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Phloem Feeding Insect Stress and Photosynthetic Gene Expression

Anna-Maria Botha[1], Leon van Eck[2], Carlo S. Jackson[3], N. Francois V. Burger[1,3] and Thia Schultz[1]
[1]*Department of Genetics, Stellenbosch University, Stellenbosch,*
[2]*Department of Soil and Crop Sciences, Colorado State University, Ft Collins, CO,*
[3]*Department of Genetics, Forestry and Agriculture Biotechnology Institute, University of Pretoria, Hillcrest, Pretoria,*
[1,3]*South Africa*
[2]*USA*

1. Introduction

The ability to photosynthesise (*i.e.,* to utilize solar energy for conversion into chemical energy) is a distinguishing characteristic unique to plants, algae and photoautotrophic bacteria. It is believed that photosynthesis was already well established at least 3.5 billion (Gyr) years ago in ancient organisms, with similar capabilities as that of modern cyanobacteria (Schidlowski, 1984, 1988; Blakenship, 1992). However, it is only much later (*i.e.,* between 2.3 to 2.7 Gyr ago), with the advent of oxygen-evolving photosynthesis, that advanced life became possible (Buick, 1992; Björn & Govindjee, 2009).

For sunlight to be converted into chemical energy, it must first be absorbed by organisms through the use of pigments. The primary light absorbing pigments, located in the thylakoid membrane of chloroplasts of eukaryotic cells, are Chlorophyll *a* (Chl *a)* and Chlorophyll *b* (Chl *b*). These pigments are located in the thylakoid membrane of the chloroplast, and absorb different light wavelengths so as to accumulate energy in the form of excited electrons. Secondary pigments, such as carotenoids (carotenes and xanthophyll), are located in the chloroplast membrane and outer membrane in order to absorb residual light wavelengths not efficiently absorbed by the primary pigments (Blankenship, 1992; Nelson & Yocum, 2006; Björn & Govindjee, 2009). This conversion of solar to chemical energy is a complex process and involves a large number of pigments and electron transfer proteins, collectively known as a photosynthetic unit (*i.e.,* photosynthetic reaction centre) (Buttner et al., 1992). In a photosynthetic system the pigments serve as an antenna, collecting light and transferring the energy to the reaction centre, where the reactions leading to chemical energy conversion take place.

The photosynthetic reaction centres, or the cores of light harvesting systems, consist of special protein-chlorophyll complexes which play a major role in the energy conversion process (Buttner et al., 1992). Oxygenic photosynthesis of chloroplasts involves two photosystems: the oxygen-evolving photosystem II (PSII) that originated from purple

bacteria and the ferredoxin reducing photosystem I (PSI) that originated from the green sulphur bacteria (Figure 1) (Xiong et al., 2000; Dent et al., 2001).

