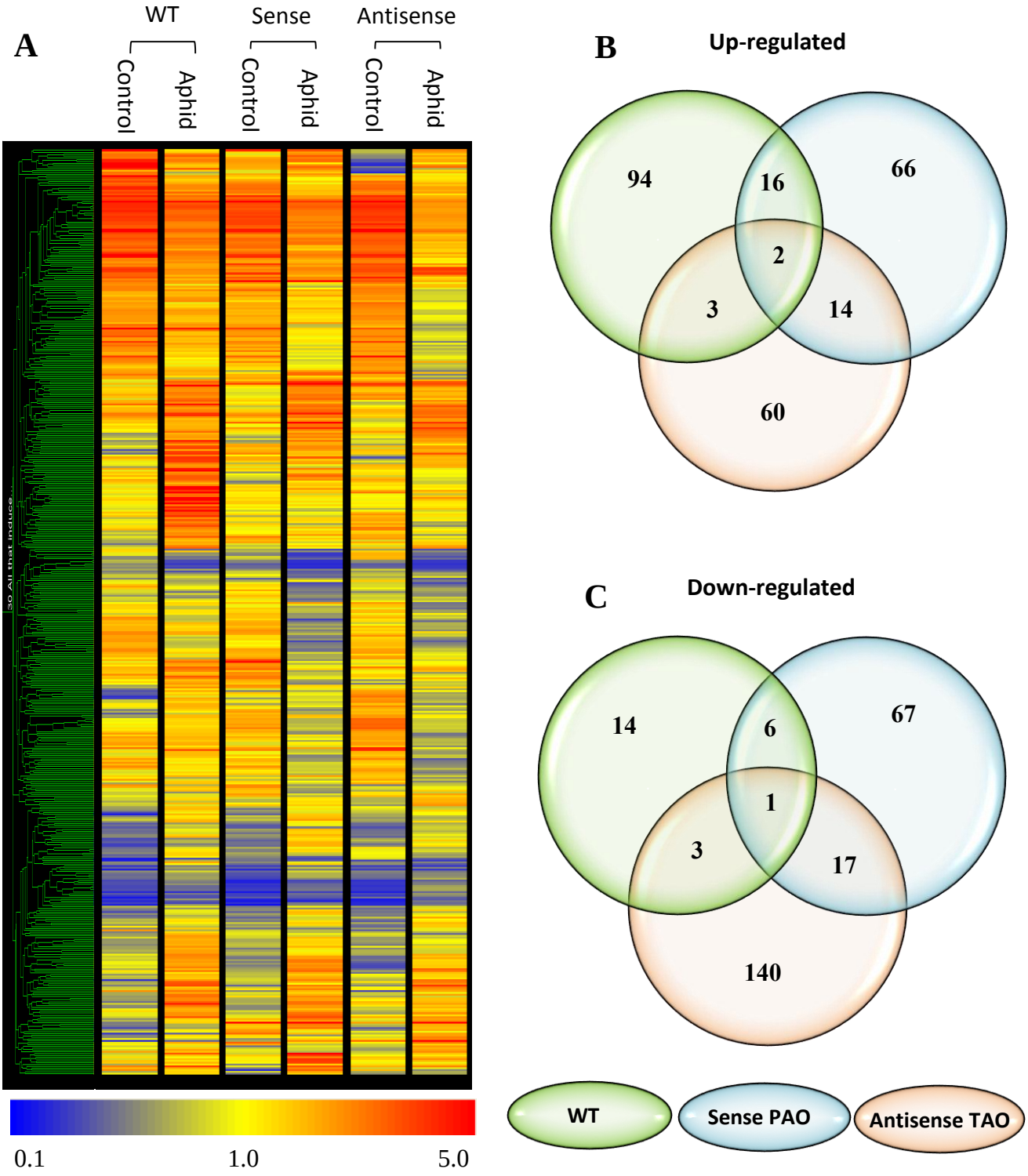


Figure 7.4 Hierarchical clustering and venn diagrams of differentially expressed transcripts in the aphid-infested leaves relative to non-infested leaves of wild type (WT) tobacco plants and transgenic lines sense (PAO) or antisense (TAO) plants pre-treated with high light (HL) for seven days. (A): Hierarchical clustering. **(B)**: Common and unique up-regulated genes. **(C)**: Common and unique down-regulated genes. The differentially expressed genes between infested and non-infested leaves were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg).

Unique transcripts that altered in response to aphid feeding in the leaves of antisense TAO plants pre-treated with HL for seven days

Of the 60 transcripts that were increased in abundance in the aphid-infested leaves of TAO plants that pre-treated with HL for seven days compared to non-infested leaves (Fig. 7.4B, C), 16 transcripts were stress-related, 14 were unknown, 12 were transcription-related and 6 were secondary metabolism-related (Fig. 7.5A).

Of the 140 transcripts that were decreased in abundance in the aphid-infested leaves of TAO plants that pre-treated with HL for seven days compared to non-infested leaves (Fig. 7.4B, C), 53 transcripts were stimulus-associated, 23 were signalling-associated, 12 were transcription-related and 5 were stress-associated (Fig. 7.5B).

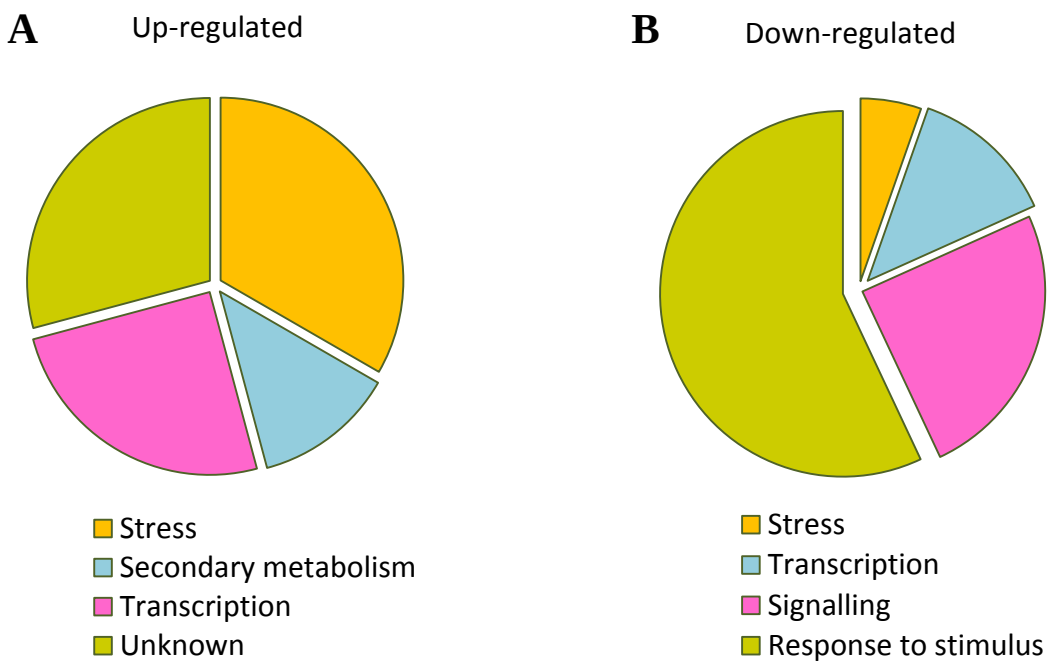


Figure 7.5 Functional groups of unique genes that induced (A) and repressed (B) in response to aphid infestation in the leaves of antisense TAO plants pre-treated with high light (HL) for seven days. To assign the genes to functional groups gene ontology (GO) annotation was carried out using agriGO software (http://bioinfo.cau.edu.cn/agriGO/).

Unique transcripts that up-regulated in response to aphid infestation in the leaves of TAO plants pre-treated with HL

Aphid infestation resulted in the induction of transcripts associated with different physiological processes (Table 7.16). For example, transcripts encoding 4-coumarate:coenzyme A ligase (4CL) were increased in abundance in response to aphid infestation in the leaves of TAO plants that had been pre-treated with HL for seven days (Table 7.16). This 4CL has important roles in lignin biosynthesis (Chapple and Carpita, 1998). In addition, 4CL is involved in the alternative pathway of lignin production, which is induced by pathogen attack (Kneusel et al., 1989; Schmitt et al., 1991). Lignin biosynthesis is increased under stress conditions (Xu et al., 2011) because it plays a crucial role in the protection of cell wall polysaccharides from pathogen-induced degradation (Vanholme et al., 2010).

Aphid-infested TAO leaves accumulated transcripts encoding a dehydration responsive element binding (DREB)-like protein under HL conditions (Table 7.16). The DREB transcription factors are involved in the regulation of a wide range of stress and hormone-related genes (Zhao et al., 2010; Lata and Prasad, 2011). Moreover, transcripts encoding a cytochrome P450 were increased in the aphid-infested TAO plants that had been pre-treated with HL (Table 7.16). This cytochrome P450 plays a central role in the biosynthesis of camalexin, a plant phytoalexin that is toxic to aphids (Kettles et al., 2013; Prince et al., 2014). In addition, this cytochrome P450 has important functions in the regulation of cross-talk between biotic and abiotic stress pathways (Narusaka et al., 2004).

The abundance of transcripts encoding different heat shock proteins were increased in response to aphid infestation in the HL-treated TAO plants (Table 7.16).

Table 7.16 List of up-regulated transcripts in response to aphid infestation in the leaves of antisense TAO plants pre-treated with high light (HL) for seven days. Plants had either been grown for three weeks under low light (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions. Sixty adult wingless aphids were transferred to upper surface of the youngest mature leaves of LL and HL pre-treated plants with a small paint brush and enclosed in a mesh (mesh size 200 μm) covered clip cage (2.5 cm diameter) for 12h. Plants with cages without aphids were used as controls for these experiments. Plants were then maintained under LL for the period of aphid infestation. The infested and non-infested leaves were collected 12h following the onset of aphid infestation and frozen in liquid nitrogen until the analysis. The differentially expressed genes between aphid-infested and non-infested leaves were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
Cell wall				
A_95_P280958	FG137079	1.11	4-coumarate:coenzyme A ligase	lignin biosynthesis related
Stress responses				
A_95_P136437	EB440199	2.44	DREB-like protein	Regulation of expression of many stress genes
A_95_P177912	TC124301	2.14	Low molecular weight heat-shock protein	
A_95_P201427	AY329066	1.89	17.6 kDa class I small heat shock protein (HS)	stress.abiotic.heat
A_95_P032981	AF211726	1.70	Retrotransposon Ty1/copia-like	sress.biotic.abiotic
A_95_P003426	DW000536	1.12	Salt responsive protein 2 (SRG2)	Salt responsive protein 2 (SRG2), stress abiotic
A_95_P099813	BP535076	1.11	Heat shock protein 81-4 (HSP81-4) chr5	Heat shock protein 81-4 (HSP81-4) chr5, stress.abiotic.heat
A_95_P155797	EB678632	1.04	Cytochrome P450	Cytochrome P450, misc.cytochrome P450

Transporter				
A_95_P023256	TC105189	1.54	SLC12A6 solute carrier family 12	potassium/chloride transporters
A_95_P097868	BP534235	1.53	Membrane protein	Membrane protein - Magnetospirillum gryphiswaldense
Metabolism				
A_95_P287973	FG155793	2.45	Tuber-specific and sucrose-responsive element	major CHO metabolism.degradation. starch.starch cleavage.beta amylase
A_95_P132077	EB432510	1.51	fatty acid desaturase A	fatty acid desaturase A
A_95_P101828	CN824875	1.44	1-aminocyclopropane-1-carboxylic acid (acc) synthase 6	1-aminocyclopropane-1-carboxylic acid (acc) synthase 6
Protein degradation				
A_95_P122362	DW002355	1.57	F-box family protein	protein.degradation.ubiquitin
A_95_P064815	BP136164	1.20	F-box family protein	protein.degradation.ubiquitin.E3.SCF.FBOX
A_95_P191097	TA15156_4097	1.14	Peptidase M50 precursor	Peptidase M50 precursor, protein.degradation

Unique transcripts that down-regulated in response to aphid infestation in the TAO leaves that had been pre-treated with HL for seven days

In total, 140 transcripts were decreased in response to aphid feeding in the TAO leaves on plants that had been pre-treated with HL for seven days (Fig. 7.4B, Table 7.17). For example, transcripts encoding GATA transcription factor 24 were decreased in response to aphid infestation in the TAO leaves after HL pre-treatment (Table 7.17). This GATA transcription factor is involved in GA signal transduction, which plays a role in the regulation of flowering time and cold tolerance (Richter et al., 2013; 2015). The expression of a gigantea protein encoding gene was down-regulated in the leaves of the TAO plants in response to aphid feeding (Table 7.17). Gigantea proteins are involved in the regulation of flowering in Arabidopsis (David et al., 2006). The levels of transcript encoding importin beta-3 were lower as a result of aphid infestation in the HL pre-treated TAO plants (Table 7.17). Importins are nuclear receptors that control transport into the nucleus (Wirthmueller et al., 2013).

Table 7.17 List of down-regulated transcripts in response to aphid infestation in the leaves of antisense TAO plants pre-treated with high light (HL) for seven days. Plants had either been grown for three weeks under low light (250 µmol m^{-2} s^{-1}) and then either maintained for a further seven days under low light growth conditions or transferred to HL (1600 µmol m^{-2} s^{-1}) for seven days. Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions. Sixty adult wingless aphids were transferred to upper surface of the youngest mature leaves of LL and HL pre-treated plants with a small paint brush and enclosed in a mesh (mesh size 200 µm) covered clip cage (2.5 cm diameter) for 12h. Plants with cages without aphids were used as controls for these experiments. Plants were then maintained under LL for the period of aphid infestation. The infested and non-infested leaves were collected 12h following the onset of aphid infestation and frozen in liquid nitrogen until the analysis. The differentially expressed genes between aphid-infested and non-infested leaves were identified by *t*-test ($p<0.05$) using (GeneSpring 7.3) with multiple testing correction of (Benjamini-Hochberg) and a fold change (FC>2).

Probe name	Accession	Fold change (log2)	Description	Function
A_95_P217182	TA20874_4097	-2.12	Disease resistance protein, verticillium wilt/ Hcr2	stress.biotic.PR-proteins
A_95_P312823	FG152581	-1.74	SBP (S-ribonuclease binding protein) family protein	unknown
A_95_P199267	EH621938	-1.61	Methylesterase inhibitor family protein	misc.invertase/pectin methylesterase inhibitor family protein
A_95_P021131	EB446515	-1.58	Alcohol oxidase-related	oxido-reductase related
A_95_P297428	EB683457	-1.56	Gigantea protein	development.unspecified
A_95_P288413	FG634212	-1.53	Importin beta-3	putative
A_95_P134382	EB436627	-1.40	Quinolinate phosphirobosyltransferase	putative, NAD biosynthetic process

A_95_P133212	EB434519	-1.12	Type-A response regulator	interesting and misceallneous
A_95_P120412	DW000313	-1.11	GATA transcription factor 24	regulation of transcription.C2C2(Zn) GATA transcription factor
A_95_P190912	EH620499	-1.04	Pseudo response regulator	protein.postranslational modification

7.2.4 Validation of microarray data by quantitative real time PCR (qRT-PCR)

In the following experiments, qRT-PCR analysis was used to confirm the findings obtained by microarray analysis. Eight transcripts were selected for this analysis based on their transcriptional patterns (Fig. 7.6A). Of these, CV017417, EH617861 and EH622485 were commonly induced under HL, CV019616, EH623458 and EH622851 were expressed in TAO plants under HL and the expression of AY329066 was increased following aphid infestation, while the expression of FG634212 was repressed in these conditions. The data shown in figure (7.6B), show that similar trends in transcript profiles were obtained by qRT-PCR and microarray analysis, the Pearson correlation coefficient being 0.9614 (Fig. 7.6B).

A

Accession	Description
CV017417	Chlorophyll a-b binding protein 1
EH617861	Disease resistance protein
EH622485	PDR-type ABC transporter 1
CV019616	Thionin-like protein (D6)
EH623458	Epi-arisotolchene synthase 110
EH622851	SAR8.2c protein
AY329066	Stress. abiotic. heat
FG634212	Putative

B

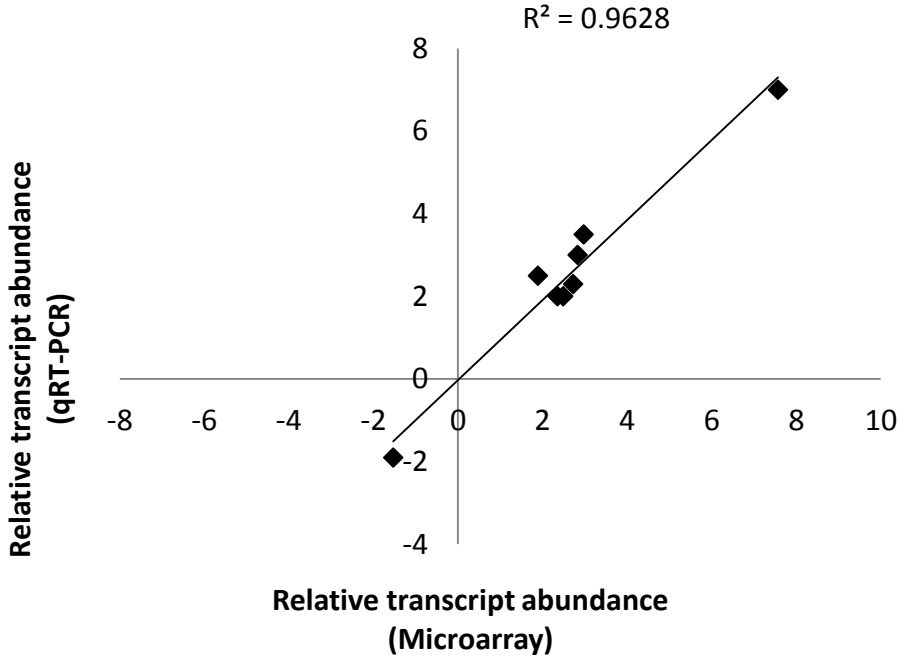


Figure 7.6 Validation of microarray data by qRT-PCR. Eight differential expressed genes between LL and HL or non-infested and infested leaves were selected to confirm the data that obtained with microarray by qRT-PCR (A). Correlation of transcript abundance obtained by microarray analysis and qRT-PCR (B).

7.3 Discussion

The studies described in this chapter were performed to investigate the differences in leaf transcript profiles between the wild type tobacco plants and the transgenic lines, in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations, in plants that had been grown under LL or HL conditions in the absence or presence of aphids. This discussion is restricted to comments on the effects of light and aphid infestation with a focus on the responses observed in the TAO lines relative to the wild type and PAO plants, because aphid resistance was only significantly increased in the TAO plants under HL compared to LL conditions.

Many transcripts were differentially expressed in the leaves of all tobacco lines in response to HL (Fig. 7.1A, B, C). The finding that large numbers of transcripts were differentially expressed in response to light intensity is consistent with other similar studies in the literature in other species. Of these, many were related to photosynthesis. For example, a large number of light-harvesting chlorophyll a-b binding proteins (LHCP)-related transcripts were increased in abundance as a result of HL treatment (Table 7.1). These findings are consistent with previous reports concerning the induction of LHCP-related transcripts under HL conditions (Kolar et al., 1995; Rossel et al., 2002; Kimura et al., 2003). The light-harvesting complexes (LHC) absorb light energy which is transported to the reaction centre of chlorophylls, where it is converted to chemical energy in photosynthesis (Jansson, 1999; Liu et al., 2013; Pietrzykowska et al., 2014). LHCI transfers light energy to PSI while LHCII transfers light energy to PSII (Kim et al., 2009). The LHC are also involved in the stacking of thylakoid membranes, a process which is also responsive to irradiance (Kim et al., 2009; Anderson et al., 2003). The expression of the *Lhc* genes is regulated by light intensity, which is recognised as a key regulator of LHCP protein accumulation (Teramoto et al. 2002; Masuda et al. 2003). The LHCs are also involved in the stacking of thylakoid membranes, a process which is also responsive to irradiance (Kim et al., 2009).

In Arabidopsis, the exposure to HL resulted in a significant increase in the abundance of transcripts encoding some LHCPs, while the expression of other LCHP-related genes was down-regulated under HL conditions (Heddad and Adamska, 2000; Kimura et al., 2003; Tanaka and Tanaka, 2005). The regulated decreases in LHCII levels under HL conditions serves to protect the PSII reaction centres form photo oxidative damage (Humbeck and Krupinska, 2003). However, transcripts associated with the photosynthetic electron transport system can be decreased in abundance in response to HL treatment (Kimura et al., 2003).

Increases in the abundance of transcripts encoding antioxidant enzymes and defence proteins are well documented in Arabidopsis plants exposed to HL (Rossel et al., 2002; Kimura et al., 2003). The transcriptome changes in response to the HL treatment reported here not only include the activation of antioxidant and photo-protection pathways, but also a large number of transcripts associated with plant responses to biotic and abiotic stresses (Fig. 7.2A, B). These findings agree with those reported previously in Arabidopsis (Rasmussen et al., 2013) and in tobacco (Chamnongpol et al., 1998).

Aphid fecundity was unaffected by irradiance level in either the wild type or PAO lines. Significant irradiance-dependent effects were only observed in the TAO plants. The light-dependent transcript changes that are specific to the TAO plants in the absence or presence of aphids can therefore provide insights into possible mechanisms contributing to the enhanced aphid resistance observed in the TAO leaves under HL compared to LL conditions. The levels of relatively large numbers of transcripts were changed as a result by growth irradiance only in the TAO lines. Of the transcripts that were either increased (508) or decreased (629) in the leaves of TAO plants grown under HL conditions relative to LL (Fig. 7.1A, B), many were associated with plant responses to biotic and abiotic stresses (Fig. 7.2A, B). Furthermore, in contrast to the transcripts that were changed in a similar light-dependent manner in all genotypes, the level of transcripts encoding proteins associated with cell wall, terpenoid biosynthesis and SAR proteins were increased in abundance specifically in the leaves of TAO plants (Tables 7.7, 7.8, 7.9 and 7.10). In particular, the changes in transcripts associated with lignin synthesis (Barakat et al., 2010; Hare, 2011) and secondary metabolism synthesis (Rogers et al., 1996; Beets and Dubery, 2011; Kettles et al., 2012) might be important in restricting the capacity of the aphids to settle, feed and reproduce.

Chapter 8. Metabolic profile of ascorbate oxidase transgenic tobacco plants grown either under low or high light in the absence or presence of aphids

8.1 Introduction

Plants have ability to acclimate their morphology, physiology and metabolism in response to environmental changes. Although the essential energy source for photosynthesis is light, excess light, which exceeds the photosynthetic capacity of the plant, can cause photoinhibition and damage to the photosynthetic apparatus (Osakabe and Osakabe, 2012). Plants growing in natural environments can be exposed to fluctuating irradiances that go from low to very high light intensities, i.e. up to full sunlight (2000 μmol m^{-2} s^{-1}). Mechanisms within the photosynthetic machinery allow rapid acclimation to high light (HL) so that excess energy to be dissipated as heat. In the longer term however, the acclimation of the photosynthetic apparatus to growth under HL involves adjustment in antenna size and composition, as well as changes in photosystem stoichiometry (Bailey et al., 2001; Walters, 2005). HL-grown plants generally have a decreased leaf size, specific leaf area, and shoot biomass mass, with fewer grana per chloroplast, lower apparent quantum efficiencies and decreased chlorophyll contents. In contrast, HL tends to increase the stomatal densities and size, and favours increased numbers of chloroplast per a cell, a larger chloroplast size and higher dark respiration rates. This reconfiguration of thylakoid membrane structure, stromal enzyme composition and general leaf morphology requires the effect perception of HL signals that result in changes to leaf transcript and metabolome profiles. Typically, the leaves of Arabidopsis plants grown under HL have more amino acids, sugars (fructose, sucrose, glucose) and TCA cycle intermediates than the the leaves of plants grown under LL (Jänkänpää et al., 2012). Leaf metabolic profiling approaches using INST-MFA indicate that the absolute rates of carboxylation and oxygenation increased following acclimation to high light intensity, but the rate of oxygenation increased more substantially (Ma et al., 2014). Arabidopsis plants that had been acclimated to HL for 9 days were reported to have thicker leaves with chloroplasts that contained more Rubisco per unit leaf area, together with a significant increase in photorespiration relative to carbon assimilation (Ma et al., 2014).

The increase in photorespiratory carbon loss in plants grown under HL was associated with increased carbon export leading to an altered sucrose to starch ratio in the leaves. *Arabidopsis thaliana* mutants that are impaired in carbohydrate transport and metabolism are compromised in their ability to acclimate to HL suggesting that cytosolic carbohydrate availability modulates acclimation to HL in *A. thaliana* (Schmitz et al., 2012).

When tomato plants were grown with optimal and limiting nitrogen availability under either HL or LL conditions, the changes in leaf carbohydrate levels were larger between the different light conditions than under the different nutrient regimes (Urbanczyk-Wochniak and Fernie, 2005). In contrast to primary metabolites, nitrogen-containing secondary metabolites tend to increase in sun-loving plants grown under low light intensities (Coelho et al. 2007). This is not the case in shade-tolerant plants that tend to accumulate secondary metabolites under LL. Light also alters plant responses to biotic stresses, and this may be linked to the enhanced expression of defence genes in plants grown under HL, as well as light-dependent effects on primary and secondary metabolism (Baldwin and Callahan, 1993; Herms and Mattson, 1992; Bolton, 2009). Moreover, in plants grown under HL, more metabolites and energy are available to drive secondary metabolism without having a negative impact on growth (Frost et al., 2008; Schwachtje and Baldwin, 2008). Exposure to biotic stresses is proposed to divert metabolites away from growth and development to defence pathways (Mole, 1994) in fitness-based resistance responses (Kessler and Baldwin, 2002). Direct and indirect defence pathways have been shown to reduce susceptibility to insect infestation. Of these, remodelling of the cell wall to make the insect feeding more difficult is an important response to insects that increases the physical barrier to attack (Thompson and Goggin, 2006). Moreover, the induction of secondary metabolism and accumulation of toxic secondary metabolites as well as polyphenol oxidases and protease inhibitors, reduces nutritive value and adversely affects insect digestion (Kessler and Baldwin, 2002; Chen, 2008).

The following studies were performed to characterise the metabolic changes caused by HL and aphid feeding in wild type tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Moreover, plants were grown for two weeks under LL (250 μmol m^{-2} s^{-1}) and then either maintained for a further seven days under LL conditions or transferred to HL (1600 μmol m^{-2} s^{-1}) for seven days. Samples were harvested to determine firstly, the metabolite composition of tobacco leaves and how this was changed in response to different apoplastic AO activity, secondly, how the leaf metabolite profile was changed as a result of HL treatment, and thirdly how these metabolite profiles were altered in response to aphid infestation.

8.2 Results

The following experiments were performed to determine the effects of light (LL and HL) availability and aphid infestation on the metabolite profiles of wild type, PAO and TAO tobacco leaves. Plants of all lines were grown for two weeks under LL and then either maintained for a further seven days under LL conditions or transferred HL for seven days. Samples were harvested from plants grown under LL and HL conditions and frozen in liquid nitrogen until analysis, as described in Chapter (2). Plants that had been pre-treated under HL conditions were then transferred back to LL growth conditions. Sixty adult wingless aphids were transferred to upper surface of the youngest mature leaves of LL and HL pre-treated plants with a small paint brush and enclosed in a mesh (mesh size 200 μm) covered clip cage (2.5 cm diameter) for 12h. Plants with cages without aphids were used as controls for these experiments. Plants were then maintained under LL for the period of aphid infestation. The infested and non-infested leaves were collected 12h following the onset of aphid infestation and frozen in liquid nitrogen until metabolite profiling analysis. In addition, other samples of LL and HL pre-treated leaves were harvested again at the 12h time point in order to determine the effects of the transition 12h from HL to LL on the abundance and composition of metabolites.

Overall, more than 80 metabolites were identified and quantified in this analysis. Metabolites were grouped according to response to genotype, light treatment and aphid infestation. The HL treatment had the most significant effect on the abundance of metabolites. In total, 54 metabolites were significantly changed in the leaves of plants grown under HL relative to LL (Table 8.1). Only 8 metabolites were changed in abundance in response to altered apoplastic AO activity in the PAO and TAO genotypes relative to the wild type (Table 8.1). In addition, the levels of 13 metabolites were significantly changed in response to aphid infestation (Table 8.1). The abundance of 8 metabolites was significantly changed in genotype-light interaction, 3 metabolites by genotype-aphid interaction, 8 metabolites by the light-aphid interaction and 9 metabolites by interactions between all factors (genotype-light-aphid; Table 8.1).

Table 8.1 Number of metabolites that were significantly changed in response to altered apoplastic AO activity (genotype; wild type, PAO, TAO), light availability and aphid infestation. Some of the metabolites that were changed in abundance in response to one of the factors (orange cells) might be also changed in response to the other factors (white cells).

	Genotype	Light	Aphid	Genotype-Light	Genotype-Aphid	Light-Aphid	Genotype-Light-Aphid
Genotype	8	4	4	2	1	0	2
Light	1	54	5	3	0	5	4
Aphid	0	0	13	1	1	2	1
Genotype-Light	0	0	0	8	1	0	0
Genotype-Aphid	0	0	0	0	3	0	0
Light-Aphid	0	0	0	0	0	8	0
Genotype-Light-Aphid	0	0	0	0	0	0	9

8.2.1 Metabolite changes in response to light availability

In total, 54 metabolites were significantly changed in the leaves of plants grown under HL relative to LL (Table 8.1; Fig. 8.1). The analysis shown in figure (8.1) illustrates that growth under HL altered the abundance of metabolites in a large number of primary and secondary pathways. Moreover, growth under HL resulted in similar changes in the leaf metabolite profiles in the wild type, PAO and TAO plants, and there were no significant differences in the leaf metabolite profiles of samples harvested under HL or 12h after the transition from HL to LL.

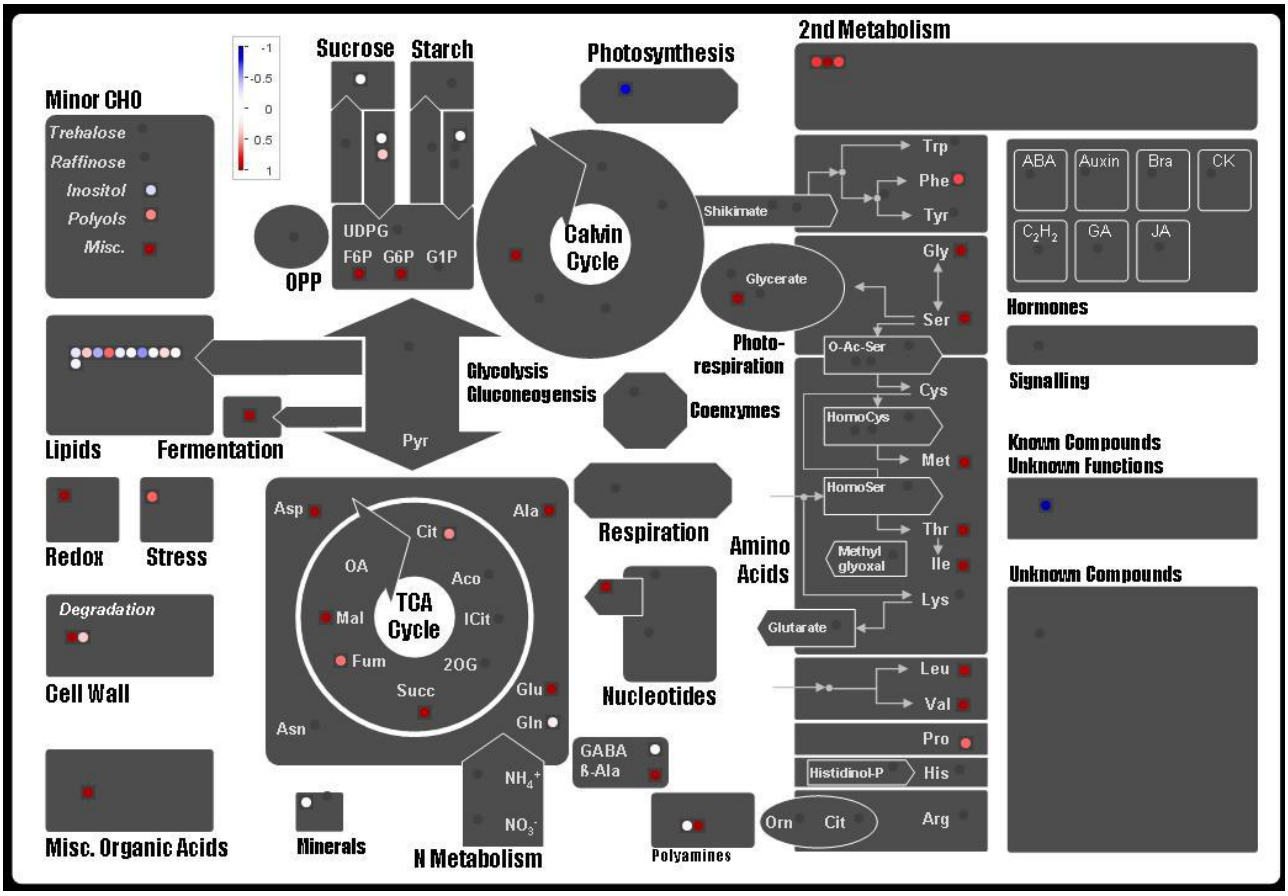


Figure 8.1 Metabolites that changed in abundance in response to high light treatment. The graph was made by putting the metabolite concentration values that analysed by GC/MS in MapMan. Each dot represents metabolite concentration in the leaves of plants that grown under high light conditions for seven days relative to low light conditions according to the scale that based on a $\log_2$ transformation. Grey dot means the value of that compound was not provided

The metabolites that changed in abundance in response to high light treatment are described in detail below:

a) Amino acids

The HL leaf profile was characterised by an increased abundance of both major amino acids (Glu, Gly, Asp) and also minor amino acids (Ala, Val, leu, Ile, Pro, Ser, Thr, Ala, Met, Phe, β-Ala) indicating a significant increase in N-rich metabolites associated with both primary and secondary metabolism (Fig. 8.2A). The HL-dependent increases in amino acids were observed in the wild type, TAO and PAO lines. The ratio of Gly/Ser was about 4 times higher in the HL than LL leaves (Fig. 8.2B, Appendix V, VI, VII).

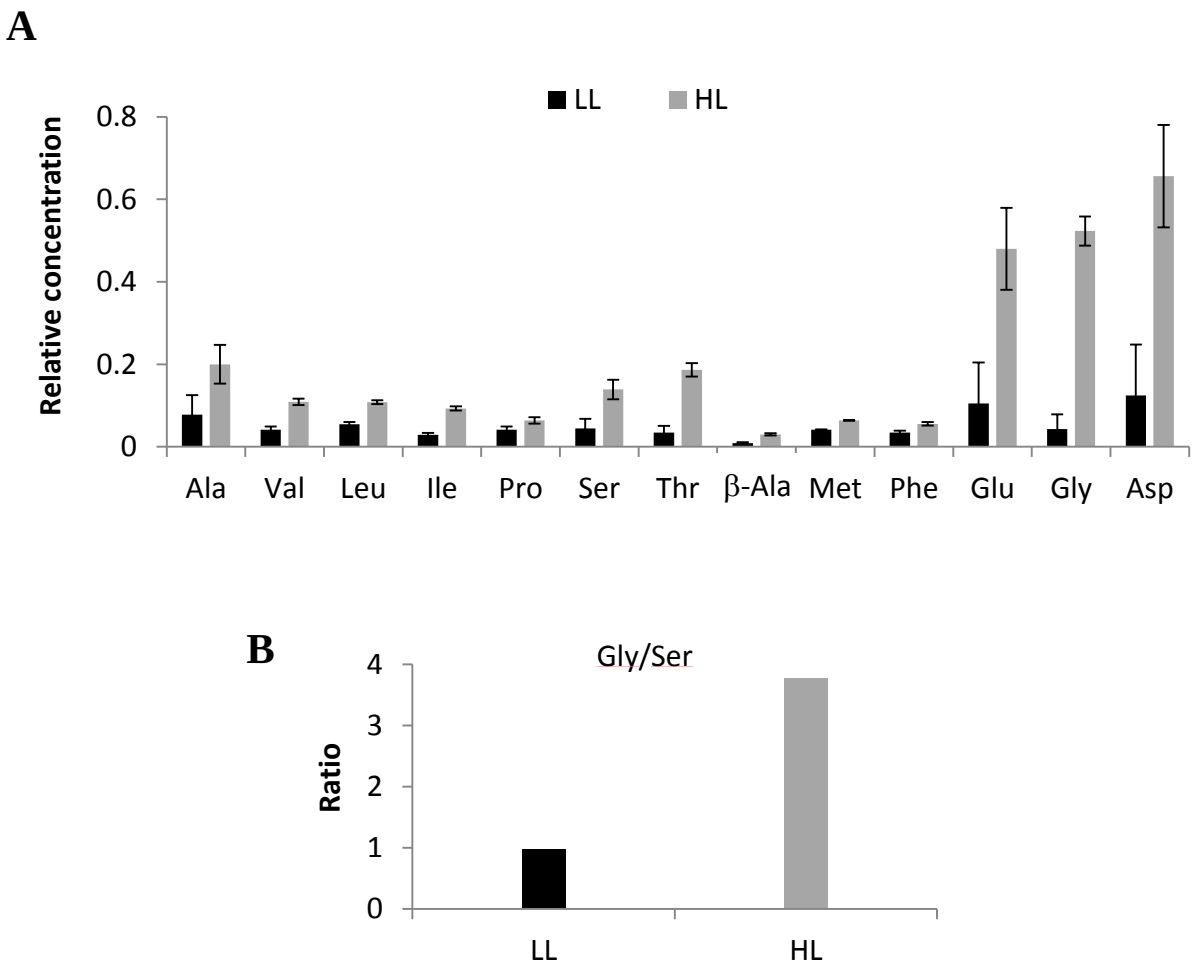


Figure 8.2 Effect of high light (HL) treatment on the levels of amino acids (A) and glycine to serine ratio (B) in the leaves of tobacco plants. Amino acids are represented using the standard 3-letter symbol. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

b) Carbohydrates

The levels of fructose, galactose, fructose-6-P and glucose-6-P were significantly higher in the HL than LL leaves but no other significant changes in leaf sugars were observed (Fig. 8.3, Appendix VIII).

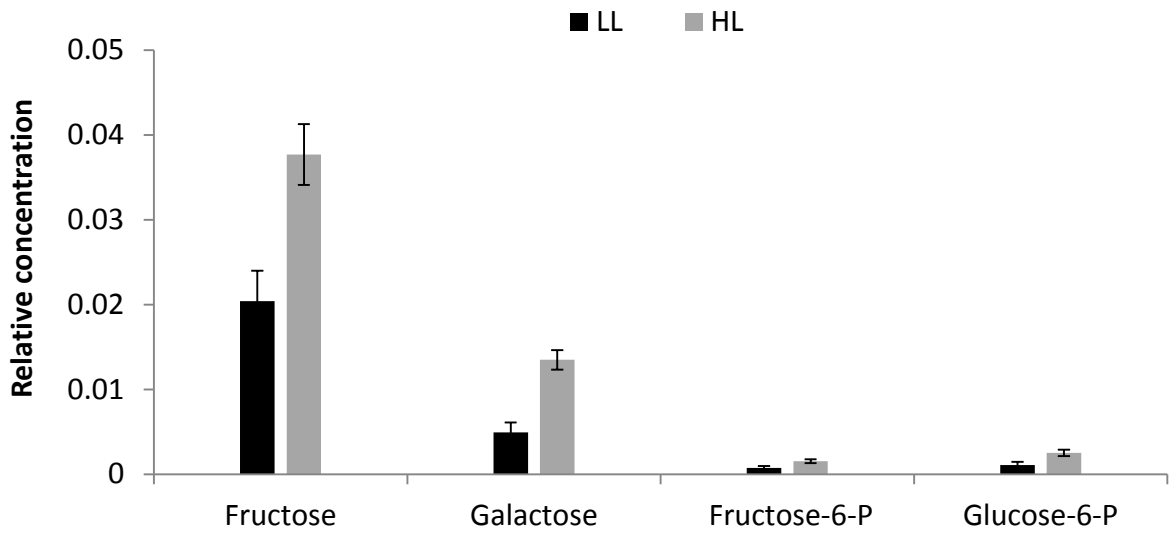


Figure 8.3 Effect of high light (HL) treatment on the levels of sugars in the leaves of tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

c) Fatty acids and fatty alcohols

In general, the fatty acid contents of the leaves were decreased under HL compared to LL, except for C16-1 and C24-0, which increased (Fig. 8.4A). In addition, the leaf contents of fatty alcohols were significantly lower in plants grown under HL compared to LL (Fig. 8.4B, Appendix XI, XII, XIII).

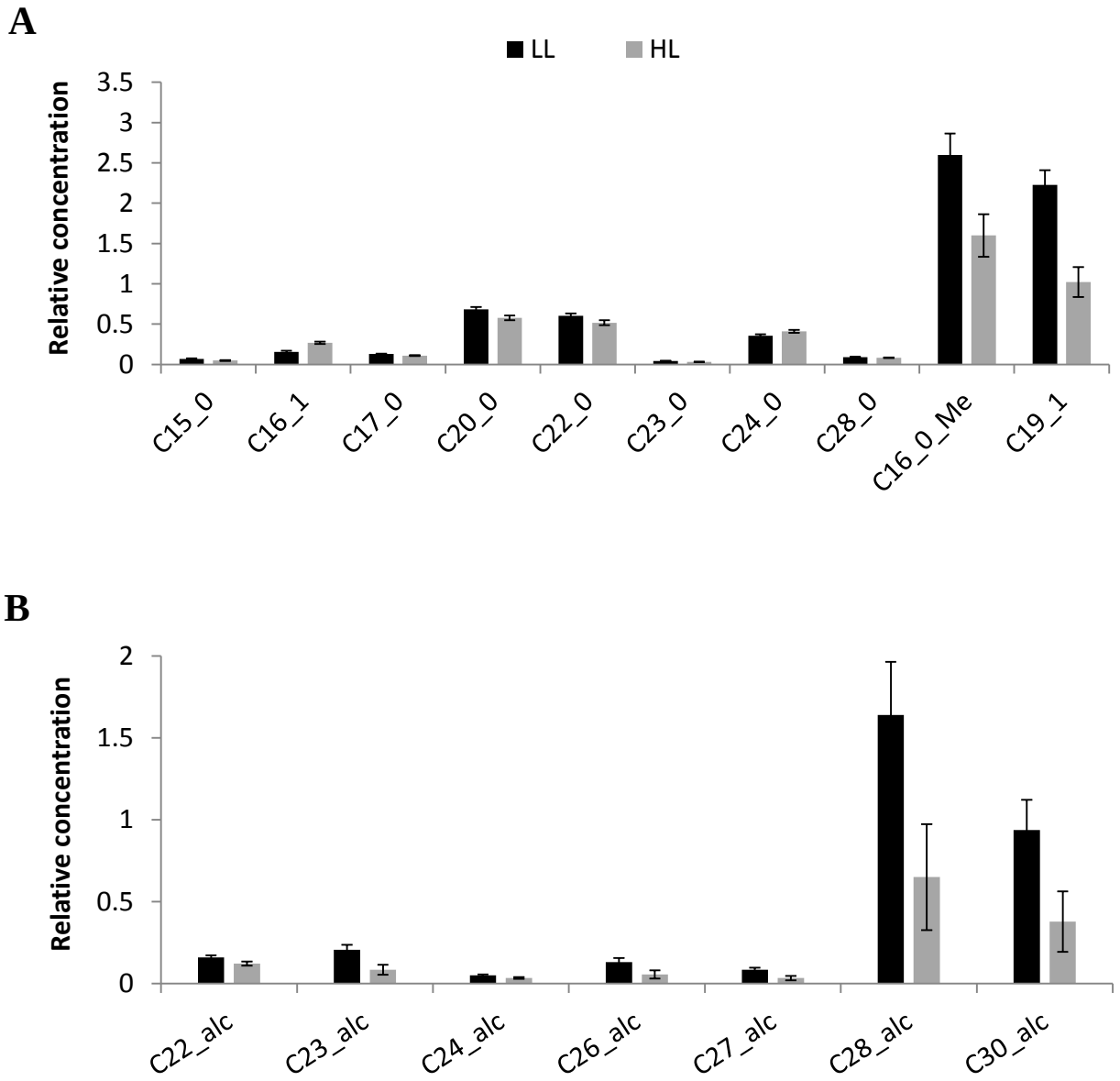


Figure 8.4 Effect of high light treatment on fatty acid (A) and fatty alcohol (B) contents in the leaves of tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

d) TCA cycle intermediates

The levels of TCA cycle intermediates, such as fumarate, succinate and malate contents were significantly higher in the leaves of HL-grown plants compared to the leaves of LL-grown plants (Fig. 8.5, Appendix IX). The levels of malate were almost three times higher under HL than LL conditions.

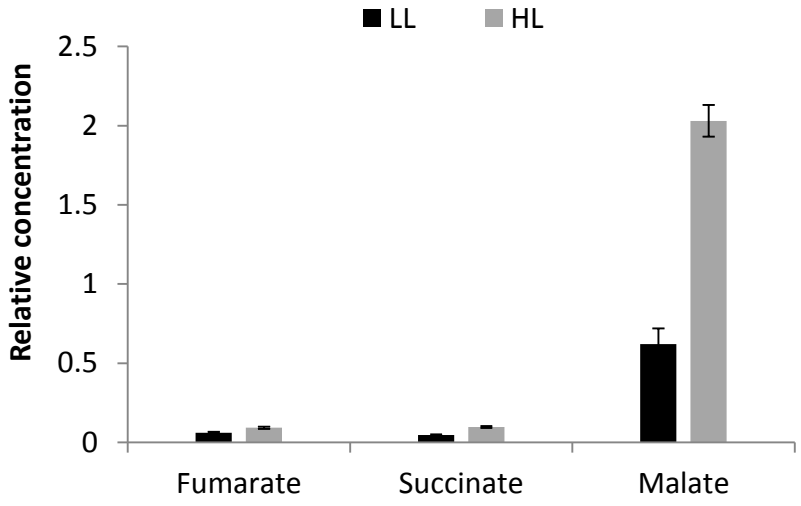


Figure 8.5 Effect of high light treatment on the abundance of TCA cycle intermediates in the leaves of tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

e) Secondary metabolites

The leaves that had been grown for seven days under HL had increased levels of caffeic acid compared to the leaves of LL grown plants (Fig. 8.6). Moreover, the HL-grown leaves had about 4 times as much chlorogenic acid, which is an important intermediate in lignin biosynthesis (Escamilla-Trevino et al., 2014), as the leaves of LL grown plants (Fig. 8.6).

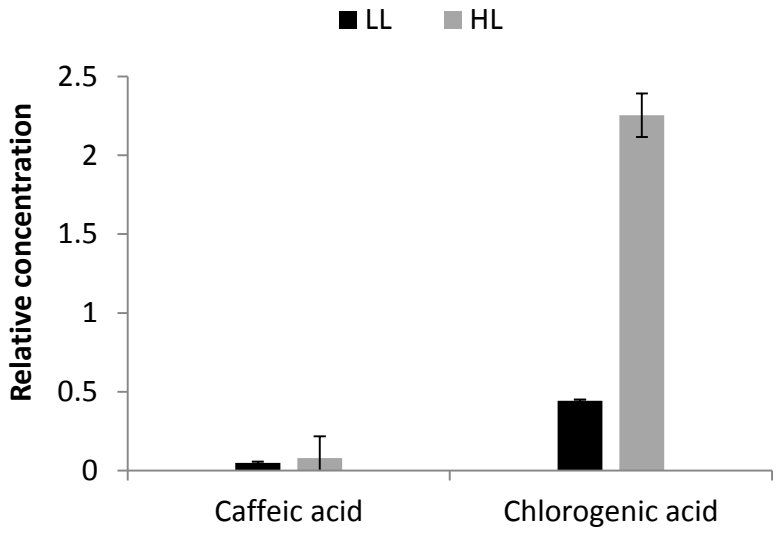


Figure 8.6 Effect of high light treatment on the abundance of caffeic and chlorogenic acids in the leaves of tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

The HL-grown leaves had lower levels of phytol A, B, C and phytil methyl ether 2 contents relative to the leaves of LL-grown plants (Fig. 8.7A, Appendix X). In contrast, the levels of threonate and oxalate, which are breakdown products of ascorbic acid, and quinate were increased in the leaves of HL-treated plants, relative to the LL-grown leaves (Fig. 8.7B). However, the abundance of dihydroxypropanoic acid was lower in the leaves of HL-treated plants compared to the leaves of LL grown plants (Fig. 8.7B).

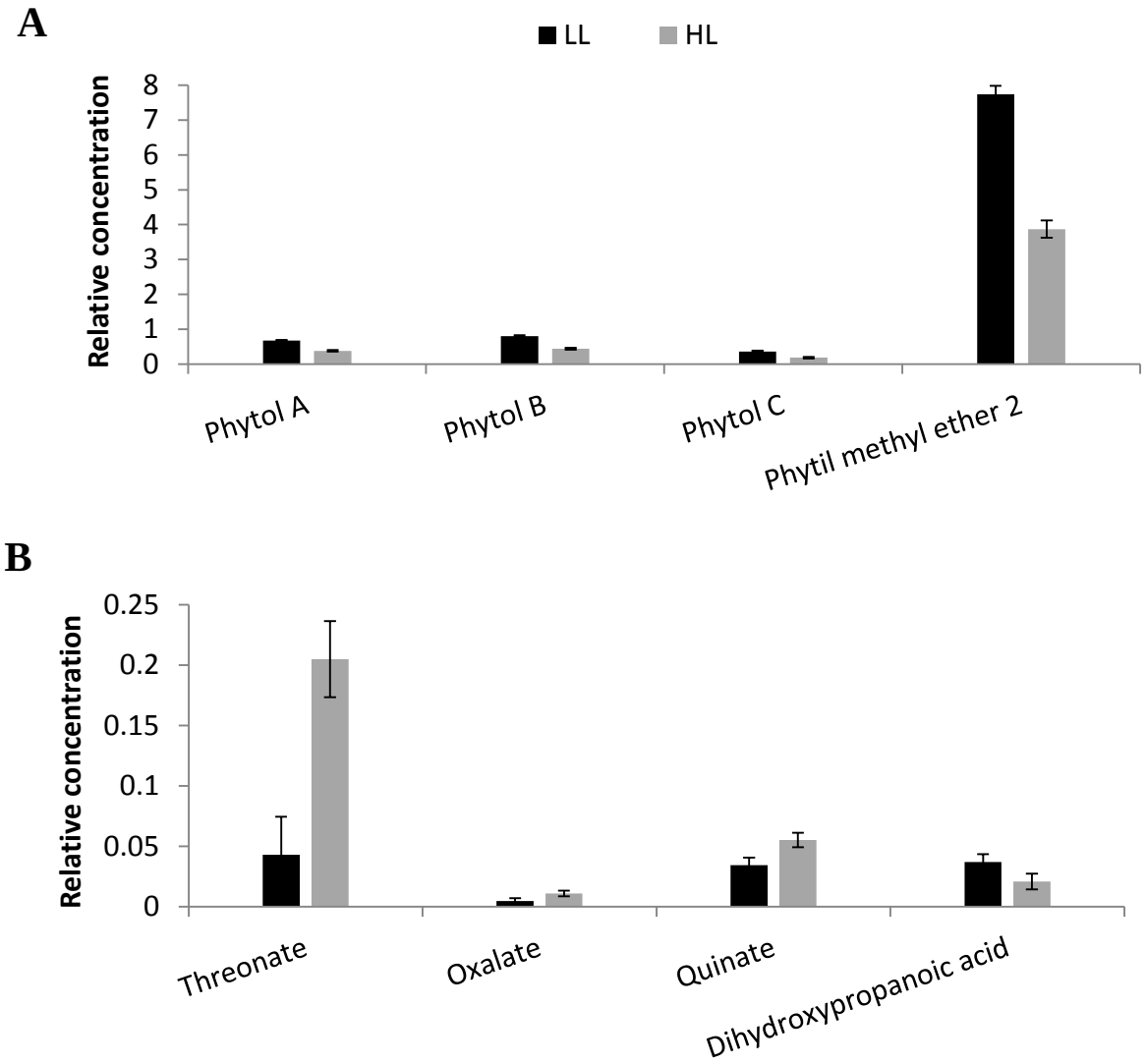


Figure 8.7 Effect of high light treatment on the levels of phytol (A), threonate, oxalate, quinate and dihydroxypropanoic acid (B) in the leaves tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

8.2.2 Metabolite changes in response to altered apoplastic AO activity (genotype)

The following differences in the metabolite profiles of the sense (PAO) or antisense (TAO) transgenic tobacco lines relative to the wild type were observed under both LL and HL conditions. The levels of 8 metabolites were changed in response to alterations in apoplastic AO activity in the transgenic lines (Table 8.1). In particular, the abundance of two amino acids, Met and β-Ala was changed as a result of altered apoplastic AO activity (Fig. 8.8).

Met levels were significantly decreased in the leaves of antisense TAO plants relative to the PAO and wild type (Fig. 8.8). In contrast, β-Ala contents were significantly higher in the leaves of sense PAO plants compared to wild type and TAO plants (Fig. 8.8).

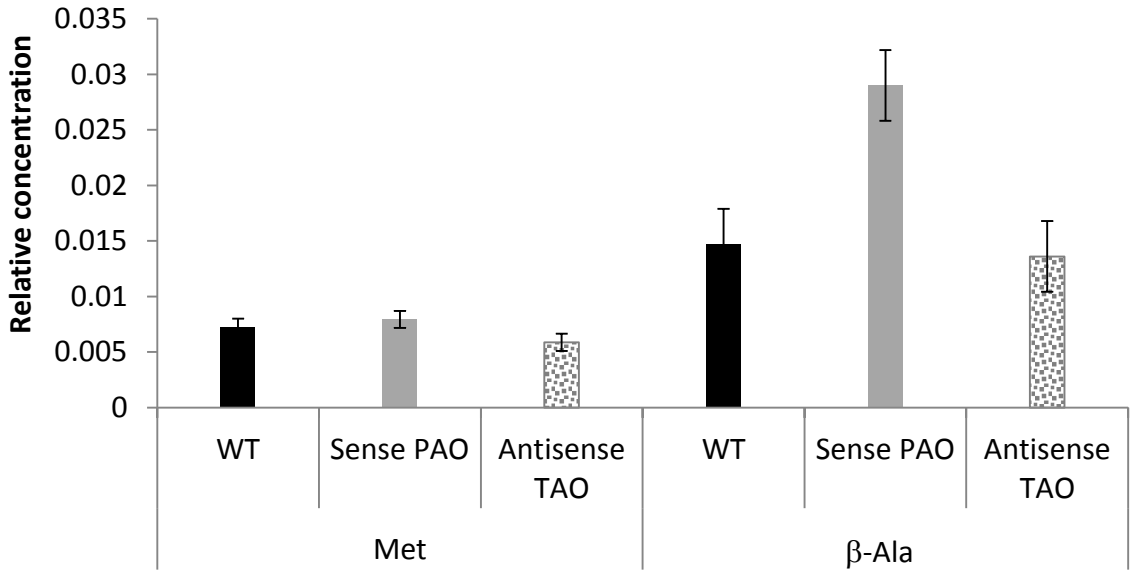


Figure 8.8 Effect of altered apoplastic AO activity on Methionine, β-Alanine contents in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Amino acids are represented using the standard 3-letter symbol. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

The abundance of phytol B and phytil methyl ether 2 were significantly higher in the leaves of antisense TAO plants relative to wild type and PAO plants (Fig. 8.9).

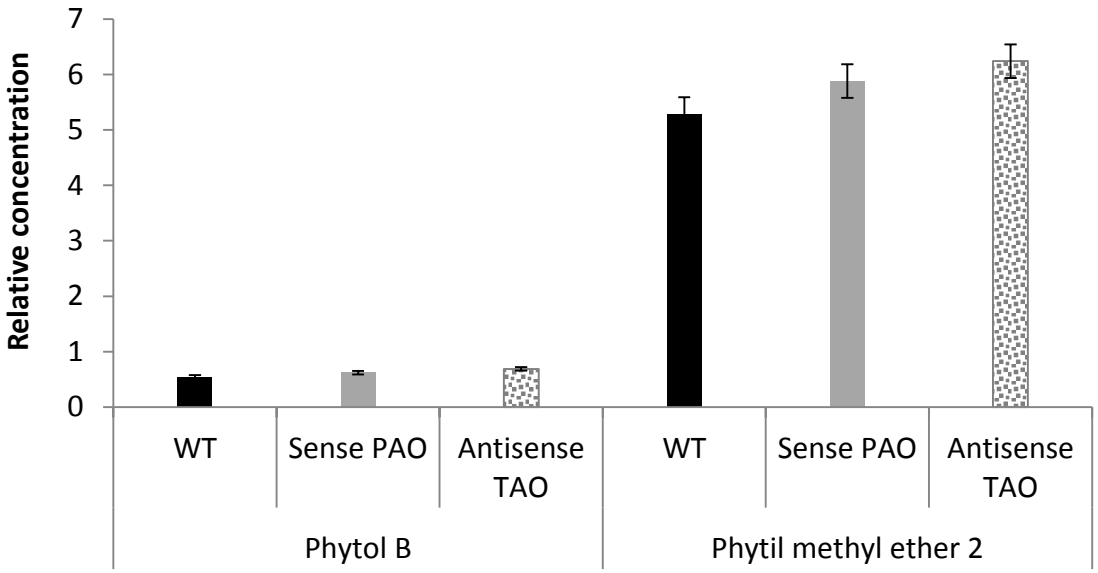


Figure 8.9 Effect of altered apoplastic AO activity on phytol B and phytil methyl ether 2 contents in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

The abundance of fatty alcohols; hexadecanol and tetracosanol were significantly higher in the leaves of PAO and TAO plants relative to wild type plants (Fig. 8.10A). The contents of N-ethyldiethanolamine, which is a product of secondary metabolism, and an unknown oligosaccharide were higher in the leaves of sense PAO plants compared to wild type and TAO plants (Fig. 8.10B). In contrast, the abundance of the unknown oligosaccharide was decreased in the leaves of antisense TAO plants relative to wild type plants (Fig. 8.10C).

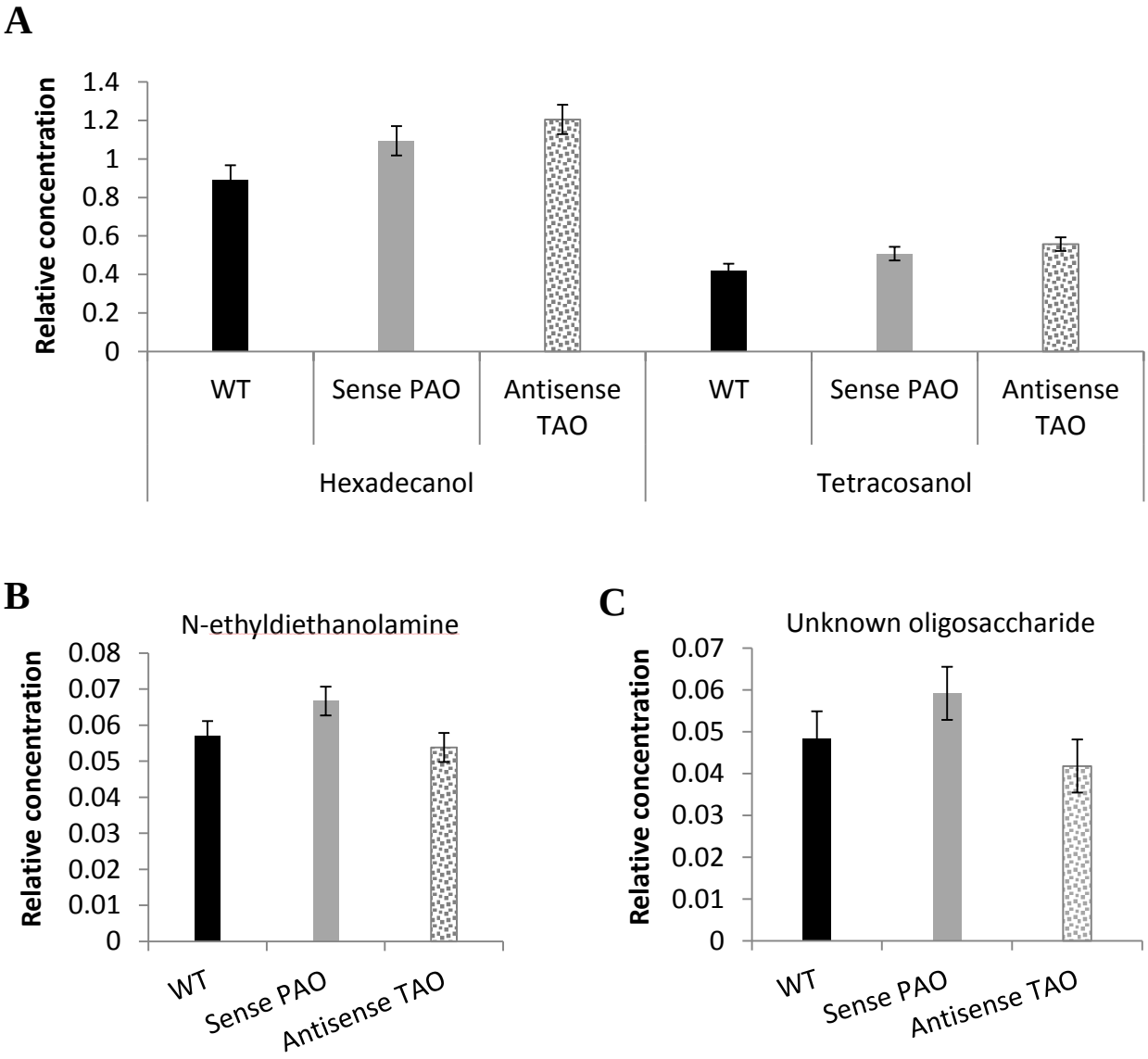


Figure 8.10 Effect of altered apoplastic AO activity on the abundance of hexadecanol and tetracosanol (A), N-ethyldiethanolamine (B) and unknown oligosaccharide (C) in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

8.2.3 Metabolite changes in response to aphid infestation

Metabolite profiles measured 12h after the onset of aphid infestation, revealed that 13 metabolites were significantly changed the abundance in all lines under both HL and LL conditions (Table 8.1). Of these, the levels of two essential amino acids Leu and Phe were significantly lower in aphid-infested leaves compared to non-infested leaves (Fig. 8.11A). In contrast, the fructose content of the leaves was greatly increased by aphid feeding compared to aphid-free leaves (Fig. 8.11B).

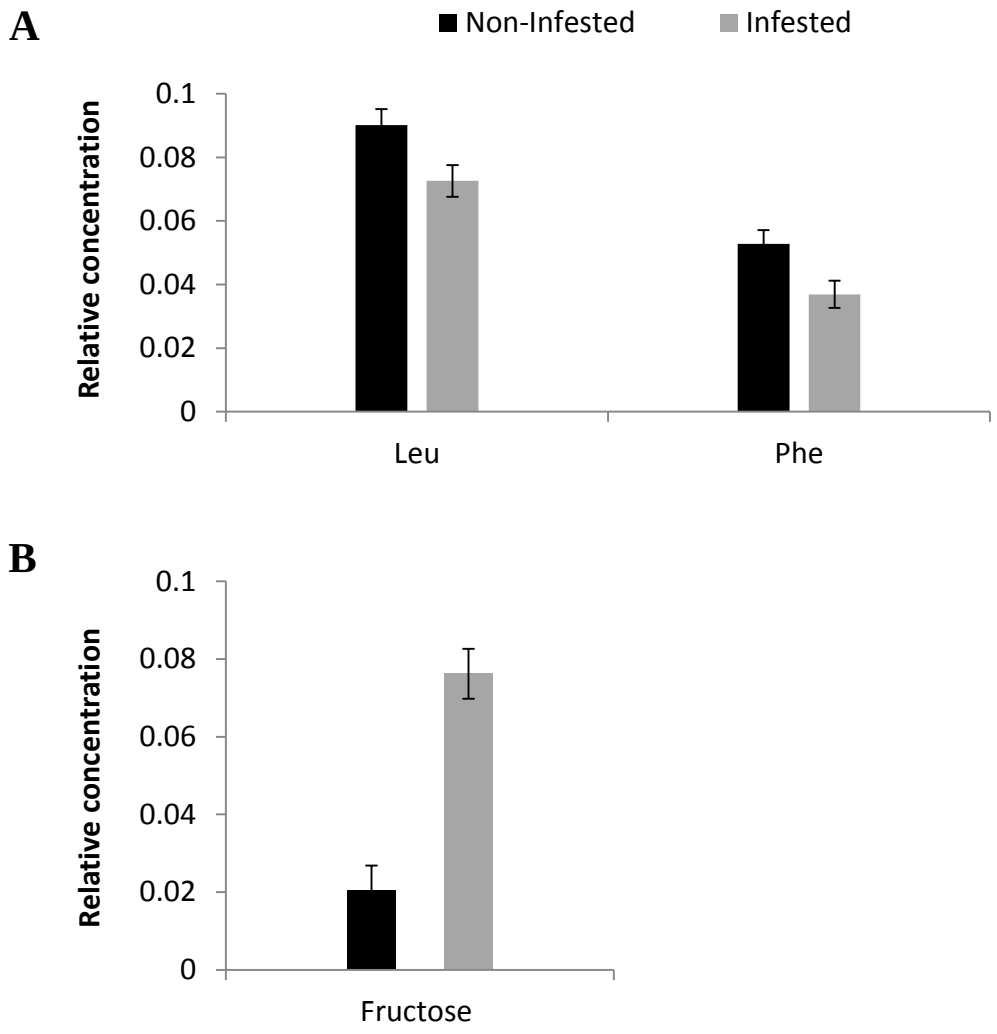


Figure 8.11 Effect of aphid feeding on the abundance of Leu and Phe (A) and Fructose (B) in the leaves of tobacco plants. Amino acids are represented using the standard 3-letter symbol. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

The abundance of phytol B was significantly higher in the aphid-infested leaves but the levels of phytol C were lower in the aphid-infested leaves relative to non-infested controls (Fig. 8.12A). Furthermore, aphid feeding resulted in a significant increase in the C14-0, C16-0-OH, C23-0 and C24-0-OH contents of the leaves compared to aphid-free leaves (Fig. 8.12B).

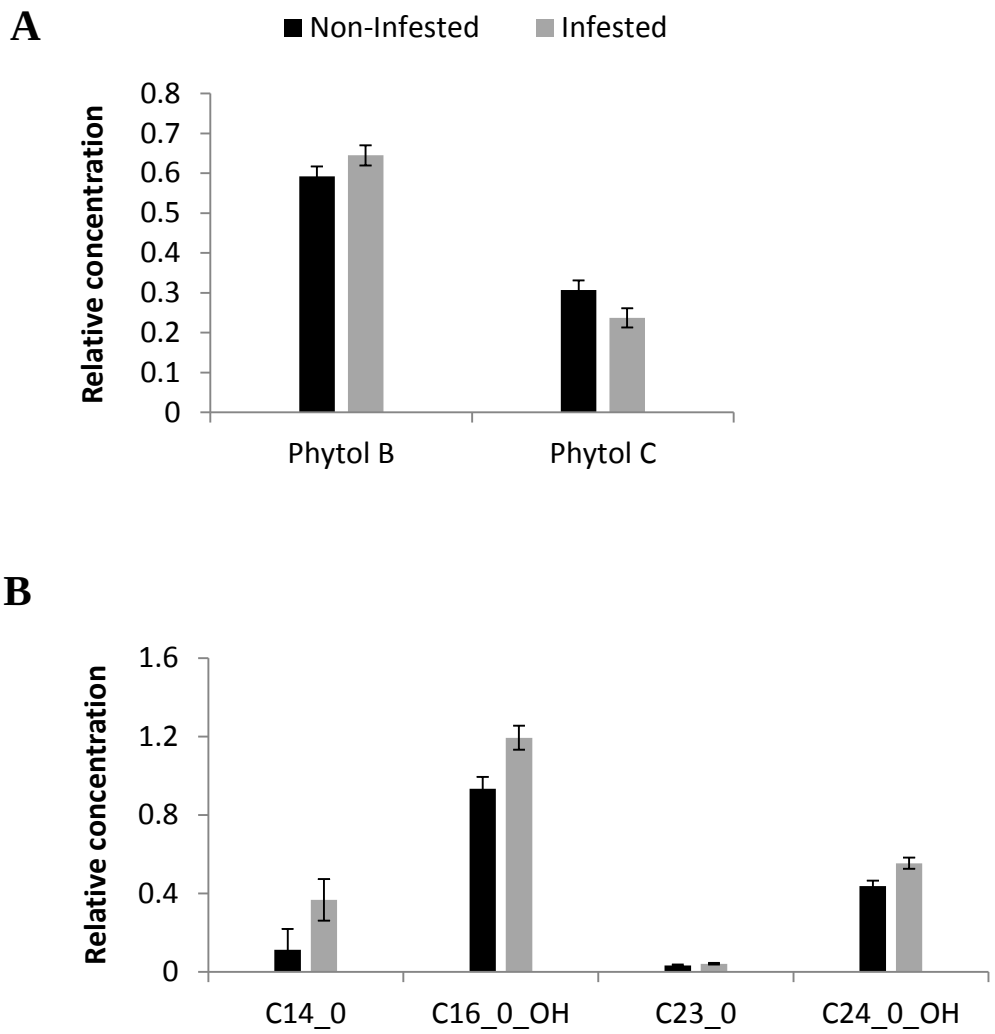


Figure 8.12 Effect of aphid infestation on phytol (A) and fatty acid (B) contents in the tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

Aphid infestation significantly increased leaf fucosterol and maltose contents relative to non-infested controls (Fig. 8.13A, B). However, the abundance of N-ethydiethanoamine was lower in the infested leaves than aphid-free leaves (Fig. 8.13C).

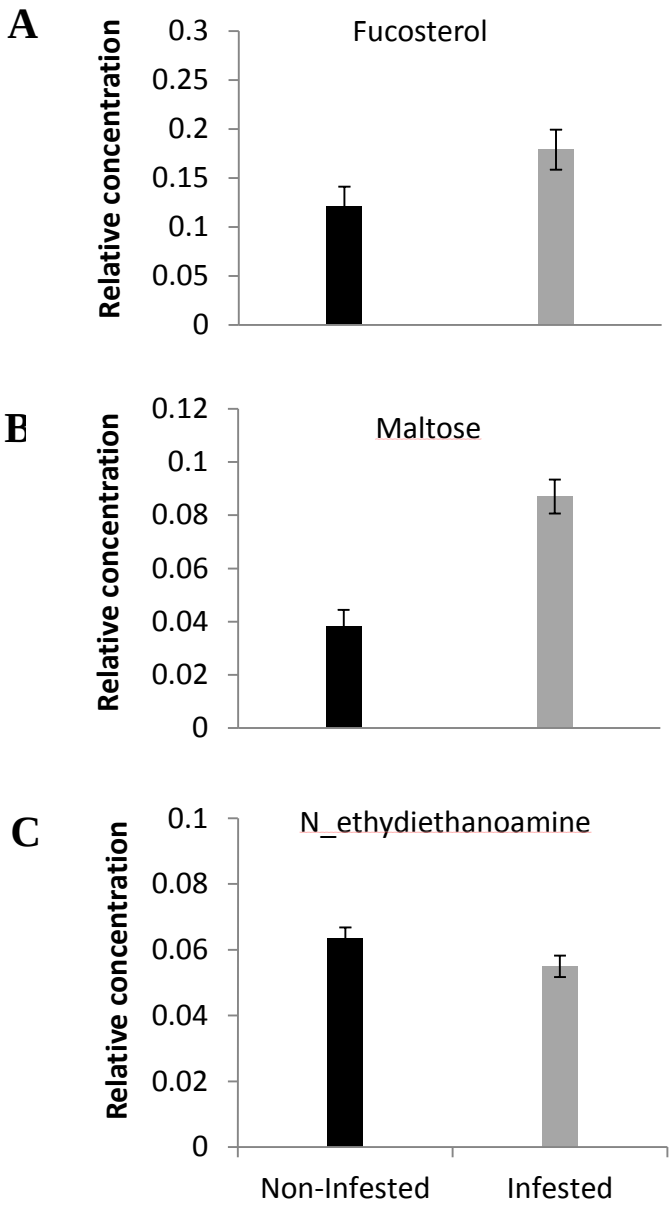


Figure 8.13 Effect of aphid feeding on the levels of fucosterol (A), maltose (B) and N-ethydiethanoamine (C) in the leaves of tobacco plants. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

8.2.4 Metabolite changes in response to the effect of genotype-high light interaction

The genotype-high light interaction effect significantly changed the abundance of 8 metabolites (Table 8.1). While leaves of all genotypes grown under HL showed significant increases in β-Ala and Asp contents compared to LL grown plants, these changes were most pronounced in the PAO leaves (Fig. 8.14).

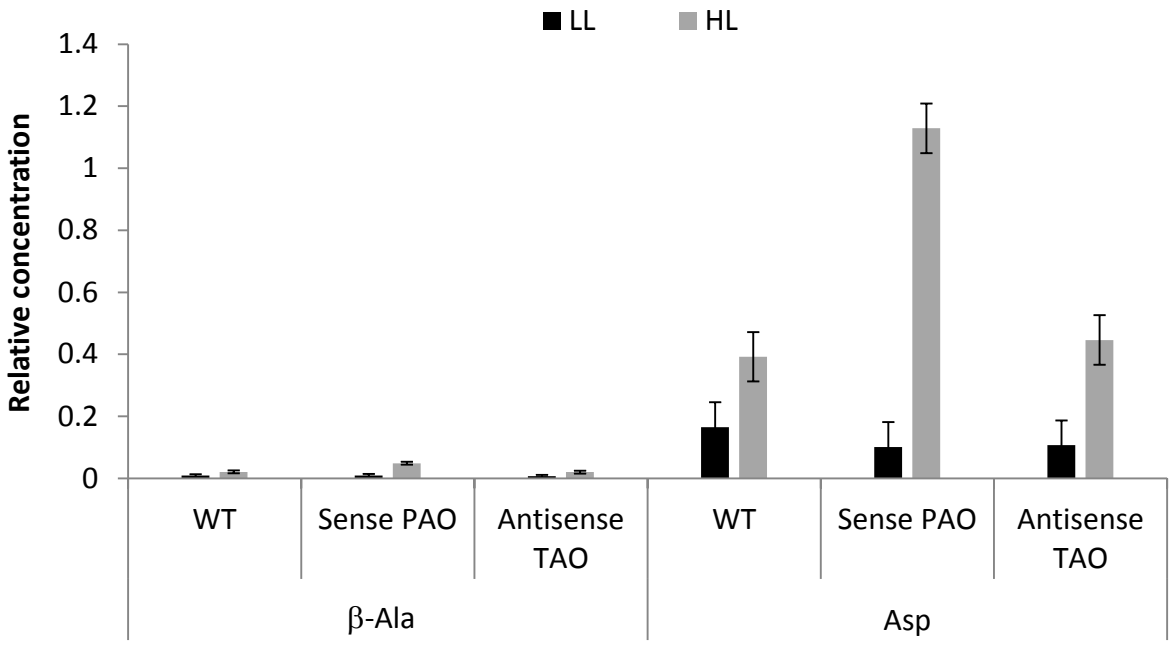


Figure 8.14 Effect of high light treatment on the abundance of β-Ala and Asp in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Amino acids are represented using the standard 3-letter symbol. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

The abundance of maltose was lower in the TAO leaves grown under LL than in the wild type and PAO lines, the levels of this metabolite were similar in all lies when the plants were grown under HL (Fig. 8.15). Moreover, while maltose levels were decreased in response to HL in the PAO and wild type leaves, this metabolite was higher in HL-grown TAO leaves than those grown under LL (Fig. 8.15).

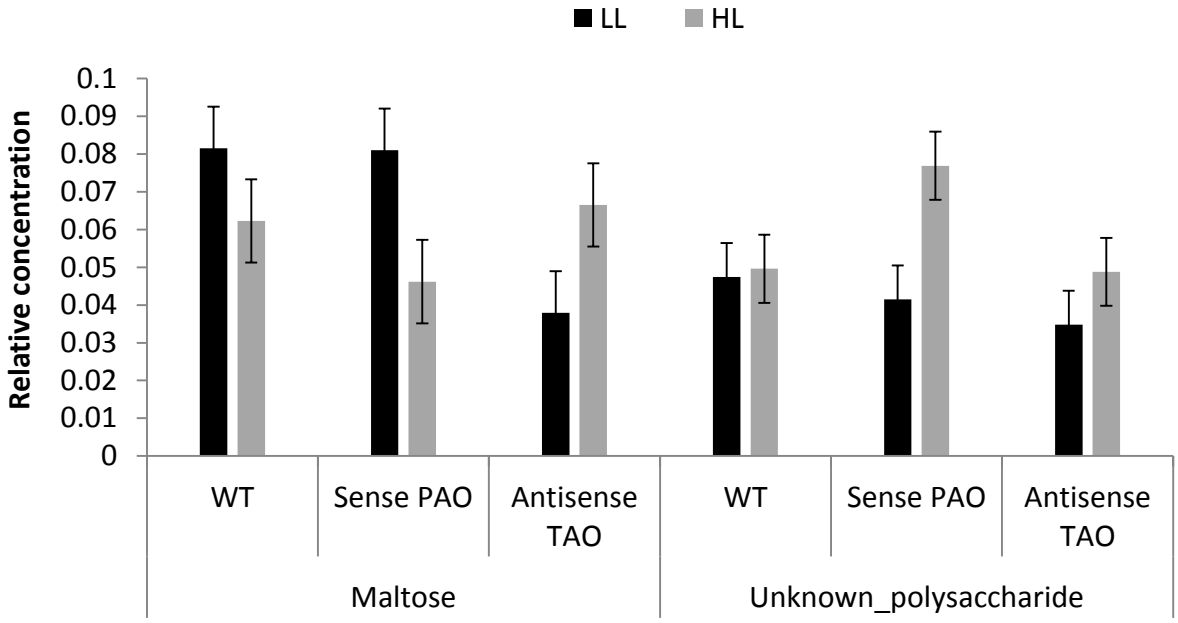


Figure 8.15 Effect of of high light treatment on the abundance of maltose and unknown polysaccharide in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

Growth under HL for seven days resulted in a significant decrease in the abundance of C18-0 only in the leaves of antisense TAO plants compared to LL grown plants (Fig. 8.16A). Moreover, leaf C20-0 contents were lower in the HL-grown wild type and TAO plants than in LL controls but values were similar in the PAO plants at LL and HL (Fig. 8.16A). Moreover, leaf citric acid contents were increased in the HL-grown PAO plants but the levels of this metabolite were similar in the wild type and TAO leaves under LL and HL (Fig. 8.16B).

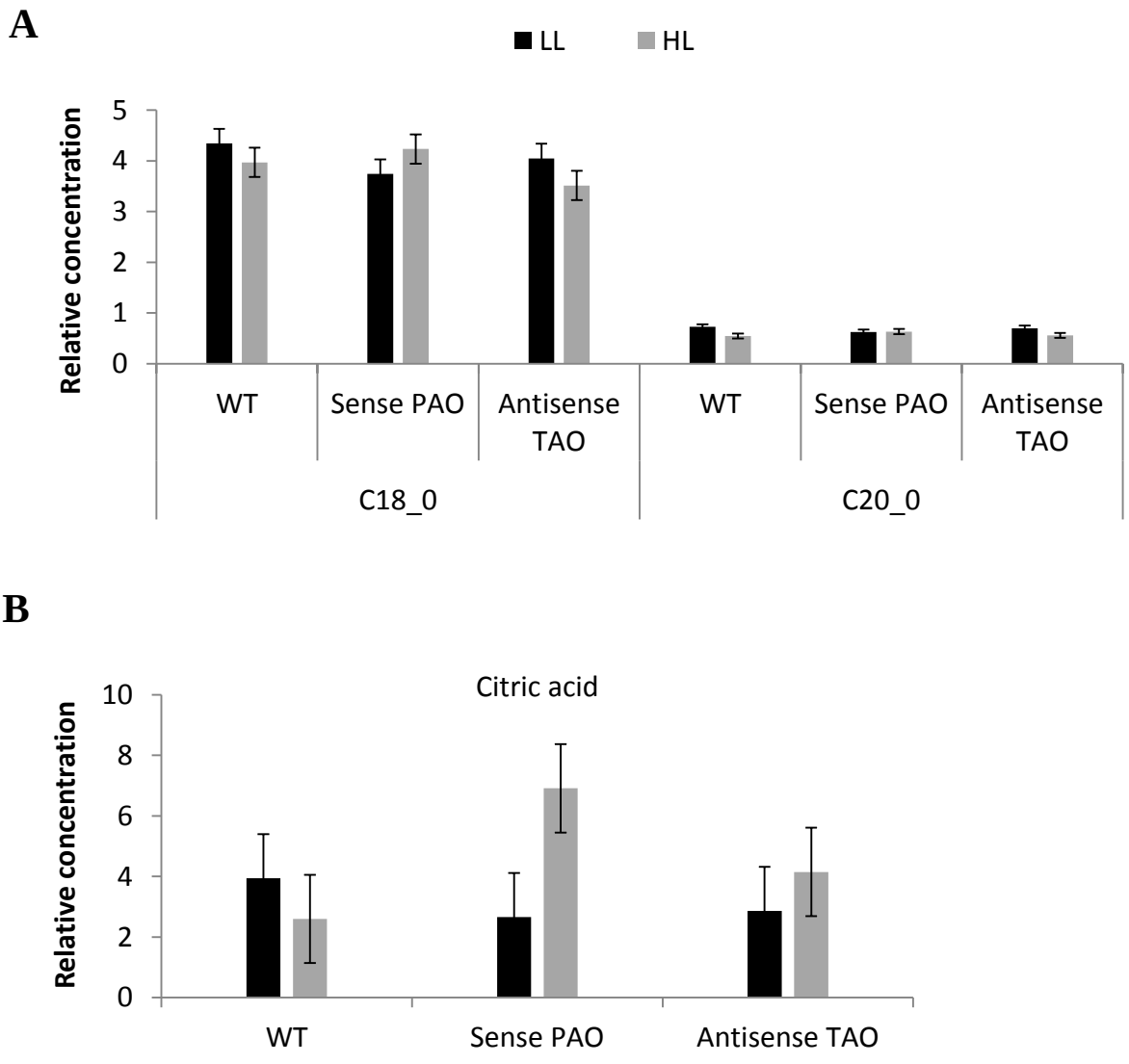


Figure 8.16 Effect of high light treatment on the abundance of fatty acid (A) and citric acid (B) in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations. Relative concentrations are the means (n = 3) and estimated as peak area relative to the internal standard. Significant differences were analysed using two-way ANOVA ($p<0.05$).

8.3 Discussion

The responses of leaf metabolism to changes in irradiance are well documented. The trends in the leaf metabolite profiles of the HL grown tobacco leaves relative to those grown under LL reported here are broadly similar to other reports in the literature (Jänkänpää et al., 2012). For example, data were presented in Chapter (6) show that HL-grown leaves had less chlorophyll contents. The lower levels of the metabolites involved in phytol production in leaves grown under HL (Fig. 8.7A) are consistent with this observation and with previously published data (Jagtap et al., 1998). Although no changes in leaf sucrose levels were found between HL and LL-grown leaves, other sugars such as fructose, galactose, fructose-6-phosphate and glucose-6-phosphate levels were significantly higher under HL conditions. The observed effects on carbon metabolites and on amino acid levels, which increased in HL-grown leaves relative to those grown in LL are consistent with increased metabolite flow through nitrogen assimilation and carbon metabolism pathways under HL. Similarly, the Gly/Ser ratio in LL-grown leaves was only a quarter the values obtained under HL (Fig. 8. 2B), suggesting that photorespiratory flow was higher under HL than LL, as described previously in other species (Wingler et al., 2000; Ma et al., 2014). Moreover, the increases in TCA cycle metabolites, particularly the large increases in malate suggest that respiratory carbon flow is also increased under HL, as has previously been observed in other species (Jänkänpää et al., 2012). HL also had a significant impact on fatty acid and secondary metabolism. It is perhaps noteworthy that the levels of chlorogenic acid were significantly higher in HL-grown leaves, suggesting that the tobacco leaves were able to invest more carbon into secondary metabolites under HL compared to LL. Moreover, the HL-dependent changes in the leaf metabolome were still significant, 12h after the transition to LL.

Only 8 metabolites were changed in response to the different AO activities of the wild type and transgenic lines (Table 8.1). Met levels were significantly decreased in the leaves of antisense TAO plants relative to the PAO and wild type (Fig. 8.8). In contrast, β-Ala contents were significantly higher in the leaves of sense PAO plants compared to wild type and TAO plants (Fig. 8.8). The abundance of phytol B and phytil methyl ether 2 were significantly higher in the leaves of antisense TAO plants relative to wild type and PAO plants (Fig. 8.9). The abundance of fatty alcohols; hexadecanol and tetracosanol were significantly higher in the leaves of PAO and TAO plants relative to wild type plants (Fig. 8.10A).

While it is likely that many of the metabolite changes that were induced by aphid infestation were below the level of detection by metabolomics profiling approaches used here, the data provide some clues to the responses of leaf metabolism to aphid feeding. Within a very short time (12h) of the onset of aphid feeding, the levels of 13 metabolites were significantly changed in abundance in all lines under both HL and LL conditions (Table 8.1). In particular, Leu and Phe were significantly lower in the leaves infested with aphids than controls. The branched-chain amino acid Leu is known to be an important component of the insect diet, which influences reproductive performance (Kerchev et al., 2011). Moreover, while aphid feeding had no significant effect on leaf sucrose levels, the large changes in fructose suggest that aphid feeding also has effects on carbon metabolism, even at the earliest stages of feeding. The aphid-induced increases in fatty acids (C14-0, C16-0-OH, C23-0 and C24-0-OH) are consistent with previously published observations. For example, aphid-infested Arabidopsis leaves had higher levels of myristic acid (C14-0) than aphid-free leaves (Kerchev et al., 2011). Myristic acid has an important role in protein post-translational regulation (Boisson et al., 2003). For example, in the N-myristoyltransferase catalase reaction, myristate is attached to the N-terminal of the target proteins such as thioredoxins and protein kinases (Boisson et al., 2003). However, it is important to note that only whole leaf metabolome responses have been characterised in these experiments, and changes in metabolites may be localized around the sites of aphid stylet penetration. Moreover, the changes in metabolites in the phloem sap that occur as a result of aphid feeding are probably much more extensive than are indicated from the data produced here and they are likely to be below the level of detection by this type of metabolite profiling

While changes in the activity of apoplastic AO activity did not greatly alter the HL-responses of the tobacco leaf metabolome, it is worthy to note that β-Ala, Asp and citric acid were all significantly increased in abundance in the PAO leaves grown under HL relative to LL (Figs. 8.14 and 8.16B), suggesting that increased apoplastic AO activity might favour changes in amino acid metabolism and associated TCA cycle activity. Moreover, the abundance of stearic acid (C18-0) was decreased in the leaves of HL-treated antisense TAO plants compared to LL grown plants (Fig. 8.16A). This finding is interesting because stearic acid can be used to produce α-linolenic acid, which is jasmonate precursor (Gfeller et al., 2010). The low level of stearic acid in the leaves of HL-treated antisense TAO plants might be caused by its use in jasmonic acid synthesis, which might be linked to the higher aphid resistance observed in the antisense TAO plants under HL conditions (Chapter 6).

Chapter 9. General discussion

Plants have co-evolved with an enormous variety of microbial pathogens and insect herbivores under conditions with different types of abiotic stress. In particular, they experience large changes in light intensities during the day as well as over the growth period. Plants therefore have a wide range of acclimatory and adaptive mechanisms to changing light levels that optimise metabolic and gene expression during stress periods in order to ensure survival. In addition, high light exposures trigger the plant innate immune system, triggering the innate immune responses associated with pathogen-associated molecular patterns that enhance defences against pathogen attack (Szechynska-Hebda et al., 2010; Karpinski et al., 2012). However, the precise role of growth light intensity on the regulation of plant responses to phloem feeding insects is poorly characterized. Moreover, little information is available in the literature concerning the dynamic aspects of plant responses to aphid attack, particularly the relationships to photosynthesis in a fluctuating light environment.

Studies on aphid infestation are usually performed in plants grown under very low and stable light conditions, which are not generally comparable to field conditions. In the studies reported in this thesis, plant responses to infestation by the green peach aphid, (*Myzus persicae*) were determined in plants that had been grown under LL, HL or that had been subjected to a HL pre-treatment and then returned to LL. These studies were performed in tobacco, which is a "sun", species that grows well under HL conditions particularly in the field and *A. thaliana,* which is often considered to be a "shade" species, and is usually grown in the laboratory under conditions of very low irradiance.

All mutants and transgenic lines that used for measurement of aphid fecundity under both LL and HL growth condition are listed in table (9.1).

Table 9.1 List of all mutants and transgenic lines that used for measurement of aphid fecundity under both low light (LL) and high light (HL) growth conditions.

LL	Resistant to aphid (All compared to LL Col-0)		LL	HL	Resistant to aphid (HL compared to LL)
Col-0	/		Col-0	Col-0	Yes
vtc2-1 (EMS)	Yes		*vtc2-1* (EMS)	*vtc2-1* (EMS)	/
vtc2 (T-DNA)	No		*vtc2* (T-DNA)	*vtc2* (T-DNA)	/
pp2a-b'γ	Yes		*pp2a-b'γ*	*pp2a-b'γ*	Yes
pp2a-b'ζ1-1	No		*pp2a-b'ζ1-1*	*pp2a-b'ζ1-1*	Yes
pp2a-b'ζ1-2	No		*pp2a-b'ζ1-2*	*pp2a-b'ζ1-2*	Yes
pp2a-b'γζ	No		*pp2a-b'γζ*	*pp2a-b'γζ*	Yes
cat2	Yes		*cat2*	*cat2*	No
cat2 pp2a-b'γ	Yes		*cat2 pp2a-b'γ*	*cat2 pp2a-b'γ*	Yes
cad2	Yes		*cad2*	*cad2*	No
cat2 cad2	Yes		*cat2 cad2*	*cat2 cad2*	No
pad2	Yes		*pad2*	*pad2*	No
clt	No		*clt*	*clt*	No
amiR-AO (3.6)	No		*amiR-AO (3.6*)	*amiR-AO (3.6*)	No
amiR-AO (8.5)	No		*amiR-AO (8.5)*	*amiR-AO (8.5)*	No
	Compared to LL WT tobacco				
WT tobacco	/		WT tobacco	WT tobacco	No
Sense PAO	No		Sense PAO	Sense PAO	No
Antisense TAO	No		Antisense TAO	Antisense TAO	Yes

Comparative studies in tobacco and *A. thaliana* were undertaken in order to identify the common signalling components. Aphid fecundity was significantly higher on tobacco grown under HL (1000 μmol m^{-2} s^{-1}) compared to plants grown under LL (250 μmol m^{-2} s^{-1}). In this situation, aphid fecundity measurements were performed on plants under HL or LL conditions. These data suggest that the metabolic status of the leaves under HL favours aphid growth and performance such that HL growth conditions enhance aphid fecundity. The performance of the aphids on the leaves under HL may be caused by the higher availability of amino acids and sugars in the insect diet. The metabolite profiling data shown in Chapter (8) show that the leaves of tobacco plants had higher levels of amino acids, fructose, galactose, fructose-6-P and glucose-6-P under HL compared to those grown under LL. Apparently, the diet rich in amino acids is sufficient to offset any of the negative effects on leaf morphology and defences that are triggered by growth under HL.

However, this is not the case when plants that had been grown for seven days under (1600 μmol m^{-2} s^{-1}) then returned to LL (250 μmol m^{-2} s^{-1}) and aphid fecundity was then measured following the transition to LL. If both the abiotic (high light) and biotic (aphid infestation) stresses were not imposed simultaneously, then a very different scenario was observed. The data presented here show that in both *A. thaliana* and in tobacco a pre-exposure to HL not only induces adaptations in photosynthesis and plant growth, but it also enhanced resistance to aphid infestation, aphid fecundity being significantly lower following a HL pre-treatment than when plants had only experienced LL growth conditions. While the metabolite profiling data shown in Chapter (8) show that the leaves of tobacco plants retain high levels of amino acids and sugars 12h after the transition to LL, it is unlikely that high levels of leaf metabolites can be maintained for long periods of growth under LL conditions. Hence, it is possible to speculate that when the dietary requirements for optimal aphid growth and developments are limiting such as might occur in leaves grown under LL conditions, then the plant defence responses triggered under HL and that are retained upon the return to LL have a negative impact on aphid fecundity. Previous studies have shown that the resistance of Arabidopsis plants to *Pseudomonas syringae* was significantly increased and the pathogen growth was dramatically inhibited, when the plants were exposed to HL (1500 μmol m^{-2} s^{-1}) for up to 24h prior to the infection, compared to plants grown under LL (100 μmol m^{-2} s^{-1}) conditions alone (Karpinski et al., 2012).

The concept that exposure to one type of stress can trigger a molecular memory of stress that leads to a general increase in plant defences against a range of stresses, is well established in plants (Pastori and Foyer, 2002; Mittler, 2006; Karpinski et al., 2012). Such cross-tolerance benefits occur because of the synergistic co-activation of non-specific stress-responsive pathways that cross biotic-abiotic stress boundaries (Pastori and Foyer, 2002; Bostock, 2005). In many cases, cross-tolerance has been linked to enhanced production of reactive oxygen species (ROS) and oxidative signalling (Foyer and Noctor, 2009). Moreover, ROS production and processing systems are intrinsically-linked to the plant response to infestation by insects, including phloem feeding insects such as aphids (Kerchev et al., 2012). A series of experiments were therefore performed to test the hypothesis that redox capacity and oxidative signalling are involved in the HL-dependent increases in aphid resistance. In these experiments a range of different mutants and transgenic plants with lower antioxidant capacity were used to examine effects on HL responses and aphid fecundity. These studies included mutants that had either a lower abundance of the low molecular weight antioxidants ascorbate or glutathione, or lower activities of the enzymic antioxidant, catalase.

In all situations where antioxidant capacity was decreased, aphid fecundity was also decreased. The experiments reported here provide new information on how the redox signalling pathways in chloroplasts (that are triggered by HL) and in the apoplast (that are modulated by AO activity) influence the ability of aphids to infest Arabidopsis and tobacco leaves, as discussed in detail below.

9.1 Oxidative stress signalling linked to PP2A-B'γ, catalase and GSH in plant responses to aphids under LL and HL

The subunit composition of PP2A phosphatases is important in the regulation of signalling networks at multiple nodes in both animals and plants (Hardie, 1990; Wu et al., 2011; Tang et al., 2011). Such multi-level action is possible because PP2A can assemble in a large number of different heterotrimeric holoenzymes that have different functional properties and therefore high specificity towards specific target phosphoproteins. Computational models of heterotrimeric PP2A complexes suggest that PP2A-B'γ and PP2A-B'ζ may bind similar PP2A-A/C dimers (Rasool et al., 2014). Hence, when PP2A-B'γ is absent, PP2A-B'ζ can act as a positive mediator and may take over to promote defensive processes. However, when both regulatory subunits are missing as in the *pp2a-b'γζ* double mutant, the situation appears to revert back to the wild type. Competitive binding of B'γ and B'ζ to PP2A may therefore modulate the subunit composition of PP2A trimers, and thereby fine-tune defence reactions in aphid infested plants (Rasool et al., 2014). Since the regulatory B subunit is essential in determining the substrate specificity and subcellular targeting of PP2A, trimeric holoenzymes with B'γ or B'ζ may regulate cellular functions in seemingly opposing ways.

Transcripts encoding protein kinases and protein phosphatases were changed in abundance following aphid attack (Kerchev et al., 2013). Protein phosphatases, which regulate protein phosphorylation and de-phosphorylation are used in cell signalling, particularly in oxidative and stress-regulated pathways (Luan, 2003; He et al., 2004; Nakagami et al., 2005; Segonzac et al., 2014), as well as in wounding responses (Rojo et al., 1998). Cellular redox signalling pathways involve crosstalk between ROS producing systems in organelles and in the plasma membrane that co-ordinate plant metabolism and defence responses (Kangasjärvi et al., 2012). The cytoplasmic regulatory B'γ subunit of the PP2A phosphatase was recently shown to be a key component that controls pathogen responses elicited by organellar ROS signals in *A. thaliana* (Trotta et al., 2011; Li et al., 2014).

Salicylic acid signalling and cell death pathways are increased in the *pp2a-b'γ* mutants (Trotta et al., 2011). While no transcripts related to callose synthesis are differentially increased in the *pp2a-b'γ* mutants relative to the wild type plants, mRNAs encoding the beta-glucanase pathogenesis-related protein (PR2), which negatively regulates the deposition of the callose, are increased relative to the wild type, together with other PR transcripts. The *pp2a-b'γ*-dependent immune responses were highly dependent on growth conditions. For example lesions were only observed when the double knockout mutants were grown under moderate light intensity, but not HL (Trotta et al., 2011; Li et al., 2014). These data show that PP2A-B'γ acts downstream of ROS signaling arising from organelles such as peroxisomes and plays a key role in the negative control of SA-linked responses in *A. thaliana* (Trotta et al., 2011; Li et al., 2014). Moreover, metabolite profiling analysis indicated that PP2A-B'γ modulates amino acid metabolism and secondary metabolism such as camalexin synthesis under oxidative stress (Li et al., 2014).

Camalexin is an important component of plant defences against bacteria, fungi and insects (Rogers et al., 1996; Beets and Dubery, 2011; Kettles et al., 2012). For example, aphid reproductive performance was decreased on the *dcl1* Arabidopsis mutants, which accumulated high levels of camalexin (Kettles et al., 2012). In contrast, aphid fecundity was significantly increased on the leaves of *pad3* mutants, which are defective in camalexin accumulation, relative to wild type plants (Kettles et al., 2012). The *pp2a-b'γ* mutants have much higher leaf camalexin levels than the wild type and they show decreased aphid fecundity under LL growth conditions. However, the *pp2a-b'γζ* double mutants accumulate more camalexin than the *pp2a-b'γ* mutants and yet aphid fecundity was similar to that observed in the wild type plants. Taken together, these findings would suggest that PP2A-B'γ negatively controls aphid resistance in *A. thaliana* under LL conditions, and that this regulatory pathway is functionally connected with PP2A-B'ζ, which in turn seems to have a positive impact on defence signalling but this control is unlikely to be related to the level of camalexin accumulation in the leaves.

Catalase catalyses a dismutation reaction, in which H_2O_2 is converted to water and oxygen (Zamocky et al., 2008). The *cat2* knockout plants that lack the photorespiratory form of catalase in the peroxisomes, show lesion development on leaves in a day length-dependent manner; i.e. they have no lesions when plants are grown under short day conditions. Lesions developed on the leaves only when the *cat2* mutants were grown under long day conditions (Queval et al., 2007; Chaouch et al., 2010). The day length-dependent effects on oxidative signalling leading to lesion formation are linked to an accumulation of SA, constitutive activation of pathogenesis-related (PR) genes and accumulation of camalexin (Li et al., 2013). The c*at2* mutants that were crossed with mutants specifically lacking the B'γ subunit showed a lesion mimic phenotype together with constitutive activation of JA and SA related defence pathways (Trotta et al., 2011; Li et al., 2014). The *cat2 pp2a-b'γ* double mutants showed lesion formation even under short day conditions, together with an accumulation of SA, PR1 and camalexin (Li et al., 2013). Thus, there is a synergistic interaction between *cat2* and *pp2a-b'γ* mutants in which PP2A-B'γ controls intracellular oxidative stress response in a day length-dependent manner (Li et al., 2013). The data shown in Chapter (4) demonstrates that growth under HL for seven days significantly increased leaf area in both *pp2a-b'γ* mutants and *cat2 pp2a-b'γ* double mutants relative to LL-grown plants. However, the HL-dependent increase in leaf area was most marked in the *pp2a-b'γ* mutants. The *cat2* mutants and *cat2 pp2a-b'γ* double mutants were more resistant to aphid infestation than the wild type plants under LL conditions. This resistance was more marked in the *cat2 pp2a-b'γ* double mutants. Moreover, a pre-exposure to HL led to a significant decrease in aphid fecundity in the *pp2a-b'γ* and *cat2 pp2a-b'γ* double mutants relative to LL grown plants. However, this light-dependent decrease in aphid performance was not observed in the *cat2* mutants.

Growth under HL led to a significant increase in the levels of transcripts encoding REDOX RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1) in the leaves of the wild type plants, *pp2a-b'γ*, *cat2* mutants and *cat2 pp2a-b'γ* double mutants relative to LL grown plants Chapter (4). This finding is consistent with the results of previous studies, which showed a 4-fold up-regulation in the levels of *RRTF1* mRNA in the leaves of HL-grown plants compared to LL conditions (Vogel et al., 2014; Matsuo et al., 2015). Similarly, aphid infestation led to significant increase in the abundance of RRTF1 in the leaves of wild type plants, *pp2a-b'γ*, *cat2* mutants and *cat2 pp2a-b'γ* double mutants grown under both LL and HL conditions compared to non-infested leaves. This result agrees with previous observations by Kerchev et al. (2013) in which the expression of *RRTF1* was up-regulated in response to aphid infestation. RRTF1 is involved in the regulation of various stress responses via generation of ROS, regulation of transcription factors and cell wall remodelling (Matsuo et al., 2015).

For example, transcripts encoding two cell wall peroxidases were increased in abundance in response to elevated RRTF1 level (Vogel et al., 2014). Thus, the increased expression of *RRTF1* may therefore be important in leading to enhanced aphid resistance observed in the *pp2a-b'γ*, *cat2* mutants and *cat2 pp2a-b'γ* double mutants.

The ALLENE OXIDE CYCLASE (AOC) family consist of four genes that encode proteins involved in JA biosynthesis (Stenzel et al., 2003; 2012). They play important roles in plant responses to wounding (Ziegler et al., 2000; Stenzel et al., 2003). *AOC3* transcripts were only increased in the leaves of *cat2 pp2a-b'γ* double mutants as a result of aphid feeding under LL conditions but not in any of the other lines used in these experiments. Growth under HL increased the levels of AOC3 transcripts in the leaves of *pp2a-b'γ*, *cat2* mutants and *cat2 pp2a-b'γ* double mutants relative to LL-grown plants. Similarly, AOC3 transcripts were increased in abundance in response to aphid infestation in the leaves of all genotypes that had been pre-treated with HL for seven days. Thus, the expression of AOC3 may therefore be important in leading to enhanced aphid resistance, particularly in the *cat2 pp2a-b'γ* double mutants

There are more than 80 MAPKKK genes in the Arabidopsis genome and the majority of them are involved in plant defence responses (Taj et al., 2010). In these studies, the expression of *MAPKKK21* was examined in detail because it had previously been shown to be responsive to aphid infestation (Kerchev et al., 2013). The data presented in Chapter (4) show that under LL the levels of *MAPKKK21* transcripts were increased in response to aphid feeding only in the leaves of *pp2a-b'γ* mutant and *cat2 pp2a-b'γ* double mutants. Growth under HL increased the levels of transcripts encoding MAPKKK21 in the leaves of wild type plants, *pp2a-b'γ* and *cat2* mutants relative to LL grown plants. The levels of MAPKKK21 transcripts were also increased in response to aphid infestation in the leaves of all genotypes that had been pre-treated with HL for seven days.

As discussed above, aphid fecundity was decreased on the leaves of *cat2* mutants and *cat2 pp2a-b'γ* double mutants relative to the wild type plants under LL, the decrease in aphid numbers being most marked on the *cat2 pp2a-b'γ* double mutants in which high levels of *AOC3* and *MAPKKK21* were observed. The *cat2 pp2a-b'γ* double mutants accumulate three times more camalexin than the leaves of *cat2* mutants, as well having increased SA signalling with higher *PR1* transcript levels (Li et al., 2013). The accumulation of camalexin in the *cat2* mutants (Chaouch et al., 2010) and *cat2 pp2a-b'γ* double mutants (Li et al., 2013) might be considered to be linked to the observed changes in aphid fecundity because secondary metabolites are toxic to aphids (Kettles et al., 2013; Prince et al., 2014).