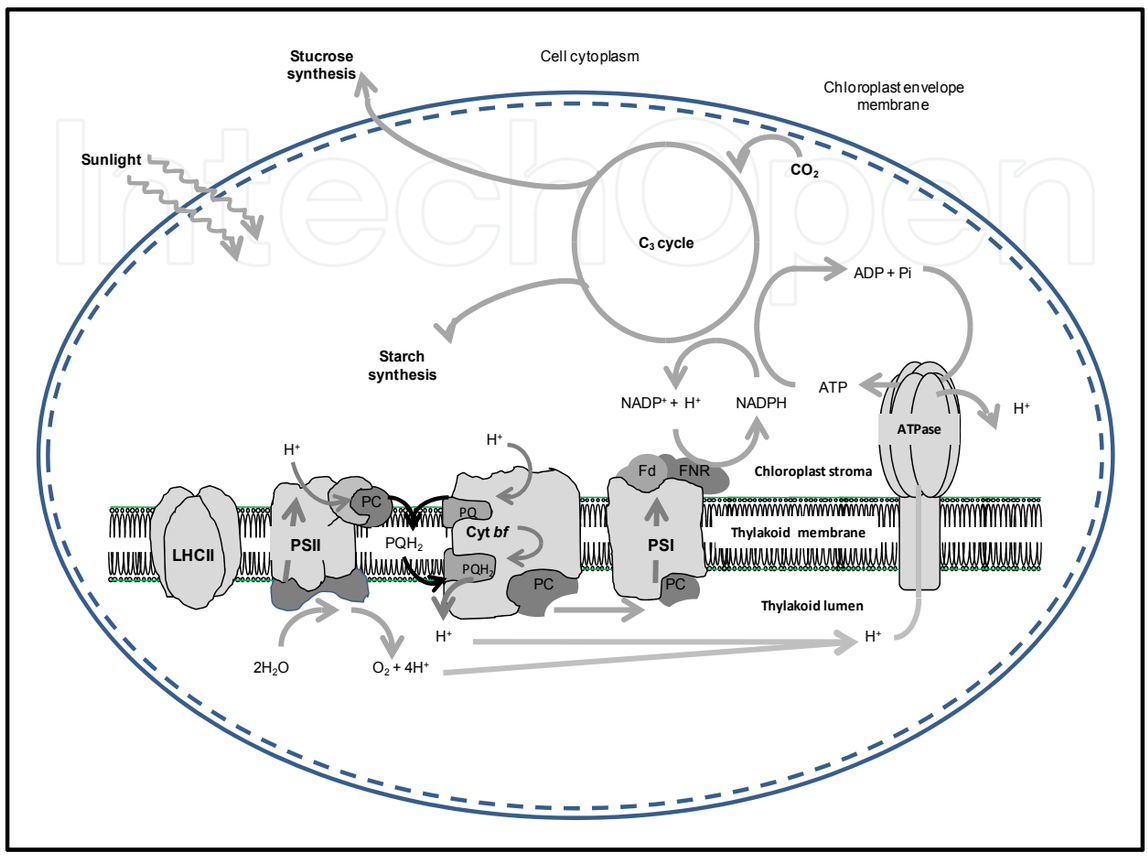

Fig. 1. Indicated are Photosystems I and II's location and their respective functions. The thylakoid membrane with PSI and PSII are indicated with the energy flow through the Calvin cycle (Modified from Dent et al., 2001).

Photosystem I (PSI) reaction centre complex consists of 6 polypeptides containing two of subunit I, which associates with P700, subunit PSI-D, subunit PSI-E, quinones and fluorenones. TMP14 thylakoid membrane phosphoprotein (14 kDa), a novel subunit of the plant PSI (Khrouchtchova et al., 2005) is found second, after PSI-D, as phosphorylation subunits of PSI (Hansson & Vener, 2003).). It is probably involved in the interaction with LHCII and together with PSI-D ensures PSI's function by accepting electrons from PSII (Khrouchtchova et al., 2005). Photosystem I P700 is bound by PsaA and PsaB in PSI and function as the primary electron donor. PSI converts photonic excitation into a charge separation, which transfers an electron from the donor P700 chlorophyll pair to the spectroscopically characterized acceptors A0, A1, FX, FA and FB in turn. Photosystem I P700 induction ensures electron excitation and reduction might force the synthesis of reactive oxygen intermediates (ROIs) for the hypersensitive response (*i.e.*, oxidative burst during plant defence) (Grotjohann & Fromme, 2005). Each PSI P700 antenna molecule consists of twenty chlorophyll *a* molecules and a cytochrome 522 heme (Bengis & Nelson, 1977). PSI utilises photons at 700 nm wavelength to excite electrons collected from its antenna

molecule P700. The electrons produced by PSI are transferred to PSII, where it is excited, captured by ferredoxin and finally used to reduce $NADP^+$ to NADPH. ATP is produced from ADP and pyrophosphate via chemiosmosis. The energy for this process is produced by three hydrogen ions, which supply the energy by passing from the thylakoid to the stroma of the chloroplast. Both ATP and NADPH are subsequently used in the light-independent reactions of the PSII complex, to convert carbon dioxide into glucose using the hydrogen atom extracted from water by PSII, and releasing oxygen as a by-product (Fromme, 1996; Nelson & Yocum, 2006).