However, as discussed above the *pp2a-b'γζ* double mutants accumulate more camalexin than the *pp2a-b'γ* mutants and yet they did not show more aphid resistance than the wild type. Hence the increased aphid resistance observed in the *cat2* and *cat2 pp2a-b'γ* double mutants is probably not linked to the extent of leaf camalexin accumulation and is more like to be related to the differences in SA-signalling pathways in the leaves. Similarly, the higher sensitivity of the *pad2* mutants to the pathogens *Pseudomonas syringae* and *Pieris brassicae* was not caused by camalexin deficiency (Glazebrook and Ausubel, 1994; Roetschi et al., 2001). The phytoalexin-deficient (*pad*) mutants (*pad1* and *pad2*), which have low camalexin content, were found to be more susceptible to these pathogens than the wild type. However, the *pad3* mutants that contain camalexin showed a similar aphid sensitivity to the wild type plants (Glazebrook and Ausubel, 1994). Hence, camalexin deficiency did not result in enhanced pathogen growth in the *pad3* mutants. The *pad1* and *pad2* mutations might therefore influence other defence pathways that are required to limit pathogen growth (Glazebrook and Ausubel, 1994). Alternatively, the camalexin precursor is accumulated in the *pad3* mutants and this metabolite might serve as a phytoalexin limiting aphid infestation (Glazebrook and Ausubel, 1994). The *pad2* mutants are defective in GSH synthesis and have only about 20% of the leaf glutathione pool found in the wild type plants (Parisy et al., 2006). Aphid fecundity was decreased in the *pad2* mutants relative to the wild type. Similarly, aphid fecundity was lower in *cad2* mutant (that is also defective in GSH synthesis) than the wild type. These data suggest that like catalase, GSH functions are important in regulating aphid resistance. However, aphid fecundity was similar in the *clt* mutants that lack the chloroplast envelope GSH transporter, to the wild type. These mutants have a lower cytosolic GSH pool and are more susceptible to pathogens. However, aphid fecundity was similar in the *clt* mutants to the wild type suggesting that cytosolic GSH pool is not important in the regulation of aphid resistance.

Catalase deficiency increases oxidative signalling through metabolic pathways linked to photorespiration. GSH plays a key role in the activation of the H_2O_2-dependent oxidative signalling pathways triggered by catalase deficiency that lead to SA-dependent signalling pathways. Blocking GSH synthesis in the *cat2* background prevented H_2O_2-induced SA accumulation and the expression of SA-genes leading to resistance to bacterial pathogens (Han et al., 2013). Moreover, the effect of GSH on oxidant-induced SA-signalling was independent of its antioxidant function (Han et al., 2013b). Aphid fecundity was similar in the *cat2, cad2* and *cat2 cad2* double mutants, suggesting that GSH-dependent oxidative signalling pathways do not influence the phytohormone defence pathways leading to aphid resistance.

9.2 Role of ascorbate in plant responses to aphid attack

Like catalase and glutathione, ascorbate plays a central role in H_2O_2 detoxification in plants (Foyer and Halliwell, 1976; Noctor and Foyer, 1998). In these studies, two different mutants, *vtc2-1* (EMS) and the *vtc2* (T-DNA), which defects in the ascorbate biosynthetic enzyme GDP-L-galactose phosphorylase/L-galactose guanylyltransferase, were used to determine the effects of low ascorbate on aphid fecundity. Aphid fecundity was decreased in the *vtc2-1* (EMS) mutant relative to the wild type plants as previously observed by Kerchev et al. (2013). These authors reported that the higher aphid resistance observed in the *vtc2-1* (EMS*)* mutants was dependent on ABSCISIC ACID INSENSITIVE-4 (ABI4) transcription factor (Kerchev et al., 2011; 2013). The data presented here show that in contrast to the *vtc2-1* (EMS) mutant, the *vtc2* (T-DNA) mutants showed a similar aphid fecundity to the wild type plants, even though both mutants show similar decreases in leaf ascorbate levels relative to the wild type. The difference in the aphid fecundity observed in the *vtc2-1* (EMS) and the *vtc2* (T-DNA) lines relative to the wild type may be related to variations in aphid-dependent defence gene expression. While aphid infestation increased the levels of transcripts encoding the WRKY62 transcription factor, which has an important role in the regulation of JA and SA cross-talk (Mao et al., 2007) and the ARABIDOPSIS ZINC-FINGER PROTEIN 1 (AZF1) in the leaves of both *vtc2-1* (EMS) and the *vtc2* (T-DNA) lines, the aphid-dependent increase was much higher in the *vtc2-1* (EMS) leaves than in the *vtc2* (T-DNA) lines. Moreover, the levels of transcripts encoding RRTF1, AOC3 and MAPKKK21 were increased in the aphid infested-leaves of the *vtc2-1* (EMS) mutant but not in the *vtc2* (T-DNA) lines relative to non-infested leaves. The *vtc2-1* (EMS) mutant (*vtc2-1*) is an intron-splice mutant, shortening the protein to less than 2/3rds that of the wild type length (Jander et al., 2002; Muller-Moule, 2008). Moreover, the *vtc2-1* level in the mutant is about 20% that of the wild type (Muller-Moule, 2008). Hence, unlike the *vtc2* (T-DNA) line, which is a knockout mutant, *vtc2-1* (EMS) shows a strong loss of function. GDP-L-galactose phosphorylase/L-galactose guanylyltransferase is found in the cytosol and the nucleus suggesting that this enzyme might have other functions in addition to ascorbate synthesis (Muller-Moule, 2008). It is therefore possible that the other functions in addition to ascorbate synthesis are differentially regulated in the two lines.

9.3 Role of apoplastic redox state on plant responses to aphid infestation under LL and HL

The effect of the redox state of the apoplast/cell wall compartment on aphid fecundity was determined using plants with different levels of the aplosatic enzyme ascorbate oxidase (AO). Aphid fecundity was compared in transgenic tobacco lines that either over-expresses a pumpkin AO (PAO) or a partial tobacco AO sequence in the antisense orientation (TAO) and wild type plants. Aphid fecundity was similar in lines when plants were grown under LL. In contrast, a HL (1600 μmol m^{-2} s^{-1}) pre-treatment for seven days prior to analysis of aphid fecundity, significantly decreased the number of aphids on the leaves of the antisense (TAO) plants, which have much lower (70% less) apoplastic ascorbate than the wild type. This finding suggests that the abundance of ascorbate in the apoplast exerts an influence on the ability of aphids to colonise tobacco leaves after a HL pre-treatment. The apoplastic AsA plays an important role in plant responses to biotic and abiotic stresses that result in an apoplastic oxidative burst (Barnes et al., 2002; Pignocchi and Foyer, 2003). It has previously been shown that AO plays important roles in plant defence responses to biotic and abiotic stresses (Sanmartin et al., 2003; Yamamoto et al., 2005; Fotopoulos et al., 2006; Pignocchi et al., 2006; Garchery et al., 2013). Low AO activity is likely to alter the extent and duration of the apoplastic oxidative burst (Pignocchi and Foyer, 2003; Foyer and Noctor, 2005a). These data suggest that apoplastic redox signalling has an impact on the chloroplast to nucleus signalling pathways that are triggered during the HL pre-treatment, and the resultant integrated signalling process produces a long-lasting change in the leaves that impairs the ability of the aphids to infest the leaves.

The apolastic AsA and DHA pools are involved in cell wall formation and loosening (Smirnoff, 2000). The lysine and arginine residues of cell wall proteins can react with DHA, a process that reduces the interaction between cell wall proteins and pectin (Lin and Varner, 1991). DHA might also influence cell wall loosening because of the reaction between DHA and the lysine side chains of cell wall proteins, which prevents the formation of Schiff's base with the reducing ends of polysaccharides (Lin and Varner, 1991). Moreover, DHA breakdown can lead to an increase in calcium oxalate crystals. A reduction in the calcium concentration of the apoplast might adversely affect calcium-pectin complexes and hence pectin cross-linking resulting in cell wall loosening (Lin and Varner, 1991). Conversely, the production of strongly oxidant hydroxyl radical (·OH) is important for cell wall growth (Kärkönen and Fry, 2006). Therefore, it is possible that AsA has a pro-oxidant function in the apoplast/cell wall compartment leading to (·OH) production, which can cause a non-enzymic scission of pectin and xyloglucan in the cell wall (Fry et al., 2001; Dumville and Fry, 2003).

The analysis of differential gene expression in the PAO and TAO tobacco lines relative to the wild type plants also provides information on the changes in gene expression that may result in the observed changes in aphid fecundity observed in these experiments. The transcript profiling analysis revealed that transcripts encoding proteins associated with terpenoid biosynthesis were increased only in the leaves of TAO plants in response to HL (Chapter 7). For example, transcripts encoding epi-arisotolchene synthase 110 were increased under HL. The epi-arisotolchene synthase family catalyses the conversion of farnesyl diphosphate to 5-epiaristolochene which is a precursor of phytoalexin capsidiol (Back et al., 1994). Capsidiol is a low molecular weight terpenoid that accumulates in tobacco plants in response to biotic stresses (Back et al., 1994; Maldonado-Bonilla et al., 2008). Secondary metabolites play key roles in plant stress responses (Edreva et al., 2008; Zavala and Ravetta, 2001; Coelho et al., 2007). Those that are involved in plant defence responses can be divided into three groups; terpenes, phenolics and nitrogen-containing compounds (Khan and Mohammad, 2011). When Arabidopsis plants experience HL, the levels of transcripts encoding proteins associated with flavonoid, anthocyanin and lignin biosynthesis are increased (Kimura et al., 2003). Terpene compounds are toxic to many herbivores (Soković et al. 2006; Attaran et al., 2008). For example, aphid reproductive performance was reduced on *Vicia fabae* plants that accumulated high levels of terpenes (Sadek et al., 2013). Hence, it is possible that the decreased aphid fecundity observed in the TAO plants that had been treated with HL was caused by an increase in aphid-toxic secondary metabolites. However, terpenes can also influence cell wall structure and composition. For example, leaves treated with monoterpene (allo-ocimene) had increased cell wall lignification (Kishimoto et al. 2006a,b). Thus, the increased abundance of transcripts encoding terpenes in the leaves of TAO plants treated with HL might have effects on cell wall lignification.

In agreement with previous studies (Coppola et al., 2013; Foyer et al., 2015), the results obtained in these studies show that aphid infestation resulted in a significant increase in the abundance of the cell wall-associated transcripts. Moreover, the levels of cell wall-associated transcripts were greatly increased in the leaves of TAO plants that had been treated with HL relative to the other lines (Chapter 7). Transcripts encoding the thionin-like protein D6, endo-1,3- β-glucosidase, aldehyde dehydrogenase 7, xyloglucan endotransglucosylase/hydrolase and cationic peroxidase were increased in abundance in the HL-treated TAO leaves. Endo-1,3- β-glucosidases play important roles in plant responses to different biotic and abiotic stresses (Rose et al., 2000). They degrade β-1,3/1,6 glucans that are localized in pathogen cell walls and function in oligosaccharide production in plant cell walls.

Cell wall oligosaccharides play an important role in signalling and the induction of plant defence responses (Ham et al., 1997). β-glucosidases, which are involved in lignin biosynthesis pathways, negatively affect insect settling and feeding (Cairns and Esen, 2010). The degradation of oligosaccharides by β-glucosidases provides monolignols, which are required for cell wall lignification (Hosel et al., 1978; Cairns and Esen, 2010). Aphid infestation lead to increased levels of transcripts encoding 4-coumarate:coenzyme A ligase (4CL) in the leaves of TAO plants that had been pre-treated with HL for seven days. 4CL has an important role in lignin biosynthesis (Chapple and Carpita, 1998; Moura et al., 2010). In addition, 4CL is also involved in an alternative pathway of lignin production, which is induced by pathogen attack (Kneusel et al., 1989; Schmitt et al., 1991).

Lignin is a phenolic heteropolymeric component of the plant secondary cell wall that plays a central role in plant responses to pathogens (Kosack and Jones, 1996; Barakat et al., 2010). It also provides essential mechanical support (Boudet, 2000) allowing water transport through the xylem (Ros Barcelo, 1997b; Boudet 2000). Lignin synthesis increases under stress conditions (Xu et al., 2011) in order to reduce water loss (Xu et al., 2011; Reina et al., 2001) and provide a physical barrier to insect attack, making settling and feeding more difficult (Barakat et al., 2010). High lignin contents are also associated with poor nutrition because the digestibility of the plant tissues is decreased (Moore and Jung, 2001; Hare, 2011). Lignin also protects cell wall polysaccharides from pathogen-induced degradation (Vanholme et al., 2010a).

Light regulates lignin biosynthesis, such that plants grown under HL have more lignin than those grown in the dark or under LL conditions (Chen et al. 2002; Andersson-Gunneras et al. 2006; Moller et al. 2006; Akgul et al. 2007). The levels of transcripts associated with lignin biosynthesis were increased in Arabidopsis plants grown under HL compared to LL (Kimura et al. 2003). Light intensity also affects the activities of enzymes involved in the lignin biosynthetic pathway. For example, the activity of cinnamyl alcohol dehydrogenase (CAD) and phenylalanine ammonia-lyase (PAL) enzymes, which are required for lignin biosynthesis, were increased in the leaves of orchid plants at high irradiances (Akgul et al. 2007). Furthermore, the HL-grown leaves had about 4 times as much chlorogenic acid, which is an important intermediate in lignin biosynthesis (Escamilla-Trevino et al., 2014), as the leaves of LL grown plants (Chapter 8).

Taken together, the information presented in this thesis suggests that the higher resistance to aphid infestation in the TAO tobacco plants grown under HL was at least in part the result of changes in cell wall structure and composition.

The levels of transcripts encoding the thionin-like protein D6 were high in the HL-treated TAO leaves. Thionins, which are cysteine-rich proteins that are localized in the cell wall, play key roles in plant defence mechanisms against fungi and insects. For example, the thionin 2.4 (Thi 2.4) that is localized in the Arabidopsis cell wall has antifungal activity (Asano et al., 2013). Thionins are considered to be antibacterial and antifungal agents because of their ability to form open pores in pathogen cell membranes and inhibit the activities of α-amylase and proteinases (Melo et al., 2002; Kang and Buchenauer, 2003; Asano et al., 2013). For example, overexpression of the oat thionin Asthi1 in transgenic rice plants increased plant resistance to *Burkholderia plantarii* and *Burkholderia glumae* (Iwai et al., 2002). Similarly, overexpression of barley α-hordothionin in transgenic sweet potato plants led to enhanced resistance to *Ceratocystis fimbriata* (Muramoto et al., 2012). The infested wheat plants with *Fusarium culmorum* accumulated high levels of thionin in their cell wall (Kang and Buchenauer, 2003).

Transcripts encoding endo-1,3- β-glucosidase were increased in abundance in the HL-treated TAO leaves. Xyloglucan endotransglucosylases/hydrolases are involved in the remodelling of the cell wall during herbivore/insect attack in a way that decreases insect settling and feeding (Divol et al., 2007). Similarly, cationic peroxidases, which are localized in the apoplast and cell wall (Young et al., 1995), accumulated in the HL-treated leaves of TAO plants. Peroxidases fulfil crucial functions in the plant cells such as the regulation of cell elongation (Goldberg et al., 1986), polysaccharide cross-linking (Fry, 1986) and wound healing (Espelie et al., 1986).

Unlike the HL-treated TAO leaves, aphid fecundity was similar in Arabidopsis *amiR-AO (3.6*) and *amiR-AO (8.5)* lines that have no detectable AO activity in their leaves. Aphid fecundity under LL conditions was similar in *amiR-AO (3.6*) and *amiR-AO (8.5)* lines to the wild type plants. The HL pre-treatment used in these studies led to a significant decrease in aphid fecundity in the wild type plants relative to LL conditions. However, aphid fecundity was similar in the *amiR-AO* lines under both LL and HL conditions. This finding suggests that the absence of AO activity and the resultant higher apoplastic AsA/DHA ratios prevent the HL-induced increase in aphid resistance that was observed in the wild type Arabidopsis leaves. Thus, while high apoplastic AsA/DHA ratios influence the HL-dependent pathways that regulate aphid resistance in tobacco and Arabidopsis, the response is markedly different between the two species. This difference may be explained by differences in the extent of HL response in the two species.

The HL treatment increased shoot growth in both tobacco and Arabidopsis leaves but leaf chlorophyll contents were decreased compared to plants grown under LL conditions. However, the HL treatment caused a significant decrease in photosynthetic CO_2 assimilation rates only in the Arabidopsis leaves. In contrast, photosynthetic CO_2 assimilation rates were only slightly inhibited in the tobacco leaves by the HL treatment. While dark-adapted Fv/Fm ratios were decreased in HL-grown tobacco and Arabidopsis leaves compared to plants grown under LL conditions, these data suggest that HL-dependent effects on photosynthesis were different in the two species. While HL led to a decrease in aphid fecundity in tobacco and Arabidopsis, further work is required to determine how HL-dependent signalling pathways interact with the apoplastic AsA/DHA-dependent signalling pathways to regulate aphid resistance.

Conclusions

These findings demonstrate that aphid infestation is not changed by HL, if aphids and HL are present together. In contrast, a HL pre-treatment is able to create a memory of abiotic stress that persists when plants are returned to LL and creates a situation that is less favourable to aphid fecundity. Moreover, decreased antioxidant capacity, whether this is achieved by deficiencies in peroxisomal catalase, ascorbate or glutathione, lead to enhanced resistance to aphid infestation. These findings suggest that increased oxidative signalling triggers pathways leading to increased aphid resistance, as illustrated in figure (9.1). The extent to which increased oxidative signalling retunes the responses is dependent on the light intensity of the environment experienced by the leaves (Fig. 9.1). While further studies are required to investigate the precise pathways and mechanisms that facilitate this response, the analysis presented here suggests that enhanced aphid resistance is linked to alterations in cell-wall linked factors, and possibly also to some changes in secondary metabolism. The data presented here also suggest that there is integration of signals from the chloroplast and the apoplast in fine tuning plant responses to aphids, as illustrated in figure (9.1). The integration of redox information from different cellular compartments finely tunes the adaptive responses of plants to aphids.

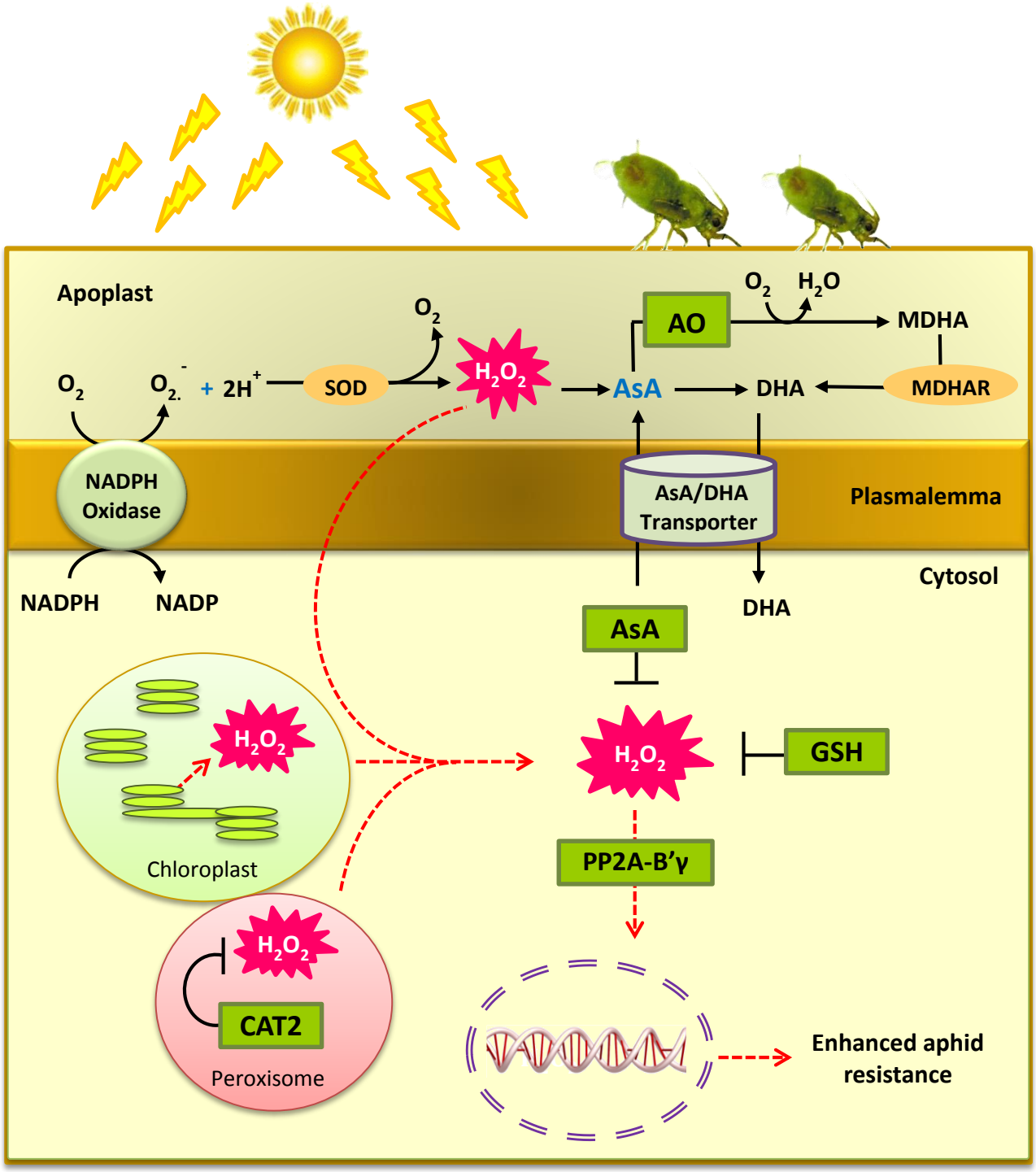


Figure 9.1 A model of the proposed role of all cell components that were studied in this project and involve in the regulation of ROS signaling under HL condition and aphid infestation. AsA: ascorbate; AO: ascorbate oxidase; MDHA: monodehydroascorbate; MDHAR: monodehydroascorbate reductase; DHA: dehydroascorbate; GSH: glutathione; PP2A-B'γ: protein phosphatase 2A; CAT2: catalase 2; SOD: superoxide dismutase; H_2O_2: hydrogen peroxide; ($O_2.^-$): superoxide.

List of References

Adamiec, M., Drath, M., and, Jackowski, G. (2008). Redox state of plastoquinone pool regulates expression of *Arabidopsis thaliana* genes in response to elevated irradiance. Acta Biochimica Polonica. **55**: 161-173.

Adie, B.A.T., Peréz-Pérez, J., Pérez-Pérez, M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A., and Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defences in *Arabidopsis*. Plant Cell. **19**: 1665-1681.

Akgul, M., Çöpür, Y. and Temiz, S. (2007). A comparison of kraft and kraft-sodium borohydrate brutia pine pulps. Building and Environment. **42:** 2586-2590.

Alexander, D., Stinson, J., Pear, J., Glascock, C., Ward, E., Goodman, R.M. and Ryals, J. (1992). A new multigene family inducible by tobacco mosaic virus or salicylic acid in tobacco. Mol. Plant Microbe Interact. **5:** 513-515.

Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H. and Shinn, P. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science **301:** 653-657.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., and Kazan, K. (2004). Antagonistic Interaction between Abscisic Acid and Jasmonate-Ethylene Signalling Pathways Modulates Defence Gene Expression and Disease Resistance in Arabidopsis. Plant Cell. **16**: 3460-3479.

Andersson, U., Heddad, M. and Adamska, I. (2003). Light stress-induced one helix protein of the chlorophyll a/b-binding family associated with photosystem I. Plant Physiol. **132:** 811-820.

Andersson-Gunneras, S., Mellerowicz, E.J., Love, J., Segerman, B., Ohmiya, Y., Coutinho, P.M., Nilsson, P., Henrissat, B., Moritz, T. and Sundberg, B. (2006). Biosynthesis of cellulose-enriched tension wood in Populus tremula: global analysis of transcripts and metabolites identifies biochemical and developmental regulators in secondary wall biosynthesis. Plant J. **46:** 349-349.

Angel, A., Song, J., Dean, C., and Howard, M. (2011). A Polycomb-based switch underlying quantitative epigenetic memory. Nature. **476**: 105-108.

Araus, J.L., Slafer, G., Royo, C. and Serret, M.D. (2008). Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Sciences. **27**: 377-412.

Arrigoni, N.O., Chinni, E., Ciraci e Mario, S., and De Tullio, C. (2003). In vivo elicitation of ascorbate oxidase activity by dioxygen and its possible role in photosynthesizing leaves. Rend. Fis. Acc. Lincei. **14**:127-134.

Asai, T., Tena, G., Plotnikova, J., Willmann, M., Chiu,W.,Gomez-Gomez, L., Boller, T., Ausubel, F., and Sheen, J. (2002). MAP kinase signalling cascade in Arabidopsis innate immunity. Nature **415**: 977-983.

Asano, T., Miwa, A., Maeda, K., Kimura, M. and Nishiuchi, T. (2013). The secreted antifungal protein thionin 2.4 in *Arabidopsis thaliana* suppresses the toxicity of a fungal fruit body lectin from *Fusarium graminearum*. PLoS Pathog. **9:** e1003581.

Aslam, M., Razaq, M., Akhter, W., Faheem, M., and Ahmad, F. (2005). Effect of sowing date of wheat on aphid (*Schizaphis gramium* RONDANI) population. Pak. Entomol. **27**: 79-82.

Atkinson, N. J. (2011). Plant molecular response to combined drought and nematode stress. PhD thesis. The University of Leeds. Institute of Integrative and Comparative Biology. Faculty of Biological Sciences.

Atkinson, N.J., and Urwin, P.E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. J. Exp. Bot. **63**: 3523-3543.

Attaran, E., Rostas, M. and Zeier, J. (2008). Pseudomonas syringae elicits emission of the terpenoid (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene in Arabidopsis leaves via jasmonate signaling and expression of the terpene synthase TPS4. Mol. Plant Microbe Interact. **21:** 1482-1497.

Back, K. and Chappell, J. (1995). Cloning and bacterial expression of a sesquiterpene cyclase from *Hyoscyamus muticus* and its molecular comparision to related terpene cyclases. Biol. Chem. **270:** 7375-7381.

Back, K., Yin, S. and Chappell, J. (1994). Expression of a plant sesquiterpene cyclase gene in *Escherichia coli.* Arch. Biochem. Biophys. **315:** 527-532.

Badejo, A.A., Wada, K., Gao, Y., Maruta, T., Sawa, Y., Shigeok,a S., Ishikawa, T. (2012). Translocation and the alternative d-galacturonate pathway contribute to increasing the ascorbate level in ripening tomato fruits together with the d-mannose/l-galactose pathway. Experimental Botany. **63**: 229-239.

Baier, M., Kandlbinder, A., Golldack, D., and Dietz, K. J. (2005). Oxidative stress and ozone: perception, signalling and response. Plant Cell Environ. **28**: 1012-1020.

Bailey, S., Walters, R.G., Jansson, S. and Horton, P. (2001). Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low-light and high-light responses. Planta. **293:** 794-801.

Baldwin, I.T., and Callahan, P. (1993). Autotoxicity and chemical defense-nicotine accumulation and carbon gain in solanaceous plants. Oecologia **94:** 534-541.

Ball, L., Accotto, G.P. and Bechtold, U. (2004). Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell. **16:** 2448-2462.

Barakat, A., Bagniewska-Zadworna, A., Frost, C.J. and Carlson, J.E. (2010). Phylogeny and expression profiling of CAD and CAD-like genes in hybrid Populus (P. deltoides 3 P. nigra): evidence from herbivore damage for subfunctionalization and functional divergence. BMC Plant Biol. **10:** 100

Bari, R., Jones, J. (2009). Role of plant hormones in plant defence responses. Plant Mol. Biol. **69**: 473-488.

Barnes, J., Zheng, Y., Lyons, T. (2002). Plant resistance to ozone: the role of ascorbate. Air Pollution and Plant Biotechnology. Springer. 235-252.

Bartoli, C.G., Pastori, G.M., Foyer, C.H. (2000) Ascorbate biosynthesis in mitochondriam is linked to the electron transport chain between complexes III and IV. Plant Physiol **123:** 335-344.

Becker, B., Holtgrefe, S., Jung, S., Wunrau, C., Kandlbinder, A., Baier, M., Dietz, K., Backhausen, J.E., and Scheibe, R. (2006). Influence of the photoperiod on redox regulation andstress responses in *Arabidopsis thaliana* L. (Heynh.) plants under long-and short day conditions. Planta. **25**: 1-14.

Becker, B., Holtgrefe, S., Jung, S., Wunrau, C., Kandlbinder, A., Baier, M., Dietz, K., Backhausen, J.E., and Scheibe, R. (2006). Influence of the photoperiod on redox regulation and stress responses in *Arabidopsis thaliana* L. (Heynh.) plants under long-and short-day conditions. Planta. **25**:1-14.

Beckers, G.J., Jaskiewicz, M., Liu, Y., Underwood, W.R., He, S.Y., Zhang, S. and Conrath, U. (2009). Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. Plant Cell. **21:** 944-953.

Beets, C. and Dubery, I. (2011). Quantification of camalexin, a phytoalexin from *Arabidopsis thaliana*: A comparison of five analytical methods. Analytical Biochemistry. **419:** 260-265.

Berger, S.L. (2007).The complex language of chromatin regulation during transcription. Nature **447**: 407-412.

Bethke, P., and Jones, R. (2001). Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. Plant J. **25**: 19-29.

Birkenmeier, G., and Ryan, C.A. (1998). Wound Signalling in Tomato Plants Evidence That ABA Is Not a Primary Signal for Defence Gene Activation. Plant Physiol. **117**: 687–693.

Blackman, R.L. and Eastop, V.F. (2000). Aphids on the world's crops: An identification and information guide: John Wiley & Sons, Chichester, UK.

Bloch Jr, C., Richardson, M. (1991). A new family of small (5 kDa) protein inhibitors of insect alpha-amylases from seeds or sorghum (Sorghum bicolar (L.) Moench) have sequence homologies with wheat γ-purothionins, FEBS Lett. **279:** 101-104.

Bloch, C. Jr. and Richardson, M. (1991). A new family of small (5 kDa) protein inhibitors of insect alpha-amylases from seeds or sorghum (*Sorghum bicolar* (L.) Moench) have sequence homologies with wheat γ-purothionins, FEBS Lett. **279:** 101-104.

Boisson, B., Giglione, C., and Meinnel, T. (2003). Unexpected Protein Families Including Cell Defense Components Feature in the N-Myristoylome of a Higher Eukaryote. J. Biol. Chem. **278:** 43418-43429.

Boller, T., and Felix, G. (2009). A renaissance of elicitors: perception of microbeassociated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol **60:** 379-406.

Bolton, M.D. (2009). Primary metabolism and plant defense fuel for the fire. Mol. Plant Microbe Inter. **22:** 487-497.

Bolwell, G.P., Bindschedler, L.V., Blee, K.A., Butt, V.S., Davies, D.R., Gardner, S.L., Gerrish, C. and Minibayeva, F. (2002). The apoplastic oxidative burst in response to biotic stress in plants: a tree component system. J. Exp. Bot. **53:** 1367-1376.

Bos, J.I.B., **Prince, D., Pitino, M., Maffei, M.E., Win, J., and Hogenhout, S.A.** (2010). A functional genomics approach identifies candidate effectors from the aphid species *Myzus persicae* (green peach aphid). PLoS Genetics **16:** e1001216.

Bostock, R.M. (2005). Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu. Rev. Phyto. **43**: 545-580.

Boudet, A.M. (2000). Lignins and lignification: selected issues. Plant Physiology and Biochemistry. **38:** 81-96.

Bowler, C., Slooten, L., Vandenbranden, S., De Ryke, R., Botterman, J., Sybesma, C., Van Montagu, M. andInze, D. (1991). Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO J. **10**: 1723-1732.

Bowler, C., Van Montagu, M., and Inze, D. (1992). Superoxide Dismutase and Stress Tolerance. Annu. Rev. Plant Physiol. **43**: 83-116.

Boyer, J.S. (1982). Plant productivity and environment. Science. **218:** 443-448.

Braendle, C., Davis, G.K., Brisson, J.A., Stern, D.L. (2006).Wing dimorphism in aphids. Heredity. **97**:192-199.

Bray, E.A. (1997). Plant responses to water deficint. Trends Plant Sci. **2**: 48-54.

Bruessow, F., Gouhier-Darimont, C., Buchala, A., Metraux, J.P., and Reymond, P. (2010). Insect eggs suppress plant defence against chewing herbivores. Plant J. **62:** 876-885.

Cadenas, E. (1989) Biochemistry of oxygen toxicity. Annu Rev Biochem **58:** 79-110.

Cairns, J. R. K. and Esen, A. (2010). b-Glucosidases. Cell. Mol. Life Sci. **67:** 3389-3405.

Chamnongpol, S., Willekens, H., Moeder, W., Langebartels, C., Sandermann, H., Van Montagu, M., Inze, D., and Van Camp, W. (1998). Defence activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. Proc. Natl. Acad. Sci.USA. **95**: 5818-5823.

Chandler, J., Wilson, A., and Dean, C. (1996). Arabidopsis mutants showing an altered response to vernalization. Plant J. **10:**637-644.

Chang, C.C.C., Slesak, I., Jorda, L., Sotnikov, A., Melzer, M., Miszalski, Z., Mullineaux, P.M., Parker, J.E., Karpinska, B. and Karpinski, S. (2009). Arabidopsis chloroplastic glutathione peroxidases play a role in cross talk between photooxidative stress and immune responses. Plant Physiology**. 150:** 670-683.

Chaouch, S., Queval, G., and Noctor, G. (2012). AtRbohF is a crucial modulator of defence-associated metabolism and a key factor in the interplay between intracellular oxidative stress and pathogenesis responses in Arabidopsis. Plant **69**: 613-627.

Chaouch, S., Queval, G., Vanderauwera, S., Mhamdi, A., Vandorpe, M., Langlois-Meurinne, M., Van Breusegem, F., Saindrenan, P. and Noctor, G. (2010). Genetic reversion of cell death in the Arabidopsis cat2 knockout mutant shows that peroxisomal H_2O_2 is coupled to biotic defense responses by isochorismate synthase 1 in a daylength-related manner. Plant Physiology. **153**: 1692-1705.

Chaouch, S., Queval, G., Vanderauwera, S., Mhamdi, A., Vandorpe, M., Langlois-Meurinne, M., Van Breusegem, F., Saindrenan, P. and Noctor, G. (2010). Genetic reversion of cell death in the Arabidopsis cat2 knockout mutant shows that peroxisomal H_2O_2 is coupled to biotic defense responses by isochorismate synthase 1 in a daylength-related manner. Plant Physiology. **153:** 1692-1705.

Chapple, C. and Carpita, N. (1998). Plant cell walls as targets for biotechnology. Current Opinion in Plant Biology. **1:** 179-185.

Chen M.S. (2008). Inducible direct plant defence against insect herbivores: a review. Insect Science **15:** 101-114.

Chen, E.L., Chen, Y.A., Chen, L.M. and Liu, Z.H. (2002). Effect of copper on peroxidase activity and lignin content in Raphanus sativus. Plant Physiology and Biochemistry. **40:** 439-444.

Chen, W., and Singh, K. (1999). The auxin, hydrogen peroxide and salicylic acid induced expression of the Arabidopsis GST6 promoter is mediated in part by an ocs element. Plant J. **19**: 667-677.

Chen, Z., and Gallie, D.R. (2005). Increasing Tolerance to Ozone by Elevating Foliar Ascorbic Acid Confers Greater Protection against Ozone Than Increasing Avoidance. Plant Physiol. **138(3)**: 1673-1689.

Chinnusamy, V., and Zhu, J.K. (2009). Epigenetic regulation of stress responses in plants. Curr. Opin. Plant Biol. **12:** 133-139.

Cho, U.S., and Xu, W. (2007). Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. Nature **445**: 4.

Chung, H.S., Koo, A.J.K., Gao, X., Jayany, S., Thines, B., Jones, A.D. and Howe, G.A. (2008). Regulation and function of Arabidopsis JASMONATE ZIM-domain genes in response to wounding and herbivory. Plant Physiol. **146:** 952-964.

Chung, M.C., Chou, S.J., Kuang, L.Y., Charng, Y.Y. and Yang, S.F. (2002). Sub- cellular localization of 1-aminocyclopropane-1-carboxylic acido xidase in apple fruit. Plant Cell Physiol. **43:** 549-554.

Clough, S.J., and Bent, A. F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana.* Plant J. **16**: 735-43.

Cobbett, C.S., May, M.J., Howden, R. and **Rolls, B.** (1998). The glutathione-deficient, cadmium-sensitive mutant, *cad2-1*, of *Arabidopsis thaliana* is deficient in g-glutamylcysteine synthetase. Plant J. **16:** 73-78.

Coelho, G.C., Rachwal, M. F.G., Dedecek, R.A., Curcio, G.R., Nietsche, K. and Schenkel, E.P. (2007). Effect of light intensity on methylxanthine contents of *Ilex paraguariensis* A. St. Hil. Biochem. Syst. Ecol. **35:** 75-80.

Colville, L., and Smirnoff, N. (2008). Antioxidant status, peroxidase activity, and PR protein transcript levels in ascorbate-deficient Arabidopsis thaliana vtc mutants. Exp. Bot. **59:** 3857-3868.

Conklin, P. L., Saracco, S.A., Norris, S.R. and Last, R. L. (2000). Identification of Ascorbic Acid-Deficient *Arabidopsis thaliana* Mutants. Genetics **154**: 847-856.

Conklin, P.L., Norris, S.R., Wheeler, G.L., Williams, E.H., Smirnoff, N., and Last R.L. (1999). Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. Proc. Natl. Acad. Sci. USA. **96:** 4198-4203.

Conklin, P.L., Williams, E.H. and Last, R.L. (1996). Environmental stress sensitivity of an ascorbic acid-deficient Arabidopsis mutant. Proc Natl Acad Sci USA. **93:** 9970-9974.

Coppola, V., Coppola, M., Rocco, M., Digilio, M.C., D'Ambrosio, C., Renzone, G., Martinelli, R. and Scaloni, A. (2013). Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. BMC Genomics. **14:** 515.

Coquoz, J.L., Buchala, A., MP, H., M´etraux, J.P. (1995). Arachidonic acid induces local but not systemic synthesis of salicylic acid and confers systemic resistance in potato plants to *Phytophthora infestans* and *Alternaria solani*. Phytopathology **85**:1219-1224.

Corpas, F.J., Sandalio, L.M., Valderrama, R., Barroso, J.B. and Del Rio, L.A. (2008). Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* L.) leaves. Journal of Plant Physiology. **165:** 1319-1330.

Corrales, A.R., Nebauer, S.G. and Carrillo, L. (2014). Characterization of tomato Cycling Dof Factors reveals conserved and new functions in the control of flowering time and abiotic stress responses. Exp. Bot. **65:** 995-1012.

Creelman, R. A., and Mullet, J. E. (1992). Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. Proc. Natl. Acad. Sci. USA. **92**: 4114-4119.

Creelman, R.A., and Mullet, J.E. (1995). Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48**: 355-381.

Cushman, J., and Bohnert, H. (2000). Genomic approaches to plant stress tolerance. Curr. Opin. Plant Biol. **3**:117-124.

Cutler, S.R., Rodriguez, P.L., Finkelstein, R.R., and Abrams, S.R. (2010). Abscisic acid: Emergence of a core signalling network. Annu. Rev. Plant Biol. **61**: 651-679.

Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K. and **Scheible, W.R.** (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. Plant Physiology. **139**(1): 5-17.

Dai, M., Zhang, C., Kania, U., Chen, F., Xue, Q., McCray, T., Li, G., Qin, G., Wakeley, M., Terzaghi, W., Wan, J., Zhao, Y., Xu, J., Friml, J., Deng, X.W., and Wang, H. (2012). A PP6-type phosphatase holoenzyme directly regulates PIN phosphorylation and auxin efflux in Arabidopsis. Plant Cell. **24(6)**: 2497-2514.

Dangl, J.L., and Jones, J.D.G. (2001). Plant pathogens and integrated defence responses to infection. Nature **411**: 826-833.

Davin, L.B. and Lewis, N.G. (2000). Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignin and lignin biosynthesis. Plant Physiol. **123**:453-461.

Davin, L.B., Lewis, N.G. (2000). Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignan and lignin biosynthesis. Plant Physiol. **123:** 453-461.

Davison, P., Hunter, C., and Horton, P. (2002). Overexpression of b-carotene hydroxylase enhances stress tolerance in Arabidopsis. Nature **418**: 203-206

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

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

De Vos, M., Van Oosten, V., Van Pecke, R., Van Pelt, J., Pozo, M., Mueller, M., Buchala A., Metraux, J., Van Loon, L., Dicke, M., and Pieterse, C. (2005). Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Mol. Plant Microbe Interact. **18:** 923-937.

De Vos, M., Van Oosten, V., VsnPecke, R., Van Plet, J., Pozo, M., Mueller, M., Buchala A., Metraux, J., Van Loon, L., Dicke, M., and Pieterse, C. (2005). Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attach. Mol. Plant Microbe Interact. **18**: 923-937.

Devoto, A., and Turner, J.G. (2005). Jasmonate-regulated Arabidopsis stress signalling network. Plant Physiol. **123**: 161-172.

Dietz, K.J. (1997). Functions and responses of the leaf apoplast under stress. Prog. Bot. **58**: 221-254.

Diezel, C., Von Dahl, C.C., Gaquerel, E., and Baldwin, I.T. (2009). Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signalling. Plant Physiol. **150**: 1576-1586.

Divol, F., Vilaine, F., Thibivilliers, S., Kusiak, C., Sauge, M.H. and Dinant, S. (2007). Involvement of the xyloglucan endotransglycosylase/ hydrolases encoded by celery XTH1 and Arabidopsis XTH33 in the phloem response to aphids. Involvement of the xyloglucan. Plant Cell Environ. **30:** 187-201.

Dorokhov, Y. L., Komarova, T.V., Petrunia, I.V., Frolova, O.Y., Pozdyshev, D.V. and Gleba,Y.Y. (2012a). Airborne signals from a wounded leaf facilitate viral spreading and induce antibacterial resistance in neighboring plants. PLoS Pathog. **8(4):** e1002640.

Dowdle, J., Ishikawa, T., Gatzek, S., Rolinski, S. and Smirnoff, N. (2007). Two genes in *Arabidopsis thaliana* encoding GDP- L–galactosephosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J. **52**: 673-689.

Dowdle, J., Ishikawa, T., Gatzek, S., Rolinski, S. and Smirnoff, N. (2007). Two genes in Arabidopsis thaliana encoding GDP-l-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J. **52**: 673-689

Dubbleden, D.K., and Oosterbeek, M. J. (1995). The availability of external affects allocation and morphology in herbaceous climbing plants. Functional Ecol. **9**:628-634.

Dumville, J.C., and Fry, S.C. (2003). Solubilisation of tomato fruit pectins by ascorbate: a possible non-enzymic mechanism of fruit softening. Planta. **217:** 951-961.

Dutta, I., Majumder, P., Saha, P., Ray, K., and Das, S. (2005). Constitutive and phloem specific expression of *Allium sativum* leaf agglutinin (ASAL) to engineer aphid (*Lipaphi serysimi*) resistance in transgenic India mustard (*Brassica juncea*). Plant Science **169**: 996-1007.

Edreva, A., Velikova, V., Tsonev, T., Dagnon, S., Gürel, A., Aktas, L. and Gesheva, E. (2008). Stress protective role of secondary metabolites; Diversity of functions and mechanisms. Gen. Appl. Plant Physiol. **34:** 67-78.

El Oirdi, M., Trapani, A. and Bouarab, K. (2010). The nature of tobacco resistance against Botrytis cinerea depends on the infection structures of the pathogen. Environ. Microbiol. **12**: 239-253

Escamilla-Trevino, L. L., Shen, H., Hernandez, T., Yin, Y., Xu, Y. and Dixon, R. A. (2014). Early lignin pathway enzymes and routes to chlorogenic acid in switchgrass (*Panicum virgatum* L.). Plant Mol Biol. **84:** 565-576.

Espelie, K.E., Franceschi, V.R. and Kolattukudy, P.E. (1986). Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound-healing potato tuber tissue. Plant Physiol. **87:** 487-492

Fan, J., Crooks, C., Creissen, G., Hill, L., Fairhurst, S., Doerner, P., and Lamb, C. (2011). Pseudomonas sax genes overcome aliphatic isothiocyanate-mediated non-host resistance in Arabidopsis. Science **331**: 1185-1188.

Fenton, B., Kasprowicz, L., Malloch, G., and Pickup, J. (2010). Reproductive performance of asexual clones of the peach-potato aphid, (*Myzus persicae*, Homoptera: Aphididae), colonising Scotland in relation to host plant and field ecology. Bull. Entomol. Res. **100:** 451-460.

Fenton, B., Woodford, J.A.T. and Malloch, G. (1998). Analysis of clonal diversity of the peach–potato aphid, Myzus persicae (sulzer), in Scotland, UK and evidence for the existence of a predominant clone. Molecular Ecology. **7(11)**: 1475-1487.

Fini, A., Guidi, L., Ferrini, F., Brunetti, C., Di Ferdinando, M., Biricolti, S., Pollastri, S., Calamai, L. and Tattini, M. (2012). Drought stress has contrasting effects on antioxidant enzymes activity and phenylpropanoid biosynthesis in *Fraxinus ornus* leaves: an excess light stress affair? Plant Physiology. **169:** 929-939.

Finkelstein, R., and Lynch, T. (2000a). The Arabidopsis abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell. **12:** 599-609.

Finkelstein, R., Lynch, T., Reeves, W., Petitfils, M. and Mostachetti, M. (2011). Accumulation of the transcription factor ABA-insensitive (ABI4) is tightly regulated post-transcriptionally. Exp. Bot. **62:** 3971-3979.

Finkelstein, R.R. (1994). Mutations at two new Arabidopsis ABA response loci are similar to the *abi3* mutations. Plant J. **5:** 765-771.

Forslund, K., Petterson, J., Bryngelsson, T., and Jonsson, L. (2000). Aphid infestation indices PR proteins differentially in barley susceptible or resistant to the bird cherry-oat aphid. Physiol. Plant. **110:** 496-502.

Fotopoulos, V., Sanmartin, M., and Kanellis, A.K. (2006). Effect of ascorbate oxidase over-expression on ascorbate recycling gene expression in response to agents imposing oxidative stress. Exp. Bot. **57**: 3933-3943.

Foyer, C. H. and Noctor, G. (2009). Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid. Redox Signal. **11**: 861-905.

Foyer, C. H., Lopez-Delgado, H., Dat, J., and Scott, I. (1997). Hydrogen peroxide- and glutathione-associated mechanisms of acclamatory stress tolerance and signalling. Plant Physiol. **100**: 241-254.

Foyer, C.H. and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta. **133**: 21-25.

Foyer, C.H., and Lelandais, M.A. (1996). Comparison of the relative rates of transport of ascorbate and glucose across the thylakoid, chloroplast and plasmalemma membranes of pea leaf mesophyll cells. Plant Physiol. **148**: 391-398.

Foyer, C.H., and Noctor, G. (2008). Redox regulation in photosynthetic organisms: signalling, acclimation, and practical implications. Antioxid. Redox Sign. **4:** 861-905

Foyer, C.H., Kerchev, P. I., and Hancock, R. D. (2012). The ABA-INSENSITIVE-4 (ABI4) transcription factor links redox, hormone and sugar signalling pathways. Plant Signal. Behav. **7(2):** 276-281.

Foyer, C.H., Rowell, J. and **Walker, D.** (1983). Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. Planta. **157:** 239-244.

Foyer, C.H., Verrall, S.R. and Hancock, R.D. (2015). Systematic analysis of phloem-feeding insect-induced transcriptional reprogramming in Arabidopsis highlights common features and reveals distinct responses to specialist and generalist insects. Experimental Botany. **66:** 495-512.

Fraire-Velazquez, S., Rodrıguez-Guerra, R. and Sanchez-Calderon, L. (2011). Abiotic and biotic stress response crosstalk in plants. In: Shanker AK, Venkateswarlu B (eds) Abiotic stress response in plants-physiological, biochemical and genetic perspectives. InTech, Rijeka, Pp 3-26.

Frenkel, M., Külheim, C., Jänkänpää, H.J., Skogström, O., Dall'Osto, L., Agren, J., Bassi, R., Moritz, T., Moen, J. and Jansson, S. (2009). Improper excess light energy dissipation in Arabidopsis results in a metabolic reprogramming. BMC Plant Biology. **26:** 9-12.

Fridovich, I. (1998). Oxygen toxicity: a radical explanation. J. Exp. Biol. **201**:1203-1209.

Fritzsche-Hoballah, M.E., and Turlings, T.C.J. (2001). Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. Evol. Ecol. Res. **3:** 553-565.

Frost, C.J., Mescher, M.C., Carlson, J.E., and De Moraes, C.M. (2008). Plant defense priming against herbivores: getting ready for a different battle. Plant Physiol. **146*:*** 818-824.

Frugoli, J.A., Zhong, H.H., Nuccio, M.L., McCourt, P., McPeek, M.A., Thomas, T.L. and McClung, C.R. (1996). Catalase is encoded by a multigene family in Arabidopsis thaliana (L.) Heynh. Plant Physiology. **112**: 327-336.

Fry, S.C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu. Rev. Plant Physiol. **37:** 165-186.

Fry, S.C. (1998). Oxidative scission of plant cell wall polysaccharides by ascorbate-nduced hydroxyl radicals. Biochem J. **332**: 507-515.

Fry, S.C., Dumville, J.C. and Miller, J.G. (2001). Fingerprinting of polysaccharides attacked by hydroxyl radicals in vitro and in the cell walls of ripening pear fruit. Biochem **357:** 729-737.

Fu, Z.Q., and Dong, X. (2013). Systemic Acquired Resistance: Turning Local Infection into Global Defence. Annu. Rev. Plant Biol. **64**: 839-863.

Fuglsang, A.T., Tulinius, G., Cui, N., and Palmgren, M.G. (2006). Protein phosphatase 2A scaffolding subunit A interacts with plasma membrane H1-ATPase C-terminus in the same region as 14-3-3 protein. Physiologia Plantarum. **128**: 334-340.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signalling networks. Curr. Opin. Plant Biol. **9:** 436-442.

Galvez-Valdivieso, G., Fryer, M.J., Lawson, T., Slattery, K., Truman, W., Smirnoff, N., Asami, T., Davies, W.J., Jones, A. M. and Baker, N.R. (2009). The high light response in Arabidopsis involves ABA signaling between vascular and bundle sheath cells. Plant Cell. **21:** 2143-2162.

Gao, Y., Badejo, A.A., Shibata, H., Sawa, Y., Maruta, T., Shigeoka, S., Page, M., Smirnoff, N. and Ishikawa, T. (2011). Expression analysis of the *VTC2* and *VTC5* genes encoding GDP-l-galactose phosphorylase, an enzyme involved in ascorbate biosynthesis, in *Arabidopsis thaliana*. Bioscience, Biotechnology, and Biochemistry. **75:** 1783-1788

Garbers, C., DeLong, A., Derue`re, J., Bernasconi, P., and Soll, D. (1996). A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in Arabidopsis. EMBO. **15**: 2115-2124.

Garchery, C., Gest, N., Do, P.T., Alhagdow, Baldet, M., Menard, P., Rothan, C., Massot, C., Gautier, H., Arrouf, J., Fernie, A.R., and Stevens, R. (2013). A diminution in ascorbate oxidase activity affects carbon allocation and improves yield in tomato under water deficit. Plant Cell Environ. **36**: 159-175.

Garcia-Mata, C., Gay, R., Sokolovski, S., Hills, A., Lamattina, L., Blatt, M.R. (2003). Nitric oxide regulates K1 and Cl2 channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Proc. Natl. Acad. Sci. USA. **100**: 11116-11121

Gendall, A.R., Levy, Y.Y., Wilson, A., and Dean, C. (2001). The *VERNALIZATION* 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell **107**: 525-535.

Gfeller, A., Dubugnon, L., Liechti, R., and Farmer E.E. (2010). Jasmonate biochemical pathway. Sci. Signal. **3(109)**: 3.

Gilkerson, J., Perez-Ruiz, J.M., Chory, J. and Callis, J. (2012). Theplastid-localized pfkB-type carbohydrate kinases FRUCTOKINASE-LIKE1and 2 are essential for growth and development of Arabidopsis thaliana. BMC Plant Biol. **12:**102.

Girousse. C., Moulia, B., Silk, W. and Bonnemain, J.L. (2005). Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibitions of longitudinal and radial expansion. Plant Physiol. **137**:1474-1484.