Photosystem II (PSII) reaction centre complex on the other hand consists of D-1 and D-2 polypeptides, five chlorophyll *a*, two pheophytin *a*, one *B*-carotene, and one or two cytochrome *b*-559 heme- molecules (Nanba & Satoh, 1987). In PSII, the P680 reaction centre captures photons, and the light energy is used for oxidation (splitting) of water molecules. Upon electron release, the water molecule is broken into oxygen gas and released into the atmosphere. The resulting hydrogen ions are then used to power ATP synthesis. The electrons, excited at the antenna molecule P680, are passed down a chain of electron-transport proteins while receiving extra electrons from PSI. More hydrogen ions are pumped across the membrane as these electrons flow down the chain providing more protons for ATP synthesis. Chloroplast ATP synthase (cpATPase) is found to be essential for photosynthesis (Maiwald et al., 2003) by playing a direct role in the translocation of protons across the membrane as a key component of the proton channel. Nine different polypeptides make up the cpATPase, which consists of intrinsic CF_0 and extrinsic CF_1 segments. The F-type ATPase CF_1 segment functions as the catalytic core and the CF_0 segment functions as the membrane proton channel (Cramer et al., 1991; Groth & Strotmann, 1999). The electrons are then transported as NADPH molecules to enzymes that build sugar from water and carbon dioxide (Nanba & Satoh, 1987).

2. Photosynthetic genes respond to biotic stressors

Plants are constantly locked in an evolutionary arms race with their biological attackers, whether they be viral, bacterial or fungal pathogens, parasitic plants or herbivorous insects, therefore imposing the need to evolve defensive strategies to overcome this onslaught. Although there are a few examples of compensatory stimulation of photosynthesis (Trumble et al., 1993), most reports suggest that a decline in photosynthetic capacity is inevitable and this may represent the "hidden fitness costs" to defence (Fouché et al., 1984; Zangerl et al., 2002, 2003; Heng-Moss et al., 2003; Bilgin et al., 2008, 2010; Nabity et al., 2009). A recent study by Bilgin et al. (2010), reported that photosynthesis associated genes were down-regulated in seven different dicotyledonous and one coniferous plant species upon exposure to twenty different forms of biotic damage, regardless of the type of biotic attack. The hosts seem able to down-regulate these genes as an adaptive response to biotic attack, since a reduction in gene expression does not necessarily translate into loss of function.

Once invasion by an attacker has been recognized, through the detection of various effectors (either pathogen-associated molecular patterns (PAMPs), microbe-associated molecular patterns (MAMPs), viral coat proteins or insect salivary elicitors), the host must balance competing demands for metabolic resources between either supporting defence versus sustaining cellular maintenance, growth and reproduction (Berger et al., 2007a,b). Plant defence can be costly in terms of plant growth and fitness (Tian et al., 2003; Zavala &

Baldwin, 2004), as in addition to the mobilization of an array of defensive strategies (*i.e.*, up-regulation of a suite of defence response genes and production of chemical defence responses), the plant usually also has to cope with a reduction in effective biomass, and a decline in photosynthetic capacity in the remaining leaf tissue (Zangerl et al., 2002; Bilgin et al., 2008, 2010; Nabity et al., 2009).

2.1 Phloem-feeding insects

Phloem-feeding insects (PFI), on the other hand selectively down-regulate the expression of only certain photosynthesis-related genes (Heidel & Baldwin, 2004; Voelckel et al., 2004; Zhu-Salzman et al., 2004; Qubbaj et al., 2005; Yuan et al., 2005; Van Eck, 2007) (Table 1), and by manipulating the host carbohydrate metabolism, induce a change in carbon flux to their own advantage (Zhu-Salzman et al., 2004).

Regulated gene	Host species	Aphid species	Platform[1]	Ref[2]
PSI P700 apoprotein	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries/cDNA arrays	1
PSI reaction centre SU2/SU IV	*Apium graviolens*	*Myzus persicae*	Oligonucleotide arrays	2
	Sorghum bicolor	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
	Arabidopsis thaliana	*Bemisia tabaci*	Oligonucleotide arrays	4
PSI antenna & assembly proteins	*Apium graviolens*	*Myzus persicae*	Oligonucleotide arrays	2
	Triticum aestivum	*Diuraphis noxia*	SSH libraries	5
PSI chain D precursor	*Sorghum bicolor*	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
PSII 5 kD protein	*Arabidopsis thaliana*	*Bemisia tabaci*	Oligonucleotide arrays	4
PSII 10 kD protein	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	6
	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2
PSII protein DI	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries	5
PSII LS1 protein	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries	7
	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2

[1] Where cDNA-AFLPs = cDNA-amplified fragment length polymorphism and SSH = suppression subtractive hybridization

[2] 1, Botha et al., 2006; 2, Divol et al., 2005; 3, Park et al., 2005; 4, Kempema et al., 2007; 5, Boyko et al., 2006; 6, Schultz, 2010; 7, Lacock et al., 2003; 8, Voelckel & Baldwin, 2004; 9, Zhu-Salzman et al., 2004; 10, Botha et al., 2010; 11, Qubbaj et al., 2005; 12, De Vos et al., 2005; 13, Van Eck, 2007; 14, Kuśnierszczyk et al., 2008; 15, Zaayman et al., 2009.

PSII O_2 evolving complex peptide	*N. attenuata*	*Myzus nitotianae*	cDNA microarrays	8
	Sorghum bicolor	*Schizaphis graminum*	Oligonucleotide arrays	9
	Triticum aestivum	*Diuraphis noxia*	SSH libraries	5
PSII type I chlorophyll *a*/*b* binding protein	*Sorghum bicolor*	*Schizaphis graminum*	Oligonucleotide arrays	9
	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2
	Sorghum bicolor	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
	Triticum aestivum	*Diuraphis noxia*	SSH libraries	5
PSII chlorophyll *a* binding protein psbB	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries	5
ATP synthase δ subunit	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries	7
	Malus domestica	*Dysaphis plantaginea*	cDNA-AFLP analysis	6, 11
	Arabidopsis thaliana	*Myzus persicae*	Oligonucleotide arrays	12
	Triticum aestivum	*Diuraphis noxia*	SSH libraries/cDNA arrays	1
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
Aconitate hydratase	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	13
Beta-glucosidase	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	6
	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2
Chloroplast carbonic anhydrase	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	6
	Sorghum bicolor	*Schizaphis graminum*	Oligonucleotide arrays	9
Chloroplast genome DNA	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries	7
Chloroplast 23S ribosomal RNA gene	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	6
Chloroplast 50S ribosomal protein L28	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10

Chloroplast precursor CH1C_ARATH 20 kDa chaperonin,	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
29 kD ribonucleoprotein chloroplast precursor	*Sorghum bicolor*	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
Ribosomal protein chloroplast-like	*Sorghum bicolor*	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
Chlorophyllase	*Apium graviolens*	*Myzus persicae*	Oligonucleotide arrays	2
Chlorophyll synthetase	*Sorghum bicolor*	*Schizaphis graminum*	Oligonucleotide arrays	9
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
Chloroplast 50S ribosomal protein L28	*Arabidopsis thaliana*	*Bemisia tabaci*	Oligonucleotide arrays	4
Chlorophyll A oxygenase (CAO)	*Apium graviolens*	*Myzus persicae*	Oligonucleotide arrays	2
Carbonic anhydrase	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide array	10
	Triticum aestivum	*Diuraphis noxia*	cDNA-AFLP analysis	6
Cytochrome P450	*Arabidopsis thaliana*	*Brevicoryne brassicae*	Oligonucleotide arrays	14
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2
	Triticum aestivum	*Diuraphis noxia*	cDNA-AFLP analysis	6
Cytochrome *c*1, *c*6 (ATC6) and/or B6	*Sorghum bicolor*	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
	Arabidopsis thaliana	*Bemisia tabaci*	Oligonucleotide arrays	4
	Sorghum bicolor	*Schizaphis graminum*	SSH libraries/cDNA arrays	3
Ferredoxin	*Sorghum bicolor*	*Schizaphis graminum*	Oligonucleotide arrays	9
	Sorghum bicolor	*Schizaphis graminum*	Oligonucleotide arrays	9
Ferredoxin-thioredoxin reductase	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10