Glazebrook, J. and Ausubel, F.M. (1994). Isolation of phytoalexinde ®cient mutants of Arabidopsis thaliana and characterization of their interactions with bacterial pathogens. Proc. Natl Acad. Sci. USA. **91:** 8955-8959.

Goldberg, R., Imberty, A., Liberman, M. and Prat, R. (1986) Relationships between peroxidatic activities and cell wall plasticity. *In* H Greppin, C Penel, T Caspar, eds, Molecular and Physiological Aspects of Plant Peroxidases. University of Geneva, Switzerland, Pp 208-220.

Gosset, V., N. Harmel, Gobel, C., Francis, F., Haubruge, E., Wathelet, J., Du Jardin, P., Feussner, I., and Fauconnier M. (2009). Attacks by a piercing-sucking insect (*Myzus persicae* Sultzer) or a chewing insect (*Leptinotarsa decemlineata* Say) on potato plants (*Solanum tuberosum* L.) induce differential changes in volatile compound release and oxylipin synthesis. J. Ex. Bot. **60:** 1231-1240.

Greb, T., Mylne, J.S., Crevillen, P., Geraldo, N., An, H.,Gendall, A.R., and Dean, C. (2007). The PHD finger protein VRN5 functions inthe epigenetic silencing of *Arabidopsis* FLC. Curr. Biol. **17**: 73-78

Green, M.A., and Fry, S.C. (2005). Vitamin C degradation in plant cells *via* enzymatic hydrolysis of 4-*O*-oxalyl-L-threonate. Nature. **433:** 83-87.

Gruszka, D. (2013). The Brassinosteroid Signaling Pathway-New Key Players and Interconnections with Other Signaling Networks Crucial for Plant Development and Stress Tolerance. Int. J. Mol. Sci. **14**: 8740-8774.

Gupta, A., Heinen, J., Holaday, A., Burke, J., and Allen, R. (1993a). Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. USA. **90**:1629–1633.

Ham, K.S., Wu, S.-C., Darvill, A.G., and Albersheim, P. (1997). Fungal pathogens secrete an inhibitor protein that distinguishes isoforms of plant pathogenesis-related endo-1,3 glucanases. Plant J. **12:** 169-179.

Hamel, L., and Beaudoin, N. (2010). Chitooligosaccharide sensing and downstream signalling: contrasted outcomes in pathogenic and beneficial plant-microbe interactions. Planta. **232**: 787-806.

Hammond-Kosack, K.E., and Jones, J.D.G. (1996). Resistance gene-dependent plant defense responses. Plant Cell. **8:** 1773-1791.

Hammond-Kosack, K.E., and Jones, J.D.G. (1997). Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 575-607.

Han, Y., Chaouch, S., Mhamdi, A., Queval, G., Zechmann, B. and Noctor, G. (2013a). Functional analysis of Arabidopsis mutants points to novel roles for glutathione in coupling H2O2 to activation of salicylic acid accumulation and signaling. Antioxid Redox Signal. **18:** 2106-2121.

Han, Y., Mhamdi, A., Chaouch, S. and Noctor, G. (2013b). Regulation of basal and oxidative stress-triggered jasmonic acid-related gene expression by glutathione. Plant Cell Environ. **36:** 1135-1146

Hardie, D.G. (1990). Roles of protein kinases and phosphatases in signal transduction. Symp. Soc .Exp. Biol. **44:** 241-255.

Hardie, J., Storer, J.R., Nottingham, S.F., Peace, L., Harrington, R., Merritt, L., Wadhams, L.J., and Wood, D.K. (1994). The interaction of sex pheromone and plant volatiles for field attraction of male bird-cherry aphid, *Rhopalosiphum padi*. Proceedings of the Brighton Crop Protection Conference: Pests and Diseases. **3:** 1223-1230.

Hare, J. D. (2011). Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annu. Rev. Entomol. **56:** 161-180.

Hashimoto, T., Nakajima, K., Ongena, G. and Yamada, Y. (1999). Two Tropinone Reductases with Distinct Stereospecificities from Cultured Roots of Hyoscyamus niger. Plant Physiol. **100(2)**: 836-845.

He, X., Anderson, J.C., Del Pozo, O., Gu, Y.Q., Tang, X., Martin, G.B. (2004). Silencing of subfamily I of protein phosphatase 2A catalytic subunits results in activation of plant defense responses and localized cell death. Plant J. **38**: 563-577.

Heddad, M. and Adamska, I. (2000). Light stress-regulated two-helix proteins in *Arabidopsis thaliana* related to the chlorophyll *a*/*b*-binding gene family. Proc Natl Acad Sci USA. **97:** 3741-3746.

Heil, M. (2009). Damaged-self recognition in plant herbivore defence. Trends in Plant Sci. **14:** 356-363.

Herms, D.A., and Mattson, W.J. (1992). The dilemma of plants: to grow or defend. Q. Rev. Biol. **67**: 283-335.

Hidalgo, A., González-Reyes, J.A., and Navas, P. (1989). Ascorbate free radical enhances vacuolization in onion root meristems. Plant, Cell & Environment. **12:** 455-460.

Higuchi, T., Suga, S., Tsuchiya, T., Hisada, H., Morishima, S., Okada, Y. and Maeshima, M. (1998). Molecular cloning, water channel activity and tissue specific expression of two isoforms of radish vacuolar aquaporin. Plant Cell Physiology. **39**: 905-913.

Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A. and Ikeuchi, M. (2001). DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. Plant Cell. **13**: 793-806.

Hildebrand, D.F., Brown, G.C., Jackson, D.M., and Hamilton-Kemp, T.R. (1993). Effects of some leaf-emitted volatile compounds on aphid population increase. J. Chem. Ecol. **19**: 1875-1887.

Hirai, M.Y., Sugiyama, K., Sawada, Y., Tohge, T., Obayashi, T., Suzuki, A., Araki, R., Sakurai, N., Suzuki, H., Aoki, K., Goda, H., Nishizawa, O.I., Shibata, D., andSaito, K. (2007). Omics-based identification of Arabidopsis Myb transcription fac tors regulating aliphatic glucosinolate biosynthesis. Proc. Natl. Acad. Sci. USA. **104:** 6478-6483.

Hirayama, T. and Shinozaki, K. (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J. **61**: 1041-1052.

Hogenhout, S.A., Ammar, E.D., Whitfield, A.E. and Redinbaugh, M.G. (2008). Insect vector interactions with persistently transmitted viruses. Annual Review of Phytopathology. **46**: 327-359.

Holmstrom, M., Hiltunen, H., Rips, S., Sipari, N., Mulo, P., Weis, E., Schaewen, A., Aro, E., and Kangasjärvi, S. (2011a). Regulatory Subunit B'γ of Protein Phosphatase 2A Prevents Unnecessary Defence Reactions under Low Light in Arabidopsis. Plant Physiol. **156**: 1464-1480.

Horemans, N., Foyer, C.H., and Asard, H. (2000). Transport and action of ascorbate at the plant plasma membrane. Trends Plant Sci. **5**: 263-267.

Horton, P., and Ruban, A. (2004). Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. Exp. Bot. **56(411)**.

Hosel, W., Surholt, E. and Borgmann, E. (1978). Characterization of beta- glucosidase isoenzymes possibly involved in lignification from chickpea (*Cicera rietinum* L.) cellsus pension cultures. Eur.J. Biochem. **84:** 487-492.

Humbeck, K. and Krupinska, K. (2003). The abundance of minor chlorophyll a/b-binding proteins CP29 and LHCI of barley (*Hordeum vulgare* L.) during leaf senescence is controlled by light. Experimental Botany. **54:** 375-383.

Huseby, S., Degerlund, M., Eriksen, G.K., Ingebrigtsen, R.A., Eilertsen, H.C. and Hansen, E. (2013). Chemical diversity as a function of temperature in six northern diatom species. Mar Drugs. **11:** 4232-4245.

Iwai, T., Kaku, H., Honkura, R., Nakamura, S. and Ochiai, H., (2002). Enhanced resistance to seed-transmitted bacterial diseases in transgenic rice plants overproducing an oat cell-wall-bound thionin. Mol Plant Microbe Interact. **15**: 515-521.

Jagtap, V., Bhargava, S., Sterb, P. and Feierabend, J. (1998). Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) moench. Exp. Bot. **49:** 1715-1721.

Jammes, F., Songa, C., Shina, D., Munemasab, S., Takedaa, K., Gua, D., Choa, D., Leea, S., Giordoa, R., Sritubtimd, S., Leonhardte, N., Ellisd, B.E., Muratab, Y., andKwaka, J. M. (2009). MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signalling. PNAS. **106(48)**.

Jammes, F., Yang, X., Xiao, S., and Kwak, J.M. (2011).Two Arabidopsis guard cell preferential MAPK genes, *MPK9* and *MPK12*, function in biotic stress response. Plant Signaling and Behavior. **6(11):** 1875-1877.

Jander, G., Norris, S.R., Rounsley, S.D., Bush, D.F., Levin, I.M., Last, R.L. (2002). Arabidopsis map-based cloning in the post-genome era. Plant Physiol. **129:** 440-450.

Jang, J.Y., Rhee, J., Chung, G.C. and Kang, H. (2012). Aquaporin as a membrane transporter of hydrogen peroxide in plant response to stresses. Plant Signaling and Behavior. **7(9)**: 1180-1181

Jänkänpää, H. J., Mishra, Y., Schroder, W.P. and Jansson, S. (2012). Metabolic profiling reveals metabolic shifts in Arabidopsis plants grown under different light conditions. Plant Cell Environ. **35:** 1824-1836.

Janssens, V., and Goris, J. (2001). Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem. J. **353**: 417-439.

Jansson, S. (1999). A guide to the Lhc genes and their relatives in Arabidopsis. Trends in Plant Sciences **4**: 236-240.

Jones, J.D., Dangl, J.L.(2006). The plant immune system. Nature **444**: 323-329.

Kaliff, M., Staal, J., Myrenas, M., and Dixelius, C. (2007). ABA is required for *Leptosphaeria maculans* resistance via ABI1- and ABI4-dependent signalling. Mol. Plant Microbe Interact. **20**: 335-345.

Kang, J., Hwang, J.U., Lee, M., Kim, Y.Y., Assmann, S.M., Martinoia, E. and Lee, Y. (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc. Natl. Acad. Sc. USA. **107:** 2355-2360.

Kang, Z. and Buchenauer, H. (2003). Immunocytochemical localization of cell wallbound thionins and hydroxyproline-rich glycoproteins in Fusarium culmoruminfected wheat spikes. Phytopathology-Phytopathologische Zeitschrift. **151**: 120-129.

Kangasjärvi, S., Neukermans, J., Li, S., Aro, E.M. and Noctor, G. (2012). Photosynthesis, photorespi- ration, and light signalling in defence responses. Exp.Bot. **63:** 1619-1636.

Karpinski, S. and Szechyńska-Hebda, M. (2012). Cellular light memory, photo electrochemical and redox retrograde signaling in plants. Biotechnology, Computational Biology and Bionanotechnology. **93(1)**: 27-39.

Karpinski, S., Gabrys, H., Mateo, A., Karpinska, B. and Mullineaux, P. M. (2003). Light perception in plant disease defence signalling. Curr. Opin. Plant Biol. **6**: 390-396.

Karpinski, S., Reynolds H., Karpinska B., Wingsle G., Creissen G. and Mullineaux P. (1999). Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Science. **284**: 654

Karpinski, S., Reynolds, H., Karpinska, B., Wingsle, G., Creissen, G., and Mullineaux, P.M. (1999). Systemic signalling and acclimation in response to excess excitation energy in Arabidopsis. Science. **284**: 654-657.

Karrer, E.E., Beachy, R.N. and Holt, C.A. (1998). Cloning of tobacco genes that elicit the hypersensitive response. Plant Mol. Biol. **36:** 681-690.

Kato, N. and Esaka, M. (1999). Changes in ascorbate oxidase gene expression and ascorbate levels in cell division and cell elongation in tobacco cells. Physiologia Plantarum. **105:** 321-329.

Kato, N., and Esaka, M. (2000). Expansion of transgenic tobacco protoplasts expressing pumpkin ascorbate oxidase is more rapid than that of wild-type protoplasts. Planta. **210**: 1018-1022.

Kerchev, P.I., Fenton, B., Foyer, C.H., and Hancock, R.D. (2012). Infestation of potato (*Solanum tuberosum* L.) by the peach-potato aphid (*Myzus persicae* Sulzer) alters cellular redox status and is influenced by ascorbate. Plant Cell Environ. **35**: 430-440.

Kerchev, P.I., Karpin´ska, B., Morris, J.A., Hussain, A., Verrall, S.R. Hedley, P.E., Fenton, B., Foyer, C.h., and Hancock, R.D. (2013). Vitamin C and the Abscisic Acid-Insensitive 4 Transcription Factor Are Important Determinants of Aphid Resistance in Arabidopsis. Antioxidants and Redox Signaling. **18(16)**: 1-15

Kerchev, P.I., Pellny,T.K.,Vivancos, P.D., Kiddle, G., Hedden, P., Driscoll, S., Vanacker, H., Verrier, P., Hancock, R.D., and Foyer C.H. (2011). The transcription factor ABI4 is required for the ascorbic acid dependent regulation of growth and regulation of jasmonate dependent defence signalling pathways in Arabidopsis. Plant Cell. **23**: 3319-3334.

Kerk, N.M., Jiang, K., and Feldman, L.J. (2000). Auxin metabolism in the root apical meristem. Plant Physiology. **122:** 925-932.

Kessler, A., and Baldwin, I.T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology. **53:** 299-328.

Kettles, G.J., Drurey, C., Schoonbeek, H.J., Maule, A.J. and Hogenhout, S.A. (2013). Resistance of Arabidopsis thaliana to the green peach aphid, *Myzus persicae*, involves camalexin and is regulated by microRNAs. New Phytol. **198:** 1178-1190.

Khandelwal, A., Elvitigala, T., Ghosh, B., and Quatrano, R.S. (2008). Arabidopsis transcriptome reveals control circuits regulating redox homeostasis and the role of an AP2 transcription factor. Plant Physiol. **148:** 2050-2058.

Khanna, R., Kronmiller, B., Maszle, D.R., Coupland, G., Holm, M., Mizuno, T., and Wu, S.H. (2009). The Arabidopsis B-box zinc finger family. Plant Cell. **21:** 3416-3420.

Kilian, J., Peschke, F., Berendzen, K.W., Harter, K., and Wanke, D. (2012). Prerequisites, performance and profits of transcriptional profiling the abiotic stress response. BiochimBiophys Acta. **1819**: 166-175.

Kim, E. H., Li, X.-P., Razeghifard, R., Anderson, J.M., Niyogi, K.K., Pogson, B.J., and Chow, W.S. (2009). The multiple roles of lightharvesting chlorophyll a/b-protein complexes define structure and pitmize function of Arabidopsis chloroplasts: a study using two chlorophyll b-less mutants. Biochim Biophys Acta. **1787**: 973-984.

Kim, J., To, T.K., Ishida, J., Morosawa, T., Kawashima, M., Matsui, A., Toyoda, T., Kimura, H., Shinozaki, K., and Seki, M. (2008). Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. Plant Cell Physiol. **49**: 1580-1588.

Kimura, M., Yoshiharu, Y.Y., Seki, M., Sakurai, T., Sato, M., Abe, T., Yoshida, S., Manabe, K., Shinozaki, K. and Matsui, M. (2003). Identification of Arabidopsis genes regulated by high light-stress using cDNA microarray. Photochem Photobiol. **77:** 226-233.

Kishimoto, K., Matsui, K., Ozawa, R. and Takabayashi, J. (2006a). Analysis of defensive responses activated by volatile alloocimene treatment in *Arabidopsis thaliana*. Phytochemistry. **67:** 1520-1529.

Kishimoto, K., Matsui, K., Ozawa, R. and Takabayashi, J. (2006b). Components of C6-aldehyde-induced resistance in *Arabidopsis thaliana* against a necrotrophic fungal pathogen, *Botrytis cinerea*. Plant Sci. **170:** 715-723.

Kliebenstein, D.J., Kroymann, J., and Mitchell-Olds, T. (2005). The glucosinolate myrosinase system in an ecological and evolutionary context. Curr. Opin. Plant Biol. **8:** 264-271.

Kneusel, R.E., Matern, U. and Nicolay, K. (1989). Formation of trans-caffeoyl CoA from trans-4-coumaroyl CoA by Zn21- dependent enzymes in cultured plant cells and its activation by an elicitor-induced pH shift. Arch Biochem Biophys. **269:** 455-462.

Kolar, C., Adam, E., Schafer, E., and Nagy, F. (1995). Expression of tobacco genes for light-harvesting chlorophyll alb binding proteins of photosystem I1 is controlled by two circadian oscillators in a developmentally regulated fashion. Proc. Natl. Acad. Sci. USA. **92:** 2174-2178.

Koncz, C., and Schell, J. (1986). The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* vector. Mol. Gen. Genet. **204:** 383-396.

Kopsell, D. A. and Sams, C. E. (2013). Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. American Society for Horticultural Science. **138 (1)**: 31-37

Kovtun, Y., Chiu, W.L., Tena, G., and Sheen, J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proceedings of the National Academy of Sciences. **97**: 2940-2945.

Kreps, J.A., Wu, Y., Chang, H.S., Zhu, T., Wang, X., and Harper, J.F. (2002). Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol. **130**: 2129-2141.

Kumar, R., Kushalappa, K., Godt, D., Pidkowich, M.S., Pastorelli, S., Hepworth, S.R. and Haughn, G.W. (2007). The Arabidopsis BEL1-LIKE HOMEODOMAIN proteins SAW1 and SAW2 act redundantly to regulate KNOX expression spatially in leaf margins. Plant Cell. **19:** 2719-2735.

Kwak, J.M., Mori, I.C., Pei, Z.M., Leonhardt, N., Torres, M.A., Dangl, J.L., Bloom, R.E., Bodde, S., Jones, J. G., and Schroeder, J.I. (2003). NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signalling in Arabidopsis. EMBO J. **22**: 2623-2633.

Lamb, R. S. (2012). Abiotic Stress Responses in Plants: A Focus on the SRO Family, Advances in Selected Plant Physiology Aspects, ISBN: 978-953-51-0557-2, InTech, Available from: http://www.intechopen.com/books/advances-in selected-plant-physiology-aspects/abioticstress-responses-in-plants-a-focus-on-the-sro-family

Lata, C. and Prasad, M. (2011) Role of DREBs in regulation of abiotic stress responses in plants. Exp. Bot. **62:** 4731-4748.

Leather, S.R., Walters, K.F.A., and Dixon, A.F.G. (1989). Factors determining the pest status of the bird-cherry oat aphid *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), europe: a study and review. Bull. Entomol. Res. **79**: 345-360.

Lee, J.H., and Kim, W.T. (2011). Regulation of abiotic stress signal transduction by E3 ubiquitin ligases in Arabidopsis. Mol.Cells. **31:** 201-208.

Lee, K. P., Kim, C., Landgraf, F. and Apel, K. (2007). EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of *Arabidopsis thaliana*. Proc. Natl Acad. Sci. **104 (10):** 270-275.

Lee, Y., Park, C.H., Ram Kim, A., Chang, S.C., Kim, S.-H., Lee, W.S., and Kim, S. K. (2011). The effect of ascorbic acid and dehydroascorbic acid on the root gravitropic response in *Arabidopsis thaliana*. Plant Physiology and Biochemistry. **49:** 909-916.

Lentzl, K.A., and Cipollini, D.F. (1998). Effect of light and simulated herbivory on growth of endangered northeastern bulrush, *Scirpus ancistrochaetus* Schuyler. Plant. Ecol. **139**: 125-131.

Levy, M., Edelbaum, O., and Sela, I. (2004). Tobacco Mosaic Virus Regulates the Expression of Its Own Resistance Gene N1. Plant Physiology. **135:** 2392-2397.

Levy, M., Wang, Q., Kaspi, R., Parrella, M.P., and Abel, S. (2005). Arabidopsis IQD1, a novel calmodulin-binding nuclear protein, stimulates glucosinolate accumulation and plant defense. Plant J. **43:** 79-96.

Li, S., Mhamdi, A., Trotta, A., Kangasjärvi, S., and 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 Phytol. **202**: 145-160.

Li, Y., Chen, L., Mu, J., and Zuo, J. (2013). LESION SIMULATING DISEASE1 interacts with catalases to regulate hypersensitive cell death in Arabidopsis. Plant Physiol. **163**: 1059-1070.

Lichtenthaler, H. K. (1986). Chlorolphylls and Carotenoids: Pigments of Photosynthetic Biomembranes. Methods in Enzymology. **148**: 350-382.

Lim, C. K. (2012). The Function of Ascorbate Oxidase in *Arabidopsis thaliana.* PhD thesis. University of Exeter. UK.

Lin, L. S., and Varner, J.E. (1991). Expression of ascorbic acid oxidase in Zucchini Squash (*Cucurbita pepo* L.). Plant Physiology. **96:** 159-165.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta CT) Method. Methods. **25:** 402-408.

Loake, G., and Grant, M. (2007). Salicylic acid in plant defence--the players and protagonists. Curr. Opin. Plant Biol. **10**: 466-472.

Lorenzo, O., Chico, J.M., Sánchez-Serrano, J.J., and Solano, R. (2004). JASMONATE INSENSITIVE1 Encodes a MYC Transcription Factor Essential to Discriminate between Different Jasmonate-Regulated Defence Responses in Arabidopsis. Plant Cell. **16**: 1938-1950.

Lu C, H. M., Guevara-Garcia, A., and Fedoroff, N. (2002). Mitogenactivated protein kinase signalling in post-germination arrest of development by abscisic acid. Proc. Natl. Acad.Sci.USA. **99**:15812-15817.

Luan, S. (2003). Protein phosphatases in plants. Annu. Rev. Plant Biol. **54**: 63-92.

Lücker, J., Bouwmeester, H.J., Schwab, W., Blaas, J., Van der Plas, L.H.W., and Verhoeven, H.A. (2001). Expression of Clarkia S-linalool synthase in transgenic petunia plants results in the accumulation of *S*-linalyl-β-D-glucopyranoside. Plant J. **27:** 315-324.

Luna, E. and Ton, J. (2012). The epigenetic machinery controlling transgenerational systemic acquired resistance. Plant Signaling and Behavior. **7:** 615-618.

Luna, E., Bruce, T.J., Roberts, M.R., Flors, V. and Ton, J. (2012). Next-generation systemic acquired resistance. Plant Physiology. **158:** 844-853.

M., Werber, M. and Weisshaar, B. (2003). Update on the basic helix-loop-helix transcription factor gene family in Arabidopsis thaliana. Plant Cell. **15:** 2497-2501.

Ma, F., Lara J., Youngb, D. and Doug K. Allen. (2014). Isotopically nonstationary 13C flux analysis of changes in Arabidopsis thaliana leaf metabolism due to high light acclimation. PNAS. **111(47)**: 169

MacKintosh, C., and Cohen, P. (1989). Identification of high levels of type 1 and type 2A protein phosphatases in higher plants. Biochem. **262**: 335-339.

MacKintosh, C., Lyon, G.J., and MacKintoshR.W. (1994). Protein phosphatase inhibitors activate anti-fungal defence responses of soybean cotyledons and cell cultures1. Plant J. **5(1)**: 137-147.

Maldonado-Bonilla, L.D., Betancourt-Jimenez, M. and Lozoya-Gloria, E. (2008). Local and systemic gene expression of sesquiterpene phytoalexin biosynthetic enzymes in plant leaves. Eur J. Plant Pathol. **121:** 439-449.

Mao, G., Meng, X., Liu, Y., Zheng, Z., Chen, Z., and Zhang, S. (2011). Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. Plant Cell. **23**: 1639-1653.

Mao, P., Duan, M., Wei, C., and Li, Y. (2007). WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate-responsive gene expression. Plant Cell Physiol. **48:** 833-842.

Marrs, K.A. (1996). The functions and regulation of glutathione s-transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47:** 127-158.

Martin, B., Collar, J. L., Tjallingii, W. F. and Fereres, A. (1997). Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of nonpersistently transmitted plant viruses. J Gen. Virol. **78:** 2701-2705.

Martin, G., Bogdanove, A., and Sessa, G. (2003). Understanding the functions of plant disease resistance proteins. Annu. Rev. Plant Biol. **54:** 23-61.

Martin, M., Kettmann, R., and Dequiedt, F. (2010). Recent insights into Protein Phosphatase 2A structure and regulation: the reasons why PP2A is no longer considered as a lazy passive housekeeping enzyme. Biotechnol. Agron. Soc. Environ. **14(1)**: 243-252.

Maruta, T., Tanouchi, A., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T. and Shigeoka, S. (2010). Arabidopsis chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. Plant Cell Physiol. **51**:190-200.

Masuda, T., Tanaka, A. and Melis, A. (2003). Chlorophyll antenna size adjustments by irradiance in Dunaliella salina involve coordinate regulation of chlorophyll a oxygenase (CAO) and Lhcb gene expression. Plant Mol. Biol. **51:** 757-771.

Mateo, A., Mühlenbock, P., Rustérucci, C., Chang, C., Miszalski, Z., Karpinska, B., Parker, J.E., Mullineaux, P.M., and Karpinski, S. (2004). *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. Plant Physiol. **136**:1-13.

Matsuo, M., Michal Johnson, J., Hieno, A., Tokizawa, M., Nomoto, M., Tada, Y., Godfrey, R., Obokata, J., Sherameti, I., Yamamoto, Y.Y., Böhmer, F.D. and Oelmüller, R. (2015). High REDOX RESPONSIVE TRANSCRIPTION FACTOR1 levels result in accumulation of reactive oxygen species in *Arabidopsis thaliana* shoots and roots. Mol. Plant. **10:** 1016.

Maughan, S.C., Pasternak, M., Cairns, N., Kiddle, G., Brach, T., Jarvis, R., Haas, F., Nieuwland, J., Lim, B., Müller, C., Salcedo-Sora, E., Kruse, C., Orsel, M., Hell, R., Miller, A.J., Bray, P., Foyer, C.H., Murray, J.A.H., Meyer, A.J. and Cobbett, C.S. (2010). Plant homologs of the *Plasmodium falciparum* chloroquinone-resistance transporter, *PfCRT*, are required for glutathione homeostasis and stress responses. Proc. Natl. Acad. Sci. USA. **107:** 2331-2336.

Mayer-Jaekel, R.E., and Hemmings, B.A. (1994). Protein phosphatase 2A, a "menage a trois". Trends Cell Biol. **4**: 287-291.

Mazid, M., Khan, T.A. and Mohammad, F. (2011). Role of secondary metabolites in defence mechanisms of plants. Biol Med. **3:** 232-249.

McClung, C.R. (1997). Regulation of catalases in Arabidopsis. Free Radical Biology and Medicine. **23**: 489-496.

Melo, F.R., Rigden, D. J. Franco, O.L. Mello,L. Ary,V. M.B. Grossi de Sa M.F., Bloch Jr, C. (2002). Inhibition of trypsin by cowpea thionin: characterization, molecular modeling, and docking. Proteins. **48**: 311-319.

Melo, F.R., Rigden, D.J., Franco, O.L., Mello, L.V., Ary, M.B., Grossi, de Sa, M.F. and Bloch, C. Jr. (2002). Inhibition of trypsin by cowpea thionin: characterization, molecular modeling, and docking. Proteins Struct. Funct. Genet. **48:** 311-319.

Mewis, I., Appel, M., Hom, A., Raina, R., and Schultz, C. (2005). Major signalling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insect. Plant Physiol. **138:** 1149-1162.

Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Van Breusegem, F. and Noctor, G. (2010). Catalases in plants : a focus on Arabidopsis mutants as stress-mimic models. Experimental Botany. **61 :** 4197-4220.

Mikkelsen, M.D., Hansen, C.H., Wittstock, U., and Halkier, B.A. (2000). Cytochrome P450 CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3 acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. J. Biol. Chem. **275:** 33712-33717.

Mikkelsen, R., Baunsgaard, L. and Blennow, A. (2004) Functional characterization of a-glucan, water dikinase, the starch phosphorylating enzyme. Biochem J. **377:** 525-532.

Miles, P.W. (1999). Aphid saliva. Biological Review.**74**: 41-85.

Millar, A.H., Mittova, V., Kiddle, G., Heazlewood, J.L., Bartoli, C.G., Theodoulou, F.L. and Foyer, C.H. (2003). Control of ascorbate synthesis by respiration and its implications for stress responses. Plant Physiology. **133**: 443-447.

Mirica, L.M., and Klinman, J.P. (2008). The nature of O_2 activation by the ethylene forming enzyme 1-aminocyclopropane-1-carboxylic acid oxidase. Proc. Natl. Acad. Sci. USA. **105*:*** 1814-1819.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. **7:** 405-410

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. Trends Plant Sci. **11**: 15-19.

Mittler, R. and Zilinskas, B.A. (1991). Purification and characterization of pea cytosolic ascorbate peroxidase. Plant Physiology. **97**: 962-968.

Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. Trends Plant Sci. **9**: 490-498.

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G., Tognetti, V.B., Vandepoele, K., Gollery, M., Shulaev, V. and Breusegem, F.V. (2011). ROS signalling: the new wave? Trends Plant Sci. **16**: 300-309.

Miyake, C., Amako, K., Shiraishi, N., and Sugimoto, T. (2009). Acclimation of tobacco leaves to high light intensity drives the plastoquinone oxidation system relationship among the fraction of open PSII centers, non photochemical quenching of Chl fluorescence and the maximum quantum yield of PSII in the dark. Plant Cell Physiol. **50(4)**: 730-743.

Mizoi, J., Shinozaki, K. and Yamaguchi Shinozaki, K. (2012). AP2/ERFfamily transcription factors in plant abiotic stress responses. Biochim. Biophys. Acta. **1819**: 86-96.

Møller, I.M., Jensen, P.E. and Hansson, A. (2007). Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol. **58:** 459-481.

Möller, R., Ball, R., Henderson, A., Modzel, G. and Find, J. (2006). Effect of light and activated charcoal on tracheary element differentiation in callus cultures of *Pinus radiata* D. Don. Plant Cell, Tissue and Organ Culture. **85:** 161-171.

Møller. I. M. (2001). Plant Mitochondria and Oxidative stress: Electron Transport, NADPH Turnover, and Metabolism of Reactive Oxygen Species. Annu. Rev. Plant Physiol. Plant Mol. Biol. **52**: 561-591

Monshausen, G.B., and Gilroy, S. (2009). Feeling green: Mechanosensing in plants. Trends Cell Biol. **19**: 228-235.

Moore, K.J. and Jung, H.G. (2001). Lignin and fiber digestion. Range Manage. **54:** 420-430.

Moran, P.J., and Thompson, G.A. (2001). Molecular Responses to Aphid Feeding in Arabidopsis in Relation to Plant Defence Pathways. Plant Physiol. **125(2)**: 1074-1085.

Moura, J.C.M.S., Bonine, C.A.V., Viana, J.D.F., Dornelas, M.C. and Mazzafera, P. (2010). Abiotic and biotic stresses and changes in the lignin content and composition in plants. Integrative Plant Biology. **52:** 360-376.

Mühlenbock, P., Szechynska-Hebda, M., Plaszczyca, M., Baudo, M., Mullineaux, P.M., Parker, J.E., Karpinska, B. and Karpinski, S. (2008). Chloroplast signalling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. Plant Cell. **20:** 2339-2356.

Mukherjee, M., Larrimore, K.E., Ahmed, N.J., Bedick, T.S., Barghouthi, N.T., Traw, M.B., and Barth, C. (2010). Ascorbic acid deficiency in Arabidopsis induces constitutive priming that is dependent on hydrogen peroxide, salicylic acid, and the NPR1 gene. Mol. Plant Microbe Interact. **23(3)**: 340-351.

Müller-Moulé, P. (2008). An expression analysis of the ascorbate biosynthesis enzyme VTC2. Plant Mol. Biol. **68:** 31-41.

Muramoto, N., Tanaka, T., Shimamura, T., Mitsukawa, N. and Hori, E. (2012). Transgenic sweet potato expressing thionin from barley gives resistance to black rot disease caused by Ceratocystis fimbriata in leaves and storage roots. Plant Cell Rep. **31**: 987-997.

Nakagami, H., Pitzschke, A., and Hirt, H. (2005). Emerging MAP kinase pathways in plant stress signalling. Trends Plant Sci. **10**: 339-346.

Nakamichi, N., Kiba, T., Henriques, R., Mizuno, T., Chua, N.H., and Sakakibara, H. (2010). PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. Plant Cell. **22:** 594-605.

Nanasato, Y., Akashi, K., and Yokota, A. (2005). Co-expression of cytochrome b561 and ascorbate oxidase in leaves of wild watermelon under drought and high light conditions. Plant Cell Physiol. **46(9)**:1515-24.

Narusaka, Y., Narusaka, M., Seki, M., Umezawa, T., Ishida, J., Nakajima, M., Enju, A. and Shinozaki, K. (2004). Crosstalk in the responses to abiotic and biotic stresses in Arabidopsis: analysis of gene expression in cytochrome P450 gene superfamily by cDNA microarray. Plant Molecular Biology. **55:** 327-342.

Ng, J.C.K. and Perry, K.L. (2004). Transmission of Plant viruses by aphid vectors. Molecular Plant Pathology. **5(5)**: 505-511.

Nicoll, D.A., Longoni, S. and Philipson, K.D. (1990). Molecular cloning and functional expression of the cardiac sarcolemmal Na^+ Ca^{2+} exchanger. Science. **250:** 562-565.

Niyogi, K.K. (1999). Photoprotection revisited: genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 333-359.

Noctor, G., and Foyer, C.H. (1998). Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**: 249-279.

Noctor, G., Veljovic-Jovanovic, S., Driscoll, S., Novitskaya, L., and Foyer, C.H. (2002). Drought and oxidative load in wheat leaves: A predominant role for photorespiration? Ann. Bot. **89**: 841-850.

Nomura, H., Komori,T.,Uemura,S., Kanda,Y., Shimotani, K. and Nakai, K., (2012). Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nat.Commun. **3**: 926.

Ogawa, T., Nishimura, K., Aoki,T., Takase, H.,Tomizawa, K. and Ashida, H., (2009). A phosphor fructo kinase B-type carbohydrate kinase family protein, NARA5 , for massive expressions of plastid-encoded photosynthetic genes in *Arabidopsis*. Plant Physiol. **151:** 114-128.

Ohara, K., Ujihara, T., Endo, T., Sato, F., and Yazaki, K. (2003). Limonene production in tobacco with *Perilla* limonene synthase cDNA. J. Ex. Bot. **54:** 2635-2642.

Ohta, M., Ohme-Takagi, M. and Shinshi, H. (2000). Three ethylene-responsive transcription factors in tobacco with distinct transactivation functions. Plant J. **22:** 29-38.

Olmos, E., Kiddle, G., Pellny, T., Kumar, S. and Foyer, C.H. (2006). Modulation of plant morphology, root architecture, and cell structure by low vitamin C in *Arabidopsis thaliana*. Exp. Bot. **57:** 1645-1655.

Osakabe, K., and Osakabe, Y. (2012). Plant Light Stress. In: eLS. John Wiley & Sons, Ltd.

Osmond, C. B., Austin, M. P., Bilings, J. A., Boyer, J. S., Dacey, W. H., Noberl, P. S., Smith, S. D., and Winner, W. E. (1987). Stress physiology and the distribution of plants. Biosience. **37**: 49.

Parisy, V., Poinssot, B. and Owsianowski, L. (2006). Identification of PAD2 as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. Plant J. **49:**159-172.

Parisy, V., Poinssot, B., Owsianowski, L., Buchala, A., Glazebrook, J. and **Mauch, F.** (2007). Identification of PAD2 as a c-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. Plant J. **49:** 159-172.

Pastori, G. M. and Foyer, C. H. (2002). Common Components, Networks, and Pathways of Cross-Tolerance to Stress. The Central Role of "Redox" and Abscisic Acid-Mediated Controls. Plant Physiol. **129**: 460-468.

Pastori, G.M., Kiddle, G., Antoniw, J., Bernard, S., Veljovic-Jovanovic, S., Verrier, P.J., Noctor, G. and Foyer, C.H. (2003). Leaf vitamin C contents modulate plant defence transcripts and regulate genes that control development through hormone signalling. Plant Cell. **15**:939-951.

Patrick J. M., and Gary, A. T. (2001). Molecular Responses to Aphid Feeding in Arabidopsis in Relation to Plant defence pathways. Plant Physiol. **125**: 1074-1085.

Pavet, V., Olmos, E., Kiddle, G., Kumar, S., Antoniw, J., Alvarez, M.E. and Foyer, C.H. (2005). Ascorbic acid deficiency activates cell death and disease resistance in Arabidopsis thaliana. Plant Physiology. **139:** 1291-1303.

Pegadaraju, V., Knepper, C., Reese, J., and Shah, J. (2005). Premature leaf senescence modulated by the Arabidopsis PHYTOALEXIN DEFICIENT4 gene is associated with defense against the phloem-feeding green peach aphid. Plant Physiol. **139:** 1927-1934.

Pei, Z.M., Mutrata, Y., Benning, G., Thomine, S., Kiusener, B., Allen, G.J, Brill, E. and Schroeder, J.I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cell. Nature. **406**: 731-734.

Pellny, T.K., Locato, V., Vivancos, P.D., Markovic, J., De Gara, L., Pallardo, F.V., and Foyer, C.H. (2009). Pyridine nucleotide cycling and control of intracellular redox state in relation to poly (ADP-ribose) polymerase activity and nuclear localization of glutathione during exponential growth of Arabidopsis cells in culture. Mol. Plant **2**: 442-456.

Pérez-Clemente, R.M., Vives, V., Zandalinas, S.I., López-Climent, M.F., Muñoz, Valeria., and Gómez-Cadenas, Aurelio. (2013). Biotechnological Approaches to Study Plant Responses to Stress. Bio. Med Research International, volume 2013, Article ID 654120.

Perrone, S.T., McDonald, K.L., Sutherland, M.W. and Guest, D.I. (2003). Superoxide release is necessary for phytoalexin accumulation in *Nicotiana tabacum* cells during the expression of cultivar-race and non-host resistance towards Phytophthora spp. Physiol. Mol. Plant Pathol. **62**: 127-135

Petrov, V. D., and Breusegem, F. V. (2012). Hydrogen peroxide-a central hub for information flow in plant cells. AoB Plants: pls014.

Pfalz, M., Vogel, H., and Kroymann, J. (2009). The gene controlling the *Indole Glucosinolate Modifier1* quantitative trait locus alters indole glucosinolate structures and aphid resistance in Arabidopis. Plant Cell. **21:** 985-999.

Pietrzykowska, M., Suorsa, M., Semchonok, D.A., Tikkanen, M., Boekema, E.J., Aro, E.M. and Jansson, S. (2014) The light-harvesting chlorophyll a/b binding proteins Lhcb1 and Lhcb2 play complementary roles during state transitions in Arabidopsis. Plant Cell. **26:** 3646-3660.

Pignocchi, C., Fletcher, J. M., Wilkinson, J. E., Barnes. J. D., and Foyer, C. H. (2003). The function of ascorbate oxidase in tobacco. Plant Physiol. **132**: 1631-1641.

Pignocchi, C., Kiddle, G., Herna´ndez, I., Asensi, A., Taybi, T., Barnes, J.D. and Foyer, C.H. (2006). Apoplast redox state modulates gene transcription leading to modified hormone signalling and defence in tobacco. Plant Physiol. **141**: 423-435.

Pitzschke, A., Djamei, A., Bitton, F., and Hirt, H. (2009a). A major role of the MEKK1-MKK1/2-MPK4 pathway in ROS signalling. Mol. Plant. **2**: 120-137.

Plochl, M., Lyons, T., Ollerenshaw, J. and Barnes, J. (2000) Simulating ozone detoxification in the leaf apoplast through the direct reaction with ascorbate. Planta. **210:** 454-467.

Pnueli, L, Hongjian, L. and Mittler, R. (2003). Growth suppression, altered stomatal responses, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1)-deficient Arabidopsis plants. Plant J. **34:** 187-203.

Polidoros, A.N., Mylona, P.V. and Scandalios, J.G. (2001). Transgenic tobacco plants expressing the maize cat2 gene have altered catalase levels that affect plant–pathogen interactions and resistance to oxidative stress. Transgenic Research. **10**: 555-569.

Porta, H., Jiménez, G., Cordoba,E., León, P., Soberón, M. and Bravo, A. (2011). Tobacco plants expressing the Cry1AbMod toxin suppress tolerance to Cry1Ab toxin of Manduca sexta cadherin-silenced larvae. Insect Biochemistry and Molecular Biology. **41:** 513-519.

Potters, G, De Gara, L., Asard, H., and Horemans, N. (2002). Ascorbate and glutathione: guardians of the cell cycle, partners in crime? Plant Physiol. Biochem. **40**: 537-548.

Potters, G., Jansen, M., Horemans, N., Guisez, Y., and Pasternak, T. (2010). Dehydroascorbate and glutathione regulate the cellular development of *Nicotiana tabacum* L. SR-1 protoplasts. In Vitro Cellular and Developmental Biology – Plant. **46:** 289-297.

Prado, E. and Tjallingii, W.F. (1994). Aphid activities during sieve element punctures. Entomologia Experimentalis Applicata. **72:** 157.

Prince, D.C., Drurey, C., Zipfel, C., and Hogenhout, S.A. (2014). Theleucine-rich repeatreceptor-likekinase BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 and the cytochrome P450 PHYTOALEXIN DEFICIENT3 contribute toinnate immunity to aphidsin *Arabidopsis*. Plant Physiol. **164:** 2207-2219.doi: 10.1104/pp.114.235598

Queval, G., Issakidis-Bourgue,t E., Hoeberichts, F.A., Vandorpe, M., Gakiere, B., Vanacker, H., Miginiac-Maslow, M., Van Breusegem, F. and Noctor, G. (2007). Conditional oxidative stress responses in the Arabidopsis photorespiratory mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death. Plant Cell. **52**: 640-657.

Ralph, S., Park, J.Y., Bohlmann, J. and Mansfield, S.D. (2006a). Dirigent proteins in conifer defense: gene discovery, phylogeny and differential wound- and insect-induced expression of a family of DIR and DIR-like genes in spruce (*Picea* spp.). Plant Mol. Biol. **60:** 21-40.

Rasmussen, S., Barah, P., Suarez-Rodriguez, M.C., Bressendorff, S., Friis, P. and Costantino, P. (2013). Transcriptome responses to combinations of stresses in Arabidopsis. Plant Physiol. **161:** 1783-1794.

Rasool, B., Karpinska, B., Konert, G., Durian, G., Denessiouk, K., Kangasjärvi, S. and Foyer, C.F. (2014). Effects of light and the regulatory Beta subunit composition of protein phosphatase 2A on the susceptibility of *Arabidopsis thaliana* to aphid (*Myzus persicae*) infestation. Front. Plant Sci. **5: 405**.

Reed, J.W. (2001). Roles and activities of Aux/IAA proteins in Arabidopsis. Trends Plant Sci. **6:** 420-425.

Reina, J.J., Dominguez, E. and Heredia, A. (2001). Water sorption-desorption in conifer cuticles: the role of lignin. Physiologia Plantarum. **112**: 372-378.

Rentel, M., Lecourieux, D., Ouaked, F., Usher, S.L., Petersen, L., and Okamoto, H. (2004). OXI1 kinase is necessary for oxidativeburst-mediated signalling in Arabidopsis. Nature. **427**: 858-861.

Richter, R., Bastakis, E. and Schwechheimer, C. (2013a). Cross-repressive interactions between SOC1 and the GATAs GNC and GNL/CGA1 in the control of greening, cold tolerance, and flowering time in Arabidopsis. Plant Physiol. **162:** 1992-2004.

Richter, R., Behringer, C., Muller, I. K. and Schwechheimer, C. (2015). The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and phytochrome interacting factors. Genes and Development. **24:** 2093-2104.

Robert-Seilaniantz, A., Navarro, L., Bari, R., and Jones, J.D.G. (2007). Pathologicalhormone imbalances. Curr. Opin. Plant Biol. **10**: 372-379.

Rodriguez, M.C., Petersen, M. and Mundy, J. (2010). Mitogen-activated protein kinase signaling in plants. Annu. Rev. Plant Biol. **61:** 621-649.

Roetschi, A., Si-Ammour, A., Belbahri, L., Mauch, F. and Mauch-Mani, B. (2001) Characterization of an Arabidopsis-Phytophthora pathosystem: resistance requires a functional PAD2 gene and is independent of salicylic acid, ethylene and jasmonic acid signalling. Plant J. **28:** 293-305.

Rogers, E. E., Glazebrook, J. and Ausubel, F.M. (1996). Mode of action of the *Arabidopsis thaliana* phytoalexin camalexin and its role in Arabidopsis-pathogen interactions. Mol. Plant Microbe Interact. **9:** 748-757.

Rojo, E., Titarenko, E., Leon, J., Berger, S., Vancanneyt, G. and Sanchez-Serrano, J.J. (1998). Reversible protein phosphorylation regulates jasmonic acid-dependent and -independent wound signal transduction pathways in *Arabidopsis thaliana*. Plant J. **13:** 153-165.

Ros Barcelo, A. (1997b). Lignifcation in plant cell walls. International Re.iew of Cytology. **176**: 87-132.

Rose, J.K.C., Ham, K.S., Wu, S.C., darvill, A.G. and Albersheim, P. (2000). Cell wall hydrolases and their inhibitor proteins: key elements in the battle between plants and their pathogens. Biochem and Biotech. **13:** 55-62.

Rossel J.B., Wilson P.B., Hussain D., Woo N.S., Gordon M.J., Mewett O.P., Howell K.A., Whelan J., Kazan K. & Pogson B.J. (2007). Systemic and intracellular response to photooxidative stress in Arabidopsis. Plant Cell. **19:** 4091-4110.

Rossel, J. B., Wilson, I. W. and Pogson, B. J. (2002). Global changes in gene expression in response to high light in Arabidopsis. Plant Physiology. **130:** 1109-1120.

Rossi, M., Goggin, F.L., Milligan, S.B., Kaloshian, I., Ullman, D.E., and Williamson, V.M. (1998). The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc. Natl. Acad. Sci. USA. **95:** 9750-9754.

Roxas, V., Smith, R. J., Allen, E., and Allen, R. (1997). Overexpression of glutathione S transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotech. **15**: 988-991.

Sadek, R. Z., Elbanna, S. M. and Semida, F. M. (2013). Aphid-host plant interaction. Open Journal of Animal Sciences. **3(2)**: 16-27.

Sakuma, Y., Liu, Q., Dubouzet, J. G., Abe, H., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2002). DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration-and cold-inducible gene expression. Biochem. Biophys. Res. Commun. **290**: 998-1009.

Sakurai, N. (1998). Dynamic function and regulation of apoplast in the plant body. Plant Research. **111:** 133-148.

Salt, D.E., Smith, R.D. and Raskin, I. (1998). Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**: 643-668.

Samac, D. A., and Smigocki, A. C. (2003). Expression of oryzacystatin I and II in alfalfa increases resistance to the root-lesion nematode. Phytopathology. **93:** 799-804.

Sancenon, V., Puig, S., Mira, H., Thiele, D.J. and Pen arrubia, L. (2003). Identification of a copper transporter family in *Arabidopsis thaliana*. Plant Mol. Biol. **51:** 577-587.

Sanmartin, M., Drogoudi, P.D., Lyons, T., Pateraki, I., Barnes, J., and Kanellis, A.K. (2003). Overexpression of ascorbate oxidase in the apoplast of transgenic tobacco results in altered ascorbate and glutathione redox states and increased sensitivity to ozone. Planta. **216**: 918-928.

Sanmartin, M., Drogoudim, P.D., Pateraki, T.L.I., Barnes, J., and Kanellis, A. K. (2002). Over-expression of ascorbate oxidase in the apoplast of transgenic tobacco results in altered ascorbate and glutathione redox states and increased sensitivity to ozone. Planta. **216**: 918-928.

Sattelmacher, B. (2001). The apoplast and its significance for plant mineral nutrition. New Phytologist. **149:** 167-192.

Schaller, F., Zerbe, P., Reinbothe, S., Reinbothe, C., Hofmann, E., and Pollmann, S. (2008). The allene oxide cyclase family of Arabidopsis thaliana: localization and cyclization. FEBS J. **275:** 2428-2441.

Scheidig, A., Fröhlich, A., Schulze, S., Lloyd, J.R. and Kossmann, J. (2002). Downregulation of a chloroplast-targeted beta-amylase leads to starch-excess phenotype in leaves. Plant J. **30**: 581-591.

Schlaich, N.L. (2007). Flavin-containing monooxygenases in plants: looking beyond detox. Trends Plant Sci. **12:** 412-418.

Schmitt, D., Pakusch, A.E. and Matern, U. (1991). Molecular cloning, induction, and taxonomic distribution of caffeoyl-CoA 3-*O*methyltransferase, an enzyme involved in disease resistance. Biol Chem. **266:** 17416-17423.

Schmitz, J., Schöttler, M.A., Krueger, S., Geimer, S., Schneider, A., Kleine, T. and Häusler, R.E. (2012). Defects in leaf carbohydrate metabolism compromise acclimation to high light and lead to a high chlorophyll fluorescence phenotype in *Arabidopsis thaliana*. BMC Plant Biology. **12:** 8.

Schwachtje, J., and Baldwin, I.T. (2008). Why does herbivore attack reconfigure primary metabolism? Plant Physiol. **146:** 845-851.

Segonzac, C., Macho, A.P., Sanmartín, M., Ntoukakis, V., Sánchez-Serrano, J.J. and Zipfel, C. (2014). Negative control of BAK1 by protein phosphatase 2A during plant innate immunity. EMBO J. **33:** 2069-2079.

Sheldon, C. C., Hills, M. J., Lister, C., Dean, C., Dennis, E. S. and Peacock, W. J. (2008). Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization. Proc. Natl. Acad. Sci. USA. **105**: 2214-2219.

Shen, W., Gomez-Cadenas, A., Routly, E.L., Simmonds, J.A., Gulick, P.J. (2001). The salt stress-inducible protein kinase gene, Esi47, from the salt-tolerant wheatgrass Lophopyrum elongatum is involved in plant hormone signaling. Plant Physiol. **125:** 1429-1441.

Sierla, M., Rahikainen, M., Salojarvi, J., Kangasjarvi, J. and Kangasjarvi, S. (2013). Apoplastic and chloroplastic redox signaling networks in plant stress responses. Antioxid. Redox Signal. **18**: 2220-2239.

Silverman, P., Seskar, M., Kanter, D., Schweizer, P.,M´etraux, J., Raskin, I. (1995). Salicylic acid in rice (biosynthesis, conjugation, and possible role). Plant Physiol. **108:** 633-639

Singh, N. K., Bracker, C. A., Hasegawa, P. M., Handa, A. K., Buckel, S., Hermodson, M. A., Pfankoch, E., Regnier, F. E. and Bressan, R. A. (1987). Characterization of Osmotin Plant. Physiol. **85**: 529-536.

Smirnoff, N. (2011) Vitamin C: the metabolism and functions of ascorbic acid in plants. In Advances in Botanical Research, Vol. 59, Biosynthesis of Vitamins in Plants Part B (eds F. Rebeille & R. Douce). Pp. 109-179. Academic Press Ltd-Elsevier Science Ltd, London, UK.

Smirnoff, N., Wheeler, G.L. (2000). Ascorbic acid in plants: biosynthesis and function. Crit. Rev. Plant Sci. **19**: 267-290.

Smith, M. and Boyko, E. (2007). The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomol. Exp. Appl. **122:** 1-16.

Smith, R.D., and Walker, J.C. (1996). Plant protein phosphatases. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47:** 101-125.

Soković, M. and Griensven, L. (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisporus. Eur. J. Plant Pathol. **116:** 211-224.

Sommer-Knudsen, J., Bacic, A., and Clarke, A.E. (1998). Hydroxyproline rich plant glycoproteins. Phytochem. **47**: 483-497.

Souer, E., van Houwelingen, A., Kloos, D., Mol, J.N.M., and Koes, R. (1996). The no apical meristem gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Plant Cell. **85:** 159-170.

Spoel, S.H., Koornneef, A., Claessens, S.M.C., Korzelius, J.P., Van Pelt, J.A., Mueller,M.J., Buchala, A.J., Métraux, J.-P., Brown, R., and Kazan, K. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defence pathways through a novel function in the cytosol. Plant Cell. **15**: 760-770.