	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	1
	Triticum aestivum	*Diuraphis noxia*	cDNA-AFLP analysis	6, 13, 15
Ferrochelatase	*Sorghum bicolor*	*Schizaphis graminum*	Oligonucleotide arrays	9
Fructose-1,6-bisphosphatase	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
	Triticum aestivum	*Diuraphis noxia*	cDNA-AFLP analysis	6
Malate dehydrogenase [NADP], chloroplast precursor	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Mg-chelate subunit chlH	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Monooxygenase 2	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	6
NADPH:quinone oxidoreductase	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Nonphototrophic hypocotyl 1b	*Apium graviolens*	*Myzus persicae*	Oligonucleotide arrays	2
Non-green plastid inner envelope membrane protein	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Phytochrome association *PAP2*	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Photolyaseblue-light receptor	*Triticum aestivum*	*Diuraphis noxia*	SSH libraries/cDNA arrays	1
Quinone oxidoreductase	*Triticum aestivum*	*Diuraphis noxia*	SSH Libraries	7
Red chlorophyll catabolic reductase gene	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	6, 13, 15
Ribulose-1,5-bisphosphate carboxylase /oxygenase LSU	*Malus domestica*	*Dysaphis plantaginea*	cDNA-AFLP analysis	11
	Nicotiana attenuata	*Myzus nicotianae*	Oligonucleotide arrays	8
	Triticum aestivum	*Diuraphis noxia*	SSH libraries/cDNA arrays	1

	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2
	Triticum aestivum	*Diuraphis noxia*	Oligonucleotide arrays	10
	Triticum aestivum	*Diuraphis noxia*	SSH libraries	7
	Triticum aestivum	*Diuraphis noxia*	cDNA-AFLP analysis	6
Ribulose-1,5-bisphosphate carboxylase/oxygenase SSU	*Nicotiana attenuata*	*Myzus nicotianae*	Oligonucleotide arrays	8
	Sorghum bicolor	*Schizaphis graminum*	Oligonucleotide arrays	9
	Apium graviolens	*Myzus persicae*	Oligonucleotide arrays	2
	Triticum aestivum	*Diuraphis noxia*	SSH libraries	7
	Triticum aestivum	*Diuraphis noxia*	SSH libraries/cDNA arrays	1
T51328 transcription initiation factor sigma5, plastid - specific	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Thioredoxin	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
TMP 14 kDa thylakoid membrane phosphoprotein	*Triticum aestivum*	*Diuraphis noxia*	cDNA-AFLP analysis	13
	Arabidopsis thaliana	*Bemisia tabaci*	Oligonucleotide arrays	4
Thylakoid luminal 15 kD protein	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Thylakoid luminal protein-related	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
Ubiquinol--cytochrome-*c* reductase	*Triticum aestivum*	*Diuraphis noxia*	Oligonucleotide arrays	10
	Triticum aestivum	*Diuraphis noxia*	cDNA-AFLP analysis	6

Table 1. Genes involved in photosynthesis under regulation after aphid feeding. Underlined genes are down-regulated, non-underlined genes are mostly up-regulated.

PFIs achieve these benefits, at some cost to their host by inducing genes, involved in carbon assimilation and mobilization, so as to increase their own sugar uptake, whilst at the same time depleting sugars and creating localized metabolic sinks (Moran & Thompson, 2001; Zhu-Salzman et al., 2004). PFIs also modify nitrogen allocation in their hosts by up-regulating genes involved in nitrogen assimilation. In particular, genes encoding enzymes required for the synthesis of tryptophan and other essential amino acids are up-regulated to fulfil to the dietary requirements of the PFI (Heidel & Baldwin, 2004; Zhu-Salzman et al., 2004; Botha et al., 2010).

2.2 Linking photosynthesis and plant defence

The linkage between photosynthesis and host defence was recently demonstrated by silencing two central photosynthetic proteins, *i.e.*, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase in *Nicotiana attenuata* using virus-induced gene silencing (VIGS) (Mitra & Baldwin, 2008). Silencing of these genes improved the performance of a native generalist (*Spodoptera littoralis*) and specialist (*Manduca sexta*) herbivorous larvae on transformed host plants (Mitra & Baldwin, 2008). Similarly, it was shown that independent silencing of the TMP 14 kDa thylakoid membrane phosphoprotein, PSI P700 apoprotein, and Fructose-1,6-bisphosphatase in near-isogenic (NILs) *Triticum aestivum* lines using VIGS, also affected host resistance to *Diuraphis noxia* in varying degrees (Jackson, 2010). In the latter study, no significant decrease in aphid fecundity was observed in the susceptible Tugela plants after silencing with any of the genes when compared with the uninfested Tugela plants (Table 2). Silencing with BSMV:TMP14 caused a significant increase in aphid fecundity when aphids were fed on the resistant Tugela-*Dn1* plants, while silencing with BSMV:FBPase caused an increase in aphid fecundity in resistant Tugela-*Dn2* plants. A significant increased aphid fecundity was also observed upon silencing of Tugela-*Dn2* plants with BSMV:P700, but in Tugela-*Dn1* plants no significant increase in aphid fecundity was observed (Table 2).