Stenzel, I., Hause, B., Miersch, O., Kurz, T., Maucher, H., Weichert, H., Ziegler, J., Feussner, I. and Wasternack, C. (2003). Jasmonate biosynthesis and the allene oxide cyclase family of Arabidopsis thaliana. Plant Mol. Biol. **51:** 895-911.

Stenzel, I., Otto, M. and Delker, C. (2012). ALLENE OXIDE CYCLASE (AOC) gene family members of Arabidopsis thaliana: tissue- and organ-specific promoter activities and in vivo heteromerization. Experimental Botany. **63:** 6125-6138.

Sunkar, R., Bartels, D. and Kirch, H.H. (2003). Overexpression of a stress-inducible aldehyde dehydrogenase gene from Arabidopsis thaliana in transgenic plants improves stress tolerance. Plant J. **35:** 452-464.

Suzuki, N., Koussevitzky, S., MittlerR and Miller, G. (2012). ROS and redox signalling in the response of plants to abiotic stress. Plant Cell and Environment. **35**: 259-270.

Suzuki, Y., Ogiso, K. (1973). Development of ascorbate oxidase activity and its isozyme pattern in the roots of pea seedlings. Physiologia Plantarum. **29**: 169-172.

Swiezewski, S., Liu, F., Magusin, A., and Dean, C. (2009). Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature. **462**: 799-802.

Szechynska-Hebda, M., Kruk, J., Gorecka, M., Karpinska, B. and Karpinski, S. (2010). Evidence for light wavelength-specific systemic photoelectrophysiological signalling and cellular light memory of excess light episode in Arabidopsis. Plant Cell. **22:** 1-18.

Tagu, D., Sabater-Munoz, B., and Simon, J.C. (2005). Deciphering reproductive polyphenism in aphids. Invert Reprod Dev. **48**:71-80.

Taj, G., Agarwal, P., Grant, M. and Kumar, A. (2010). MAPK machinery in plants: recognition and response to different stresses through multiple signal transduction pathways. Plant Signal Behav. **5:** 1370-13788.

Takahama, U. (1993a) Redox state of ascorbic acid in the apoplast of stems of *Kalanchoe diagremontiana.* Physiol. Plant. **89**: 791-798.

Takahashi, F., Mizoguchi, T., Yoshida, R., Ichimura, K., and Shinozaki, K. (2011). Calmodulin-dependent activation of MAP kinase for ROS homeostasis in Arabidopsis. Mol. Cell. **41:** 649-660.

Tameling, W.I., Elzinga, S.D., Darmin, P.S., Vossen, J.H., Takken, F.L., Haring, M.A. and Cornelissen, B.J. (2002). The tomato R gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. Plant Cell. **14:** 2929-2939.

Tanaka, R., and Tanaka, A. (2005). Effects of chlorophyllide a oxygenase overexpression on light acclimation in *Arabidopsis thaliana*. Photosynth. Res. **85:** 327-340.

Tang, W., Yuan, M., Wang, R., Yang, Y., Wang, C., Oses-Prieto, J. A., Kim, T. W., Zhou, H. W., Deng, Z., and Gampala, S. S. (2011). PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nat. Cell Biol. **13**: 124-131.

Teige, M., Scheikl, E., Eulgem, T., Doczi, R., Ichimura, K., Shinozaki, K., Dang, J.L. and Hirt, H. (2004). The MKK2 pathway mediates cold and salt stress signalling in Arabidopsis. Mol. Cell. **15**: 141-152

Teramoto, H., Nakamori, A., Minagawa, J. and Ono, T. (2002). Light-intensity-dependent expression of Lhc gene family encoding light-harvesting chlorophyll-a/b proteins of Photosystem II in *Chlamydomonas reinhardtii*. Plant Physiol. **130:** 325-333.

Thangstad, O.P., Gilde, B., Chadchawan, S., Seem, M., Husebye, H., Bradley, D., and Bones, A.M. (2004). Cell specific cross-species expression of myrosinases in *Brassica napus, Arabidopsis thaliana* and *Nicotiana tabacum.* Plant Mol. Biol. **54:** 597-611.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A. and Browse, J. (2007). JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature. **448:** 661-665.

Thompson, G., and Goggin, F. (2006). Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. Exp. Bot. **57:** 755-766.

Tjallingii, W.F. (2006). Salivary secretion by aphids interacting with proteins of phloem wound responses. Exp. Bot. **57**: 739-754.

Tjallingii, W.F., and Hogen Esch, T. (1993). Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol. entomol. **18**: 317-328.

Ton, J., Flors, V., and Mauch-Mani, B. (2009). The multifaceted role of ABA in disease resistance. Trends Plant Sci. **14**: 310-317.

Torii, K.U. (2004). Leucine-rich repeat receptor kinases in plants: Structure, function, and signal transduction pathways. Int. Rev. Cytol. **234:** 1-46.

Torres, M.A., and Dangl, J.L. (2005). Functions of the respiratory burst oxidase inbiotic interactions, abiotic stress and development. Curr. Opin. Plant Biol. **8**: 397-403.

Torres, M.A., Dangl, J.L., and Jones, J.D. (2002). Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defence response. Proc. Natl. Acad. Sci. USA. **99**: 517-522.

Torres, M.A., Jones, J.D., and Dangl, J.L. (2006). Reactive oxygen species signalling in response to pathogens. Plant Physiol. **141**: 373-378.

Traber, M.G., and Stevens, J.F. (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radic. Biol. Med. **51**: 1000-1013

Trotta, A., Konert, G., Rahikainen, M., Aro, E., and Kangasjärvi, S. (2011b). Knock-down of protein phosphatase 2A subunit B'γ promotes phosphorylation of CALRETICULIN 1 in *Arabidopsis thaliana*. Plant Signaling and Behavior. **6(11)**: 1665-1668.

Trotta, A., Wrzaczek, M., Scharte, J., Tikkanen, M., Konert, G., Rahikainen, M., Holmstrom, M., Hiltunen, H. M., Rips, S. and Sipari, N. (2011a). Regulatory subunit B'γ gamma of protein phosphatase 2A prevents unnecessary defense reactions under low light in Arabidopsis. Plant Physiology. **156**: 1464-1480

Trotta, A., Wrzaczek, M., Scharte, J., Tikkanen, M., Konert, G., Rahikainen, M., Tsuji, H., Saika, H., Tsutsumi, N., Hirai, A., and Nakazono, M. (2006). Dynamic and reversible changes in histone H3-Lys4 methylation and H3 acetylation occurring at submergence-inducible genes in rice. Plant Cell Physiol. **47**: 995-1003.

Turnbull, J.J., Nakajima, J., Welford, R.W.D., Yamazaki, M., Saito, K., and Schofield, C.J. (2004). Mechanistic studies on three 2-oxoglutarate-dependent oxygenases of flavonoid biosynthesis: anthocyanidin synthase, flavonol synthase, and flavanone 3beta-hydroxylase. J. Biol. Chem. **279*:*** 1206-1216.

Urban, P., Mignotte, C., Kazmaier, M., Delorme, F., and Pompon, D. (1997). Cloning, yeast expression, and characterization of the coupling of two distantly related *Arabidopsis thaliana* NADPH cytochrome P450 reductases with P450 CYP73A5. Biol Chem. **272**: 19176-19186

Urbanczyk-Wochniak, E. and Fernie, A.R. (2005). Metabolic profiling reveals altered nitrogen nutrient regimes have diverse effects on the metabolism of hydroponically grown tomato (*Solanum lycopersicum*) plants. Experimental Botany. **56:** 309-321.

Urbanska, A., Tjallingii, W.F., Dixon, A., and Leszczynski, B. (1998). Phenol oxidizing enzymes in the grain aphid's saliva. Entomol. Exp. Appl. **86:** 197-203.

Van Camp, W., Capiau, K., Van Montagu, M., Inze, D., and Slooten, L. (1996). Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. Plant Physiol. **112**:1703-1714.

Van Emden, H.F., Eastop, V.F., Hughes, R.D., and Way, M.J. (1969). The ecology of *Myzus persicae*. Annu. Rev. Entomol. **14**: 197-270.

Vanacker, H., Harbinson, J., Ruisch, J., Carver, T., Foyer, C.H. (1998). Antioxidant defences of the apoplast. Protoplasma. **205**: 129-140.

Vancanneyt, G., Sanz, C., Farmaki, T., Paneque, M., Ortego, F., Castañera, P., and Sánchez-Serrano, J.J. (2001). Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. Proc. Natl. Acad. Sci. USA. **98:** 8139-8144.

Vandenabeele, S., S. Vanderauwera, M. Vuylsteke, S. Rombauts, C. Langebartels H.K. Seidlitz, M. Zabeau, M. Van Montagu, D. Inzé, and F. Van Breusegem. (2004). Catalase deficiency drastically affects gene expression induced by high light in *Arabidopsis thaliana.* Plant J. **39**: 45-58.

Vanderauwera, S., Zimmermann, P., Rombauts, S., Vandenabeele, S., Langebartels, C., Gruissem, W., Inzé, D. and Van Breusegem, F. (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol **139:** 806-821.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., Boerjan, W. (2010a). Lignin biosynthesis and structure. Plant Physiology. **153:** 895-905.

Velázquez, S. F., Guerra, R., and Calderón, L. (2011). Abiotic and Biotic StressResponse Crosstalk in Plants, Abiotic Stress Response in Plants - Physiological, Biochemical and Genetic Perspectives. ISBN: 978-953-307-672-0, InTech, Available from: http://www.intechopen.com/books/abiotic-stress-response-in-plants-physiological-biochemical-and-geneticperspectives abiotic-and-biotic-stress response -crosstalk-in-plants

Veljovic-Jovanovic, S.D., Pignocchi, C., Noctor, G., and Foyer, C.H. (2001). Low ascorbic acid in the *vtc-1* mutant of arabidopsis is associated with decreased growth and intracellular redistribution of the antioxidant system. Plant Physiol. **127(2)**: 426-435.

Venkatachalam, P., Thulaseedharan, A. and Raghothama, K. (2007). Identification of expression profiles of tapping panel dryness (TPD) associated genes from the latex of rubber tree (*Hevea brasiliensis* Muell. Arg.). Planta. **226:** 499-515.

Verhage, A., Wees, V.S.C.M., and Pieterse C.M.J. (2010). Plant immunity: It's the hormones talking, but what do they say? Plant Physiol. **154**: 536-540.

Villada, E., González, E., López-Sesé, A., Castiel, A., and Gómez-Guillamón, M. (2009). Hypersensitive response to *Aphis gossypii* Glover in melon genotypes carrying the Vat gene. J. Ex. Bot. **60:** 3269-3277.

Vlot, A.C., Dempsey, D.M.A., and Klessig, D.F. (2009). Salicylic acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. **47**: 177-206.

Vogel, M.O., Moore, M., König, K., Pecher, P., Alsharafa, K., Lee, J. and Dietz, K. J. (2014). Fast retrograde signaling in response to high light involves metabolite export, MITOGEN-ACTIVATED PROTEIN KINASE6, and AP2/ERF transcription factors in Arabidopsis. Plant Cell. **26:** 1151-1165.

Walters, R.G. (2005). Towards an understanding of photosynthetic acclimation. Exp. Bot. **56:** 435-447.

Wan, J., Zhang, X., Neece, D., Ramonell, K., Clough, S., Kim, S., Stacey, M., and Stacey, G. (2008). A LysM Receptor-Like Kinase Plays a Critical Role in Chitin Signaling and Fungal Resistance in Arabidopsis. Plant Cell. **20:** 471-481.

Wang, W., Zhao, X., Pan, Y., Zhu, L., Fu, B. and Li, Z. (2011). DNA methylation changes detected by methylation-sensitive amplified polymorphism in two contrasting rice genotypes under salt stress. Genet. Genomics. **38**: 419-424.

Wang, Y., Tao, X., Tang, X., Xiao, L. Sun, J., Yan, X., Li, D., Deng, H. and MA, X. (2013). Comparative transcriptome analysis of tomato (*Solanum lycopersicum*) in response to exogenous abscisic acid. BMC Genomics. **14:**841

Wasilewska, A., Vlad, F., Sirichandra, C., Redko, Y., Jammes, F., Valon, C., Frey, N., and Leung, J. (2008). An Update on Abscisic Acid Signalling in Plants and More. Molecular Plant. **1**: 198-217

Wassink, E. C., and Stolwijk, J. A. J. (1956). Effects of Light Quality on Plant Growth. Annu. Rev. of Plant Physiol. **7:** 373-400.

Wheeler, G.L., Jones, M.A., and Smirnoff, N. (1998). Thebiosyn-thetic pathway of vitamin C in higher plants. Nature. **393**: 365-369.

Widodo, Patterson, J.H., Newbigin, E., Tester, M., Bacic, A. and Roessner, U. (2009). Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. Experimental Botany. **60:** 4089-4103.

Wienkoop, S., Morgenthal, K., Wolschin, F., Scholz, M., Selbig, J. and Weckwerth, W. (2008). Integration of metabolomic and proteomic phenotypes. Molecular and Cellular Proteomics. **7:** 1725-1736.

Will, T., and Van Bel, A.J. (2006). Physical and chemical interactions between aphids and plants. Exp. Bot. **57**: 729-737.

Will, T., Kornemann, S.R., Furch, A.C.U., Tjallingii, W.F., and van Bel, A. J. E. (2009). Aphid watery saliva counteracts sieve-tube occlusion: a universal phenomenon? Exp. Biol. **212**: 3305-3312.

Wingler, A., Lea, P.J., Quick, W.P. and Leegood, R.C. (2000). Photorespiration: metabolic pathways and their role in stress protection. Philos Trans R Soc Lond B Biol. Sci. **355:** 1517-1529

Wirthmueller, L., Roth, C., Banfield, M.J. and Wiermer, M. (2013) Hop-on hop-off: importin-a-guided tours to the nucleus in innate immune signaling. Front. Plant Sci. **4:** 149.

Wu, G., Wang, X., Li, X., Kamiya, Y., Otegui, M.S., and Chory, J. (2011). Methylation of a phosphatase specifies dephosphorylation and degradation of activated brassinosteroid receptors. Sci.Signal. **4:** nal.2001258.

Wulff-Zottele, C., Gatzke, N., Kopka, J., Orellana, A., Hoefgen, R., Fisahn, J. and Hesse, H. (2010). Photosynthesis and metabolism interact during acclimation of *Arabidopsis thaliana* to high irradiance and sulphur depletion. Plant, Cell and Environment. **33:** 1974-1988.

Xia, X., Zhou, Y., Shi, K., Zhou, J., Foyer, C. H. and Yu, J. (2015). Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. Experimental Botany. **66(10):** 2839-2856.

Xing, D., Zhao, H., Xu, R., and Li, Q.Q. (2008). Arabidopsis PCFS4, a homologue of yeast polyadenylation factor Pcf11p, regulates FCA alternative processing and promotes flowering time. Plant J. **54**: 899-910.

Xiong, L., Schumaker, K.S. and Zhu, J-K. (2002). Cell signalling during cold, drought, and salt stress. Plant Cell. **14**: 165-183.

Xu, L., Zhu, L.F., Tu, L.L., Liu, L.L., Yuan, D.J. and Jin, L. (2011). Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahlia* as revealed by RNA-Seq-dependent transcriptiona lanalysis and histochemistry. Exp. Bot. **62:** 5607-5621.

Yamamoto, A., Nazmul, M. d., Bhuiyan, H., Waditee, R., Tanaka, Y., Esaka, M., Oba, K., Jagendorf, A.T., and Takabe, T. (2005). Suppressed expression of the apoplasticascorbateoxidase gene increases salt tolerance in tobacco and Arabidopsis plants. Exp. Bot. **56**: 1785-1796.

Yokota, E. and and Shimmen, T. (2011). Plant Myosins. The Plant Cytoskeleton, Advances in Plant Biology. 2 DOI 10.1007.

Young, S.A., Cuo, A., Cuikema, J.A., White, F.F. and Leach, J.E. (1995). Rice cationic peroxidase accumulates in xylem vessels during incompatible interactions with *Xanthomonas oryzae* pv *oryzae*. Plant Physiology. **107:** 1333-1341.

Yu, D., Liu, Y., Fan, B., Klessig, D.F., and Chen, Z. (1997). Is the high basal level of salicylic acid important for disease resistance in potato? Plant Physiol. **115**:343-349.

Zabala, D.T., Bennet, M.M.H., Truman, W.H., and Grant, M.R. (2009). Antagonism between salicylic acid and abscisic acid reflects early host-pathogen conflict and moulds plant defence responses. Plant J. **59**: 375-386.

Zamocky, M., Furtmuller, P.G. and Obinger, C. (2008). Evolution of catalases from bacteria to humans. Antioxidants and Redox Signaling. **10**: 1527-1547.

Zarate, S.I., Kempema, L.A., and Walling, L.L. (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defences. Plant Physiol. **143**: 866-875.

Zavala, J. A., Ravetta, D.A. (2001). Allocation of photoassimilates to biomass, resin and carbohydrates in *Grindelia chiloensis* as affected by light intensity. Field Crop Res. **69**: 143-149.

Zhao, L., Hu, Y., Chong, K. and Wang, T. (2010). ARAG1, an ABAresponsive DREB gene, plays a role in seed germination and drought tolerance of rice. Ann. Bot. **105:** 401-409.

Zhao, Y., Zhou, Z. and Xing, D. (2014). Phytochrome B-mediated activation of lipoxygenase modulates an excess red light-induced defence response in Arabidopsis. Experimental Botany. **65 (17):** 4907-4918.

Zheng, X., and Vanhuystee, R.B. (1992). Anionic peroxidase catalyzed ascorbic acid and IAA oxidation in the presence of hydrogen peroxide: a defense system against peroxidative stress in peanut plant. Phytochemistry. **31**: 1895-1898.

Zhou, H.W., Nussbaumer, C., Chao, Y., and DeLong, A. (2004). Disparate Roles for the Regulatory A Subunit Isoforms in Arabidopsis Protein Phosphatase 2A. Plant Cell. **16**: 709-722.

Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Miersch, O., Hamberg, M., Grimm, M., Ganal, M. and Wasternack, C. (2000) Molecular cloning of allene oxide cyclase: the enzyme establishing the stereochemistry of octadecanoids and jasmonates. Biol. Chem. **275:** 19132-19138.

Zimmermann, P., Heinlein, C., Orendi, G. and Zentgraf, U. (2006). Senescence-specific regulation of catalase in *Arabidopsis thaliana* (L.) Heynh. Plant Cell and Environment. **29**: 1049-1056.

Zipfel, C. (2014). Negative control of BAK1 by protein phosphatase 2A during plant innate immunity. EMBO J. **33**: 2069-2079.

Appendix

Appendix I List of differentially expressed transcripts under high light (HL) conditions relative to low light (LL) in the leaves of wild type (WT) tobacco plants and in transgenic lines in which AO was expressed in either the sense (PAO) or antisense (TAO) orientations.

Probe ID	Fold change (log2)
A_95_P002906	7.88
A_95_P003266	7.63
A_95_P176552	7.59
A_95_P105332	7.57
A_95_P107827	7.50
A_95_P003231	7.45
A_95_P078000	7.42
A_95_P125507	6.52
A_95_P110677	6.31
A_95_P105757	6.30
A_95_P006596	6.18
A_95_P297428	6.10
A_95_P003611	6.10
A_95_P181152	5.95
A_95_P026581	5.91
A_95_P112032	5.86
A_95_P260066	5.81
A_95_P109547	5.79
A_95_P006166	5.73
A_95_P154182	5.72
A_95_P003321	5.56
A_95_P005681	5.54
A_95_P179537	5.36
A_95_P106117	5.35
A_95_P112052	5.31
A_95_P129812	5.27
A_95_P092958	5.27
A_95_P106947	5.22
A_95_P179542	5.14
A_95_P108943	5.14
A_95_P002941	5.13
A_95_P258191	5.13
A_95_P025311	5.13
A_95_P002821	5.13
A_95_P105557	5.09
A_95_P136022	5.09
A_95_P008206	5.08
A_95_P102582	5.03

Probe ID	Fold change (log2)
A_95_P221247	4.95
A_95_P002696	4.91
A_95_P104072	4.88
A_95_P180197	4.86
A_95_P107847	4.85
A_95_P003156	4.84
A_95_P022336	4.82
A_95_P154192	4.82
A_95_P275243	4.80
A_95_P005261	4.77
A_95_P159232	4.77
A_95_P111232	4.72
A_95_P223057	4.68
A_95_P113437	4.63
A_95_P005072	4.58
A_95_P288413	4.56
A_95_P031196	4.55
A_95_P106022	4.53
A_95_P002901	4.50
A_95_P111667	4.44
A_95_P103857	4.44
A_95_P002466	4.43
A_95_P009366	4.40
A_95_P118182	4.39
A_95_P006996	4.31
A_95_P177727	4.29
A_95_P026346	4.29
A_95_P002581	4.28
A_95_P114722	4.27
A_95_P203912	4.26
A_95_P108792	4.22
A_95_P106552	4.19
A_95_P107182	4.18
A_95_P106952	4.18
A_95_P110782	4.09
A_95_P253064	4.08
A_95_P083910	4.07
A_95_P227104	4.06

Probe ID	Fold change (log2)
A_95_P118527	4.06
A_95_P213197	4.06
A_95_P161687	4.05
A_95_P218372	4.04
A_95_P226084	4.04
A_95_P012686	4.03
A_95_P130862	4.00
A_95_P188122	3.99
A_95_P285293	3.99
A_95_P145737	3.98
A_95_P183892	3.97
A_95_P283653	3.96
A_95_P044476	3.95
A_95_P112912	3.94
A_95_P232369	3.94
A_95_P267686	3.94
A_95_P103327	3.93
A_95_P109267	3.91
A_95_P226444	3.87
A_95_P273726	3.83
A_95_P181702	3.82
A_95_P009796	3.81
A_95_P284928	3.79
A_95_P095268	3.75
A_95_P108772	3.75
A_95_P006301	3.75
A_95_P247017	3.73
A_95_P153692	3.72
A_95_P106982	3.72
A_95_P230954	3.72
A_95_P000121	3.71
A_95_P049656	3.70
A_95_P144672	3.69
A_95_P022491	3.68
A_95_P117147	3.65
A_95_P159957	3.63
A_95_P082265	3.63
A_95_P001666	3.62

Probe ID	Fold change (log2)
A_95_P209492	3.62
A_95_P028491	3.60
A_95_P003166	3.58
A_95_P136437	3.58
A_95_P104037	3.58
A_95_P181697	3.57
A_95_P015601	3.56
A_95_P283558	3.56
A_95_P267766	3.56
A_95_P161117	3.55
A_95_P176527	3.55
A_95_P211952	3.55
A_95_P020531	3.53
A_95_P105167	3.53
A_95_P094278	3.53
A_95_P176522	3.53
A_95_P030291	3.52
A_95_P105297	3.51
A_95_P209247	3.51
A_95_P130412	3.50
A_95_P113187	3.50
A_95_P180202	3.50
A_95_P020166	3.48
A_95_P136712	3.47
A_95_P185737	3.46
A_95_P179347	3.46
A_95_P106872	3.45
A_95_P111727	3.45
A_95_P307228	3.44
A_95_P106837	3.43
A_95_P080475	3.43
A_95_P233959	3.41
A_95_P283403	3.41
A_95_P106502	3.40
A_95_P184047	3.39
A_95_P114107	3.39
A_95_P111737	3.38
A_95_P145557	3.38

Probe ID	Fold change (log2)
A_95_P182112	3.37
A_95_P192272	3.37
A_95_P009701	3.36
A_95_P187637	3.35
A_95_P231164	3.35
A_95_P020491	3.35
A_95_P014836	3.35
A_95_P247242	3.34
A_95_P159082	3.33
A_95_P027736	3.33
A_95_P111202	3.32
A_95_P129992	3.32
A_95_P140787	3.31
A_95_P009011	3.31
(+)eQC-40	3.30
A_95_P114342	3.30
A_95_P287563	3.29
A_95_P177572	3.27
A_95_P247927	3.27
A_95_P027786	3.26
A_95_P215257	3.25
A_95_P093798	3.24
A_95_P234064	3.24
A_95_P262226	3.23
A_95_P012501	3.22
A_95_P260071	3.22
A_95_P001231	3.21
A_95_P299143	3.20
A_95_P253029	3.19
A_95_P091768	3.18
A_95_P242612	3.17
A_95_P000971	3.17
A_95_P028291	3.16
A_95_P102902	3.15
A_95_P113277	3.14
A_95_P178767	3.14
A_95_P009221	3.14
A_95_P285748	3.13

Probe ID	Fold change (log2)
A_95_P003871	3.13
A_95_P004521	3.12
A_95_P019016	3.11
A_95_P305843	3.11
A_95_P196247	3.10
A_95_P060000	3.08
A_95_P138867	3.08
A_95_P199267	3.07
A_95_P240579	3.06
A_95_P106487	3.06
A_95_P000771	3.04
A_95_P002626	3.04
A_95_P303953	3.04
A_95_P115072	3.02
A_95_P101503	3.02
A_95_P182022	3.02
A_95_P109762	3.01
A_95_P220112	3.01
A_95_P203282	3.00
A_95_P016346	3.00
A_95_P210372	3.00
A_95_P263376	2.99
A_95_P103082	2.99
A_95_P159547	2.99
A_95_P273666	2.97
A_95_P176997	2.97
A_95_P157492	2.96
A_95_P206577	2.96
A_95_P194542	2.95
A_95_P222822	2.94
A_95_P048241	2.94
A_95_P288388	2.94
A_95_P273201	2.93
A_95_P254204	2.93
A_95_P005136	2.92
A_95_P012641	2.91
A_95_P028616	2.91
A_95_P256184	2.91

Probe ID	Fold change (log2)
A_95_P039471	2.91
A_95_P114717	2.90
A_95_P150732	2.88
A_95_P105552	2.88
A_95_P233239	2.87
A_95_P025081	2.87
A_95_P177002	2.87
A_95_P029586	2.86
A_95_P106637	2.85
A_95_P185067	2.84
A_95_P182117	2.84
A_95_P023776	2.83
A_95_P017046	2.83
A_95_P107032	2.82
A_95_P105232	2.82
A_95_P212662	2.82
A_95_P002546	2.81
A_95_P250462	2.81
A_95_P147452	2.80
A_95_P249137	2.80
A_95_P003656	2.80
A_95_P265181	2.79
A_95_P062910	2.79
A_95_P108877	2.79
A_95_P106782	2.79
A_95_P248862	2.79
A_95_P129172	2.78
A_95_P255914	2.78
A_95_P032931	2.77
A_95_P114372	2.77
A_95_P106807	2.76
A_95_P186307	2.76
A_95_P041566	2.76
A_95_P136317	2.76
A_95_P113967	2.75
A_95_P026596	2.74
A_95_P193792	2.73
A_95_P127477	2.73

Probe ID	Fold change (log2)
A_95_P045706	2.73
A_95_P254529	2.73
A_95_P004526	2.72
A_95_P161912	2.72
A_95_P116307	2.71
A_95_P010131	2.71
A_95_P021856	2.71
A_95_P019626	2.71
A_95_P006776	2.70
A_95_P103362	2.70
A_95_P015286	2.69
A_95_P110457	2.69
A_95_P129157	2.69
A_95_P105487	2.69
A_95_P278458	2.68
A_95_P177912	2.68
A_95_P163312	2.68
A_95_P113852	2.68
A_95_P104477	2.67
A_95_P204557	2.67
A_95_P003171	2.67
A_95_P114147	2.67
A_95_P034039	2.65
A_95_P154087	2.65
A_95_P183832	2.65
A_95_P089588	2.64
A_95_P000331	2.64
A_95_P198372	2.63
A_95_P163817	2.63
A_95_P152072	2.63
A_95_P097983	2.62
A_95_P107677	2.62
A_95_P058611	2.62
A_95_P113337	2.62
A_95_P196332	2.61
A_95_P216597	2.61
A_95_P242412	2.60
A_95_P221527	2.60

Probe ID	Fold change (log2)
A_95_P293953	2.60
A_95_P155642	2.59
A_95_P107994	2.59
A_95_P115787	2.59
A_95_P036603	2.58
A_95_P206357	2.58
A_95_P114402	2.56
A_95_P211842	2.55
A_95_P106322	2.55
A_95_P268586	2.55
A_95_P112707	2.54
A_95_P136387	2.54
A_95_P289628	2.54
A_95_P162502	2.53
A_95_P019576	2.53
A_95_P152692	2.52
A_95_P160622	2.52
A_95_P132842	2.52
A_95_P122747	2.51
A_95_P258416	2.51
A_95_P010676	2.51
A_95_P177822	2.50
A_95_P001566	2.48
A_95_P107272	2.48
A_95_P101928	2.47
A_95_P106512	2.47
A_95_P016511	2.47
A_95_P151517	2.46
A_95_P025876	2.46
A_95_P034034	2.46
A_95_P099723	2.46
A_95_P007511	2.46
A_95_P102202	2.45
A_95_P015876	2.45
A_95_P118888	2.44
A_95_P031006	2.44
A_95_P176202	2.44
A_95_P286013	2.44

Probe ID	Fold change (log2)
A_95_P177857	2.43
A_95_P031591	2.43
A_95_P164432	2.43
A_95_P217182	2.42
A_95_P020886	2.42
A_95_P096208	2.41
A_95_P002561	2.41
A_95_P060205	2.41
A_95_P072990	2.41
A_95_P259376	2.41
A_95_P285443	2.41
A_95_P311148	2.41
A_95_P052066	2.40
A_95_P211852	2.40
A_95_P099203	2.40
A_95_P257114	2.40
A_95_P178782	2.40
A_95_P254344	2.39
A_95_P210862	2.39
A_95_P194092	2.39
A_95_P030286	2.38
A_95_P186802	2.38
A_95_P211187	2.38
A_95_P021006	2.38
A_95_P079150	2.37
A_95_P108332	2.37
A_95_P305928	2.37
A_95_P095513	2.37
A_95_P190912	2.37
A_95_P008301	2.37
A_95_P113582	2.36
A_95_P094703	2.36
A_95_P152722	2.36
A_95_P276723	2.36
A_95_P017301	2.35
A_95_P302208	2.35
A_95_P164302	2.34
A_95_P003426	2.34

Probe ID	Fold change (log2)
A_95_P003006	2.34
A_95_P063510	2.34
A_95_P094163	2.33
A_95_P290233	2.33
A_95_P112757	2.33
A_95_P010261	2.32
A_95_P005701	2.32
A_95_P181452	2.32
A_95_P141837	2.31
A_95_P000146	2.31
A_95_P106042	2.30
A_95_P181457	2.30
A_95_P191097	2.30
A_95_P015871	2.30
A_95_P018951	2.29
A_95_P005626	2.29
A_95_P006901	2.29
A_95_P089883	2.29
A_95_P021131	2.29
A_95_P000166	2.29
A_95_P077395	2.29
A_95_P112417	2.29
A_95_P178317	2.29
A_95_P112802	2.29
A_95_P283728	2.29
A_95_P123017	2.28
A_95_P190817	2.28
A_95_P112677	2.28
A_95_P109597	2.28
A_95_P034698	2.28
A_95_P147862	2.27
A_95_P022206	2.27
A_95_P105132	2.27
A_95_P292563	2.26
A_95_P270636	2.26
A_95_P096748	2.26
A_95_P286453	2.26
A_95_P191792	2.26

Probe ID	Fold change (log2)
A_95_P149487	2.26
A_95_P047006	2.25
A_95_P283868	2.25
A_95_P209127	2.24
A_95_P122217	2.24
A_95_P010626	2.24
A_95_P003206	2.24
A_95_P001236	2.23
A_95_P030181	2.23
A_95_P115702	2.23
A_95_P155837	2.23
A_95_P114452	2.23
A_95_P298518	2.22
A_95_P051391	2.22
A_95_P077205	2.22
A_95_P114797	2.21
A_95_P180582	2.21
A_95_P190152	2.21
A_95_P043466	2.21
A_95_P285958	2.21
A_95_P002381	2.21
A_95_P028996	2.20
A_95_P004201	2.20
A_95_P214407	2.20
A_95_P212457	2.20
A_95_P006506	2.20
A_95_P246952	2.19
A_95_P050261	2.19
A_95_P070265	2.19
A_95_P113877	2.18
A_95_P303808	2.17
A_95_P139062	2.17
A_95_P106537	2.17
A_95_P051041	2.17
A_95_P101338	2.17
A_95_P306578	2.16
A_95_P263506	2.16
A_95_P089078	2.15

Probe ID	Fold change (log2)
A_95_P016341	2.15
A_95_P193912	2.15
A_95_P148932	2.15
A_95_P110492	2.15
A_95_P014526	2.15
A_95_P031801	2.15
A_95_P107422	2.15
A_95_P121147	2.15
A_95_P160707	2.14
A_95_P240793	2.14
A_95_P196392	2.14
A_95_P207287	2.14
A_95_P215592	2.14
A_95_P239709	2.14
A_95_P103057	2.13
A_95_P291733	2.13
A_95_P132717	2.13
A_95_P112772	2.13
A_95_P259746	2.12
A_95_P102367	2.12
A_95_P007411	2.12
A_95_P151682	2.12
A_95_P004706	2.12
A_95_P006051	2.12
A_95_P302173	2.12
A_95_P199552	2.12
A_95_P110747	2.12
A_95_P108662	2.12
A_95_P023016	2.11
A_95_P076350	2.11
A_95_P113432	2.11
A_95_P229449	2.11
A_95_P160962	2.11
A_95_P117837	2.10
A_95_P221762	2.10
A_95_P091238	2.10
A_95_P144232	2.10
A_95_P158767	2.10

Probe ID	Fold change (log2)
A_95_P159202	2.09
A_95_P239369	2.09
A_95_P016146	2.09
A_95_P154807	2.09
A_95_P159802	2.09
A_95_P112227	2.09
A_95_P207657	2.09
A_95_P161637	2.08
A_95_P026636	2.08
A_95_P003626	2.07
A_95_P200827	2.07
A_95_P061270	2.07
A_95_P112022	2.07
A_95_P189682	2.07
A_95_P159767	2.07
A_95_P013771	2.06
A_95_P096038	2.06
A_95_P160477	2.06
A_95_P254654	2.06
A_95_P157967	2.05
A_95_P267666	2.05
A_95_P273761	2.05
A_95_P034953	2.05
A_95_P103392	2.05
A_95_P236709	2.04
A_95_P164287	2.04
A_95_P116527	2.04
A_95_P207067	2.04
A_95_P092983	2.04
A_95_P298383	2.03
A_95_P130602	2.03
A_95_P200822	2.03
A_95_P201617	2.03
A_95_P199562	2.02
A_95_P100318	2.02
A_95_P087258	2.02
A_95_P103172	2.02
A_95_P269761	2.02

Probe ID	Fold change (log2)
A_95_P058771	2.02
A_95_P131712	2.02
A_95_P215977	2.01
A_95_P027721	2.01
A_95_P300603	2.01
A_95_P103872	2.01
A_95_P223672	2.00
A_95_P164862	2.00
A_95_P005981	2.00
A_95_P004391	2.00
A_95_P110142	1.99
A_95_P034903	1.99
A_95_P260116	1.99
A_95_P117092	1.98
A_95_P223362	1.98
A_95_P196907	1.98
A_95_P227339	1.98
A_95_P268421	1.98
A_95_P072100	1.98
A_95_P000536	1.98
A_95_P242427	1.97
A_95_P097063	1.97
A_95_P164182	1.97
A_95_P275618	1.96
A_95_P161342	1.96
A_95_P164582	1.96
A_95_P305663	1.95
A_95_P106417	1.95
A_95_P112937	1.95
A_95_P290014	1.95
A_95_P295023	1.95
A_95_P247207	1.94
A_95_P258851	1.94
A_95_P298073	1.94
A_95_P164222	1.94
A_95_P076905	1.94
A_95_P066990	1.94
A_95_P016106	1.94

Probe ID	Fold change (log2)	Probe ID	Fold change (log2)	Probe ID	Fold change (log2)
A_95_P028416	1.94	A_95_P199832	1.88	A_95_P112777	1.82
A_95_P254914	1.94	A_95_P199832	1.88	A_95_P294153	1.82
A_95_P187112	1.93	A_95_P199832	1.88	A_95_P016531	1.82
A_95_P012936	1.93	A_95_P199832	1.88	A_95_P125357	1.82
A_95_P146707	1.93	A_95_P199832	1.88	A_95_P078055	1.82
A_95_P188127	1.93	A_95_P199832	1.88	A_95_P252934	1.82
A_95_P224277	1.93	A_95_P199832	1.88	A_95_P143732	1.81
A_95_P002476	1.93	A_95_P199832	1.88	A_95_P148192	1.81
A_95_P198552	1.92	A_95_P199832	1.88	A_95_P174682	1.81
A_95_P026201	1.92	A_95_P199832	1.88	A_95_P159237	1.81
A_95_P000541	1.92	A_95_P199832	1.88	A_95_P017966	1.81
A_95_P148122	1.92	A_95_P199832	1.88	A_95_P012296	1.81
A_95_P010506	1.92	A_95_P199832	1.88	A_95_P001981	1.81
A_95_P255714	1.92	A_95_P199832	1.88	A_95_P118653	1.81
A_95_P007276	1.92	A_95_P199832	1.88	A_95_P162822	1.81
A_95_P287318	1.91	A_95_P199832	1.88	A_95_P097993	1.81
A_95_P298028	1.91	A_95_P199832	1.88	A_95_P129032	1.80
A_95_P091298	1.91	A_95_P199832	1.88	A_95_P130677	1.80
A_95_P055446	1.91	A_95_P199832	1.88	A_95_P191617	1.80
A_95_P274858	1.91	A_95_P199832	1.88	A_95_P210307	1.80
A_95_P008441	1.91	A_95_P199832	1.88	A_95_P185012	1.80
A_95_P162187	1.90	A_95_P199832	1.88	A_95_P264196	1.80
A_95_P190962	1.90	A_95_P199832	1.88	A_95_P219462	1.79
A_95_P121357	1.90	A_95_P199832	1.88	A_95_P197557	1.79
A_95_P091943	1.90	A_95_P199832	1.88	A_95_P204127	1.79
A_95_P161812	1.90	A_95_P199832	1.88	A_95_P132942	1.79
A_95_P107769	1.90	A_95_P199832	1.88	A_95_P115897	1.79
A_95_P088268	1.90	A_95_P199832	1.88	A_95_P283429	1.79
A_95_P113417	1.90	A_95_P199832	1.88	A_95_P140897	1.79
A_95_P209087	1.90	A_95_P199832	1.88	A_95_P141517	1.79
A_95_P163447	1.90	A_95_P199832	1.88	A_95_P114967	1.79
A_95_P002746	1.89	A_95_P199832	1.88	A_95_P282428	1.78
A_95_P111097	1.89	A_95_P199832	1.88	A_95_P107957	1.78
A_95_P105692	1.89	A_95_P199832	1.88	A_95_P193932	1.78
A_95_P102287	1.89	A_95_P199832	1.88	A_95_P205602	1.78
A_95_P212232	1.88	A_95_P199832	1.88	A_95_P000381	1.78
A_95_P259126	1.88	A_95_P199832	1.88	A_95_P216762	1.78
A_95_P263316	1.88	A_95_P199832	1.88	A_95_P181542	1.77

Probe ID	Fold change (log2)
A_95_P102712	1.77
A_95_P015281	1.77
A_95_P114657	1.77
A_95_P110137	1.77
A_95_P113207	1.76
A_95_P152152	1.76
A_95_P154742	1.76
A_95_P105282	1.76
A_95_P199442	1.76
A_95_P030586	1.76
A_95_P023116	1.76
A_95_P291608	1.76
A_95_P091883	1.76
A_95_P100008	1.75
A_95_P023071	1.75
A_95_P130292	1.75
A_95_P264501	1.75
A_95_P209592	1.75
A_95_P041996	1.75
A_95_P246497	1.74
A_95_P009051	1.74
A_95_P275823	1.74
A_95_P142352	1.74
A_95_P124057	1.74
A_95_P270446	1.74
A_95_P101443	1.74
A_95_P140917	1.74
A_95_P030011	1.74
A_95_P074255	1.74
A_95_P004286	1.74
A_95_P120937	1.73
A_95_P113072	1.73
A_95_P260206	1.73
A_95_P283473	1.73
A_95_P185872	1.73
A_95_P248162	1.73
A_95_P091818	1.73
A_95_P003031	1.73

Probe ID	Fold change (log2)
A_95_P004671	1.73
A_95_P216457	1.73
A_95_P218462	1.73
A_95_P000436	1.72
A_95_P110517	1.72
A_95_P155197	1.72
A_95_P284723	1.72
A_95_P041456	1.72
A_95_P022806	1.72
A_95_P153227	1.72
A_95_P011261	1.72
A_95_P111567	1.72
A_95_P004106	1.72
A_95_P235069	1.71
A_95_P104382	1.71
A_95_P153457	1.71
A_95_P164272	1.71
A_95_P131372	1.71
A_95_P109322	1.71
A_95_P059625	1.71
A_95_P006791	1.71
A_95_P261691	1.71
A_95_P018816	1.71
A_95_P286468	1.70
A_95_P205727	1.70
A_95_P006266	1.70
A_95_P127342	1.70
A_95_P156942	1.70
A_95_P260091	1.70
A_95_P028356	1.70
A_95_P000196	1.69
A_95_P154882	1.69
A_95_P164947	1.69
A_95_P004336	1.69
A_95_P249267	1.69
A_95_P161182	1.69
A_95_P110932	1.69
A_95_P304493	1.69

Probe ID	Fold change (log2)
A_95_P275453	1.69
A_95_P112217	1.68
A_95_P246232	1.68
A_95_P009996	1.68
A_95_P109002	1.68
A_95_P162182	1.68
A_95_P008576	1.68
A_95_P080920	1.68
A_95_P301143	1.68
A_95_P013206	1.68
A_95_P155622	1.68
A_95_P153937	1.67
A_95_P205807	1.67
A_95_P070685	1.67
A_95_P019621	1.67
A_95_P254164	1.67
A_95_P286778	1.67
A_95_P209942	1.67
A_95_P093233	1.66
A_95_P158072	1.66
A_95_P105667	1.66
A_95_P117437	1.65
A_95_P108597	1.65
A_95_P032421	1.65
A_95_P101308	1.65
A_95_P193607	1.65
A_95_P018721	1.65
A_95_P213182	1.65
A_95_P108017	1.65
A_95_P205707	1.65
A_95_P198877	1.65
A_95_P190187	1.65
A_95_P164387	1.65
A_95_P108637	1.64
A_95_P109532	1.64
A_95_P145632	1.64
A_95_P011926	1.64
A_95_P273681	1.64

Probe ID	Fold change (log2)
A_95_P030766	1.63
A_95_P002756	1.63
A_95_P132822	1.63
A_95_P082520	1.63
A_95_P003371	1.63
A_95_P205577	1.63
A_95_P161427	1.63
A_95_P008951	1.63
A_95_P019771	1.63
A_95_P024551	1.62
A_95_P260141	1.62
A_95_P146877	1.62
A_95_P147837	1.62
A_95_P142222	1.62
A_95_P316163	1.62
A_95_P271791	1.62
A_95_P090158	1.62
A_95_P219027	1.61
A_95_P018351	1.61
A_95_P182162	1.61
A_95_P077825	1.61
A_95_P141577	1.61
A_95_P130977	1.61
A_95_P078710	1.61
A_95_P104482	1.61
A_95_P071855	1.61
A_95_P135667	1.61
A_95_P219012	1.61
A_95_P110717	1.61
A_95_P164152	1.61
A_95_P109852	1.60
A_95_P215057	1.60
A_95_P201622	1.60
A_95_P228729	1.60
A_95_P005201	1.60
A_95_P162752	1.60
A_95_P206483	1.60
A_95_P000716	1.60

Probe ID	Fold change (log2)
A_95_P117547	1.60
A_95_P032446	1.59
A_95_P146492	1.59
A_95_P253514	1.59
A_95_P025791	1.59
A_95_P216582	1.59
A_95_P011252	1.59
A_95_P224867	1.59
A_95_P198587	1.59
A_95_P195077	1.59
A_95_P184552	1.59
A_95_P106712	1.59
A_95_P203942	1.58
A_95_P111617	1.58
A_95_P309068	1.58
A_95_P145282	1.58
A_95_P067955	1.58
A_95_P105497	1.58
A_95_P160647	1.58
A_95_P107922	1.58
A_95_P158497	1.58
A_95_P146867	1.58
A_95_P140707	1.58
A_95_P161932	1.58
A_95_P102027	1.58
A_95_P062020	1.58
A_95_P024461	1.58
A_95_P001376	1.57
A_95_P121297	1.57
A_95_P102522	1.57
A_95_P221617	1.57
A_95_P253074	1.57
A_95_P217322	1.57
A_95_P233904	1.56
A_95_P095688	1.56
A_95_P000776	1.56
A_95_P155492	1.56
A_95_P227134	1.56

Probe ID	Fold change (log2)
A_95_P298453	1.56
A_95_P162442	1.56
A_95_P110772	1.56
A_95_P303668	1.56
A_95_P091873	1.56
A_95_P307993	1.56
A_95_P308598	1.55
A_95_P114747	1.55
A_95_P282258	1.55
A_95_P089373	1.55
A_95_P079520	1.55
A_95_P302468	1.55
A_95_P109697	1.55
A_95_P282568	1.55
A_95_P111127	1.55
A_95_P154752	1.55
A_95_P113802	1.55
A_95_P085840	1.54
A_95_P293388	1.54
A_95_P157097	1.54
A_95_P204707	1.54
A_95_P199202	1.54
A_95_P113692	1.54
A_95_P183757	1.54
A_95_P252789	1.53
A_95_P102177	1.53
A_95_P262981	1.53
A_95_P240174	1.53
A_95_P252549	1.53
A_95_P223157	1.53
A_95_P211512	1.53
A_95_P007061	1.53
A_95_P180212	1.53
A_95_P109247	1.53
A_95_P049626	1.52
A_95_P146182	1.52
A_95_P041091	1.52
A_95_P079720	1.52

Probe ID	Fold change (log2)
A_95_P225437	1.52
A_95_P279538	1.52
A_95_P061700	1.52
A_95_P255834	1.52
A_95_P221952	1.52
A_95_P054701	1.52
A_95_P105832	1.52
A_95_P131762	1.52
A_95_P123167	1.51
A_95_P112157	1.51
A_95_P103617	1.51
A_95_P160122	1.51
A_95_P108272	1.51
A_95_P300498	1.51
A_95_P010526	1.51
A_95_P269776	1.51
A_95_P113327	1.51
A_95_P155697	1.51
A_95_P138087	1.51
A_95_P019531	1.51
A_95_P197167	1.50
A_95_P160902	1.50
A_95_P092793	1.50
A_95_P028226	1.50
A_95_P139542	1.50
A_95_P186242	1.50
A_95_P180577	1.50
A_95_P111707	1.50
A_95_P022971	1.50
A_95_P016471	1.50
A_95_P135117	1.50
A_95_P003241	1.50
A_95_P261911	1.49
A_95_P016221	1.49
A_95_P266326	1.49
A_95_P210277	1.49
A_95_P197867	1.49
A_95_P291358	1.49

Probe ID	Fold change (log2)
A_95_P162452	1.49
A_95_P291748	1.49
A_95_P022401	1.49
A_95_P160947	1.49
A_95_P193602	1.49
A_95_P141192	1.49
A_95_P002681	1.49
A_95_P185877	1.48
A_95_P003621	1.48
A_95_P114762	1.48
A_95_P136282	1.48
A_95_P101973	1.48
A_95_P188972	1.48
A_95_P052471	1.48
A_95_P164602	1.48
A_95_P109997	1.48
A_95_P146232	1.48
A_95_P000371	1.48
A_95_P002096	1.48
A_95_P299113	1.48
A_95_P070620	1.48
A_95_P215217	1.48
A_95_P147082	1.47
A_95_P136827	1.47
A_95_P035638	1.47
A_95_P164732	1.47
A_95_P303273	1.47
A_95_P297453	1.47
A_95_P262326	1.47
A_95_P177967	1.47
A_95_P289503	1.47
A_95_P105197	1.47
A_95_P161892	1.47
A_95_P007286	1.46
A_95_P218212	1.46
A_95_P019601	1.46
A_95_P004431	1.46
A_95_P180217	1.46

Probe ID	Fold change (log2)
A_95_P213302	1.46
A_95_P105032	1.46
A_95_P142762	1.46
A_95_P076850	1.46
A_95_P234189	1.46
A_95_P022991	1.46
A_95_P104702	1.45
A_95_P261496	1.45
A_95_P002806	1.45
A_95_P111402	1.45
A_95_P006056	1.45
A_95_P144402	1.45
A_95_P227699	1.45
A_95_P006676	1.45
A_95_P062715	1.45
A_95_P279848	1.44
A_95_P114537	1.44
A_95_P305273	1.44
A_95_P265266	1.44
A_95_P077755	1.44
A_95_P153197	1.44
A_95_P206922	1.44
A_95_P178707	1.44
A_95_P125897	1.44
A_95_P014536	1.44
A_95_P029936	1.44
A_95_P149772	1.44
A_95_P239514	1.44
A_95_P241400	1.44
A_95_P047746	1.44
A_95_P001716	1.44
A_95_P183897	1.43
A_95_P026911	1.43
A_95_P106157	1.43
A_95_P219817	1.43
A_95_P204522	1.43
A_95_P067045	1.43
A_95_P146782	1.43

Probe ID	Fold change (log2)
A_95_P268326	1.43
A_95_P027091	1.43
A_95_P189627	1.43
A_95_P215122	1.43
A_95_P055536	1.43
A_95_P113972	1.43
A_95_P233549	1.42
A_95_P163192	1.42
A_95_P235119	1.42
A_95_P147257	1.42
A_95_P041361	1.42
A_95_P191497	1.42
A_95_P221732	1.42
A_95_P090143	1.42
A_95_P208892	1.42
A_95_P083170	1.42
A_95_P029706	1.42
A_95_P070455	1.42
A_95_P295838	1.42
A_95_P218947	1.42
A_95_P043331	1.41
A_95_P002871	1.41
A_95_P177207	1.41
A_95_P034164	1.41
A_95_P034269	1.41
A_95_P002176	1.41
A_95_P077380	1.41
A_95_P144932	1.41
A_95_P070900	1.41
A_95_P248197	1.41
A_95_P306083	1.41
A_95_P028906	1.40
A_95_P194127	1.40
A_95_P102182	1.40
A_95_P162937	1.40
A_95_P100928	1.40
A_95_P001726	1.40
A_95_P309158	1.40

Probe ID	Fold change (log2)
A_95_P276453	1.40
A_95_P305818	1.40
A_95_P224377	1.40
A_95_P023501	1.40
A_95_P289963	1.40
A_95_P106532	1.40
A_95_P106277	1.39
A_95_P311593	1.39
A_95_P016691	1.39
A_95_P180587	1.39
A_95_P114562	1.39
A_95_P027256	1.39
A_95_P249767	1.39
A_95_P099953	1.39
A_95_P235114	1.39
A_95_P186297	1.39
A_95_P026391	1.39
A_95_P255849	1.39
A_95_P271051	1.39
A_95_P001156	1.39
A_95_P214602	1.38
A_95_P157842	1.38
A_95_P270831	1.38
A_95_P134002	1.38
A_95_P115112	1.38
A_95_P229549	1.38
A_95_P220092	1.38
A_95_P086813	1.38
A_95_P266311	1.38
A_95_P004746	1.38
A_95_P005326	1.38
A_95_P051491	1.38
A_95_P231759	1.37
A_95_P027336	1.37
A_95_P025031	1.37
A_95_P303148	1.37
A_95_P008676	1.37
A_95_P031441	1.37

Probe ID	Fold change (log2)
A_95_P229579	1.37
A_95_P019606	1.37
A_95_P272466	1.37
A_95_P112337	1.37
A_95_P201137	1.37
A_95_P264406	1.36
A_95_P005611	1.36
A_95_P164282	1.36
A_95_P103502	1.36
A_95_P141032	1.36
A_95_P203457	1.36
A_95_P124862	1.36
A_95_P032891	1.36
A_95_P210297	1.36
A_95_P119697	1.36
A_95_P197592	1.36
A_95_P235964	1.36
A_95_P255189	1.35
A_95_P206812	1.35
A_95_P079400	1.35
A_95_P192207	1.35
A_95_P135532	1.35
A_95_P268451	1.35
A_95_P030056	1.35
A_95_P023101	1.35
A_95_P141167	1.35
A_95_P012171	1.35
A_95_P247422	1.35
A_95_P007796	1.35
A_95_P290523	1.34
A_95_P013361	1.34
A_95_P130872	1.34
A_95_P113847	1.34
A_95_P195572	1.34
A_95_P023846	1.34
A_95_P185327	1.34
A_95_P156677	1.34
A_95_P233114	1.34

Probe ID	Fold change (log2)
A_95_P308223	1.34
A_95_P154977	1.33
A_95_P226449	1.33
A_95_P163167	1.33
A_95_P213252	1.33
A_95_P103637	1.33
A_95_P101909	1.33
A_95_P103902	1.33
A_95_P300443	1.33
A_95_P132132	1.33
A_95_P237784	1.33
A_95_P252864	1.33
A_95_P121982	1.33
A_95_P189262	1.33
A_95_P107802	1.33
A_95_P031091	1.33
A_95_P028286	1.32
A_95_P221567	1.32
A_95_P006336	1.32
A_95_P004401	1.32
A_95_P006851	1.32
A_95_P004976	1.32
A_95_P289478	1.32
A_95_P015526	1.32
A_95_P017511	1.32
A_95_P263836	1.32
A_95_P033664	1.32
A_95_P207722	1.31
A_95_P163622	1.31
A_95_P001326	1.31
A_95_P120347	1.31
A_95_P123037	1.31
A_95_P042386	1.31
A_95_P126942	1.31
A_95_P001741	1.31
A_95_P054941	1.31
A_95_P006966	1.31
A_95_P307073	1.31

Probe ID	Fold change (log2)
A_95_P213362	1.31
A_95_P106082	1.31
A_95_P134497	1.31
A_95_P068095	1.31
A_95_P191597	1.30
A_95_P109367	1.30
A_95_P194347	1.30
A_95_P162022	1.30
A_95_P110007	1.30
A_95_P143787	1.30
A_95_P163532	1.30
A_95_P156937	1.30
A_95_P287903	1.30
A_95_P053281	1.30
A_95_P147347	1.30
A_95_P000191	1.30
A_95_P034748	1.29
A_95_P092438	1.29
A_95_P094643	1.29
A_95_P203392	1.29
A_95_P161077	1.29
A_95_P007331	1.29
A_95_P032141	1.29
A_95_P026091	1.29
A_95_P113567	1.29
A_95_P100938	1.29
A_95_P070990	1.29
A_95_P267781	1.29
A_95_P013231	1.29
A_95_P008836	1.29
A_95_P048216	1.29
A_95_P235214	1.28
A_95_P271376	1.28
A_95_P177442	1.28
A_95_P033979	1.28
A_95_P103367	1.28
A_95_P034314	1.28
A_95_P195147	1.28

Probe ID	Fold change (log2)
A_95_P010971	1.28
A_95_P313023	1.28
A_95_P023936	1.28
A_95_P125087	1.28
A_95_P183022	1.28
A_95_P054436	1.28
A_95_P269481	1.28
A_95_P178972	1.28
A_95_P249937	1.27
A_95_P200702	1.27
A_95_P011681	1.27
A_95_P089918	1.27
A_95_P261561	1.27
A_95_P012341	1.27
A_95_P080310	1.27
A_95_P144122	1.27
A_95_P254994	1.27
A_95_P268381	1.27
A_95_P055106	1.27
A_95_P136927	1.27
A_95_P012116	1.27
A_95_P177837	1.27
A_95_P102827	1.27
A_95_P103412	1.27
A_95_P311288	1.27
A_95_P154982	1.27
A_95_P283313	1.27
A_95_P277818	1.26
A_95_P102312	1.26
A_95_P164262	1.26
A_95_P105127	1.26
A_95_P214937	1.26
A_95_P115107	1.26
A_95_P241500	1.26
A_95_P206517	1.26
A_95_P005863	1.26
A_95_P032271	1.26
A_95_P157387	1.26

Probe ID	Fold change (log2)
A_95_P033319	1.26
A_95_P220317	1.26
A_95_P214867	1.26
A_95_P155792	1.26
A_95_P146752	1.26
A_95_P149157	1.25
A_95_P157402	1.25
A_95_P114727	1.25
A_95_P019776	1.25
A_95_P176577	1.25
A_95_P004596	1.25
A_95_P118767	1.25
A_95_P110842	1.25
A_95_P201977	1.25
A_95_P243307	1.25
A_95_P276353	1.25
A_95_P102677	1.25
A_95_P001321	1.24
A_95_P214027	1.24
A_95_P136152	1.24
A_95_P012221	1.24
A_95_P152637	1.24
A_95_P103297	1.24
A_95_P086250	1.24
A_95_P145897	1.24
A_95_P153502	1.24
A_95_P238859	1.24
A_95_P096373	1.24
A_95_P272936	1.24
A_95_P179847	1.24
A_95_P308923	1.24
A_95_P136212	1.24
A_95_P231029	1.24
A_95_P292508	1.24
A_95_P027231	1.24
A_95_P018301	1.24
A_95_P064645	1.24
A_95_P159652	1.24

Probe ID	Fold change (log2)
A_95_P253589	1.24
A_95_P006116	1.24
A_95_P209562	1.24
A_95_P024386	1.24
A_95_P268441	1.23
A_95_P093178	1.23
A_95_P000851	1.23
A_95_P105342	1.23
A_95_P004381	1.23
A_95_P271701	1.23
A_95_P017701	1.23
A_95_P130542	1.23
A_95_P137227	1.23
A_95_P108767	1.23
A_95_P140992	1.23
A_95_P112582	1.22
A_95_P164107	1.22
A_95_P196387	1.22
A_95_P009846	1.22
A_95_P164177	1.22
A_95_P305203	1.22
A_95_P147872	1.22
A_95_P156577	1.22
A_95_P093883	1.22
A_95_P156082	1.22
A_95_P188977	1.22
A_95_P191992	1.22
A_95_P228109	1.22
A_95_P188242	1.22
A_95_P009401	1.22
A_95_P010671	1.21
A_95_P004456	1.21
A_95_P105517	1.21
A_95_P005766	1.21
A_95_P001706	1.21
A_95_P125282	1.21
A_95_P191682	1.21
A_95_P034843	1.21

Probe ID	Fold change (log2)
A_95_P256334	1.21
A_95_P094738	1.21
A_95_P293663	1.21
A_95_P207942	1.20
A_95_P128037	1.20
A_95_P163902	1.20
A_95_P242052	1.20
A_95_P308118	1.20
A_95_P020331	1.20
A_95_P311698	1.20
A_95_P038396	1.20
A_95_P203302	1.20
A_95_P160292	1.20
A_95_P208867	1.20
A_95_P246477	1.19
A_95_P267416	1.19
A_95_P293273	1.19
A_95_P159457	1.19
A_95_P305173	1.19
A_95_P303558	1.19
A_95_P161997	1.19
A_95_P086673	1.19
A_95_P159452	1.19
A_95_P120722	1.19
A_95_P246042	1.19
A_95_P009016	1.19
A_95_P034344	1.19
A_95_P137067	1.19
A_95_P031001	1.19
A_95_P002406	1.19
A_95_P041911	1.18
A_95_P034070	1.18
A_95_P058231	1.18
A_95_P049291	1.18
A_95_P061615	1.18
A_95_P189857	1.18
A_95_P187967	1.18
A_95_P304878	1.18

Probe ID	Fold change (log2)
A_95_P128282	1.18
A_95_P183872	1.18
A_95_P179097	1.18
A_95_P104352	1.18
A_95_P152667	1.18
A_95_P019816	1.18
A_95_P193862	1.18
A_95_P177032	1.18
A_95_P063065	1.18
A_95_P111637	1.18
A_95_P162072	1.18
A_95_P205252	1.18
A_95_P216467	1.18
A_95_P016966	1.18
A_95_P019436	1.18
A_95_P161432	1.18
A_95_P114512	1.18
A_95_P210807	1.18
A_95_P234229	1.18
A_95_P117457	1.18
A_95_P151172	1.18
A_95_P237654	1.18
A_95_P223747	1.18
A_95_P119707	1.18
A_95_P007676	1.18
A_95_P213972	1.17
A_95_P127177	1.17
A_95_P158982	1.17
A_95_P195122	1.17
A_95_P021041	1.17
A_95_P105122	1.17
A_95_P104862	1.17
A_95_P248737	1.17
A_95_P111072	1.17
A_95_P000361	1.17
A_95_P054191	1.17
A_95_P310088	1.17
A_95_P181562	1.17

Probe ID	Fold change (log2)
A_95_P004441	1.17
A_95_P179182	1.17
A_95_P110952	1.17
A_95_P214642	1.17
A_95_P286123	1.17
A_95_P113062	1.17
A_95_P000451	1.17
A_95_P130742	1.17
A_95_P103087	1.17
A_95_P202952	1.17
A_95_P224967	1.17
A_95_P259636	1.17
A_95_P138062	1.17
A_95_P100198	1.17
A_95_P156247	1.17
A_95_P159597	1.17
A_95_P001061	1.17
A_95_P228559	1.17
A_95_P113557	1.16
A_95_P000491	1.16
A_95_P140562	1.16
A_95_P162912	1.16
A_95_P226539	1.16
A_95_P295688	1.16
A_95_P149062	1.16
A_95_P114917	1.16
A_95_P161722	1.16
A_95_P192487	1.16
A_95_P155347	1.16
A_95_P008521	1.16
A_95_P154602	1.16
A_95_P109197	1.16
A_95_P068020	1.16
A_95_P113822	1.16
A_95_P235189	1.16
A_95_P183827	1.15
A_95_P176632	1.15
A_95_P000301	1.15

Probe ID	Fold change (log2)
A_95_P019891	1.15
A_95_P006226	1.15
A_95_P147607	1.15
A_95_P147952	1.15
A_95_P087993	1.15
A_95_P003181	1.15
A_95_P113507	1.15
A_95_P297288	1.15
A_95_P021401	1.15
A_95_P016206	1.15
A_95_P130877	1.15
A_95_P211332	1.15
A_95_P237684	1.15
A_95_P305788	1.15
A_95_P119112	1.15
A_95_P143337	1.14
A_95_P177397	1.14
A_95_P092308	1.14
A_95_P292133	1.14
A_95_P014276	1.14
A_95_P131637	1.14
A_95_P240454	1.14
A_95_P164307	1.14
A_95_P091738	1.14
A_95_P083550	1.14
A_95_P062905	1.14
A_95_P031551	1.14
A_95_P164777	1.14
A_95_P203007	1.14
A_95_P004306	1.14
A_95_P031266	1.14
A_95_P234299	1.14
A_95_P115347	1.14
A_95_P093713	1.14
A_95_P038651	1.14
A_95_P311968	1.13
A_95_P052646	1.13
A_95_P308893	1.13

Probe ID	Fold change (log2)
A_95_P241445	1.13
A_95_P234824	1.13
A_95_P131087	1.13
A_95_P150027	1.13
A_95_P029376	1.13
A_95_P001331	1.13
A_95_P235569	1.13
A_95_P066120	1.13
A_95_P034858	1.13
A_95_P024661	1.13
A_95_P177962	1.13
A_95_P113037	1.13
A_95_P092493	1.13
A_95_P140677	1.13
A_95_P152902	1.13
A_95_P013126	1.13
A_95_P153962	1.13
A_95_P047686	1.12
A_95_P148672	1.12
A_95_P207437	1.12
A_95_P156092	1.12
A_95_P201752	1.12
A_95_P072395	1.12
A_95_P015951	1.12
A_95_P122677	1.12
A_95_P001171	1.12
A_95_P145757	1.12
A_95_P184087	1.12
A_95_P160437	1.11
A_95_P150257	1.11
A_95_P160562	1.11
A_95_P176777	1.11
A_95_P005381	1.11
A_95_P156807	1.11
A_95_P123897	1.11
A_95_P013961	1.11
A_95_P218242	1.11
A_95_P117372	1.11

Probe ID	Fold change (log2)
A_95_P247557	1.11
A_95_P165007	1.11
A_95_P217937	1.11
A_95_P125182	1.11
A_95_P067010	1.11
A_95_P108827	1.11
A_95_P111837	1.11
A_95_P221062	1.11
A_95_P238019	1.11
A_95_P023546	1.11
A_95_P249712	1.11
A_95_P178392	1.11
A_95_P211882	1.11
A_95_P041351	1.11
A_95_P144712	1.10
A_95_P029981	1.10
A_95_P295908	1.10
A_95_P000596	1.10
A_95_P286283	1.10
A_95_P022446	1.10
A_95_P030971	1.10
A_95_P124167	1.10
A_95_P181262	1.10
A_95_P105957	1.10
A_95_P194607	1.10
A_95_P038961	1.10
A_95_P010116	1.10
A_95_P258281	1.10
A_95_P069890	1.10
A_95_P021756	1.10
A_95_P101754	1.10
A_95_P228684	1.10
A_95_P258952	1.10
A_95_P158972	1.10
A_95_P308648	1.10
A_95_P052606	1.10
A_95_P182762	1.10
A_95_P154892	1.10

Probe ID	Fold change (log2)
A_95_P106387	1.10
A_95_P033061	1.09
A_95_P147602	1.09
A_95_P254854	1.09
A_95_P152327	1.09
A_95_P091538	1.09
A_95_P309503	1.09
A_95_P003176	1.09
A_95_P164217	1.09
A_95_P016086	1.09
A_95_P182742	1.09
A_95_P208032	1.09
A_95_P030891	1.09
A_95_P011871	1.09
A_95_P158012	1.09
A_95_P164842	1.08
A_95_P151932	1.08
A_95_P006111	1.08
A_95_P110052	1.08
A_95_P077440	1.08
A_95_P107577	1.08
A_95_P037671	1.08
A_95_P103042	1.08
A_95_P302478	1.08
A_95_P228499	1.08
A_95_P052011	1.08
A_95_P000961	1.08
A_95_P150997	1.08
A_95_P157827	1.08
A_95_P148227	1.07
A_95_P048676	1.07
A_95_P191752	1.07
A_95_P126247	1.07
A_95_P163727	1.07
A_95_P205757	1.07
A_95_P159467	1.07
A_95_P130197	1.07
A_95_P063390	1.07

Probe ID	Fold change (log2)
A_95_P253309	1.07
A_95_P031241	1.07
A_95_P157712	1.07
A_95_P027946	1.07
A_95_P256804	1.07
A_95_P061985	1.07
A_95_P105257	1.07
A_95_P109202	1.07
A_95_P092353	1.07
A_95_P102947	1.07
A_95_P257189	1.07
A_95_P026966	1.07
A_95_P299778	1.07
A_95_P105422	1.07
A_95_P181252	1.06
A_95_P078170	1.06
A_95_P268316	1.06
A_95_P133212	1.06
A_95_P111137	1.06
A_95_P156657	1.06
A_95_P034658	1.06
A_95_P018851	1.06
A_95_P208362	1.06
A_95_P082790	1.06
A_95_P229694	1.06
A_95_P202662	1.06
A_95_P214092	1.06
A_95_P107572	1.06
A_95_P150937	1.06
A_95_P163907	1.06
A_95_P191987	1.06
A_95_P198707	1.06
A_95_P259821	1.05
A_95_P205362	1.05
A_95_P045886	1.05
A_95_P206217	1.05
A_95_P007911	1.05
A_95_P173222	1.05

Probe ID	Fold change (log2)
A_95_P304463	1.05
A_95_P307368	1.05
A_95_P242632	1.05
A_95_P200317	1.05
A_95_P004421	1.05
A_95_P144572	1.05
A_95_P132157	1.05
A_95_P182737	1.05
A_95_P255879	1.05
A_95_P133332	1.05
A_95_P000246	1.05
A_95_P027912	1.05
A_95_P254924	1.05
A_95_P003641	1.05
A_95_P159542	1.05
A_95_P081025	1.04
A_95_P107842	1.04
A_95_P110192	1.04
A_95_P125217	1.04
A_95_P057926	1.04
A_95_P202092	1.04
A_95_P110222	1.04
A_95_P208692	1.04
A_95_P109447	1.04
A_95_P211477	1.04
A_95_P139122	1.04
A_95_P047486	1.04
A_95_P124827	1.04
A_95_P164137	1.04
A_95_P145137	1.04
A_95_P108797	1.04
A_95_P157837	1.04
A_95_P158887	1.04
A_95_P215317	1.04
A_95_P007596	1.04
A_95_P077960	1.04
A_95_P019876	1.03
A_95_P007492	1.03

Probe ID	Fold change (log2)
A_95_P041721	1.03
A_95_P052571	1.03
A_95_P004626	1.03
A_95_P164872	1.03
A_95_P018716	1.03
A_95_P005396	1.03
A_95_P017861	1.03
A_95_P120827	1.03
A_95_P234449	1.03
A_95_P205112	1.03
A_95_P201732	1.03
A_95_P102857	1.03
A_95_P181257	1.03
A_95_P033269	1.03
A_95_P151787	1.03
A_95_P071775	1.03
A_95_P273031	1.03
A_95_P029006	1.03
A_95_P068270	1.03
A_95_P304728	1.03
A_95_P201952	1.03
A_95_P062470	1.03
A_95_P295328	1.02
A_95_P155857	1.02
A_95_P002521	1.02
A_95_P000841	1.02
A_95_P299383	1.02
A_95_P308613	1.02
A_95_P203907	1.02
A_95_P257104	1.02
A_95_P107622	1.02
A_95_P112432	1.02
A_95_P032861	1.02
A_95_P004621	1.02
A_95_P217237	1.02
A_95_P116447	1.02
A_95_P259281	1.02
A_95_P104267	1.02

Probe ID	Fold change (log2)
A_95_P156387	1.02
A_95_P102453	1.02
A_95_P293838	1.02
A_95_P187347	1.02
A_95_P153892	1.02
A_95_P024841	1.02
A_95_P176782	1.02
A_95_P109172	1.02
A_95_P308888	1.02
A_95_P088893	1.02
A_95_P026026	1.02
A_95_P202567	1.02
A_95_P110407	1.02
A_95_P305158	1.02
A_95_P253799	1.02
A_95_P108037	1.01
A_95_P193877	1.01
A_95_P163307	1.01
A_95_P164592	1.01
A_95_P108457	1.01
A_95_P077015	1.01
A_95_P106697	1.01
A_95_P164362	1.01
A_95_P253699	1.01
A_95_P176282	1.01
A_95_P211037	1.01
A_95_P201367	1.01
A_95_P163667	1.01
A_95_P013171	1.01
A_95_P208947	1.01
A_95_P164247	1.01
A_95_P008986	1.01
A_95_P082235	1.01
A_95_P133882	1.01
A_95_P251949	1.01
A_95_P313333	1.01
A_95_P111322	1.01
A_95_P048956	1.01

Probe ID	Fold change (log2)
A_95_P105732	1.01
A_95_P162157	1.01
A_95_P296428	1.01
A_95_P113789	1.00
A_95_P030311	1.00
A_95_P015191	1.00
A_95_P270856	1.00
A_95_P178817	1.00
A_95_P041766	1.00
A_95_P130337	1.00
A_95_P094214	1.00
A_95_P064630	1.00
A_95_P113867	1.00
A_95_P089758	1.00
A_95_P316278	1.00
A_95_P220937	1.00
A_95_P009641	1.00
A_95_P216907	1.00
A_95_P010161	1.00
A_95_P105387	1.00
A_95_P209452	1.00
A_95_P030906	1.00
A_95_P009236	1.00
A_95_P159827	1.00
A_95_P308948	1.00
A_95_P012111	1.00
A_95_P098663	1.00
A_95_P109722	1.00
A_95_P036508	1.00
A_95_P241945	0.99
A_95_P084585	0.99
A_95_P111117	0.99
A_95_P156267	0.99
A_95_P018586	0.99
A_95_P164782	0.99
A_95_P269076	0.99
A_95_P011821	0.99
A_95_P291793	0.99

Probe ID	Fold change (log2)
A_95_P187972	0.99
A_95_P164972	0.99
A_95_P116512	0.99
A_95_P311353	0.99
A_95_P306538	0.99
A_95_P004321	0.99
A_95_P090738	0.99
A_95_P142507	0.99
A_95_P245427	0.98
A_95_P297388	0.98
A_95_P069095	0.98
A_95_P180412	0.98
A_95_P264156	0.98
A_95_P037248	0.98
A_95_P196967	0.98
A_95_P258581	0.98
A_95_P162522	0.98
A_95_P100268	0.98
A_95_P000630	0.98
A_95_P103757	0.98
A_95_P276193	0.98
A_95_P162902	0.98
A_95_P113022	0.98
A_95_P007441	0.98
A_95_P068530	0.97
A_95_P123222	0.97
A_95_P000456	0.97
A_95_P210357	0.97
A_95_P112822	0.97
A_95_P222867	0.97
A_95_P213267	0.97
A_95_P138687	0.97
A_95_P109317	0.97
A_95_P006976	0.97
A_95_P160802	0.97
A_95_P061160	0.97
A_95_P095398	0.97
A_95_P105082	0.97

Probe ID	Fold change (log2)
A_95_P029521	0.97
A_95_P279278	0.97
A_95_P234114	0.97
A_95_P263821	0.96
A_95_P246877	0.96
A_95_P185317	0.96
A_95_P140702	0.96
A_95_P121637	0.96
A_95_P128222	0.96
A_95_P309213	0.96
A_95_P017221	0.96
A_95_P229894	0.96
A_95_P003201	0.96
A_95_P010836	0.96
A_95_P154787	0.96
A_95_P101818	0.96
A_95_P202027	0.96
A_95_P260486	0.96
A_95_P162172	0.96
A_95_P105772	0.96
A_95_P185302	0.96
A_95_P003081	0.96
A_95_P164717	0.96
A_95_P152917	0.96
A_95_P121687	0.96
A_95_P185092	0.96
A_95_P025341	0.96
A_95_P104232	0.96
A_95_P023856	0.96
A_95_P104317	0.95
A_95_P182572	0.95
A_95_P258411	0.95
A_95_P102302	0.95
A_95_P253304	0.95
A_95_P005166	0.95
A_95_P198152	0.95
A_95_P109397	0.95
A_95_P200697	0.95

Probe ID	Fold change (log2)
A_95_P018106	0.95
A_95_P224447	0.95
A_95_P120362	0.95
A_95_P137217	0.95
A_95_P210447	0.95
A_95_P055156	0.95
A_95_P137987	0.95
A_95_P104287	0.94
A_95_P191087	0.94
A_95_P159527	0.94
A_95_P161712	0.94
A_95_P254624	0.94
A_95_P249797	0.94
A_95_P220772	0.94
A_95_P285978	0.94
A_95_P222378	0.94
A_95_P031326	0.94
A_95_P026131	0.94
A_95_P139112	0.94
A_95_P215672	0.94
A_95_P293618	0.94
A_95_P016616	0.93
A_95_P137797	0.93
A_95_P195707	0.93
A_95_P223427	0.93
A_95_P083875	0.93
A_95_P091958	0.93
A_95_P114782	0.93
A_95_P145997	0.93
A_95_P180332	0.93
A_95_P203677	0.93
A_95_P145037	0.93
A_95_P305588	0.93
A_95_P138547	0.92
A_95_P240369	0.92
A_95_P135457	0.92
A_95_P150062	0.92
A_95_P243632	0.92

Probe ID	Fold change (log2)
A_95_P069550	0.92
A_95_P271731	0.92
A_95_P074345	0.92
A_95_P024616	0.92
A_95_P242492	0.92
A_95_P253219	0.92
A_95_P117482	0.92
A_95_P201362	0.92
A_95_P290128	0.92
A_95_P185867	0.92
A_95_P006256	0.92
A_95_P163492	0.92
A_95_P299068	0.92
A_95_P048906	0.92
A_95_P146097	0.92
A_95_P071415	0.92
A_95_P162012	0.91
A_95_P046371	0.91
A_95_P090453	0.91
A_95_P149722	0.91
A_95_P156592	0.91
A_95_P095323	0.91
A_95_P150297	0.91
A_95_P203147	0.91
A_95_P185502	0.91
A_95_P100293	0.91
A_95_P201007	0.91
A_95_P283578	0.91
A_95_P232254	0.91
A_95_P007396	0.91
A_95_P295468	0.91
A_95_P015591	0.91
A_95_P014846	0.91
A_95_P215237	0.91
A_95_P308758	0.91
A_95_P225452	0.91
A_95_P214767	0.90
A_95_P025316	0.90

Probe ID	Fold change (log2)
A_95_P112517	0.90
A_95_P086440	0.90
A_95_P109097	0.90
A_95_P160657	0.90
A_95_P107552	0.90
A_95_P209227	0.90
A_95_P222362	0.90
A_95_P093108	0.90
A_95_P033999	0.90
A_95_P141952	0.90
A_95_P058271	0.90
A_95_P110442	0.90
A_95_P279938	0.90
A_95_P191267	0.90
A_95_P187417	0.90
A_95_P290163	0.90
A_95_P003881	0.90
A_95_P162662	0.89
A_95_P008316	0.89
A_95_P134057	0.89
A_95_P143157	0.89
A_95_P203932	0.89
A_95_P005501	0.89
A_95_P052481	0.89
A_95_P004581	0.89
A_95_P183207	0.89
A_95_P160667	0.89
A_95_P233289	0.89
A_95_P242962	0.89
A_95_P005346	0.89
A_95_P285223	0.89
A_95_P264931	0.89
A_95_P002236	0.89
A_95_P231389	0.89
A_95_P159272	0.89
A_95_P207447	0.89
A_95_P072755	0.89
A_95_P163392	0.88

Probe ID	Fold change (log2)
A_95_P198022	0.88
A_95_P148357	0.88
A_95_P190607	0.88
A_95_P254419	0.88
A_95_P241639	0.88
A_95_P282643	0.88
A_95_P249002	0.88
A_95_P127827	0.88
A_95_P052206	0.88
A_95_P124337	0.88
A_95_P159437	0.88
A_95_P285558	0.88
A_95_P269491	0.88
A_95_P027241	0.88
A_95_P066805	0.88
A_95_P156027	0.88
A_95_P213912	0.88
A_95_P106127	0.88
A_95_P112497	0.88
A_95_P121027	0.88
A_95_P164047	0.87
A_95_P101393	0.87
A_95_P123492	0.87
A_95_P107312	0.87
A_95_P031291	0.87
A_95_P001161	0.87
A_95_P257359	0.87
A_95_P291828	0.87
A_95_P020991	0.87
A_95_P000576	0.87
A_95_P005211	0.87
A_95_P121662	0.87
A_95_P132757	0.87
A_95_P273371	0.87
A_95_P134427	0.87
A_95_P207047	0.87
A_95_P305833	0.86
A_95_P083625	0.86

Probe ID	Fold change (log2)
A_95_P025261	0.86
A_95_P155352	0.86
A_95_P069650	0.86
A_95_P197577	0.86
A_95_P059446	0.86
A_95_P151917	0.86
A_95_P061220	0.86
A_95_P084280	0.86
A_95_P208202	0.86
A_95_P157317	0.86
A_95_P089363	0.86
A_95_P190477	0.86
A_95_P141902	0.86
A_95_P000976	0.85
A_95_P253794	0.85
A_95_P221417	0.85
A_95_P148597	0.85
A_95_P019981	0.85
A_95_P164712	0.85
A_95_P103267	0.85
A_95_P161277	0.85
A_95_P026401	0.85
A_95_P114192	0.85
A_95_P299798	0.85
A_95_P158137	0.85
A_95_P022406	0.85
A_95_P116842	0.85
A_95_P201882	0.85
A_95_P000621	0.85
A_95_P157367	0.85
A_95_P234579	0.85
A_95_P004466	0.85
A_95_P019061	0.85
A_95_P162632	0.85
A_95_P226679	0.84
A_95_P105607	0.84
A_95_P113222	0.84
A_95_P160167	0.84

Probe ID	Fold change (log2)
A_95_P211567	0.84
A_95_P009376	0.84
A_95_P179112	0.84
A_95_P030036	0.84
A_95_P279608	0.84
A_95_P207642	0.84
A_95_P216542	0.84
A_95_P253434	0.84
A_95_P019226	0.84
A_95_P246972	0.84
A_95_P104802	0.84
A_95_P160847	0.84
A_95_P183432	0.84
A_95_P104782	0.84
A_95_P120417	0.83
A_95_P022706	0.83
A_95_P202467	0.83
A_95_P002851	0.83
A_95_P290673	0.83
A_95_P058516	0.83
A_95_P195847	0.83
A_95_P144957	0.83
A_95_P000611	0.83
A_95_P091613	0.83
A_95_P087503	0.83
A_95_P271686	0.83
A_95_P240364	0.83
A_95_P013591	0.83
A_95_P106102	0.82
A_95_P185657	0.82
A_95_P248747	0.82
A_95_P185152	0.82
A_95_P003136	0.82
A_95_P033754	0.82
A_95_P284138	0.82
A_95_P254394	0.82
A_95_P025386	0.82
A_95_P028046	0.82

Probe ID	Fold change (log2)
A_95_P149537	0.81
A_95_P302543	0.81
A_95_P295503	0.81
A_95_P051721	0.81
A_95_P134782	0.81
A_95_P102472	0.81
A_95_P087498	0.81
A_95_P220962	0.81
A_95_P215127	0.81
A_95_P092383	0.81
A_95_P205007	0.81
A_95_P198722	0.81
A_95_P191702	0.81
A_95_P119872	0.81
A_95_P222587	0.81
A_95_P096758	0.80
A_95_P085205	0.80
A_95_P024041	0.80
A_95_P043551	0.80
A_95_P284758	0.80
A_95_P006876	0.80
A_95_P049781	0.80
A_95_P132622	0.80
A_95_P148147	0.80
A_95_P035458	0.80
A_95_P256349	0.80
A_95_P218912	0.80
A_95_P106752	0.80
A_95_P208727	0.80
A_95_P213332	0.80
A_95_P006131	0.80
A_95_P120112	0.80
A_95_P021536	0.80
A_95_P023461	0.79
A_95_P271946	0.79
A_95_P255994	0.79
A_95_P135952	0.79
A_95_P248302	0.79

Probe ID	Fold change (log2)
A_95_P303898	0.79
A_95_P200302	0.79
A_95_P009126	0.79
A_95_P149987	0.79
A_95_P155167	0.79
A_95_P110622	0.79
A_95_P030076	0.79
A_95_P030041	0.79
A_95_P273111	0.79
A_95_P157012	0.79
A_95_P306908	0.79
A_95_P164692	0.79
A_95_P247742	0.79
A_95_P189412	0.79
A_95_P197652	0.79
A_95_P007456	0.79
A_95_P119352	0.78
A_95_P074720	0.78
A_95_P137167	0.78
A_95_P143972	0.78
A_95_P278333	0.78
A_95_P266721	0.78
A_95_P257079	0.78
A_95_P268851	0.78
A_95_P054581	0.78
A_95_P019471	0.78
A_95_P019376	0.78
A_95_P260196	0.78
A_95_P042226	0.77
A_95_P205217	0.77
A_95_P154357	0.77
A_95_P162637	0.77
A_95_P153577	0.77
A_95_P284423	0.77
A_95_P047591	0.77
A_95_P149397	0.77
A_95_P051821	0.77
A_95_P142922	0.77

Probe ID	Fold change (log2)
A_95_P205492	0.77
A_95_P243602	0.77
A_95_P219597	0.76
A_95_P003786	0.76
A_95_P127252	0.76
A_95_P239254	0.76
A_95_P095553	0.76
A_95_P152197	0.76
A_95_P257574	0.76
A_95_P025886	0.76
A_95_P012026	0.76
A_95_P051106	0.76
A_95_P102502	0.76
A_95_P230484	0.76
A_95_P162857	0.76
A_95_P155007	0.76
A_95_P205537	0.76
A_95_P143852	0.75
A_95_P018206	0.75
A_95_P104052	0.75
A_95_P093998	0.75
A_95_P176822	0.75
A_95_P131502	0.75
A_95_P065750	0.75
A_95_P298528	0.75
A_95_P258471	0.75
A_95_P148662	0.75
A_95_P096583	0.75
A_95_P147447	0.75
A_95_P224542	0.75
A_95_P058881	0.75
A_95_P032591	0.75
A_95_P118672	0.74
A_95_P053231	0.74
A_95_P239644	0.74
A_95_P152762	0.74
A_95_P112827	0.74
A_95_P025241	0.74

Probe ID	Fold change (log2)
A_95_P103527	0.74
A_95_P105077	0.74
A_95_P217667	0.74
A_95_P241265	0.74
A_95_P161377	0.74
A_95_P121682	0.74
A_95_P095648	0.73
A_95_P001101	0.73
A_95_P220757	0.73
A_95_P147767	0.73
A_95_P022251	0.73
A_95_P161532	0.73
A_95_P146832	0.73
A_95_P243177	0.73
A_95_P241305	0.73
A_95_P011121	0.73
A_95_P025236	0.73
A_95_P132162	0.73
A_95_P037493	0.72
A_95_P046161	0.72
A_95_P239420	0.72
A_95_P112402	0.72
A_95_P137047	0.72
A_95_P104567	0.72
A_95_P114152	0.72
A_95_P129977	0.72
A_95_P216802	0.72
A_95_P164207	0.72
A_95_P105687	0.72
A_95_P249817	0.72
A_95_P159127	0.72
A_95_P056071	0.72
A_95_P248877	0.71
A_95_P082445	0.71
A_95_P156147	0.71
A_95_P309473	0.71
A_95_P028341	0.71
A_95_P099043	0.71

Probe ID	Fold change (log2)
A_95_P251672	0.71
A_95_P162702	0.71
A_95_P274158	0.71
A_95_P294333	0.71
A_95_P151632	0.71
A_95_P212272	0.71
A_95_P239404	0.71
A_95_P304098	0.70
A_95_P211147	0.70
A_95_P164887	0.70
A_95_P216352	0.70
A_95_P070820	0.70
A_95_P128872	0.70
A_95_P201352	0.70
A_95_P019351	0.70
A_95_P086175	0.70
A_95_P185282	0.70
A_95_P261216	0.70
A_95_P312823	0.70
A_95_P119957	0.70
A_95_P205037	0.70
A_95_P024681	0.70
A_95_P164547	0.70
A_95_P271256	0.70
A_95_P020031	0.70
A_95_P079440	0.70
A_95_P028266	0.70
A_95_P107322	0.69
A_95_P003466	0.69
A_95_P215947	0.69
A_95_P061110	0.69
A_95_P229054	0.69
A_95_P080815	0.69
A_95_P012356	0.69
A_95_P195057	0.69
A_95_P134717	0.69
A_95_P116402	0.69
A_95_P248132	0.69

Probe ID	Fold change (log2)
A_95_P072220	0.69
A_95_P210882	0.69
A_95_P119807	0.69
A_95_P113167	0.69
A_95_P208002	0.69
A_95_P093028	0.69
A_95_P237479	0.69
A_95_P253544	0.69
A_95_P176722	0.69
A_95_P150987	0.69
A_95_P090033	0.68
A_95_P016011	0.68
A_95_P132867	0.68
A_95_P111152	0.68
A_95_P135837	0.68
A_95_P275033	0.68
A_95_P252819	0.68
A_95_P001896	0.68
A_95_P062580	0.68
A_95_P092953	0.68
A_95_P009251	0.68
A_95_P215302	0.68
A_95_P135227	0.68
A_95_P008351	0.68
A_95_P296998	0.68
A_95_P028071	0.67
A_95_P006416	0.67
A_95_P079970	0.67
A_95_P311803	0.67
A_95_P144072	0.67
A_95_P085905	0.67
A_95_P109122	0.67
A_95_P013526	0.67
A_95_P064530	0.67
A_95_P042466	0.67
A_95_P020026	0.67
A_95_P138032	0.67
A_95_P296563	0.67

Probe ID	Fold change (log2)
A_95_P279593	0.67
A_95_P209607	0.67
A_95_P228799	0.67
A_95_P026826	0.67
A_95_P059950	0.67
A_95_P163282	0.67
A_95_P218637	0.67
A_95_P023006	0.67
A_95_P273686	0.67
A_95_P002716	0.66
A_95_P265461	0.66
A_95_P162577	0.66
A_95_P127997	0.66
A_95_P149997	0.66
A_95_P176012	0.66
A_95_P192587	0.66
A_95_P102447	0.66
A_95_P046626	0.66
A_95_P302878	0.66
A_95_P114577	0.66
A_95_P128097	0.66
A_95_P013991	0.66
A_95_P294293	0.66
A_95_P208137	0.65
A_95_P086918	0.65
A_95_P068655	0.65
A_95_P109732	0.65
A_95_P220027	0.65
A_95_P140892	0.65
A_95_P236729	0.65
A_95_P220887	0.65
A_95_P159627	0.65
A_95_P039036	0.65
A_95_P212937	0.65
A_95_P069640	0.65
A_95_P264901	0.65
A_95_P104467	0.64
A_95_P160207	0.64

Probe ID	Fold change (log2)
A_95_P184142	0.64
A_95_P160582	0.64
A_95_P206457	0.64
A_95_P223897	0.64
A_95_P263471	0.64
A_95_P218202	0.64
A_95_P217437	0.64
A_95_P017526	0.64
A_95_P181497	0.64
A_95_P267791	0.64
A_95_P269356	0.64
A_95_P159072	0.63
A_95_P145082	0.63
A_95_P013731	0.63
A_95_P256344	0.63
A_95_P126337	0.63
A_95_P127692	0.63
A_95_P222157	0.63
A_95_P143857	0.63
A_95_P184327	0.63
A_95_P308863	0.63
A_95_P110037	0.62
A_95_P218332	0.62
A_95_P055396	0.62
A_95_P116932	0.62
A_95_P195812	0.62
A_95_P228689	0.62
A_95_P137177	0.62
A_95_P124297	0.62
A_95_P144607	0.62
A_95_P149142	0.62
A_95_P008531	0.62
A_95_P202947	0.61
A_95_P117607	0.61
A_95_P226124	0.61
A_95_P010321	0.61
A_95_P195177	0.61
A_95_P164622	0.61

Probe ID	Fold change (log2)
A_95_P123607	0.61
A_95_P305403	0.61
A_95_P256194	0.61
A_95_P214467	0.60
A_95_P238509	0.60
A_95_P273316	0.60
A_95_P010376	0.60
A_95_P234939	0.60
A_95_P062880	0.60
A_95_P297738	0.60
A_95_P149842	0.60
A_95_P153122	0.60
A_95_P257791	0.60
A_95_P027016	0.60
A_95_P196732	0.60
A_95_P028741	0.60
A_95_P083335	0.60
A_95_P110827	0.60
A_95_P063495	0.59
A_95_P027056	0.59
A_95_P189267	0.59
A_95_P023871	0.59
A_95_P025466	0.59
A_95_P036983	0.59
A_95_P110122	0.59
A_95_P201472	0.59
A_95_P001806	0.59
A_95_P023216	0.59
A_95_P065990	0.59
A_95_P233344	0.59
A_95_P283413	0.59
A_95_P204932	0.59
A_95_P287373	0.58
A_95_P051216	0.58
A_95_P263226	0.58
A_95_P159997	0.58
A_95_P235909	0.58
A_95_P163502	0.58

Probe ID	Fold change (log2)
A_95_P273391	0.58
A_95_P013866	0.58
A_95_P134302	0.58
A_95_P063250	0.58
A_95_P124537	0.58
A_95_P253629	0.58
A_95_P108367	0.58
A_95_P188287	0.57
A_95_P308218	0.57
A_95_P097073	0.57
A_95_P214142	0.57
A_95_P139842	0.57
A_95_P251817	0.57
A_95_P160452	0.57
A_95_P294953	0.57
A_95_P288358	0.57
A_95_P229404	0.57
A_95_P016396	0.57
A_95_P185727	0.56
A_95_P056566	0.56
A_95_P013471	0.56
A_95_P278708	0.56
A_95_P110397	0.56
A_95_P145447	0.56
A_95_P129292	0.56
A_95_P055346	0.56
A_95_P161542	0.56
A_95_P202042	0.56
A_95_P105372	0.56
A_95_P020966	0.55
A_95_P193447	0.55
A_95_P149692	0.55
A_95_P160062	0.55
A_95_P159427	0.55
A_95_P153997	0.55
A_95_P011571	0.55
A_95_P230559	0.54
A_95_P023986	0.54

Probe ID	Fold change (log2)
A_95_P268951	0.54
A_95_P213327	0.54
A_95_P158347	0.54
A_95_P009981	0.54
A_95_P098963	0.54
A_95_P022621	0.54
A_95_P075235	0.53
A_95_P037033	0.53
A_95_P273576	0.53
A_95_P188882	0.53
A_95_P224727	0.53
A_95_P187232	0.53
A_95_P277723	0.53
A_95_P293988	0.53
A_95_P112627	0.53
A_95_P279723	0.53
A_95_P245872	0.52
A_95_P032596	0.52
A_95_P186737	0.52
A_95_P074585	0.52
A_95_P058121	0.52
A_95_P232964	0.52
A_95_P188772	0.52
A_95_P109807	0.51
A_95_P296863	0.51
A_95_P134847	0.51
A_95_P021526	0.51
A_95_P084140	0.51
A_95_P277583	0.51
A_95_P054811	0.51
A_95_P208127	0.51
A_95_P079785	0.51
A_95_P309648	0.50
A_95_P163052	0.50
A_95_P121512	0.50
A_95_P111157	0.50
A_95_P110312	0.50
A_95_P049426	0.50

Probe ID	Fold change (log2)
A_95_P078340	0.50
A_95_P275418	0.50
A_95_P238389	0.49
A_95_P202097	0.49
A_95_P200117	0.49
A_95_P129567	0.49
A_95_P102932	0.49
A_95_P132197	0.49
A_95_P032576	0.49
A_95_P018806	0.49
A_95_P010511	0.49
A_95_P069145	0.49
A_95_P282343	0.49
A_95_P154057	0.49
A_95_P266526	0.49
A_95_P192252	0.48
A_95_P281923	0.48
A_95_P083590	0.48
A_95_P057651	0.48
A_95_P286518	0.48
A_95_P034194	0.48
A_95_P261031	0.48
A_95_P116567	0.47
A_95_P272416	0.47
A_95_P108842	0.47
A_95_P302318	0.47
A_95_P288208	0.47
A_95_P090353	0.47
A_95_P028086	0.47
A_95_P147222	0.47
A_95_P050101	0.47
A_95_P106662	0.47
A_95_P016916	0.47
A_95_P044326	0.47
A_95_P085675	0.46
A_95_P245747	0.46
A_95_P200737	0.46
A_95_P131272	0.46

Probe ID	Fold change (log2)
A_95_P024061	0.46
A_95_P019596	0.46
A_95_P028066	0.46
A_95_P062495	0.46
A_95_P127552	0.46
A_95_P270056	0.46
A_95_P268086	0.46
A_95_P150422	0.45
A_95_P247147	0.45
A_95_P066285	0.45
A_95_P242607	0.45
A_95_P038946	0.45
A_95_P129437	0.45
A_95_P123212	0.45
A_95_P120092	0.45
A_95_P185157	0.45
A_95_P198872	0.45
A_95_P255184	0.45
A_95_P052231	0.45
A_95_P136412	0.45
A_95_P267841	0.44
A_95_P241300	0.44
A_95_P182817	0.44
A_95_P193142	0.44
A_95_P163717	0.44
A_95_P311513	0.43
A_95_P176627	0.43
A_95_P195153	0.43
A_95_P131252	0.43
A_95_P193372	0.43
A_95_P303093	0.43
A_95_P121977	0.43
A_95_P180377	0.43
A_95_P063880	0.43
A_95_P018771	0.42
A_95_P133892	0.42
A_95_P043891	0.42
A_95_P041141	0.42

Probe ID	Fold change (log2)
A_95_P225682	0.42
A_95_P263076	0.42
A_95_P123532	0.41
A_95_P200212	0.41
A_95_P125422	0.41
A_95_P127867	0.41
A_95_P099933	0.41
A_95_P082600	0.41
A_95_P106362	0.41
A_95_P033964	0.40
A_95_P222117	0.40
A_95_P249622	0.40
A_95_P135032	0.38
A_95_P103132	0.38
A_95_P096543	0.38
A_95_P158892	0.38
A_95_P017031	0.38
A_95_P137347	0.38
A_95_P309643	0.38
A_95_P309468	0.38
A_95_P146762	0.38
A_95_P112082	0.37
A_95_P065550	0.37
A_95_P133067	0.37
A_95_P120517	0.37
A_95_P130507	0.37
A_95_P132437	0.37
A_95_P286553	0.37
A_95_P088678	0.37
A_95_P307588	0.37
A_95_P143582	0.36
A_95_P161512	0.36
A_95_P132372	0.36
A_95_P141242	0.36
A_95_P053486	0.36
A_95_P285153	0.36
A_95_P064810	0.36
A_95_P233499	0.36

Probe ID	Fold change (log2)
A_95_P248797	0.36
A_95_P025561	0.36
A_95_P201427	0.36
A_95_P091573	0.35
A_95_P112142	0.35
A_95_P129562	0.35
A_95_P150002	0.35
A_95_P131397	0.35
A_95_P094793	0.35
A_95_P134842	0.35
A_95_P127032	0.34
A_95_P079590	0.34
A_95_P120952	0.34
A_95_P060460	0.34
A_95_P193382	0.34
A_95_P152807	0.34
A_95_P158867	0.33
A_95_P238699	0.33
A_95_P306293	0.33
A_95_P252569	0.33
A_95_P047236	0.33
A_95_P111312	0.33
A_95_P233224	0.33
A_95_P300968	0.33
A_95_P311638	0.33
A_95_P115097	0.33
A_95_P093858	0.32
A_95_P148292	0.32
A_95_P152142	0.32
A_95_P226939	0.32
A_95_P289508	0.32
A_95_P234734	0.31
A_95_P099813	0.31
A_95_P255989	0.31
A_95_P240698	0.31
A_95_P016211	0.31
A_95_P263541	0.30
A_95_P135992	0.30

Probe ID	Fold change (log2)
A_95_P040536	0.30
A_95_P088658	0.30
A_95_P286178	0.29
A_95_P189942	0.29
A_95_P168736	0.29
A_95_P196642	0.29
A_95_P143087	0.29
A_95_P006856	0.29
A_95_P009921	0.29
A_95_P005556	0.28
A_95_P168676	0.28
A_95_P260711	0.28
A_95_P124067	0.27
A_95_P085765	0.27
A_95_P153957	0.27
A_95_P034623	0.27
A_95_P115177	0.27
A_95_P142757	0.26
A_95_P295018	0.26
A_95_P005361	0.26
A_95_P034963	0.26
A_95_P151882	0.26
A_95_P024146	0.25
A_95_P256989	0.25
A_95_P184147	0.25
A_95_P019246	0.25
A_95_P188662	0.25
A_95_P002741	0.25
A_95_P178107	0.25
A_95_P115427	0.25
A_95_P126887	0.24
A_95_P093578	0.24
A_95_P141067	0.24
A_95_P206712	0.24
A_95_P238939	0.24
A_95_P007776	0.23
A_95_P212482	0.23
A_95_P272586	0.23

Probe ID	Fold change (log2)
A_95_P017341	0.22
A_95_P110327	0.22
A_95_P290028	0.22
A_95_P238474	0.22
A_95_P304348	0.22
A_95_P020526	0.22
A_95_P243652	0.22
A_95_P143042	0.21
A_95_P279398	0.21
A_95_P235534	0.21
A_95_P001511	0.21
A_95_P275903	0.21
A_95_P224312	0.21
A_95_P119602	0.21
A_95_P132117	0.20
A_95_P089073	0.20
A_95_P227619	0.20
A_95_P292753	0.20
A_95_P279438	0.20
A_95_P069080	0.19
A_95_P268266	0.19
A_95_P269681	0.19
A_95_P023246	0.19
A_95_P158652	0.19
A_95_P057566	0.18
A_95_P005571	0.18
A_95_P079925	0.18
A_95_P218477	0.18
A_95_P127167	0.17
A_95_P042706	0.17
A_95_P217912	0.17
A_95_P020051	0.17
A_95_P122232	0.17
A_95_P207032	0.16
A_95_P147842	0.16
A_95_P077615	0.16
A_95_P113682	0.15
A_95_P173657	0.15

Probe ID	Fold change (log2)
A_95_P132032	0.15
A_95_P043391	0.15
A_95_P304118	0.15
A_95_P050016	0.15
A_95_P077670	0.15
A_95_P159222	0.15
A_95_P154577	0.15
A_95_P147692	0.15
A_95_P109237	0.14
A_95_P037771	0.14
A_95_P209442	0.14
A_95_P259071	0.14
A_95_P053186	0.14
A_95_P145187	0.14
A_95_P022781	0.14
A_95_P216327	0.14
A_95_P082785	0.13
A_95_P073190	0.13
A_95_P308673	0.13
A_95_P058806	0.12
A_95_P031761	0.12
A_95_P017126	0.12
A_95_P044321	0.12
A_95_P102232	0.11
A_95_P000496	0.11
A_95_P179292	0.11
A_95_P120412	0.10
A_95_P219747	0.09
A_95_P298013	0.09
A_95_P101773	0.09
A_95_P188587	0.09
A_95_P228979	0.09
A_95_P308853	0.08
A_95_P144807	0.08
A_95_P188457	0.08
A_95_P140767	0.08
A_95_P092428	0.08
A_95_P003326	0.08

Probe ID	Fold change (log2)
A_95_P023581	0.08
A_95_P104662	0.08
A_95_P286628	0.07
A_95_P251152	0.07
A_95_P006866	0.07
A_95_P163827	0.07
A_95_P077295	0.07
A_95_P162717	0.06
A_95_P088728	0.06
A_95_P254084	0.06
A_95_P131357	0.06
A_95_P312788	0.06
A_95_P121602	0.06
A_95_P144517	0.05
A_95_P068560	0.05
A_95_P055391	0.04
A_95_P196213	0.04
A_95_P181482	0.04
A_95_P152837	0.04
A_95_P141092	0.03
A_95_P103202	0.03
A_95_P253834	0.03
A_95_P151957	0.03
A_95_P282713	0.03
A_95_P209217	0.03
A_95_P159537	0.03
A_95_P298898	0.02
A_95_P117712	0.02
A_95_P076280	0.02
A_95_P311688	0.02
A_95_P011826	0.02
A_95_P027376	0.02
A_95_P008701	0.02
A_95_P140622	0.02
A_95_P274323	0.01
A_95_P086978	0.01
A_95_P164857	0.01
A_95_P096733	0.01

Probe ID	Fold change (log2)
A_95_P085220	0.01
A_95_P007551	0.00
A_95_P143292	0.00
A_95_P236344	0.00
A_95_P300263	0.00
A_95_P139072	0.00
A_95_P187572	-0.01
A_95_P130962	-0.01
A_95_P230574	-0.01
A_95_P153802	-0.01
A_95_P297778	-0.01
A_95_P276543	-0.01
A_95_P177112	-0.01
A_95_P135742	-0.02
A_95_P042376	-0.02
A_95_P123392	-0.02
A_95_P211467	-0.03
A_95_P139502	-0.03
A_95_P122802	-0.04
A_95_P291753	-0.04
A_95_P145917	-0.04
A_95_P242222	-0.04
A_95_P188537	-0.04
A_95_P222512	-0.04
A_95_P032706	-0.04
A_95_P004276	-0.05
A_95_P139947	-0.06
A_95_P030516	-0.06
A_95_P121867	-0.06
A_95_P065640	-0.06
A_95_P272721	-0.06
A_95_P163797	-0.06
A_95_P010356	-0.06
A_95_P197852	-0.06
A_95_P233094	-0.06
A_95_P207927	-0.07
A_95_P201762	-0.07
A_95_P134167	-0.07

Probe ID	Fold change (log2)
A_95_P058326	-0.08
A_95_P299998	-0.08
A_95_P156182	-0.08
A_95_P276923	-0.08
A_95_P108587	-0.08
A_95_P152962	-0.08
A_95_P223472	-0.08
A_95_P025346	-0.09
A_95_P145232	-0.09
A_95_P061190	-0.09
A_95_P058061	-0.10
A_95_P295253	-0.10
A_95_P124747	-0.10
A_95_P043471	-0.10
A_95_P263601	-0.10
A_95_P157502	-0.10
A_95_P137852	-0.11
A_95_P154012	-0.11
A_95_P164077	-0.11
A_95_P142152	-0.11
A_95_P141302	-0.11
A_95_P107237	-0.11
A_95_P133267	-0.12
A_95_P123877	-0.12
A_95_P284188	-0.12
A_95_P208797	-0.12
A_95_P138282	-0.12
A_95_P147402	-0.13
A_95_P087783	-0.13
A_95_P036623	-0.13
A_95_P057866	-0.13
A_95_P221817	-0.13
A_95_P145942	-0.13
A_95_P010646	-0.13
A_95_P066290	-0.14
A_95_P212697	-0.14
A_95_P003966	-0.14
A_95_P217307	-0.15