Treatment	**Susceptible NIL**	**Resistant NILs**	
	Tugela	**Tugela-Dn1[a]**	**Tugela-Dn2[b]**
Control	31.0 ± 1.82	14.3 ± 2.08	21.0 ± 2.64
BSMV:00	30.25± 2.62	15.0 ± 1.00	20.3 ± 2.08
BSMV:TMP14	34.4 ± 3.58	21.8 ± 2.63*	23.6 ± 2.06
BSMV:FBPase	33.8 ± 2.93	17.1 ± 2.04	26.2 ± 2.78*
BSMV:P700	34.0 ± 1.54	12.2 ± 1.17	24.8 ± 2.31*

*Significantly different from control ($P < 0.05$)
[a]SA1684/6* 'Tugela' (*Dn1*$^+$) – confers antibiosis to *Diuraphis noxia* (Wang et al., 2004)
[b]SA2199/6* 'Tugela' (*Dn2*$^+$) – confers tolerance to *Diuraphis noxia* (Wang et al., 2004)

Table 2. Summary of *Diuraphis noxia* fecundities when feeding on three near isogenic wheat lines before and after gene silencing. Aphid fecundity is indicated as number of aphids per plant 10 d.p.i. (n=3)(From Jackson, 2010).

2.3 Does the photosynthetic compensation of the wheat host form part of *Diuraphis noxia*'s defence strategy?

Diuraphis noxia feeding on susceptible wheat causes chlorosis (*i.e.,* longitudinal chlorotic streaking) and leaf rolling in the leaves of susceptible wheat (Figure 2). Leaf chlorophyll content is reduced by *D. noxia* infestation (Heng-Moss et al., 2003; Botha et al., 2006). This results in decreased photosynthetic potential and the eventual collapse of the plant (Burd & Burton, 1992).

Fig. 2. Wheat expressing the longitudinal chlorotic streaking phenotype associated with *Diuraphis noxia* susceptibility.

Aphid damage has historically been ascribed to a phytotoxin injected during feeding, which is responsible for chloroplast disintegration (Fouché et al., 1984). Although, such a phytotoxin has never been described or isolated, ultrastructural studies revealed limited chloroplast breakdown in the leaves of resistant cultivars after aphid feeding (Van der Westhuizen et al., 1998). Since cell fluorescence data has shown that *D. noxia* feeding causes reduced photosynthetic capacity even in intact chloroplasts (Haile et al., 1999), this chloroplast rupture mechanism seems unlikely. *D. noxia* feeding probably induces malfunctioning of the photosynthetic apparatus in the stacked region of the thylakoid membrane, but the exact site of interference has not been determined (Burd & Elliott, 1996; Heng-Moss et al., 2003). Chlorosis induced by *D. noxia* differs significantly from normal

chlorophyll degradation during leaf senescence (Ni et al., 2001). *D. noxia* feeding stimulates an increase in the activity of Mg-dechelatase, a catabolic enzyme that converts chlorophyllide *a* to pheophorbide *a* as the final step in the chlorophyllase pathway (Ni et al., 2001; Wang et al., 2004)

Total chlorophyll concentration assays indicate that *D. noxia* feeding causes a marked decrease in chlorophyll levels in Tugela, but that the reduction in the antibiotic near-isogenic line (NIL) Tugela-*Dn1* is much less severe (Botha et al., 2006). Since the phenotypes afforded by different *Dn* genes vary — *Dn1* confers antibiosis, *Dn2* tolerance and *Dn5* a combination of antibiosis and antixenosis (Wang et al., 2004) — it appears that the presence of these genes activate transcription of defence-related genes differently (Botha et al., 2008). Antibiotic Betta-*Dn1* plants are also unable to compensate for chlorophyll loss, which has been attributed to an increase in defence compound production. Tolerant Betta-*Dn2* plants have very stable chlorophyll content during *D. noxia* feeding, suggesting that they can compensate for chlorophyll loss in some way (Heng-Moss et al., 2003).