Probe ID	Fold change (log2)
A_95_P112577	-0.15
A_95_P080365	-0.15
A_95_P185547	-0.15
A_95_P122522	-0.15
A_95_P254469	-0.16
A_95_P210212	-0.16
A_95_P135467	-0.16
A_95_P093503	-0.17
A_95_P053116	-0.18
A_95_P110112	-0.18
A_95_P098703	-0.18
A_95_P045286	-0.18
A_95_P122637	-0.18
A_95_P242737	-0.19
A_95_P027586	-0.19
A_95_P000346	-0.19
A_95_P025801	-0.19
A_95_P157747	-0.19
A_95_P215572	-0.19
A_95_P242342	-0.20
A_95_P283838	-0.21
A_95_P090368	-0.21
A_95_P045291	-0.21
A_95_P315993	-0.21
A_95_P161692	-0.21
A_95_P214382	-0.22
A_95_P081565	-0.22
A_95_P176387	-0.22
A_95_P125952	-0.22
A_95_P110402	-0.22
A_95_P138217	-0.23
A_95_P081895	-0.23
A_95_P273521	-0.23
A_95_P164502	-0.23
A_95_P005049	-0.23
A_95_P132222	-0.24
A_95_P280543	-0.24
A_95_P025151	-0.24

Probe ID	Fold change (log2)
A_95_P313153	-0.24
A_95_P223627	-0.24
A_95_P122447	-0.24
A_95_P086395	-0.24
A_95_P153717	-0.24
A_95_P024651	-0.24
A_95_P024656	-0.25
A_95_P145677	-0.25
A_95_P296198	-0.25
A_95_P275128	-0.25
A_95_P239819	-0.25
A_95_P013886	-0.26
A_95_P259766	-0.26
A_95_P091348	-0.26
A_95_P041446	-0.26
A_95_P042401	-0.26
A_95_P017386	-0.26
A_95_P238349	-0.27
A_95_P003376	-0.27
A_95_P105337	-0.27
A_95_P125097	-0.27
A_95_P001441	-0.28
A_95_P005316	-0.28
A_95_P021861	-0.28
A_95_P206487	-0.28
A_95_P106892	-0.28
A_95_P161472	-0.28
A_95_P267621	-0.28
A_95_P211362	-0.29
A_95_P147087	-0.29
A_95_P021046	-0.29
A_95_P100838	-0.29
A_95_P160382	-0.29
A_95_P040591	-0.29
A_95_P140902	-0.29
A_95_P186032	-0.29
A_95_P312288	-0.30
A_95_P034139	-0.30

Probe ID	Fold change (log2)
A_95_P184507	-0.31
A_95_P266251	-0.31
A_95_P077680	-0.32
A_95_P215627	-0.32
A_95_P012721	-0.32
A_95_P195437	-0.33
A_95_P162592	-0.33
A_95_P018061	-0.33
A_95_P030522	-0.33
A_95_P062640	-0.33
A_95_P194627	-0.33
A_95_P007521	-0.34
A_95_P157742	-0.34
A_95_P001521	-0.34
A_95_P295628	-0.35
A_95_P097393	-0.35
A_95_P023231	-0.36
A_95_P215717	-0.36
A_95_P145962	-0.37
A_95_P222102	-0.37
A_95_P271266	-0.37
A_95_P212127	-0.37
A_95_P004311	-0.38
A_95_P138192	-0.38
A_95_P187812	-0.38
A_95_P017356	-0.38
A_95_P132082	-0.38
A_95_P144057	-0.38
A_95_P126792	-0.39
A_95_P159422	-0.39
A_95_P296448	-0.39
A_95_P296208	-0.39
A_95_P268576	-0.39
A_95_P030091	-0.39
A_95_P159117	-0.39
A_95_P308433	-0.39
A_95_P012206	-0.40
A_95_P137587	-0.40

Probe ID	Fold change (log2)
A_95_P154292	-0.40
A_95_P205262	-0.41
A_95_P006566	-0.41
A_95_P002641	-0.41
A_95_P214962	-0.41
A_95_P114802	-0.41
A_95_P198322	-0.42
A_95_P122362	-0.42
A_95_P255529	-0.42
A_95_P245137	-0.42
A_95_P223552	-0.42
A_95_P232589	-0.42
A_95_P069755	-0.43
A_95_P199627	-0.43
A_95_P132992	-0.43
A_95_P139387	-0.43
A_95_P205532	-0.43
A_95_P024946	-0.44
A_95_P041591	-0.44
A_95_P189972	-0.44
A_95_P135827	-0.44
A_95_P145767	-0.44
A_95_P075040	-0.44
A_95_P238229	-0.44
A_95_P112392	-0.44
A_95_P172031	-0.44
A_95_P127247	-0.44
A_95_P063080	-0.45
A_95_P216337	-0.45
A_95_P073585	-0.45
A_95_P019411	-0.45
A_95_P066420	-0.45
A_95_P092813	-0.45
A_95_P007481	-0.45
A_95_P080590	-0.46
A_95_P199242	-0.46
A_95_P309433	-0.46
A_95_P017676	-0.46

Probe ID	Fold change (log2)
A_95_P288473	-0.46
A_95_P019541	-0.46
A_95_P095303	-0.46
A_95_P256999	-0.46
A_95_P044061	-0.46
A_95_P258326	-0.46
A_95_P006331	-0.47
A_95_P161287	-0.47
A_95_P259086	-0.47
A_95_P008601	-0.47
A_95_P117292	-0.49
A_95_P122972	-0.50
A_95_P253964	-0.50
A_95_P182412	-0.51
A_95_P286498	-0.51
A_95_P244737	-0.51
A_95_P136617	-0.51
A_95_P271651	-0.52
A_95_P128117	-0.52
A_95_P031921	-0.52
A_95_P018461	-0.52
A_95_P177242	-0.52
A_95_P294263	-0.52
A_95_P222127	-0.52
A_95_P210172	-0.52
A_95_P283673	-0.52
A_95_P026906	-0.53
A_95_P022376	-0.53
A_95_P075980	-0.53
A_95_P072605	-0.53
A_95_P136527	-0.53
A_95_P005976	-0.53
A_95_P067985	-0.53
A_95_P020311	-0.54
A_95_P153047	-0.54
A_95_P238164	-0.54
A_95_P195447	-0.54
A_95_P029271	-0.54

Probe ID	Fold change (log2)
A_95_P241580	-0.54
A_95_P155437	-0.54
A_95_P272391	-0.54
A_95_P218067	-0.54
A_95_P030646	-0.54
A_95_P138762	-0.55
A_95_P121032	-0.55
A_95_P117417	-0.55
A_95_P162607	-0.55
A_95_P119512	-0.55
A_95_P039796	-0.55
A_95_P093458	-0.55
A_95_P015096	-0.55
A_95_P313188	-0.56
A_95_P150667	-0.56
A_95_P265766	-0.56
A_95_P276438	-0.56
A_95_P223222	-0.56
A_95_P189022	-0.57
A_95_P222342	-0.57
A_95_P126072	-0.57
A_95_P249167	-0.57
A_95_P189027	-0.58
A_95_P285733	-0.58
A_95_P265736	-0.58
A_95_P005331	-0.58
A_95_P176942	-0.58
A_95_P267701	-0.58
A_95_P248332	-0.59
A_95_P250727	-0.59
A_95_P021321	-0.59
A_95_P034753	-0.60
A_95_P122732	-0.60
A_95_P252939	-0.60
A_95_P126382	-0.60
A_95_P138267	-0.60
A_95_P214182	-0.61
A_95_P224852	-0.61

Probe ID	Fold change (log2)
A_95_P007231	-0.61
A_95_P007801	-0.61
A_95_P006356	-0.61
A_95_P023396	-0.61
A_95_P096248	-0.62
A_95_P176842	-0.62
A_95_P205497	-0.62
A_95_P188517	-0.62
A_95_P018636	-0.62
A_95_P002376	-0.62
A_95_P121452	-0.62
A_95_P008756	-0.62
A_95_P295898	-0.62
A_95_P150788	-0.62
A_95_P143897	-0.62
A_95_P256454	-0.63
A_95_P283398	-0.63
A_95_P122857	-0.63
A_95_P280383	-0.63
A_95_P287463	-0.63
A_95_P019926	-0.64
A_95_P147207	-0.64
A_95_P177972	-0.64
A_95_P016216	-0.64
A_95_P019716	-0.64
A_95_P177977	-0.64
A_95_P215012	-0.64
A_95_P033274	-0.64
A_95_P029511	-0.65
A_95_P135267	-0.65
A_95_P092713	-0.65
A_95_P307658	-0.65
A_95_P139547	-0.66
A_95_P126182	-0.66
A_95_P107902	-0.66
A_95_P015516	-0.66
A_95_P163397	-0.66
A_95_P255014	-0.66

Probe ID	Fold change (log2)
A_95_P154517	-0.66
A_95_P139382	-0.66
A_95_P213407	-0.66
A_95_P090408	-0.66
A_95_P296788	-0.67
A_95_P258396	-0.67
A_95_P162727	-0.67
A_95_P195372	-0.67
A_95_P217357	-0.67
A_95_P132317	-0.67
A_95_P288233	-0.67
A_95_P212207	-0.67
A_95_P177147	-0.67
A_95_P013876	-0.67
A_95_P154352	-0.68
A_95_P008086	-0.68
A_95_P262681	-0.68
A_95_P162127	-0.68
A_95_P272296	-0.68
A_95_P246692	-0.68
A_95_P216152	-0.68
A_95_P245082	-0.68
A_95_P241490	-0.68
A_95_P017521	-0.68
A_95_P067730	-0.68
A_95_P000746	-0.68
A_95_P125982	-0.69
A_95_P217452	-0.69
A_95_P020801	-0.69
A_95_P137507	-0.69
A_95_P268436	-0.69
A_95_P125662	-0.69
A_95_P008886	-0.70
A_95_P008721	-0.70
A_95_P164237	-0.70
A_95_P155662	-0.70
A_95_P221002	-0.70
A_95_P239999	-0.70

Probe ID	Fold change (log2)
A_95_P200107	-0.70
A_95_P127202	-0.70
A_95_P000781	-0.71
A_95_P010271	-0.71
A_95_P195377	-0.71
A_95_P159022	-0.71
A_95_P245352	-0.71
A_95_P159717	-0.71
A_95_P120637	-0.71
A_95_P274063	-0.72
A_95_P211627	-0.72
A_95_P178702	-0.72
A_95_P180947	-0.72
A_95_P010781	-0.72
A_95_P045186	-0.72
A_95_P135882	-0.72
A_95_P300028	-0.72
A_95_P146387	-0.72
A_95_P208887	-0.72
A_95_P202337	-0.72
A_95_P027541	-0.72
A_95_P099663	-0.73
A_95_P179592	-0.73
A_95_P008651	-0.73
A_95_P200742	-0.73
A_95_P016181	-0.73
A_95_P188412	-0.73
A_95_P143442	-0.73
A_95_P135277	-0.74
A_95_P185447	-0.74
A_95_P097523	-0.74
A_95_P161057	-0.74
A_95_P008181	-0.74
A_95_P291378	-0.74
A_95_P206467	-0.74
A_95_P195282	-0.74
A_95_P134512	-0.74
A_95_P105527	-0.75

Probe ID	Fold change (log2)
A_95_P214342	-0.75
A_95_P176962	-0.75
A_95_P003606	-0.75
A_95_P121957	-0.75
A_95_P178212	-0.75
A_95_P017821	-0.75
A_95_P135657	-0.75
A_95_P007876	-0.75
A_95_P000486	-0.76
A_95_P179467	-0.76
A_95_P115557	-0.76
A_95_P182917	-0.76
A_95_P093493	-0.76
A_95_P022071	-0.76
A_95_P024971	-0.76
A_95_P264691	-0.76
A_95_P022366	-0.76
A_95_P145122	-0.76
A_95_P018981	-0.76
A_95_P088413	-0.77
A_95_P128497	-0.77
A_95_P177012	-0.77
A_95_P136077	-0.77
A_95_P145892	-0.77
A_95_P031686	-0.77
A_95_P270791	-0.77
A_95_P109342	-0.77
A_95_P015181	-0.77
A_95_P194217	-0.78
A_95_P041106	-0.78
A_95_P020171	-0.78
A_95_P123317	-0.78
A_95_P009596	-0.78
A_95_P150312	-0.78
A_95_P135712	-0.78
A_95_P262136	-0.78
A_95_P119217	-0.78
A_95_P014381	-0.78

Probe ID	Fold change (log2)
A_95_P184642	-0.78
A_95_P100398	-0.78
A_95_P003671	-0.79
A_95_P189767	-0.79
A_95_P021961	-0.79
A_95_P084700	-0.79
A_95_P150262	-0.80
A_95_P029676	-0.80
A_95_P132232	-0.80
A_95_P125232	-0.80
A_95_P143797	-0.80
A_95_P019196	-0.80
A_95_P026561	-0.80
A_95_P176692	-0.80
A_95_P164383	-0.80
A_95_P151767	-0.81
A_95_P210312	-0.81
A_95_P013911	-0.81
A_95_P008381	-0.81
A_95_P265991	-0.81
A_95_P108197	-0.81
A_95_P226944	-0.81
A_95_P131117	-0.81
A_95_P014321	-0.81
A_95_P109142	-0.81
A_95_P179042	-0.81
A_95_P257249	-0.82
A_95_P104307	-0.82
A_95_P103992	-0.82
A_95_P201467	-0.82
A_95_P183752	-0.82
A_95_P020496	-0.82
A_95_P264776	-0.82
A_95_P117942	-0.82
A_95_P228424	-0.82
A_95_P139882	-0.82
A_95_P231129	-0.82
A_95_P151857	-0.82

Probe ID	Fold change (log2)
A_95_P014141	-0.82
A_95_P185362	-0.82
A_95_P177742	-0.83
A_95_P137142	-0.83
A_95_P000906	-0.83
A_95_P122147	-0.83
A_95_P153212	-0.83
A_95_P228709	-0.83
A_95_P218402	-0.83
A_95_P117972	-0.83
A_95_P068365	-0.84
A_95_P039651	-0.84
A_95_P180082	-0.84
A_95_P119392	-0.84
A_95_P139042	-0.84
A_95_P251082	-0.85
A_95_P074265	-0.85
A_95_P070130	-0.85
A_95_P185102	-0.85
A_95_P181162	-0.85
A_95_P212907	-0.85
A_95_P202067	-0.85
A_95_P146282	-0.85
A_95_P176332	-0.85
A_95_P258156	-0.85
A_95_P176682	-0.86
A_95_P273281	-0.86
A_95_P230889	-0.86
A_95_P184837	-0.86
A_95_P005116	-0.86
A_95_P027856	-0.86
A_95_P029251	-0.86
A_95_P098228	-0.86
A_95_P003336	-0.86
A_95_P116397	-0.86
A_95_P009406	-0.86
A_95_P247582	-0.86
A_95_P090213	-0.86

Probe ID	Fold change (log2)
A_95_P026426	-0.87
A_95_P202537	-0.87
A_95_P056851	-0.87
A_95_P163832	-0.87
A_95_P002356	-0.87
A_95_P269956	-0.87
A_95_P003661	-0.87
A_95_P183437	-0.87
A_95_P117897	-0.87
A_95_P023751	-0.87
A_95_P129297	-0.87
A_95_P258211	-0.87
A_95_P028026	-0.87
A_95_P112927	-0.88
A_95_P212252	-0.88
A_95_P289293	-0.88
A_95_P178277	-0.88
A_95_P109387	-0.88
A_95_P216252	-0.88
A_95_P116087	-0.88
A_95_P160107	-0.88
A_95_P001661	-0.88
A_95_P243112	-0.88
A_95_P285843	-0.88
A_95_P187547	-0.88
A_95_P283523	-0.89
A_95_P115677	-0.89
A_95_P000616	-0.89
A_95_P182992	-0.89
A_95_P078870	-0.89
A_95_P147727	-0.89
A_95_P000931	-0.89
A_95_P142057	-0.89
A_95_P220502	-0.89
A_95_P109982	-0.89
A_95_P135297	-0.89
A_95_P024191	-0.89
A_95_P008061	-0.89

Probe ID	Fold change (log2)
A_95_P108737	-0.89
A_95_P289053	-0.90
A_95_P112522	-0.90
A_95_P125242	-0.90
A_95_P286903	-0.90
A_95_P139202	-0.90
A_95_P144812	-0.90
A_95_P264721	-0.90
A_95_P275218	-0.90
A_95_P006451	-0.90
A_95_P022451	-0.90
A_95_P198157	-0.90
A_95_P199882	-0.90
A_95_P025336	-0.91
A_95_P215602	-0.91
A_95_P177577	-0.91
A_95_P147427	-0.91
A_95_P113422	-0.91
A_95_P187177	-0.91
A_95_P070280	-0.91
A_95_P177622	-0.91
A_95_P152482	-0.91
A_95_P182402	-0.92
A_95_P304563	-0.92
A_95_P234649	-0.92
A_95_P107512	-0.92
A_95_P153947	-0.92
A_95_P110177	-0.92
A_95_P122402	-0.92
A_95_P065840	-0.92
A_95_P003616	-0.92
A_95_P217352	-0.92
A_95_P070145	-0.92
A_95_P039956	-0.92
A_95_P011341	-0.93
A_95_P026241	-0.93
A_95_P187172	-0.93
A_95_P024986	-0.93

Probe ID	Fold change (log2)
A_95_P206067	-0.93
A_95_P287388	-0.93
A_95_P122322	-0.93
A_95_P023711	-0.93
A_95_P207682	-0.93
A_95_P209527	-0.93
A_95_P112367	-0.93
A_95_P199157	-0.94
A_95_P104442	-0.94
A_95_P008781	-0.94
A_95_P038326	-0.94
A_95_P307693	-0.94
A_95_P091473	-0.94
A_95_P034304	-0.94
A_95_P140907	-0.94
A_95_P285953	-0.94
A_95_P252539	-0.94
A_95_P135342	-0.94
A_95_P141772	-0.94
A_95_P026396	-0.94
A_95_P217062	-0.94
A_95_P176887	-0.95
A_95_P307778	-0.95
A_95_P301378	-0.95
A_95_P110357	-0.95
A_95_P082035	-0.95
A_95_P117702	-0.95
A_95_P098048	-0.95
A_95_P239694	-0.95
A_95_P144827	-0.96
A_95_P140642	-0.96
A_95_P189592	-0.96
A_95_P144622	-0.96
A_95_P087278	-0.96
A_95_P054216	-0.96
A_95_P096808	-0.96
A_95_P093668	-0.96
A_95_P287638	-0.97

Probe ID	Fold change (log2)
A_95_P006031	-0.97
A_95_P094008	-0.97
A_95_P201302	-0.97
A_95_P263046	-0.97
A_95_P008431	-0.97
A_95_P217927	-0.97
A_95_P195017	-0.97
A_95_P144117	-0.97
A_95_P162027	-0.97
A_95_P146922	-0.97
A_95_P161612	-0.97
A_95_P245302	-0.98
A_95_P024526	-0.98
A_95_P132637	-0.98
A_95_P201747	-0.98
A_95_P068280	-0.98
A_95_P211897	-0.98
A_95_P307538	-0.98
A_95_P284033	-0.98
A_95_P076430	-0.98
A_95_P087548	-0.98
A_95_P141262	-0.98
A_95_P116577	-0.98
A_95_P190842	-0.98
A_95_P138732	-0.99
A_95_P251157	-0.99
A_95_P159932	-0.99
A_95_P275193	-0.99
A_95_P288428	-0.99
A_95_P283173	-0.99
A_95_P106402	-0.99
A_95_P156497	-1.00
A_95_P280108	-1.00
A_95_P226359	-1.00
A_95_P189287	-1.00
A_95_P316878	-1.00
A_95_P311188	-1.00
A_95_P013836	-1.00

Probe ID	Fold change (log2)
A_95_P236189	-1.00
A_95_P128897	-1.00
A_95_P294748	-1.01
A_95_P203612	-1.01
A_95_P157847	-1.01
A_95_P071880	-1.01
A_95_P177117	-1.01
A_95_P010601	-1.01
A_95_P033629	-1.01
A_95_P233559	-1.01
A_95_P219357	-1.01
A_95_P128122	-1.01
A_95_P121087	-1.01
A_95_P303813	-1.01
A_95_P010396	-1.01
A_95_P148137	-1.01
A_95_P136227	-1.01
A_95_P092628	-1.01
A_95_P180907	-1.01
A_95_P208757	-1.01
A_95_P130392	-1.01
A_95_P013711	-1.01
A_95_P054301	-1.01
A_95_P159387	-1.01
A_95_P213697	-1.01
A_95_P116417	-1.02
A_95_P295008	-1.02
A_95_P243517	-1.02
A_95_P013036	-1.02
A_95_P188272	-1.02
A_95_P150722	-1.02
A_95_P275038	-1.02
A_95_P294433	-1.02
A_95_P223012	-1.02
A_95_P021921	-1.02
A_95_P133282	-1.02
A_95_P014931	-1.02
A_95_P126707	-1.02

Probe ID	Fold change (log2)
A_95_P227109	-1.02
A_95_P310558	-1.02
A_95_P135707	-1.02
A_95_P297393	-1.02
A_95_P050936	-1.02
A_95_P124522	-1.02
A_95_P156712	-1.02
A_95_P214477	-1.02
A_95_P216217	-1.03
A_95_P143987	-1.03
A_95_P030876	-1.03
A_95_P307353	-1.03
A_95_P012241	-1.03
A_95_P100228	-1.03
A_95_P109192	-1.03
A_95_P019591	-1.03
A_95_P051746	-1.03
A_95_P224912	-1.03
A_95_P133057	-1.03
A_95_P186707	-1.03
A_95_P087393	-1.03
A_95_P308108	-1.03
A_95_P134772	-1.03
A_95_P273871	-1.03
A_95_P142467	-1.04
A_95_P137552	-1.04
A_95_P121447	-1.04
A_95_P152887	-1.04
A_95_P183282	-1.04
A_95_P114312	-1.04
A_95_P022296	-1.04
A_95_P010186	-1.04
A_95_P003451	-1.04
A_95_P272086	-1.04
A_95_P078715	-1.04
A_95_P128417	-1.04
A_95_P032406	-1.04
A_95_P155147	-1.04

Probe ID	Fold change (log2)
A_95_P006476	-1.04
A_95_P232874	-1.04
A_95_P036678	-1.04
A_95_P152497	-1.04
A_95_P271291	-1.04
A_95_P131732	-1.04
A_95_P018041	-1.04
A_95_P129367	-1.05
A_95_P009936	-1.05
A_95_P296413	-1.05
A_95_P184812	-1.05
A_95_P101828	-1.05
A_95_P212587	-1.05
A_95_P193027	-1.05
A_95_P095868	-1.05
A_95_P260661	-1.05
A_95_P126667	-1.05
A_95_P019496	-1.05
A_95_P119157	-1.05
A_95_P209647	-1.05
A_95_P246552	-1.05
A_95_P046776	-1.05
A_95_P206647	-1.05
A_95_P193007	-1.05
A_95_P015686	-1.05
A_95_P238254	-1.06
A_95_P081055	-1.06
A_95_P119967	-1.06
A_95_P254304	-1.06
A_95_P140737	-1.06
A_95_P310268	-1.06
A_95_P122262	-1.06
A_95_P157327	-1.06
A_95_P220872	-1.06
A_95_P209472	-1.06
A_95_P149662	-1.06
A_95_P059585	-1.06
A_95_P086415	-1.06

Probe ID	Fold change (log2)
A_95_P243827	-1.06
A_95_P067750	-1.06
A_95_P120367	-1.06
A_95_P287828	-1.06
A_95_P148972	-1.06
A_95_P001456	-1.07
A_95_P070275	-1.07
A_95_P195227	-1.07
A_95_P226799	-1.07
A_95_P195092	-1.07
A_95_P188387	-1.07
A_95_P018156	-1.07
A_95_P260101	-1.07
A_95_P308038	-1.07
A_95_P210437	-1.07
A_95_P242117	-1.07
A_95_P120232	-1.07
A_95_P142862	-1.07
A_95_P012456	-1.07
A_95_P219002	-1.07
A_95_P211162	-1.07
A_95_P186647	-1.08
A_95_P044726	-1.08
A_95_P272801	-1.08
A_95_P268166	-1.08
A_95_P021236	-1.08
A_95_P006196	-1.08
A_95_P041046	-1.08
A_95_P021936	-1.08
A_95_P269821	-1.08
A_95_P108372	-1.08
A_95_P269521	-1.08
A_95_P159897	-1.08
A_95_P232349	-1.08
A_95_P215412	-1.08
A_95_P118437	-1.08
A_95_P016131	-1.08
A_95_P002151	-1.08

Probe ID	Fold change (log2)
A_95_P291803	-1.09
A_95_P142137	-1.09
A_95_P005551	-1.09
A_95_P012671	-1.09
A_95_P017076	-1.09
A_95_P004141	-1.09
A_95_P089638	-1.09
A_95_P200282	-1.09
A_95_P006986	-1.09
A_95_P130052	-1.09
A_95_P105547	-1.09
A_95_P031861	-1.09
A_95_P007256	-1.10
A_95_P259461	-1.10
A_95_P296203	-1.10
A_95_P098498	-1.10
A_95_P131782	-1.10
A_95_P302598	-1.10
A_95_P102742	-1.10
A_95_P093628	-1.10
A_95_P145392	-1.10
A_95_P293768	-1.10
A_95_P187722	-1.10
A_95_P147587	-1.10
A_95_P079190	-1.10
A_95_P213627	-1.10
A_95_P215817	-1.11
A_95_P100948	-1.11
A_95_P271026	-1.11
A_95_P139312	-1.11
A_95_P089143	-1.11
A_95_P026836	-1.11
A_95_P105117	-1.11
A_95_P107647	-1.11
A_95_P191667	-1.11
A_95_P177842	-1.11
A_95_P242035	-1.11
A_95_P308543	-1.11

Probe ID	Fold change (log2)
A_95_P078250	-1.11
A_95_P009881	-1.11
A_95_P115197	-1.11
A_95_P221688	-1.11
A_95_P239894	-1.11
A_95_P196522	-1.12
A_95_P215582	-1.12
A_95_P020786	-1.12
A_95_P086135	-1.12
A_95_P147092	-1.12
A_95_P146027	-1.12
A_95_P031346	-1.12
A_95_P002161	-1.12
A_95_P025761	-1.12
A_95_P242867	-1.12
A_95_P192557	-1.12
A_95_P213822	-1.12
A_95_P290148	-1.12
A_95_P090953	-1.13
A_95_P001846	-1.13
A_95_P194162	-1.13
A_95_P093793	-1.13
A_95_P000271	-1.13
A_95_P303508	-1.13
A_95_P282833	-1.13
A_95_P199477	-1.13
A_95_P138682	-1.13
A_95_P014411	-1.13
A_95_P280913	-1.13
A_95_P048841	-1.13
A_95_P226864	-1.13
A_95_P046006	-1.14
A_95_P264616	-1.14
A_95_P105072	-1.14
A_95_P116667	-1.14
A_95_P145657	-1.14
A_95_P188842	-1.14
A_95_P011136	-1.14

Probe ID	Fold change (log2)
A_95_P245842	-1.14
A_95_P120352	-1.14
A_95_P267161	-1.14
A_95_P030111	-1.14
A_95_P141762	-1.14
A_95_P254604	-1.15
A_95_P197362	-1.15
A_95_P149052	-1.15
A_95_P246017	-1.15
A_95_P136752	-1.15
A_95_P216637	-1.15
A_95_P102267	-1.15
A_95_P118607	-1.15
A_95_P023631	-1.15
A_95_P108107	-1.15
A_95_P234364	-1.15
A_95_P142462	-1.15
A_95_P121042	-1.15
A_95_P004741	-1.16
A_95_P178407	-1.16
A_95_P031866	-1.16
A_95_P010436	-1.16
A_95_P228029	-1.16
A_95_P195532	-1.16
A_95_P007931	-1.16
A_95_P289943	-1.16
A_95_P118537	-1.16
A_95_P170556	-1.16
A_95_P096548	-1.16
A_95_P014831	-1.16
A_95_P284898	-1.16
A_95_P024086	-1.16
A_95_P222112	-1.16
A_95_P159872	-1.16
A_95_P120482	-1.17
A_95_P154732	-1.17
A_95_P153737	-1.17
A_95_P063780	-1.17

Probe ID	Fold change (log2)
A_95_P198262	-1.17
A_95_P046916	-1.17
A_95_P314723	-1.17
A_95_P013146	-1.17
A_95_P012486	-1.17
A_95_P229194	-1.17
A_95_P312103	-1.17
A_95_P179717	-1.17
A_95_P214077	-1.17
A_95_P251562	-1.18
A_95_P183107	-1.18
A_95_P113172	-1.18
A_95_P200597	-1.18
A_95_P197842	-1.18
A_95_P221642	-1.18
A_95_P001431	-1.18
A_95_P176892	-1.18
A_95_P208412	-1.18
A_95_P209302	-1.18
A_95_P244112	-1.18
A_95_P120882	-1.18
A_95_P129712	-1.18
A_95_P274253	-1.18
A_95_P295963	-1.18
A_95_P000411	-1.18
A_95_P013976	-1.18
A_95_P217982	-1.19
A_95_P300343	-1.19
A_95_P206947	-1.19
A_95_P015886	-1.19
A_95_P166822	-1.19
A_95_P316613	-1.19
A_95_P297918	-1.19
A_95_P184762	-1.19
A_95_P074175	-1.19
A_95_P115887	-1.19
A_95_P252129	-1.19
A_95_P150682	-1.19

Probe ID	Fold change (log2)
A_95_P001781	-1.19
A_95_P261631	-1.19
A_95_P225912	-1.19
A_95_P212647	-1.19
A_95_P223507	-1.19
A_95_P127632	-1.19
A_95_P026991	-1.20
A_95_P231234	-1.20
A_95_P134157	-1.20
A_95_P272646	-1.20
A_95_P238334	-1.20
A_95_P155452	-1.20
A_95_P222402	-1.20
A_95_P300638	-1.20
A_95_P185767	-1.20
A_95_P160907	-1.20
A_95_P020921	-1.20
A_95_P248917	-1.20
A_95_P226999	-1.20
A_95_P151022	-1.20
A_95_P191262	-1.20
A_95_P024381	-1.20
A_95_P080420	-1.21
A_95_P112167	-1.21
A_95_P269906	-1.21
A_95_P215512	-1.21
A_95_P135547	-1.21
A_95_P133712	-1.21
A_95_P022671	-1.21
A_95_P036438	-1.21
A_95_P203847	-1.21
A_95_P252079	-1.22
A_95_P089613	-1.22
A_95_P179797	-1.22
A_95_P158557	-1.22
A_95_P267096	-1.22
A_95_P027326	-1.22
A_95_P109067	-1.22

Probe ID	Fold change (log2)
A_95_P247747	-1.22
A_95_P285388	-1.22
A_95_P301098	-1.22
A_95_P224592	-1.22
A_95_P039076	-1.22
A_95_P238959	-1.22
A_95_P267872	-1.22
A_95_P020756	-1.22
A_95_P123527	-1.23
A_95_P014781	-1.23
A_95_P300353	-1.23
A_95_P128792	-1.23
A_95_P206407	-1.23
A_95_P029551	-1.23
A_95_P132767	-1.23
A_95_P268081	-1.23
A_95_P122387	-1.23
A_95_P149607	-1.23
A_95_P138632	-1.23
A_95_P259856	-1.23
A_95_P199362	-1.23
A_95_P042321	-1.23
A_95_P101958	-1.23
A_95_P132022	-1.24
A_95_P177007	-1.24
A_95_P303173	-1.24
A_95_P034618	-1.24
A_95_P133177	-1.24
A_95_P139257	-1.24
A_95_P300998	-1.24
A_95_P198617	-1.24
A_95_P150947	-1.24
A_95_P135137	-1.25
A_95_P188742	-1.25
A_95_P217317	-1.25
A_95_P109312	-1.25
A_95_P130732	-1.25
A_95_P005016	-1.25

Probe ID	Fold change (log2)
A_95_P209467	-1.26
A_95_P090053	-1.26
A_95_P122667	-1.26
A_95_P186367	-1.26
A_95_P126207	-1.26
A_95_P000818	-1.26
A_95_P306628	-1.26
A_95_P268211	-1.26
A_95_P045936	-1.26
A_95_P223582	-1.26
A_95_P093868	-1.27
A_95_P289578	-1.27
A_95_P204492	-1.27
A_95_P286618	-1.27
A_95_P016501	-1.27
A_95_P017671	-1.27
A_95_P157687	-1.27
A_95_P131042	-1.27
A_95_P011026	-1.27
A_95_P239054	-1.27
A_95_P270506	-1.27
A_95_P236489	-1.28
A_95_P228659	-1.28
A_95_P268611	-1.28
A_95_P159967	-1.28
A_95_P200247	-1.28
A_95_P028511	-1.28
A_95_P137342	-1.28
A_95_P152187	-1.28
A_95_P049916	-1.29
A_95_P114132	-1.29
A_95_P253894	-1.29
A_95_P010251	-1.29
A_95_P081095	-1.29
A_95_P197237	-1.29
A_95_P023441	-1.29
A_95_P012511	-1.29
A_95_P270741	-1.29

Probe ID	Fold change (log2)
A_95_P189597	-1.29
A_95_P100828	-1.29
A_95_P262931	-1.29
A_95_P018101	-1.29
A_95_P258876	-1.29
A_95_P146112	-1.30
A_95_P172985	-1.30
A_95_P195302	-1.30
A_95_P114507	-1.30
A_95_P272716	-1.30
A_95_P221712	-1.30
A_95_P115122	-1.30
A_95_P019881	-1.30
A_95_P037478	-1.30
A_95_P029746	-1.30
A_95_P263151	-1.31
A_95_P304478	-1.31
A_95_P031961	-1.31
A_95_P295673	-1.31
A_95_P009411	-1.31
A_95_P121332	-1.31
A_95_P100178	-1.31
A_95_P294043	-1.31
A_95_P002111	-1.31
A_95_P093103	-1.31
A_95_P121462	-1.31
A_95_P054981	-1.31
A_95_P199367	-1.32
A_95_P150087	-1.32
A_95_P065155	-1.32
A_95_P276123	-1.32
A_95_P029541	-1.32
A_95_P161352	-1.32
A_95_P131432	-1.32
A_95_P156862	-1.32
A_95_P025356	-1.33
A_95_P136327	-1.33
A_95_P023911	-1.33

Probe ID	Fold change (log2)
A_95_P032466	-1.33
A_95_P097608	-1.33
A_95_P301163	-1.33
A_95_P016581	-1.33
A_95_P289948	-1.33
A_95_P016841	-1.33
A_95_P116312	-1.33
A_95_P152517	-1.33
A_95_P257294	-1.34
A_95_P269926	-1.34
A_95_P004096	-1.34
A_95_P075550	-1.34
A_95_P254444	-1.34
A_95_P232784	-1.34
A_95_P154252	-1.34
A_95_P224882	-1.34
A_95_P094553	-1.34
A_95_P050891	-1.34
A_95_P192282	-1.34
A_95_P132527	-1.35
A_95_P119912	-1.35
A_95_P281483	-1.35
A_95_P220122	-1.35
A_95_P097258	-1.35
A_95_P107932	-1.35
A_95_P141447	-1.35
A_95_P278453	-1.35
A_95_P133547	-1.35
A_95_P181857	-1.35
A_95_P132352	-1.36
A_95_P283818	-1.36
A_95_P176897	-1.36
A_95_P010391	-1.36
A_95_P304413	-1.36
A_95_P135852	-1.36
A_95_P216297	-1.36
A_95_P137267	-1.36
A_95_P151142	-1.36

Probe ID	Fold change (log2)
A_95_P206622	-1.36
A_95_P292248	-1.36
A_95_P088108	-1.36
A_95_P247832	-1.36
A_95_P132957	-1.37
A_95_P239759	-1.37
A_95_P296013	-1.37
A_95_P136717	-1.37
A_95_P187482	-1.38
A_95_P253124	-1.38
A_95_P021126	-1.38
A_95_P262676	-1.38
A_95_P016121	-1.38
A_95_P258811	-1.38
A_95_P224047	-1.38
A_95_P182237	-1.38
A_95_P071820	-1.39
A_95_P179207	-1.39
A_95_P246662	-1.39
A_95_P210502	-1.39
A_95_P182702	-1.39
A_95_P148872	-1.39
A_95_P012816	-1.39
A_95_P270776	-1.40
A_95_P296613	-1.40
A_95_P027966	-1.40
A_95_P206062	-1.40
A_95_P007981	-1.40
A_95_P189652	-1.40
A_95_P125887	-1.40
A_95_P120617	-1.40
A_95_P190897	-1.40
A_95_P186982	-1.41
A_95_P156352	-1.41
A_95_P301548	-1.41
A_95_P024341	-1.41
A_95_P203137	-1.41
A_95_P207257	-1.41

Probe ID	Fold change (log2)
A_95_P308128	-1.41
A_95_P146512	-1.41
A_95_P208092	-1.41
A_95_P133202	-1.42
A_95_P098403	-1.42
A_95_P234194	-1.42
A_95_P136982	-1.42
A_95_P023791	-1.42
A_95_P290878	-1.42
A_95_P157192	-1.42
A_95_P023606	-1.42
A_95_P222817	-1.43
A_95_P061130	-1.43
A_95_P311663	-1.43
A_95_P182502	-1.43
A_95_P267611	-1.43
A_95_P189097	-1.43
A_95_P029231	-1.43
A_95_P128457	-1.43
A_95_P138982	-1.44
A_95_P126672	-1.44
A_95_P118097	-1.44
A_95_P017186	-1.44
A_95_P060240	-1.44
A_95_P055046	-1.44
A_95_P271356	-1.44
A_95_P242842	-1.45
A_95_P210462	-1.45
A_95_P070805	-1.45
A_95_P144842	-1.45
A_95_P299498	-1.45
A_95_P209382	-1.45
A_95_P008151	-1.45
A_95_P274988	-1.45
A_95_P272681	-1.45
A_95_P151107	-1.46
A_95_P150902	-1.46
A_95_P222812	-1.46

Probe ID	Fold change (log2)
A_95_P018916	-1.46
A_95_P110317	-1.46
A_95_P204257	-1.47
A_95_P113807	-1.47
A_95_P182907	-1.47
A_95_P213427	-1.47
A_95_P051641	-1.47
A_95_P150207	-1.47
A_95_P097475	-1.47
A_95_P255489	-1.48
A_95_P222367	-1.48
A_95_P217702	-1.48
A_95_P052666	-1.48
A_95_P144372	-1.48
A_95_P050241	-1.49
A_95_P193672	-1.49
A_95_P127157	-1.49
A_95_P020156	-1.49
A_95_P212932	-1.49
A_95_P209037	-1.50
A_95_P302793	-1.50
A_95_P274073	-1.50
A_95_P251022	-1.50
A_95_P139747	-1.50
A_95_P007421	-1.50
A_95_P304458	-1.50
A_95_P019846	-1.50
A_95_P144352	-1.50
A_95_P185997	-1.50
A_95_P261271	-1.50
A_95_P148782	-1.51
A_95_P132337	-1.51
A_95_P008856	-1.51
A_95_P078655	-1.51
A_95_P210497	-1.52
A_95_P190892	-1.52
A_95_P130807	-1.52
A_95_P221632	-1.52

Probe ID	Fold change (log2)
A_95_P282498	-1.52
A_95_P116122	-1.52
A_95_P246892	-1.53
A_95_P272726	-1.53
A_95_P155817	-1.53
A_95_P213647	-1.53
A_95_P126242	-1.53
A_95_P279363	-1.53
A_95_P126342	-1.53
A_95_P308198	-1.53
A_95_P075025	-1.53
A_95_P218807	-1.53
A_95_P001146	-1.54
A_95_P022216	-1.54
A_95_P231779	-1.54
A_95_P215647	-1.55
A_95_P223782	-1.55
A_95_P313053	-1.55
A_95_P140392	-1.55
A_95_P157162	-1.55
A_95_P124342	-1.55
A_95_P136882	-1.56
A_95_P199387	-1.56
A_95_P283783	-1.56
A_95_P189947	-1.56
A_95_P201462	-1.57
A_95_P022021	-1.57
A_95_P179237	-1.57
A_95_P250517	-1.57
A_95_P267561	-1.57
A_95_P272651	-1.57
A_95_P026741	-1.58
A_95_P113082	-1.58
A_95_P126152	-1.58
A_95_P075030	-1.58
A_95_P120692	-1.58
A_95_P047821	-1.58
A_95_P016431	-1.59

Probe ID	Fold change (log2)
A_95_P142042	-1.59
A_95_P133372	-1.59
A_95_P066335	-1.59
A_95_P121117	-1.60
A_95_P090188	-1.60
A_95_P296253	-1.61
A_95_P245227	-1.61
A_95_P160957	-1.61
A_95_P245132	-1.61
A_95_P179242	-1.62
A_95_P024376	-1.62
A_95_P216267	-1.62
A_95_P039671	-1.62
A_95_P285703	-1.62
A_95_P005351	-1.62
A_95_P160532	-1.63
A_95_P137637	-1.63
A_95_P124917	-1.63
A_95_P221307	-1.64
A_95_P216182	-1.64
A_95_P294353	-1.65
A_95_P119572	-1.65
A_95_P218747	-1.65
A_95_P310498	-1.65
A_95_P061740	-1.65
A_95_P215922	-1.66
A_95_P204762	-1.66
A_95_P259611	-1.66
A_95_P138712	-1.66
A_95_P014126	-1.67
A_95_P307333	-1.67
A_95_P222732	-1.67
A_95_P201152	-1.67
A_95_P290598	-1.68
A_95_P258886	-1.68
A_95_P007297	-1.68
A_95_P283663	-1.69
A_95_P121642	-1.69

Probe ID	Fold change (log2)
A_95_P016606	-1.70
A_95_P107332	-1.70
A_95_P154547	-1.70
A_95_P252479	-1.70
A_95_P131967	-1.70
A_95_P136287	-1.71
A_95_P096513	-1.72
A_95_P003001	-1.72
A_95_P310338	-1.73
A_95_P134252	-1.73
A_95_P181372	-1.73
A_95_P222642	-1.73
A_95_P183537	-1.74
A_95_P222927	-1.74
A_95_P203397	-1.74
A_95_P208737	-1.75
A_95_P159307	-1.75
A_95_P008921	-1.75
A_95_P259441	-1.75
A_95_P259246	-1.75
A_95_P225812	-1.75
A_95_P141212	-1.76
A_95_P305423	-1.77
A_95_P228674	-1.77
A_95_P259496	-1.77
A_95_P081475	-1.78
A_95_P133687	-1.78
A_95_P145097	-1.79
A_95_P137432	-1.79
A_95_P178437	-1.79
A_95_P013531	-1.79
A_95_P158732	-1.79
A_95_P180542	-1.79
A_95_P268006	-1.79
A_95_P208298	-1.79
A_95_P000446	-1.80
A_95_P133327	-1.80
A_95_P260721	-1.80

Probe ID	Fold change (log2)
A_95_P258921	-1.80
A_95_P215687	-1.80
A_95_P127862	-1.80
A_95_P141972	-1.80
A_95_P157457	-1.81
A_95_P009551	-1.81
A_95_P293738	-1.82
A_95_P141187	-1.82
A_95_P121107	-1.82
A_95_P131992	-1.82
A_95_P086773	-1.82
A_95_P268371	-1.83
A_95_P013931	-1.83
A_95_P134072	-1.84
A_95_P307443	-1.84
A_95_P128382	-1.84
A_95_P134702	-1.85
A_95_P141497	-1.85
A_95_P164202	-1.85
A_95_P160772	-1.87
A_95_P124147	-1.87
A_95_P249212	-1.87
A_95_P259351	-1.87
A_95_P217697	-1.87
A_95_P018796	-1.87
A_95_P247817	-1.88
A_95_P206687	-1.88
A_95_P133442	-1.89
A_95_P157682	-1.89
A_95_P002021	-1.90
A_95_P092568	-1.90
A_95_P004116	-1.90
A_95_P149492	-1.90
A_95_P131817	-1.90
A_95_P289898	-1.90
A_95_P107967	-1.91
A_95_P133822	-1.93
A_95_P245877	-1.93

Probe ID	Fold change (log2)
A_95_P220392	-1.93
A_95_P128597	-1.93
A_95_P221472	-1.93
A_95_P069660	-1.94
A_95_P091693	-1.94
A_95_P224652	-1.94
A_95_P253749	-1.94
A_95_P030441	-1.94
A_95_P206897	-1.95
A_95_P214337	-1.95
A_95_P000261	-1.95
A_95_P246757	-1.95
A_95_P153367	-1.96
A_95_P025461	-1.97
A_95_P244762	-1.97
A_95_P033499	-1.97
A_95_P139307	-1.98
A_95_P102517	-1.98
A_95_P151002	-1.99
A_95_P010951	-1.99
A_95_P138807	-1.99
A_95_P245462	-2.00
A_95_P140577	-2.02
A_95_P123677	-2.02
A_95_P184312	-2.03
A_95_P160027	-2.03
A_95_P120757	-2.04
A_95_P055161	-2.04
A_95_P150412	-2.04
A_95_P228844	-2.04
A_95_P131077	-2.04
A_95_P138597	-2.06
A_95_P239854	-2.08
A_95_P145682	-2.08
A_95_P026431	-2.09
A_95_P148322	-2.09
A_95_P125512	-2.10
A_95_P069140	-2.11

Probe ID	Fold change (log2)
A_95_P015846	-2.11
A_95_P144822	-2.11
A_95_P132682	-2.12
A_95_P092728	-2.12
A_95_P219072	-2.12
A_95_P246257	-2.13
A_95_P041871	-2.14
A_95_P207647	-2.14
A_95_P126987	-2.14
A_95_P110882	-2.14
A_95_P181847	-2.15
A_95_P280588	-2.15
A_95_P266246	-2.15
A_95_P150797	-2.15
A_95_P024806	-2.16
A_95_P017646	-2.17
A_95_P135262	-2.17
A_95_P178962	-2.17
A_95_P009371	-2.17
A_95_P230499	-2.19
A_95_P223337	-2.19
A_95_P249012	-2.20
A_95_P267946	-2.20
A_95_P083150	-2.21
A_95_P254659	-2.22
A_95_P062265	-2.22
A_95_P013386	-2.23
A_95_P019661	-2.23
A_95_P130217	-2.24
A_95_P138388	-2.25
A_95_P079770	-2.25
A_95_P163472	-2.25
A_95_P129802	-2.26
A_95_P221507	-2.26
A_95_P128357	-2.27
A_95_P105587	-2.27
A_95_P007186	-2.30
A_95_P127142	-2.30

Probe ID	Fold change (log2)
A_95_P133297	-2.30
A_95_P016781	-2.31
A_95_P283088	-2.32
A_95_P211207	-2.33
A_95_P119577	-2.33
A_95_P023756	-2.33
A_95_P181177	-2.34
A_95_P140967	-2.34
A_95_P017311	-2.34
A_95_P186117	-2.35
A_95_P286198	-2.35
A_95_P274383	-2.35
A_95_P142097	-2.36
A_95_P083515	-2.36
A_95_P137077	-2.36
A_95_P163972	-2.36
A_95_P227329	-2.37
A_95_P189012	-2.37
A_95_P157472	-2.38
A_95_P268306	-2.38
A_95_P202977	-2.38
A_95_P259206	-2.39
A_95_P133937	-2.39
A_95_P186302	-2.39
A_95_P054931	-2.40
A_95_P146827	-2.40
A_95_P119722	-2.40
A_95_P028611	-2.40
A_95_P278668	-2.41
A_95_P204907	-2.42
A_95_P208632	-2.42
A_95_P016506	-2.42
A_95_P131032	-2.43
A_95_P001651	-2.43
A_95_P000116	-2.44
A_95_P016921	-2.46
A_95_P024336	-2.49
A_95_P127602	-2.49

Probe ID	Fold change (log2)
A_95_P217932	-2.50
A_95_P161772	-2.54
A_95_P170234	-2.54
A_95_P294718	-2.54
A_95_P137892	-2.54
A_95_P227989	-2.56
A_95_P138977	-2.58
A_95_P302408	-2.62
A_95_P146747	-2.64
A_95_P033409	-2.64
A_95_P134592	-2.66
A_95_P151027	-2.68
A_95_P099508	-2.69
A_95_P151192	-2.69
A_95_P289078	-2.69
A_95_P132382	-2.69
A_95_P061925	-2.69
A_95_P280958	-2.69
A_95_P119297	-2.69
A_95_P159657	-2.72
A_95_P157627	-2.73
A_95_P132047	-2.74
A_95_P147077	-2.74
A_95_P138467	-2.74
A_95_P159567	-2.75
A_95_P184747	-2.76
A_95_P183412	-2.76
A_95_P202227	-2.81
A_95_P010541	-2.81
A_95_P016776	-2.83
A_95_P023256	-2.84
A_95_P194728	-2.84
A_95_P310868	-2.84
A_95_P025286	-2.85
A_95_P231404	-2.85
A_95_P121412	-2.92
A_95_P153072	-2.93
A_95_P133182	-2.98

Probe ID	Fold change (log2)
A_95_P217832	-3.00
A_95_P140632	-3.01
A_95_P029306	-3.01
A_95_P132077	-3.02
A_95_P150317	-3.09
A_95_P104612	-3.11
A_95_P017226	-3.14
A_95_P138747	-3.15
A_95_P131572	-3.16
A_95_P016256	-3.21
A_95_P134087	-3.21
A_95_P133917	-3.24
A_95_P136702	-3.26
A_95_P158472	-3.26
A_95_P299948	-3.27
A_95_P200927	-3.28
A_95_P180422	-3.31
A_95_P180427	-3.35
A_95_P270191	-3.35
A_95_P156357	-3.36
A_95_P155797	-3.37
A_95_P123627	-3.38
A_95_P011581	-3.39
A_95_P234719	-3.42
A_95_P177477	-3.44
A_95_P121057	-3.47
A_95_P223997	-3.47
A_95_P209912	-3.52
A_95_P156432	-3.52
A_95_P141122	-3.53
A_95_P154557	-3.62
A_95_P184752	-3.67
A_95_P026211	-3.79
A_95_P226494	-3.82
A_95_P192782	-3.84
A_95_P225342	-3.86
A_95_P053051	-3.90
A_95_P001001	-4.01

Probe ID	Fold change (log2)
A_95_P138802	-4.07
A_95_P195322	-4.13
A_95_P294843	-4.16
A_95_P011106	-4.17
A_95_P150367	-4.18
A_95_P275008	-4.20
A_95_P003566	-4.35
A_95_P003551	-4.36
A_95_P030776	-4.39
A_95_P212112	-4.46
A_95_P129257	-4.59
A_95_P196157	-4.60
A_95_P041366	-4.66
A_95_P010996	-4.66
A_95_P196562	-4.72
A_95_P206522	-4.91
A_95_P287758	-4.91
A_95_P127192	-6.71

Appendix II List of differentially expressed transcripts in the aphid-infested leaves relative to non-infested leaves of wild type (WT) tobacco plants and transgenic lines sense (PAO) or antisense (TAO) plants pre-treated with high light (HL) for seven days.

Probe ID	Fold change (log2)
A_95_P053051	4.08
A_95_P155527	3.31
A_95_P212112	3.22
A_95_P110232	2.49
A_95_P259871	2.32
A_95_P004436	2.17
A_95_P258216	2.15
A_95_P304073	2.10
A_95_P297533	2.08
A_95_P283783	2.02
A_95_P086135	1.99
A_95_P103907	1.98
A_95_P154667	1.97
A_95_P278853	1.94
A_95_P270026	1.91
A_95_P199242	1.90
A_95_P283398	1.90
A_95_P150412	1.89
A_95_P133032	1.88
A_95_P270826	1.85
A_95_P307748	1.85
A_95_P282593	1.82
A_95_P132767	1.82
A_95_P032576	1.80
A_95_P192137	1.77
A_95_P275008	1.76
A_95_P193832	1.76
A_95_P032981	1.76
A_95_P199477	1.75
A_95_P184497	1.74
A_95_P100353	1.71
A_95_P221532	1.71
A_95_P176892	1.70
A_95_P206522	1.70
A_95_P044891	1.70
A_95_P092078	1.68
A_95_P212252	1.67
A_95_P078655	1.64

Probe ID	Fold change (log2)
A_95_P211187	1.57
A_95_P184507	1.56
A_95_P074255	1.53
A_95_P179237	1.49
A_95_P308853	1.48
A_95_P240179	1.47
A_95_P161512	1.46
A_95_P158752	1.45
A_95_P204257	1.43
A_95_P010996	1.42
A_95_P221167	1.41
A_95_P287973	1.39
A_95_P133822	1.38
A_95_P227989	1.37
A_95_P015882	1.37
A_95_P308233	1.36
A_95_P176897	1.35
A_95_P159217	1.32
A_95_P023806	1.31
A_95_P071630	1.31
A_95_P060295	1.29
A_95_P020126	1.29
A_95_P238739	1.28
A_95_P184987	1.27
A_95_P265346	1.26
A_95_P222402	1.26
A_95_P253304	1.26
A_95_P312593	1.26
A_95_P269956	1.25
A_95_P276038	1.25
A_95_P299948	1.25
A_95_P300648	1.24
A_95_P150367	1.23
A_95_P233549	1.22
A_95_P201732	1.20
A_95_P250517	1.19
A_95_P179397	1.18
A_95_P163832	1.18

Probe ID	Fold change (log2)
A_95_P054931	1.17
A_95_P082155	1.17
A_95_P177912	1.17
A_95_P227424	1.16
A_95_P001761	1.15
A_95_P270506	1.14
A_95_P192817	1.14
A_95_P202067	1.14
A_95_P151002	1.14
A_95_P163062	1.14
A_95_P001846	1.12
A_95_P270191	1.12
A_95_P024806	1.11
A_95_P014126	1.11
A_95_P037003	1.11
A_95_P280803	1.10
A_95_P142862	1.10
A_95_P125822	1.10
A_95_P129172	1.09
A_95_P280588	1.09
A_95_P213452	1.09
A_95_P133182	1.08
A_95_P177857	1.08
A_95_P150682	1.08
A_95_P308223	1.08
A_95_P006306	1.06
A_95_P024021	1.06
A_95_P071090	1.05
A_95_P199882	1.05
A_95_P261076	1.05
A_95_P096048	1.05
A_95_P041351	1.04
A_95_P255529	1.04
A_95_P186117	1.04
A_95_P125887	1.03
A_95_P029376	1.03
A_95_P035573	1.02
A_95_P138762	1.02

Probe ID	Fold change (log2)
A_95_P316878	1.01
A_95_P058461	1.01
A_95_P307353	1.01
A_95_P218577	1.01
A_95_P119602	1.00
A_95_P150552	1.00
A_95_P306068	1.00
A_95_P186982	1.00
A_95_P201427	0.99
A_95_P176887	0.98
A_95_P023256	0.98
A_95_P132077	0.97
A_95_P304728	0.95
A_95_P207882	0.94
A_95_P031226	0.92
A_95_P073445	0.89
A_95_P101828	0.89
A_95_P223997	0.85
A_95_P233519	0.85
A_95_P032946	0.85
A_95_P115772	0.84
A_95_P129257	0.79
A_95_P315568	0.79
A_95_P016256	0.78
A_95_P029746	0.78
A_95_P099813	0.78
A_95_P177072	0.78
A_95_P017356	0.76
A_95_P202537	0.76
A_95_P155797	0.76
A_95_P159967	0.76
A_95_P217727	0.76
A_95_P108722	0.75
A_95_P294033	0.75
A_95_P025151	0.75
A_95_P019171	0.74
A_95_P090158	0.74
A_95_P131962	0.74
A_95_P077840	0.71
A_95_P191097	0.69
A_95_P039956	0.67
A_95_P098853	0.67
A_95_P154517	0.66
A_95_P058111	0.66
A_95_P090428	0.65
A_95_P003426	0.65
A_95_P215922	0.65
A_95_P156432	0.65
A_95_P041046	0.65
A_95_P152512	0.63

Probe ID	Fold change (log2)
A_95_P244172	0.62
A_95_P064815	0.62
A_95_P004636	0.61
A_95_P007466	0.60
A_95_P273846	0.60
A_95_P194542	0.59
A_95_P057296	0.59
A_95_P029106	0.58
A_95_P161472	0.55
A_95_P255714	0.54
A_95_P208107	0.53
A_95_P221817	0.51
A_95_P003611	0.50
A_95_P160472	0.49
A_95_P164172	0.49
A_95_P283413	0.49
A_95_P260756	0.49
A_95_P034149	0.48
A_95_P023776	0.48
A_95_P232964	0.47
A_95_P254439	0.46
A_95_P068110	0.45
A_95_P134382	0.44
A_95_P292628	0.43
A_95_P084295	0.43
A_95_P161772	0.42
A_95_P200592	0.40
A_95_P076095	0.40
A_95_P075035	0.39
A_95_P099733	0.39
A_95_P258856	0.39
A_95_P226084	0.39
A_95_P090018	0.38
A_95_P109177	0.35
A_95_P078405	0.34
A_95_P279813	0.33
A_95_P079360	0.33
A_95_P200092	0.33
A_95_P071335	0.33
A_95_P288738	0.30
A_95_P132827	0.29
A_95_P272886	0.28
A_95_P155007	0.28
A_95_P287758	0.27
A_95_P075165	0.27
A_95_P122362	0.26
A_95_P051856	0.26
A_95_P220887	0.25
A_95_P100243	0.25
A_95_P029241	0.24

Probe ID	Fold change (log2)
A_95_P301328	0.23
A_95_P144232	0.21
A_95_P176722	0.21
A_95_P069145	0.21
A_95_P290703	0.20
A_95_P242222	0.17
A_95_P256319	0.16
A_95_P077300	0.14
A_95_P116527	0.13
A_95_P299543	0.12
A_95_P154557	0.10
A_95_P127817	0.09
A_95_P212272	0.08
A_95_P096213	0.07
A_95_P218722	0.07
A_95_P127632	0.06
A_95_P280958	0.05
A_95_P022756	0.05
A_95_P275743	0.04
A_95_P198552	0.03
A_95_P197507	0.02
A_95_P065440	0.01
A_95_P077395	0.01
A_95_P050896	-0.01
A_95_P019531	-0.01
A_95_P026386	-0.05
A_95_P036088	-0.05
A_95_P084355	-0.05
A_95_P089223	-0.06
A_95_P119062	-0.06
A_95_P103057	-0.07
A_95_P156632	-0.07
A_95_P209942	-0.08
A_95_P218477	-0.09
A_95_P069095	-0.10
A_95_P275903	-0.11
A_95_P278358	-0.12
A_95_P066255	-0.12
A_95_P312823	-0.13
A_95_P145737	-0.13
A_95_P164972	-0.13
A_95_P046776	-0.13
A_95_P247712	-0.14
A_95_P117147	-0.14
A_95_P200562	-0.14
A_95_P309693	-0.14
A_95_P065915	-0.15
A_95_P209492	-0.15
A_95_P261686	-0.15
A_95_P185277	-0.16

Probe ID	Fold change (log2)
A_95_P106972	-0.16
A_95_P226449	-0.18
A_95_P199272	-0.18
A_95_P217322	-0.19
A_95_P097868	-0.19
A_95_P288143	-0.19
A_95_P136712	-0.20
A_95_P015621	-0.20
A_95_P156942	-0.20
A_95_P026141	-0.22
A_95_P111727	-0.23
A_95_P119967	-0.24
A_95_P008676	-0.24
A_95_P298073	-0.25
A_95_P252989	-0.25
A_95_P000311	-0.25
A_95_P242612	-0.26
A_95_P303228	-0.27
A_95_P301498	-0.27
A_95_P088268	-0.28
A_95_P189262	-0.28
A_95_P120347	-0.28
A_95_P083405	-0.29
A_95_P009236	-0.29
A_95_P144547	-0.30
A_95_P209247	-0.30
A_95_P082520	-0.31
A_95_P214467	-0.31
A_95_P184642	-0.31
A_95_P246757	-0.32
A_95_P216762	-0.33
A_95_P289628	-0.33
A_95_P085660	-0.34
A_95_P115347	-0.34
A_95_P280168	-0.34
A_95_P065955	-0.34
A_95_P233094	-0.35
A_95_P076810	-0.35
A_95_P273031	-0.36
A_95_P005696	-0.36
A_95_P058596	-0.36
A_95_P221247	-0.38
A_95_P210357	-0.38
A_95_P213932	-0.38
A_95_P146182	-0.38
A_95_P115292	-0.39
A_95_P279283	-0.39
A_95_P163432	-0.39
A_95_P305158	-0.40
A_95_P048161	-0.41

Probe ID	Fold change (log2)
A_95_P245517	-0.41
A_95_P119112	-0.42
A_95_P306473	-0.42
A_95_P083310	-0.42
A_95_P187637	-0.42
A_95_P180202	-0.43
A_95_P000196	-0.43
A_95_P060915	-0.43
A_95_P235114	-0.43
A_95_P157942	-0.44
A_95_P184552	-0.45
A_95_P262471	-0.45
A_95_P041866	-0.47
A_95_P159997	-0.47
A_95_P120412	-0.47
A_95_P145677	-0.47
A_95_P283653	-0.50
A_95_P268266	-0.51
A_95_P004431	-0.51
A_95_P133212	-0.52
A_95_P017541	-0.52
A_95_P273201	-0.53
A_95_P004366	-0.54
A_95_P300943	-0.54
A_95_P232369	-0.54
A_95_P163717	-0.56
A_95_P151107	-0.56
A_95_P203072	-0.58
A_95_P197557	-0.58
A_95_P114157	-0.59
A_95_P217182	-0.60
A_95_P222557	-0.60
A_95_P267686	-0.60
A_95_P109267	-0.61
A_95_P041601	-0.61
A_95_P015281	-0.65
A_95_P255149	-0.65
A_95_P118182	-0.66
A_95_P205232	-0.66
A_95_P154192	-0.67
A_95_P148192	-0.68
A_95_P308218	-0.68
A_95_P297428	-0.69
A_95_P029246	-0.69
A_95_P184312	-0.70
A_95_P045031	-0.70
A_95_P141517	-0.71
A_95_P136437	-0.71
A_95_P034613	-0.73
A_95_P105832	-0.74

Probe ID	Fold change (log2)
A_95_P010321	-0.74
A_95_P103517	-0.75
A_95_P008836	-0.75
A_95_P253064	-0.75
A_95_P291608	-0.77
A_95_P140897	-0.79
A_95_P096208	-0.79
A_95_P211882	-0.81
A_95_P094278	-0.83
A_95_P089783	-0.83
A_95_P047036	-0.85
A_95_P021131	-0.86
A_95_P055766	-0.86
A_95_P083910	-0.87
A_95_P131712	-0.87
A_95_P270446	-0.87
A_95_P199267	-0.87
A_95_P161687	-0.89
A_95_P037228	-0.89
A_95_P006331	-0.90
A_95_P278008	-0.91
A_95_P104232	-0.91
A_95_P180197	-0.93
A_95_P190912	-0.94
A_95_P152167	-0.94
A_95_P089373	-0.98
A_95_P136022	-0.99
A_95_P032281	-1.00
A_95_P132542	-1.00
A_95_P121977	-1.01
A_95_P121407	-1.01
A_95_P030816	-1.02
A_95_P110842	-1.02
A_95_P131237	-1.02
A_95_P050806	-1.03
A_95_P273761	-1.03
A_95_P058806	-1.04
A_95_P144997	-1.04
A_95_P298028	-1.04
A_95_P112067	-1.04
A_95_P141217	-1.04
A_95_P116222	-1.05
A_95_P247062	-1.05
A_95_P016446	-1.05
A_95_P103082	-1.05
A_95_P212912	-1.06
A_95_P218167	-1.06
A_95_P241945	-1.07
A_95_P136942	-1.07
A_95_P113277	-1.07

Probe ID	Fold change (log2)
A_95_P116822	-1.08
A_95_P072723	-1.08
A_95_P222222	-1.08
A_95_P300638	-1.08
A_95_P113337	-1.09
A_95_P097993	-1.09
A_95_P170234	-1.09
A_95_P086440	-1.10
A_95_P012671	-1.10
A_95_P096983	-1.12
A_95_P035173	-1.12
A_95_P102453	-1.12
A_95_P023036	-1.12
A_95_P113022	-1.13
A_95_P104042	-1.13
A_95_P113807	-1.14
A_95_P009701	-1.15
A_95_P039546	-1.16
A_95_P025341	-1.16
A_95_P263046	-1.17
A_95_P107022	-1.17
A_95_P209072	-1.17
A_95_P288413	-1.18
A_95_P152767	-1.19
A_95_P261691	-1.20
A_95_P212097	-1.20
A_95_P146542	-1.20
A_95_P214027	-1.21
A_95_P085645	-1.21
A_95_P116797	-1.21
A_95_P005501	-1.22
A_95_P140787	-1.22
A_95_P005766	-1.23
A_95_P124057	-1.23
A_95_P004456	-1.23
A_95_P046051	-1.24
A_95_P041996	-1.24
A_95_P110192	-1.24
A_95_P067025	-1.25
A_95_P064565	-1.25
A_95_P002096	-1.25
A_95_P047236	-1.25
A_95_P023276	-1.25
A_95_P069175	-1.26
A_95_P289503	-1.26
A_95_P012816	-1.26
A_95_P219177	-1.27
A_95_P134087	-1.30
A_95_P218967	-1.30
A_95_P176632	-1.30

Probe ID	Fold change (log2)
A_95_P134957	-1.31
A_95_P198372	-1.34
A_95_P061985	-1.34
A_95_P274383	-1.34
A_95_P256814	-1.35
A_95_P022391	-1.36
A_95_P076730	-1.36
A_95_P056611	-1.37
A_95_P253434	-1.38
A_95_P275243	-1.38
A_95_P114917	-1.39
A_95_P184652	-1.40
A_95_P241445	-1.41
A_95_P205007	-1.41
A_95_P052011	-1.42
A_95_P275453	-1.43
A_95_P239514	-1.43
A_95_P186302	-1.44
A_95_P113387	-1.46
A_95_P017311	-1.46
A_95_P277163	-1.47
A_95_P303283	-1.52
A_95_P284723	-1.59
A_95_P127167	-1.59
A_95_P038811	-1.61
A_95_P263026	-1.66
A_95_P311303	-1.66
A_95_P096873	-1.67
A_95_P250462	-1.73
A_95_P088658	-1.77
A_95_P007131	-1.77
A_95_P025876	-1.79
A_95_P252939	-1.83
A_95_P036508	-1.83
A_95_P017031	-1.91
A_95_P130372	-1.92
A_95_P118472	-2.17
A_95_P089588	-2.58
A_95_P077795	-2.69

Apendix III Polar metabolites identified using GC-MS grouped by retention index, with listing of selection ions for the integration of peaks. GC-MS = Gas Chromatography-Mass Spectrometry; *m/z* = mass to ion ratio; RRi = relative retention index; U = unknown; UC = unknown carbohydrate, UP = unknown polysaccharide; USA = unknown sugar alcohol.

Metabolite	***m/z***	**RRi**
alanine	116.1	1095
oxalic acid	190.2, 219.2	1122
valine	144.1	1216
U1233	174.2	1233
urea	189.2	1244
ethanolamine	174.2	1266
phosphate	299.3	1270
leucine	158.1	1272
glycerol	205.2	1275
isoleucine	158.1	1291
proline	142.1	1293
glycine	174.2	1300
succinic acid	247.2	1315
U1324	299.3	1324
2,3-dihydroxypropanoic acid	189.2, 292.3	1333
fumaric acid	245.2	1359
serine	204.2	1366
2-piperidinecarboxylic acid	156.1	1369
dihydroxydihydrofuranone	247.2	1380
U1376	141.1	1375
threonine	218.2	1393
b-alanine	174.2, 248.2	1438
homoserine	218.2	1461
malic acid	233.2	1499
U1509	243.2	1509
methionine	176.2	1525
oxoproline 1	156.1	1526
aspartic acid	232.2	1527
g-aminobutyric acid	174.2	1535

oxoproline 2	156.1	1541
threonic acid	292.3	1562
U1567	218.2, 261.2	1567
U1570	185.2	1570
U1586	227.2, 301.3, 344.3	1586
U1585	218.2, 261.2	1585
U1593	156.1	1593
U1598	188.2, 216.2	1598
glutamic acid	246.2	1618
phenylalanine	192.2, 218.2	1623
asparagine 1	188.2	1625
trihydroxypentanoic acid	245.2	1649
USA1656	204.2	1656
USA1663	204.2	1663
asparagine 2	188.2	1670
U1702	217.2	1702
U1703	275.3	1703
glutamine 1	227.2	1736
putrescine	174.2	1742
U1751	261.2, 292.3, 465.5	1751
U1755	167.1, 216.2	1755
USA1768	292.3, 293.3	1768
α-glycerophosphate	299.3	1767
glutamine 2	156.1	1781
U1786	334.4	1786
U1791	128.1, 264.2, 429.5	1791
U1801	359.4	1801
U1809	217.2, 437.5	1809
U1816	461.5	1816
unoximated fructose 1	204.2, 217.2, 437.5	1820
citric acid	273.2, 363.4, 465.5	1824
unoximated fructose 2	204.2, 217.2, 437.5	1853
U1858	174.2	1858
quinic acid	345.4	1860
U1871	188.2	1871

fructose 1	307.3	1873
fructose 2	307.3	1882
allantoin 1	331.3	1885
mannose	319.3	1887
galactose	319.3	1891
unoximated glucose 1	204.2	1894
glucose 1	319.3	1896
unoximated glucose 2	204.2	1903
allantoin 2	331.3	1906
glucose 2	319.3	1914
histidine	154.1, 254.2	1919
lysine	174.2	1923
mannitol	319.3	1927
sorbitol	319.3	1933
tyrosine	218.2	1939
U1948	203.2, 232.2, 449.5	1948
U1953	217.2, 361.4	1953
unoximated glucose 3	191.2, 204.2	1973
UC2020	204.2, 319.3	2020
galactaric acid	292.3, 333.3	2036
inositol	217.2, 305.3	2086
UC2105	205.2, 245.2, 319.3	2105
U2125	229.2, 331.3	2125
caffeic acid	219.2, 396.4	2138
U2190	130.1, 218.2	2190
tryptophan	202.2, 291.3	2212
spermidine	144.1	2251
fructose-6-phosphate	315.3	2300
galactosyl glycerol	204.2, 337.3	2309
glucose-6-phosphate	387.4	2313
U2322	257.2, 303.3, 347.3, 437.5	2322
U2367	204.2. 217.2, 292.3, 375.4, 451.5	2367
U2467	446.5	2467
UC2477a	204.2, 217.2	2477
U2477b	260.2	2477

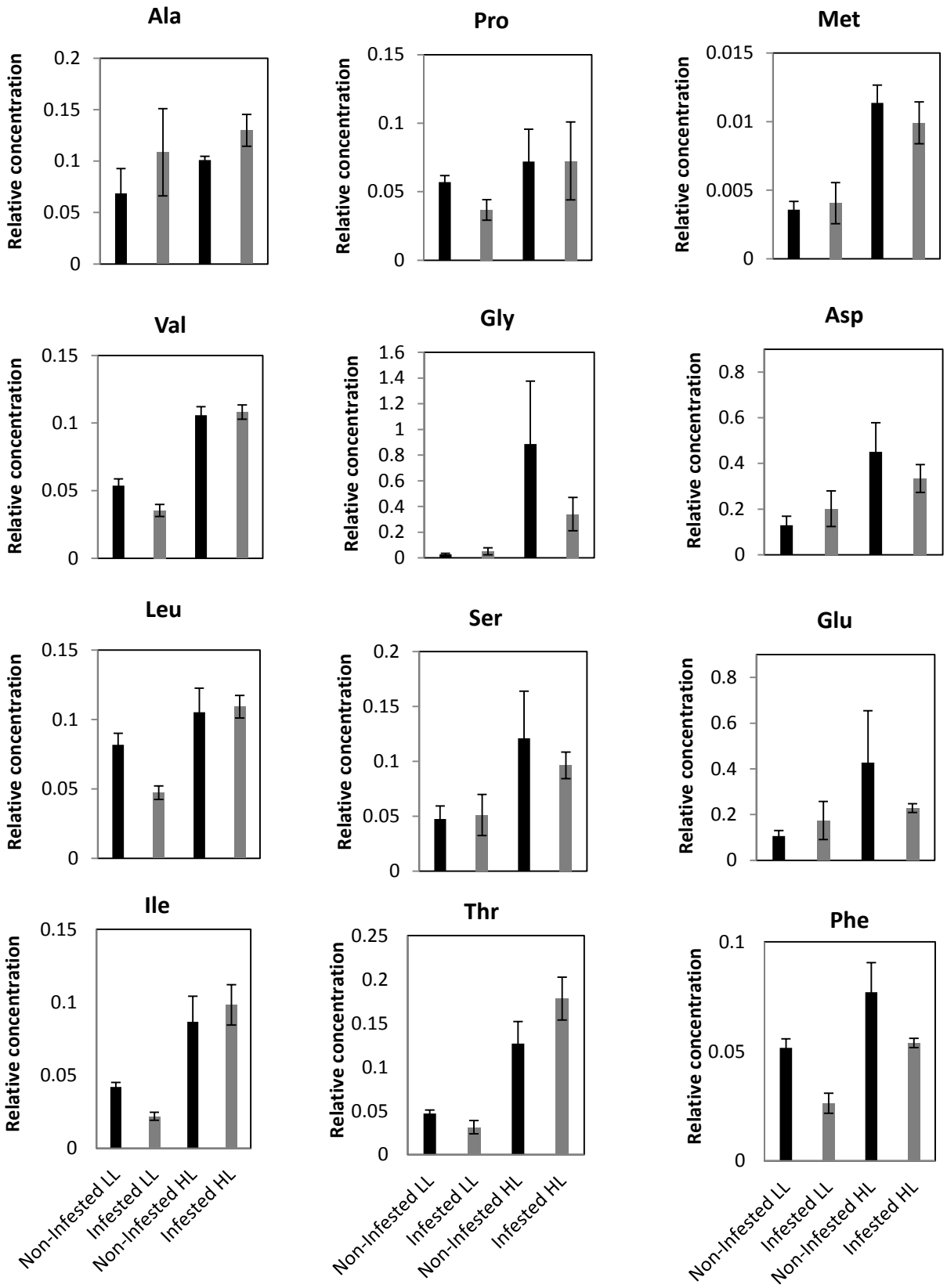
UC2477c	257.2, 303.3, 347.3, 437.5	2477
U2495	446.5	2495
U2502	260.2	2502
sucrose	217.2, 361.4	2637
maltose	204.2, 217.2, 361.4	2736
galactinol	204.2	2973
UP2993	204.2	2993
chlorogenic acid	255.2, 345.3	3107
UP3118	204.2, 217.2	3118

Appendix IV Non-polar metabolites quantified using GC-MS grouped by retention index with listing of selection ions for the integration of peaks. α = alpha; β = beta; Δ = delta; GC-MS = Gas Chromatography-Mass Spectrometry; *m/z* = mass to ion ratio; RRi = relative retention index; U = unknown.

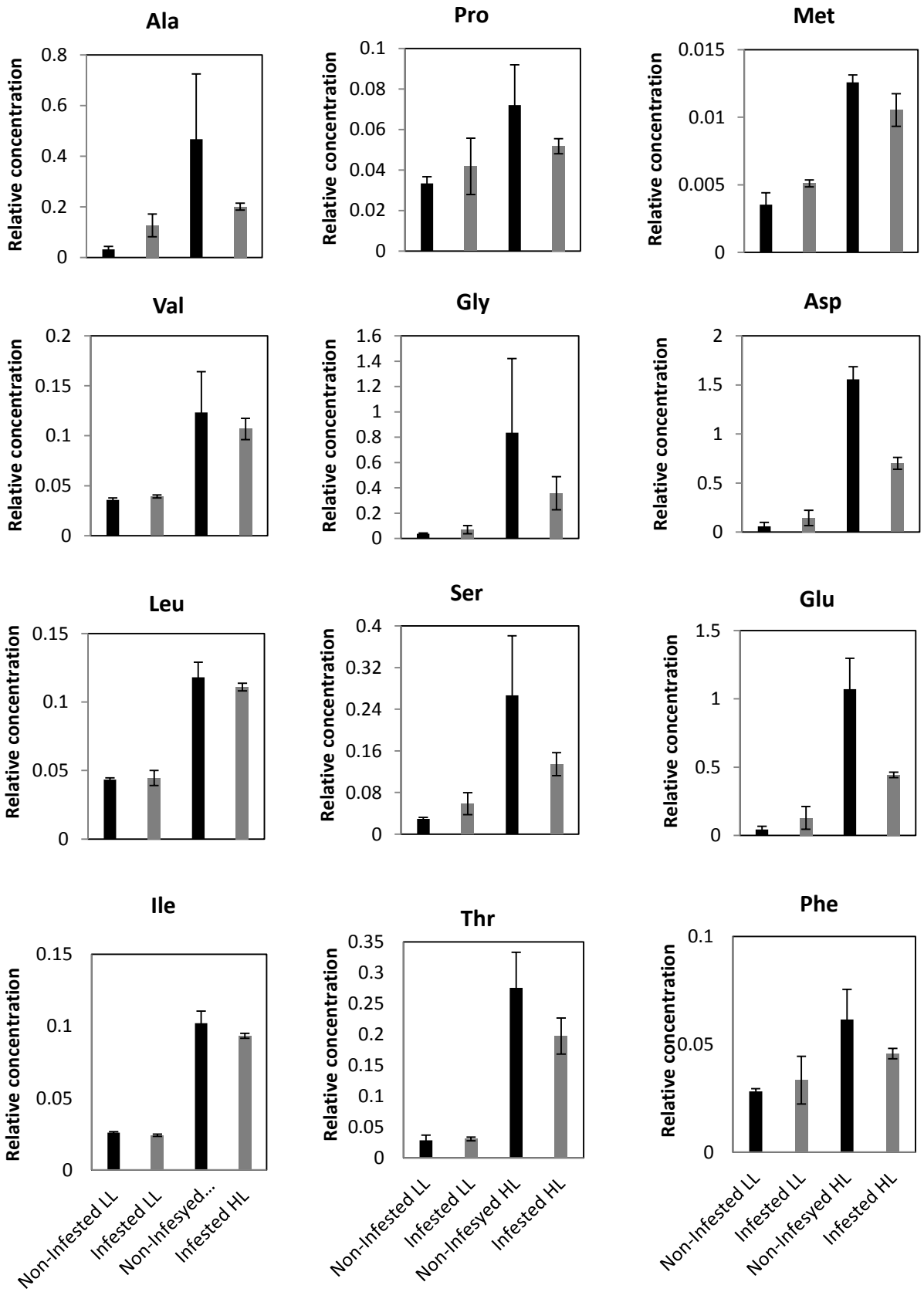
Metabolite	***m/z***	**RRi**
U1595	201.1	1595
U1680	314	1680
n-tetradecanoic acid	242.4	1735
U1762	239.3	1762
br-pentadecanoic acid	74	1799
pentadecenoic acid	74	1819
OCH3OH cinnamic acid 1	250.2	1829
n-pentadecanoic acid	256.1	1835
U1845	239.2	1845
U1895	75	1895
hexadecenoic acid	236.3	1911
n-hexadecanoic acid	270.2	1931
OCH3OH cinnamic acid 2	250.2	1947
15-methylhexadecanoic acid	284.4	1989
n-heptadecanoic acid	284.3	2025
linoleic acid	294.2	2098
α-linolenic acid	292.3	2103
octadecenoic acid	264.4	2109
2-OH hexadecanoic acid	343.3	2126
n-octadecanoic acid	298.4	2131
nonadecenoic acid	278.3	2168
U2263	292.3	2263
n-tricosane	155.2	2308
n-eicosanoic acid	326.5	2330
n-heneicosanoic acid	340.3	2430
n-heneicosanol	369.5	2453
U2457	271.3	2457
U2466	259.3	2466
U2510	259.3	2510

n-docosanoic acid	354.5	2537
n-docosanol	383.5	2557
n-tricosanoic acid	368.6	2640
n-tricosanol	397.5	2660
n-tetracosanoic acid	382.5	2743
n-tetracosanol	411.5	2760
n-pentacosanoic acid	396.5	2840
2-OH tetracosanoic acid	411.6	2913
n-hexacosanoic acid	410.5	2937
n-hexacosanol	439.5	2947
n-heptacosanol	453.6	3037
n-octacosanoic acid	438.5	3135
n-octacosanol	467.7	3142
solanidenol	150.2	3183
demissidenol	150.2	3191
n-nonacosanoic acid	452.8	3232
n-nonacosanol	481.6	3239
stigmasterol	484.7	3258
fucosterol	296.3	3318
β-sitosterol	357.5	3321
Δ5-avenasterol	296.3	3333
n-triacontanoic acid	466.8	3336
n-triacontanol	495.7	3336
Δ5,24(25)-stigmastadienol	296.4	3355

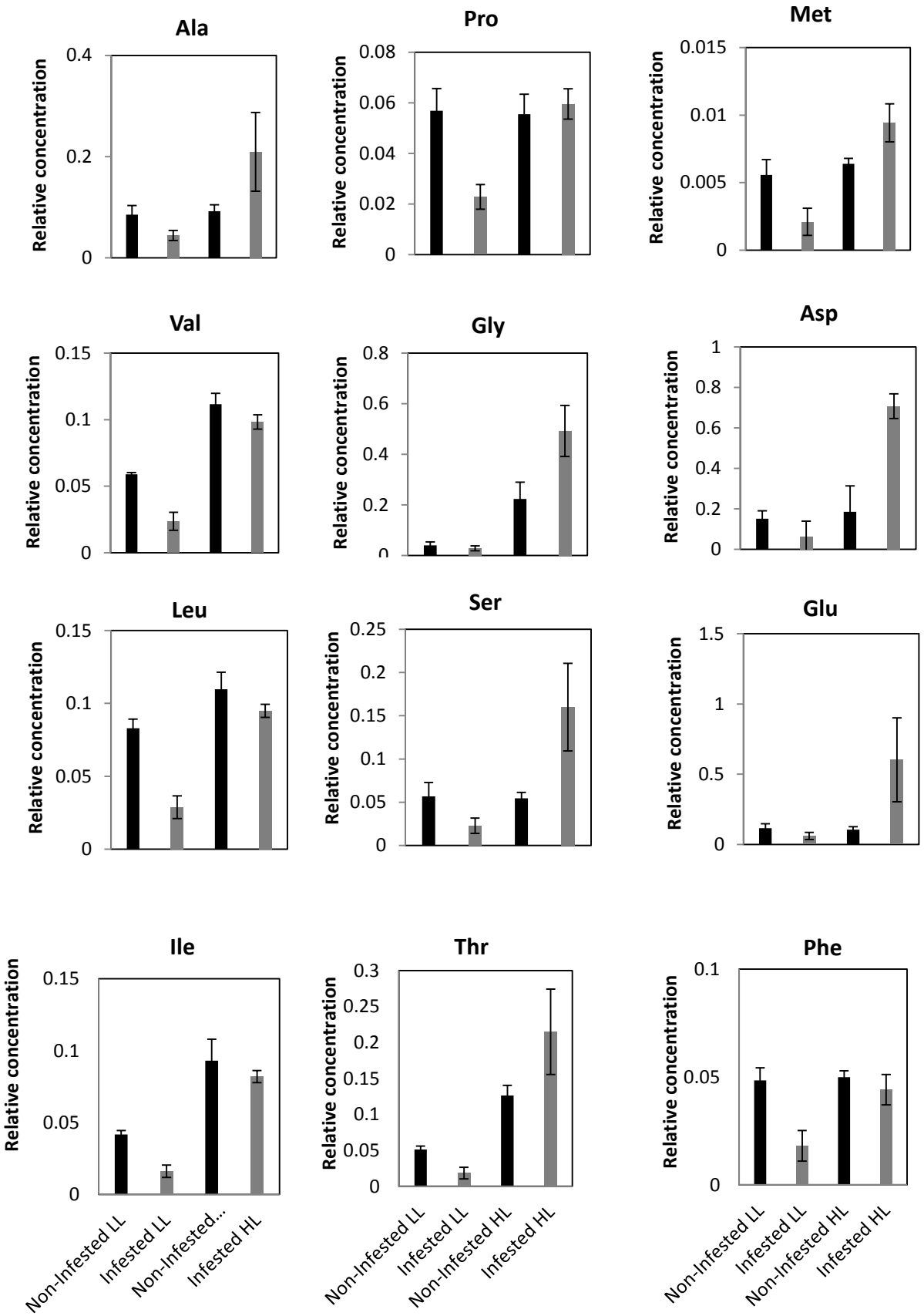
Appendix V Amino acid changes in response to light availability in the infested and non-infested leaves of wild type tobacco plants



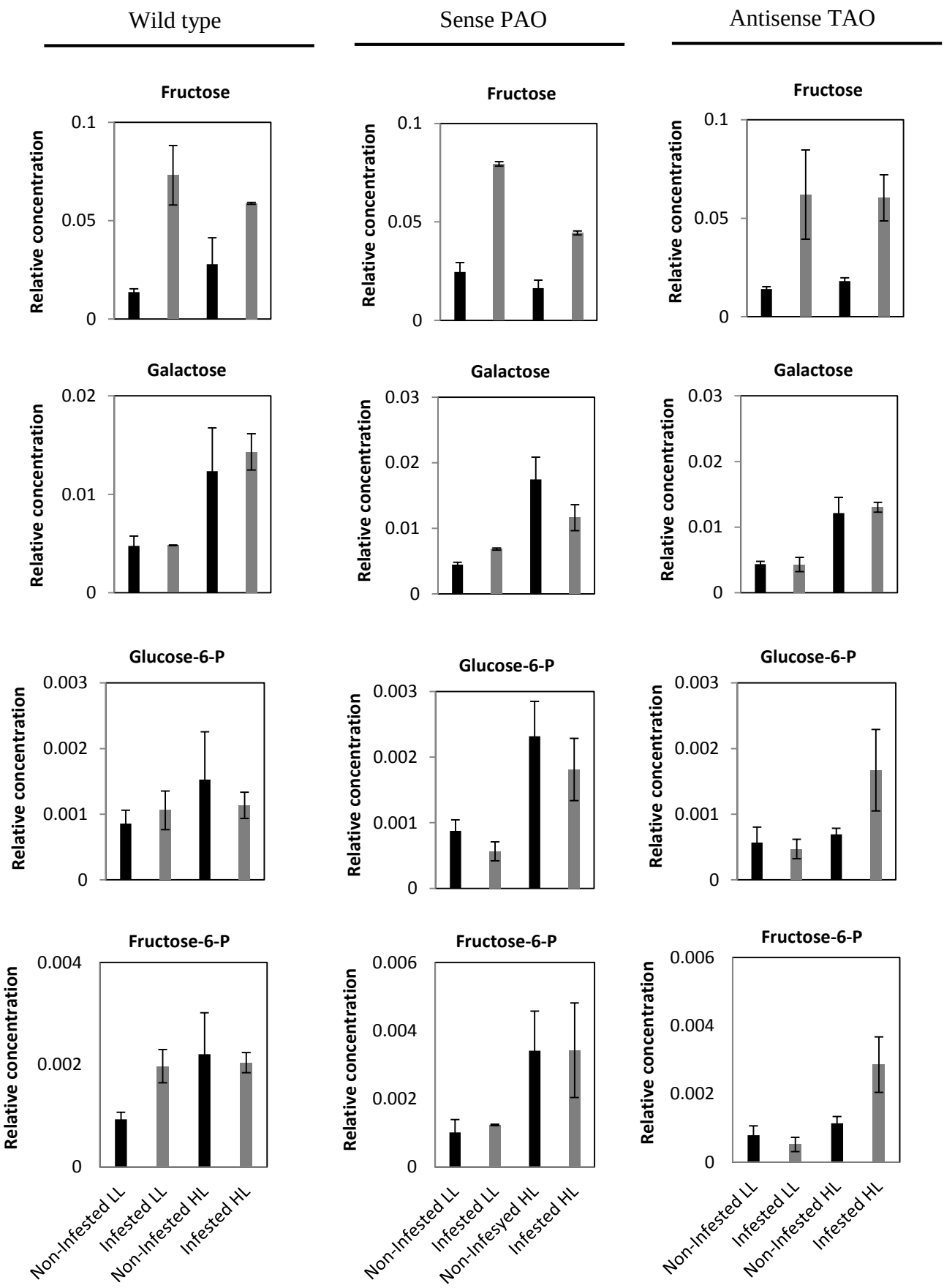
Appendix VI Amino acid changes in response to light availability in the infested and non-infested leaves of sense PAO plants



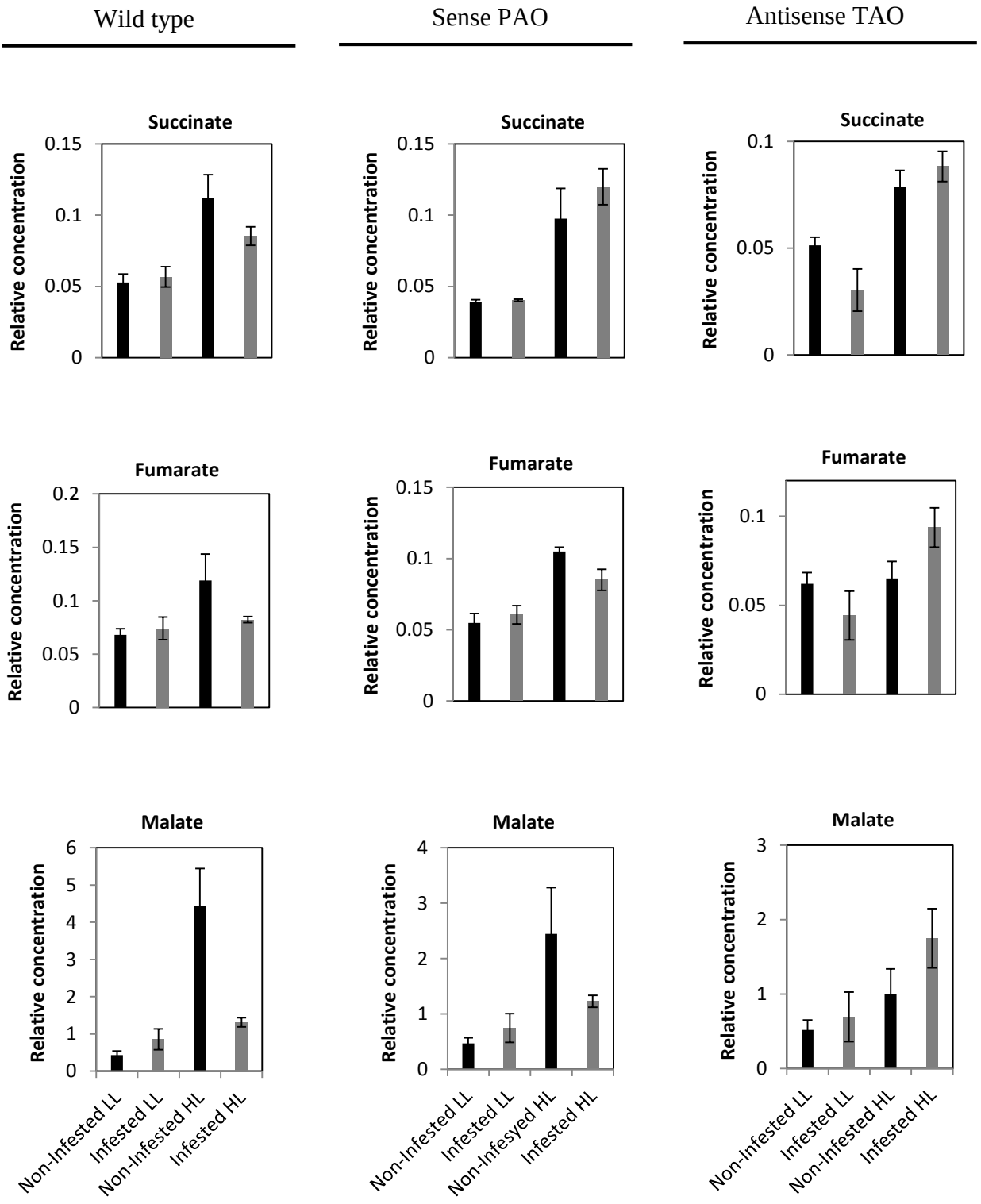
Appendix VII Amino acid changes in response to light availability in the infested and non-infested leaves of antisense TAO plants



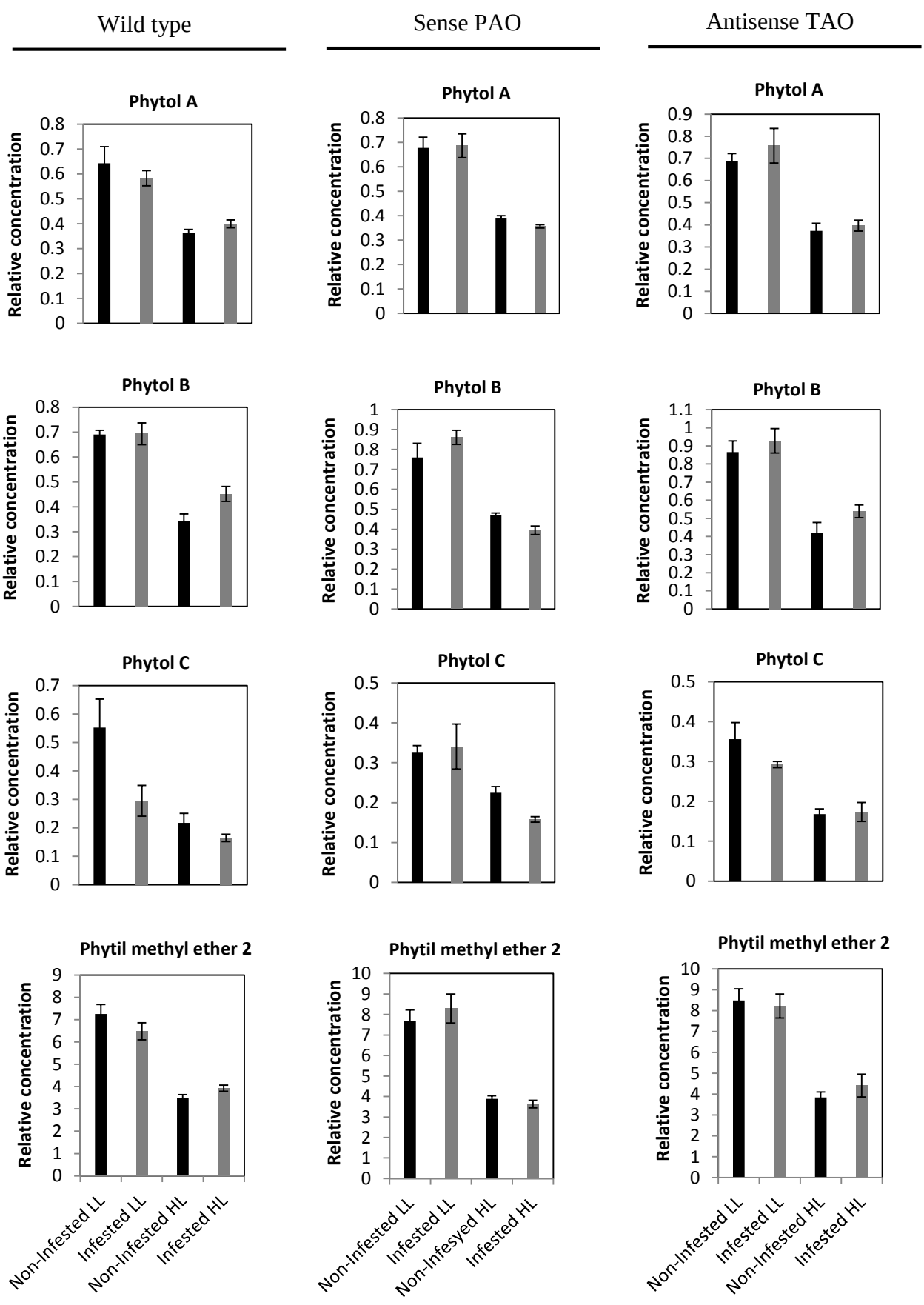
Appendix VIII Carbohydrate changes in response to light availability in the infested and non-infested leaves of wild type, sense PAO and antisense TAO tobacco plants



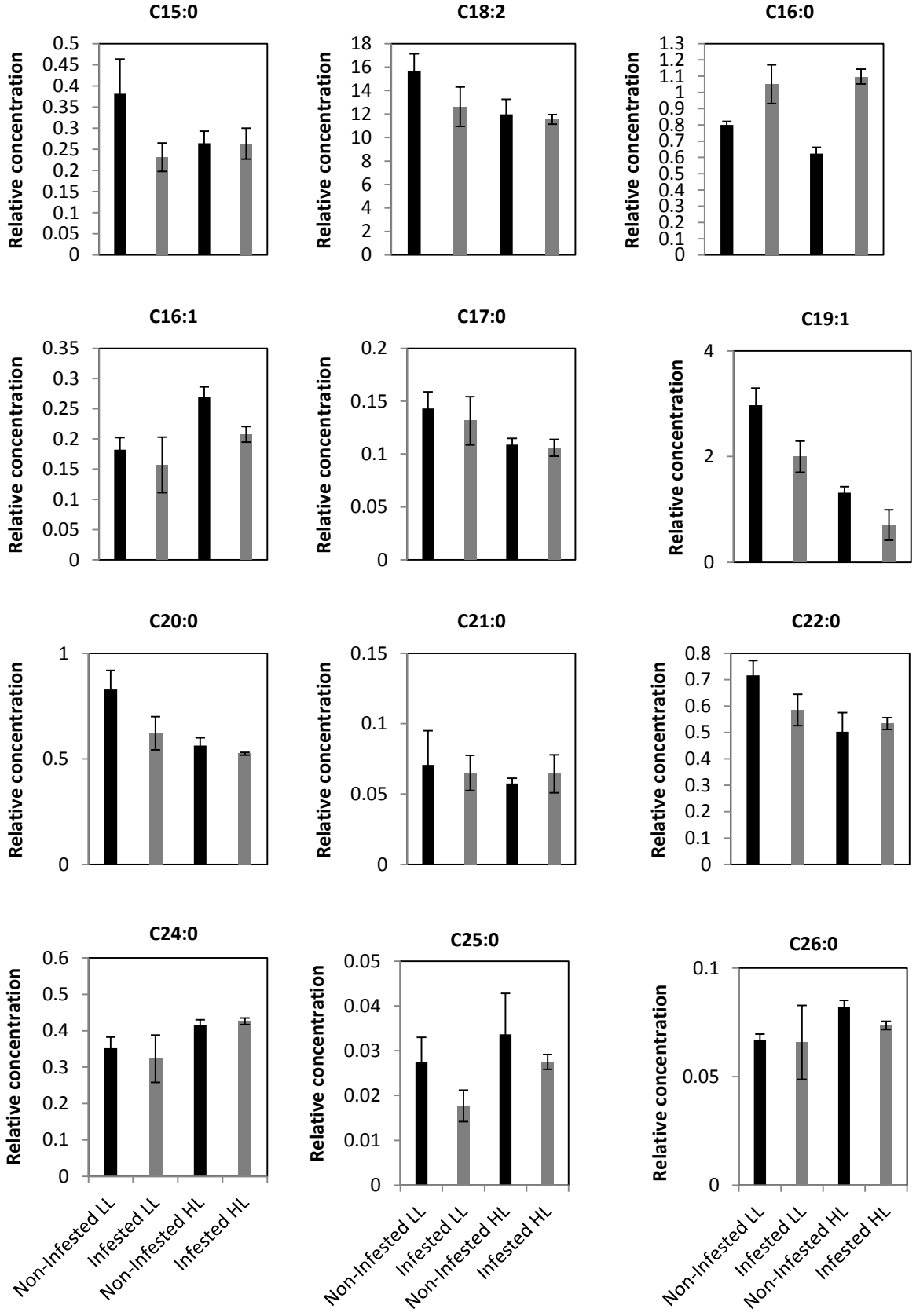
Appendix IX TCA cycle intermediates changes in response to light availability in the infested and non-infested leaves of wild type, sense PAO and antisense TAO tobacco plants



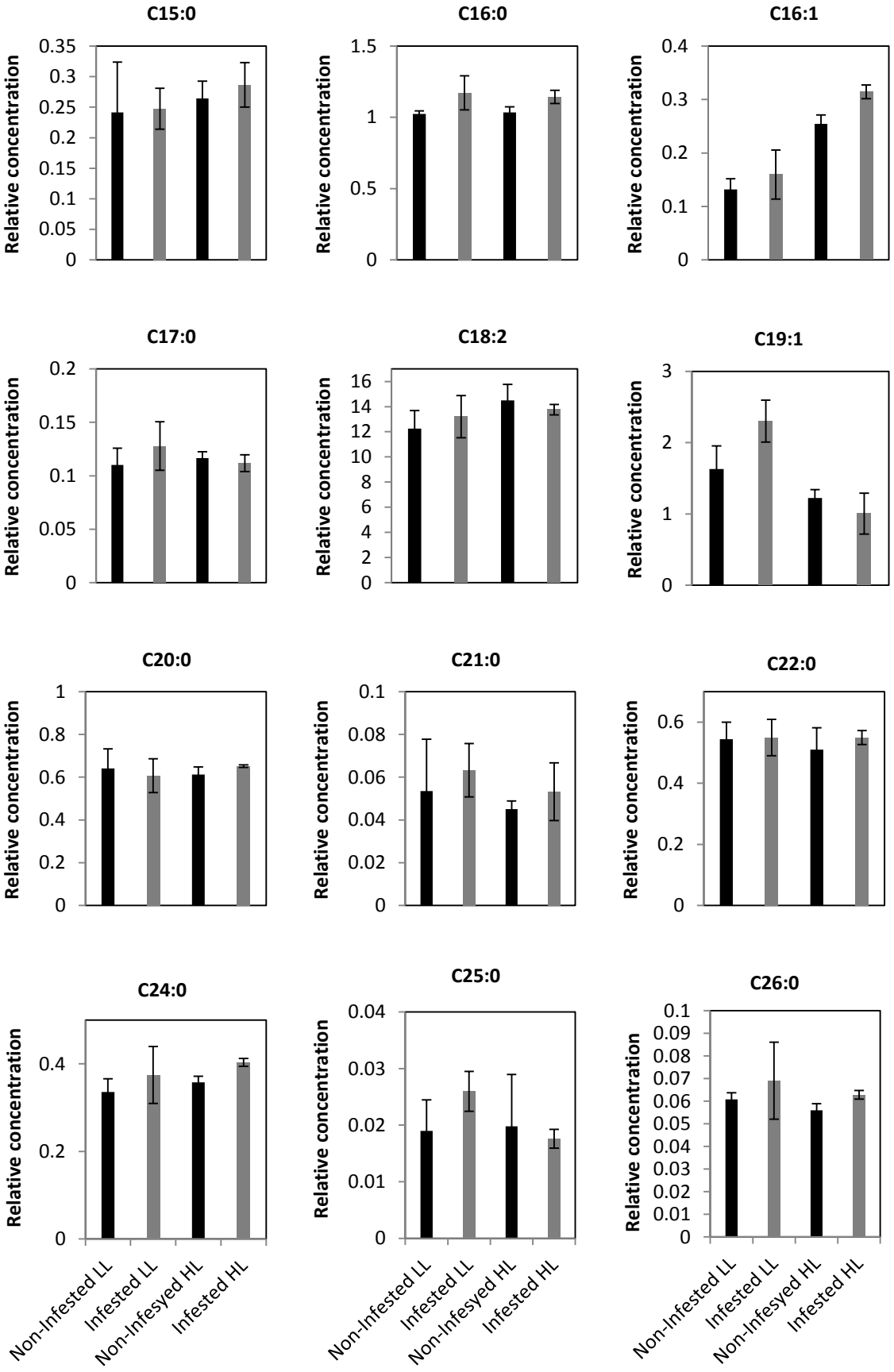
Appendix X Phytol changes in response to light availability in the infested and non-infested leaves of wild type, sense PAO and antisense TAO tobacco plants



Appendix XI Fatty acid changes in response to light availability in the infested and non-infested leaves of wild type tobacco plants



Appendix XII Fatty acid changes in response to light availability in the infested and non-infested leaves of sense PAO plants



Appendix XIII Fatty acid changes in response to light availability in the infested and non-infested leaves of antisense TAO plants