Chlorosis due to *D. noxia* infestation is thought to originate from interference with electron transport (Burd & Elliott, 1996; Haile et al., 1999; Heng-Moss et al., 2003; Botha et al., 2006). Susceptible wheat shows decreased levels of chlorophyll *a* upon infestation by *D. noxia* (Burd & Elliott, 1996; Ni et al., 2001; Wang et al., 2004) which indicates damage to PSI (Botha et al., 2006). If this is indeed the case, it has serious implications for susceptible wheat under aphid attack. PSI catalyzes the electron transport from plastocyanin to ferredoxin (Haldrup et al., 2003). This reduced ferredoxin pool is mostly employed in generating NADPH for CO_2 assimilation, but is also used in regulating the activity of, among others, CF_1-ATPase and several enzymes in the Calvin cycle (Ruelland & Miginiac-Maslow, 1999). Under-reduced ferredoxin directly diminishes the plant's ability to synthesize ATP and carbohydrates. Studies by Van Eck (2007) using qRT-PCR analysis of the CF_1-ATPase response to aphid feeding indicated an increased demand for ATPase transcripts as infestation progressed (Figure 3).

Since damaged PSI can no longer act as electron acceptor from PSII via the cytochrome $b_{6\text{-}f}$ complex, inefficient reoxidation of the reduced plastoquinone occurs, halting state transitions and resulting in an over-reduction of PSII (Burd & Elliott, 1996). This leads to photoinactivation of PSII, and thus the irreversible decline in functional PSII complexes, because the absorbed light energy exceeds the amount that can be employed in electron transport (Kornyeyev et al., 2006). An acute induction of a TMP 14 kDa thylakoid membrane phosphoprotein, a putative component of PSI, was observed in Tugela-*Dn2* after infestation with RWA (Table 1, Figure 3) which indicates transcriptionally regulated photosynthetic compensation (Van Eck, 2007).

An induction of TMP14 could be a strategy to overcome pest attack in order to keep PSI stable and energy production going, while a reduction of TMP14 might force energy to flow in a different direction. Thus, up-regulation of PSI complexes would ensure the integrity of electron transport from PSII during state 2 as well as increased levels of NADPH and possibly increased CO_2 assimilation. In growth tolerance experiments, the tolerant PI 262660 line containing the *Dn2* gene maintained vigorous growth during aphid infestation when compared to the susceptible Arapahoe and antibiotic PI 137739 line (Haile et al., 1999). It is suggested that increased photosynthetic capacity via up-regulation of photosystem components may provide a mechanism for passive resistance against *D. noxia* feeding (Botha et al., 2006).

2.4 Regulating plant homeostasis

The production of ROIs is a by-product of normal cellular processes, such as photosynthesis and respiration, but can also be produced in response to a variety of environmental conditions, *i.e.*, light, cold, drought, as well as pathogen and pest attack. The latter event is known as the hypersensitive response and has proven to be effective against sedentary insects, such as PFIs that target a specific tissue. However, for an effective hypersensitive response-based programmed cell death to occur, a cascade of events have to occur, including production of ROIs and associated downstream defensive responses. Indeed, increases in the activity of oxidative enzymes such as peroxidases, polyphenol oxidases and lipoxygenases were observed after *Diuraphis nox*ia feeding (Van der Westhuizen et al., 1998; Ni et al., 2000, 2001; Ni & Quisenberry, 2003). This increase occurs not only at the site of feeding, but it also spreads systemically (Van der Westhuizen et al. 1998). Thus, if this spreading is not kept at bay, it can be lethal to the host. ROIs are partially reduced forms of atmospheric oxygen (O_2), and typically result from the excitation of O_2 to form singlet oxygen O_2^1 or from the transfer of one, two or three electrons to O_2 to form, respectively, a superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) or a hydroxyl radical (HO^-). Unlike atmospheric oxygen, ROIs are capable of unrestricted oxidation of various cellular components and can lead to the oxidative destruction of the cell (Asada, 1999). A variety of mechanisms exist for the dissipation of excess excitation that may give rise to the generation of reductants and the production of ROIs, act as signalling agents, or to serve as alternative

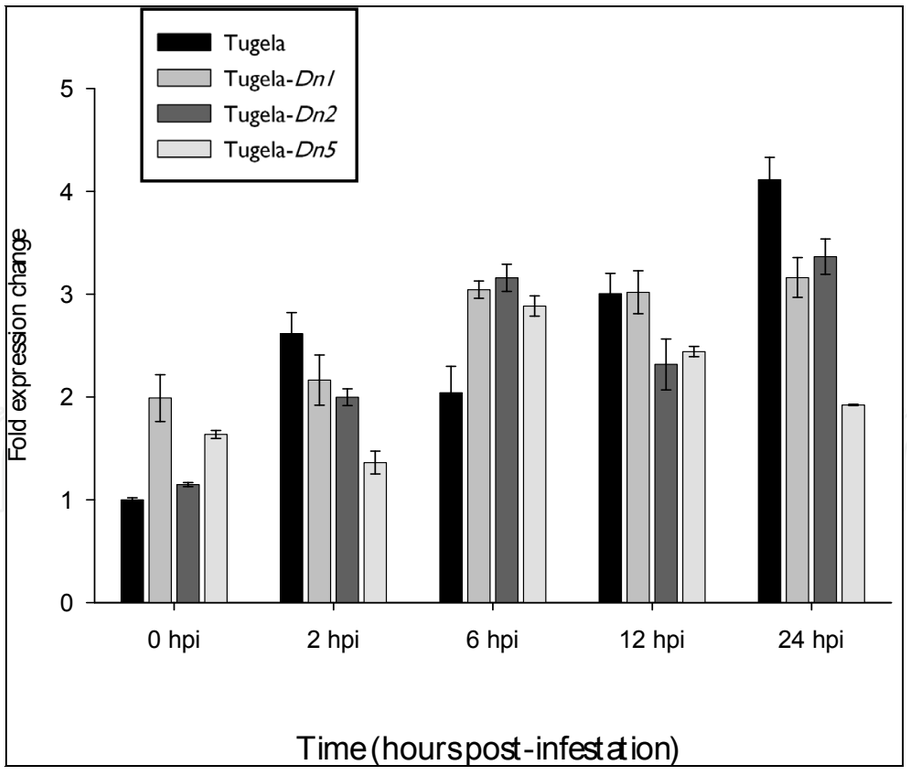

Fig. 3. qRT-PCR expression profiles of ATPase, where the expression level is calculated relative to the expression level of the uninfested, susceptible Tugela (at 0 h.p.i.) sample and is normalized to the expression of the unregulated chloroplast 16S rRNA transcript.

electron acceptors to avoid over-reduction and potentially the generation of toxic intermediates (Avenson et al., 2005; Mullineaux & Karpinski, 2002; Foyer & Noctor, 2005). It is thus suggested that cellular homeostasis will be maintained as long as the mechanisms for redox poising are in place, otherwise uncontrolled cellular damage will follow leading to death of the host (Schelbe et al., 2005).

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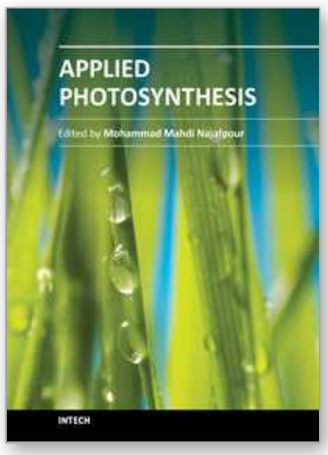

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Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. This book is aimed at providing applied aspects of photosynthesis. Different research groups have collected their valuable results from the study of this interesting process. In this book, there are two sections: Fundamental and Applied aspects. All sections have been written by experts in their fields. The book chapters present different and new subjects, from photosynthetic inhibitors, to interaction between flowering initiation and photosynthesis.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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