UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Profiling of wounding and *Diuraphis noxia* induced transcripts in hexaploid wheat using cDNA-AFLP analysis

BY

Thia Schultz

Submitted in partial fulfillment of the requirements for the degree

Magister Scientiae

In the Faculty of Natural and Agricultural Sciences

Department of Genetics

University of Pretoria

Pretoria

April 2010

Under the supervision of

Prof. AM Botha-Oberholster

Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the Degree Magister Scientiae to the University of Pretoria, contains my own independent work and has not been submitted for any other degree at any other University.

T Schultz

2009

Table of Contents

Chapter 3 Transcript profiling of wounded and Russian wheat aphid-infested Betta wheat NILs

ACKNOWLEDGEMENTS

The following people and organizations receive my earnest appreciation for their assistance during the completion of this research:

- Prof. AM Botha-Oberholster for her determination, support and enthusiasm without which this would not have been possible
- My colleagues in the Cereal Genomics Research Group: Adrene Laubscher, Mmampe Aphane, Francois Burger, Lerato Matsaunyane, Dirk Swanevelder, Leon van Eyk and Rosie van Zyl
- The Department of Genetics and the Forestry and Agricultural Biotechnology Institute (FABI) for providing infrastructure
- The Small Grain Institute (SGI), Bethlehem, for providing the germplasm used in this study
- The Winter Cereal Trust (WCT), National Research Foundation of South Africa (NRF) and the Human Resources and Technology for Industry Programme (THRIP) for financial assistance for this project
- My mother for her patience and quiet support

List of Abbreviations

AFLP	amplified fragment length polymorphism
CC	coiled coil
cDNA-AFLP	complementary DNA AFLP
Dn	*Diuraphis noxia*
GST	Glutathion-S-transferase
HR	hypersensitive response
ISR	induced systemic resistance
JA	jasmonic acid
LRR	leucine rich repeat
LZ	leucine zipper
MAPK	mitogen activated phosphokinase
MeJA	methyl jasmonate
MeSA	methyl salicylate
NBS-LRR	nucleotide-binding site leucine-rich repeat
NILs	near isogenic lines
NO	nitric oxide
OGA	oligogalacturonide acids
PCD	programmed cell death
PCR	polymerase chain reaction
PI	proteinase inhibitor
qRT-PCR	quantitative real-time PCR
ROI	reactive oxygen intermediates
ROS	reactive oxygen species
RWA	Russian wheat aphid
SA	salicylic acid

SAR	systemic acquired resistance
TDF	transcript-derived fragment
TIR	toll/interleukin-1/resistance

List of Figures

Figure 3.4.16: Graph (A) shows relative expression of the TDF 104 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine; image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M254.4) that was excised.

Figure 3.4.17: Graph (A) shows relative expression of the TDF 126 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine; image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M289.7) that was excised.

Figure 3.4.18: Graph (A) shows relative expression of the TDF 129 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine; image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M286.9) that was excised.

Figure 3.4.19: Graph (A) shows relative expression of the TDF 235 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine; image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M287.6) that was excised.

Figure 3.4.20: Graph of the qRT-PCR results showing fold change of TDF 92.

Figure 3.4.21: Graph of the qRT-PCR results showing fold change of TDF 104.

Figure 3.4.22: Graph of the qRT-PCR results showing fold change of TDF 126.

Figure 3.4.23: Graph of the qRT-PCR results showing fold change of TDF 129.

Figure 3.4.24: Graph of the qRT-PCR results showing fold change of TDF 235.

List of tables

Chapter 1 Introduction

The second most cultivated crop in the world next to rice is wheat. The demand for wheat and its products is rising in developing countries, and South Africa imported 1.2 Million Rand worth of wheat in 2008 as it only produces 66 % of the wheat needed for domestic consumption. One of the insects that cause large scale damage to wheat crops, resulting in a decrease in yield, is the Russian wheat aphid (*Diuraphis noxia*: Kurdjomov). Biotechnological advances hold obvious advantages in order to gain an understanding of the RWA-wheat interaction as genetic resistance does exist. In order to breed more resistant crops the causal agents of resistance need to be elucidated. Also, new biotypes of RWA have emerged, especially in areas were only one resistance gene was used. Thus more than one type of resistance is needed to combat the arms race.

The foundation of knowledge of many of the resistance responses known is sound, but it is not always possible to apply what is found in a model system to your plant of interest. Much of the research done to date has been on the model plant *Arabidopsis* which is a dicotyledonous species. Since the cereals belong to the monocotyledonous species, knowledge gained on *Arabidopsis* is not always applicable to cereals, i.e. rice and wheat. Also, the genomes of cereals are more complex and resistance loci, being under constant selection pressure, change continuously. Resistance loci are also evolutionarily divergent between monocots and dicots (Pan *et al*. 2000).

Also, there are still very large knowledge gaps about the underlying genetic mechanisms involved in the RWA-wheat interaction. The protein elicitor from RWA is yet to be elucidated, as well as the *Dn* genes. Thus far, the *Dn* gene and mode of resistance seem to be highly dependant on chloroplast function and speed of recognition (Botha *et al*. 2006; Heng-Moss *et al*. 2003; Macedo *et al*. 2003). In order to breed sustainable resistance against the RWA pest without applying too much selection pressure, various resistance genes must be incorporated into new wheat lines. As most of the resistance genes have not been characterized in any way other than chromosomal placement (Liu *et al*. 2001) and type of plant resistance conveyed, it is essential to study the biological systems involved in the different types of resistance in order to elucidate the genes involved.

Chapter 2 of this dissertation is comprised of an overview of literature pertaining to the wheat-RWA interaction. The symptoms of RWA infestation, a section on the aphid saliva and their possible connections are covered. General plant defence strategies are discussed as well as known genes conveying resistance. The modes of resistance conveyed by the different defence genes i.e. antibiosis, tolerance and antixenosis are explained.

Chapter 3 describes the use of transcript profiling to identify and isolate transcripts thought to be induced by wounding and RWA feeding in Betta wheat and it's near isogenic lines (NILs) BettaDN and Betta *Dn2*. These transcripts are analyzed using bioinformatics tools and further in depth analysis was done on five of the isolated transcripts, using quantitative PCR. Putative functions were assigned to identified transcripts in light of the wounding or RWA-defence related differences seen.

Chapter 4 is a summary of results including the possible pathways involved in the RWA resistance response in *Dn1* and *Dn2* containing wheat plants.

Appendix contains tables of relevant information used in the analysis of the clones such as the normalized cDNA-AFLP data and putative biological pathways. Unknown sequences were submitted to GenBank EST database (www.ncbi.nlm.nih.gov).

Preface

The findings presented in this dissertation represent the results of a study undertaken between Sept 2007 and July 2009 in the Department of Genetics, University of Pretoria, under the supervision of Prof. AM Botha-Oberholster. Chapter 3 is being prepared as a manuscript to be submitted for review to the journal *Cell and Plant Physiology*.

The following outputs resulted from the dissertation:

Sequences submitted to GenBank had the following accession numbers: GR881173, GR881174, GR881175, GR881176, GR881177, GR881178, GR881179, GR881180, GR881181, GR881182, GR881183, GR881184, GR881185, GR881186, GR881187, GR881188, GR881189.

Conference posters presented:

Schultz, T., Botha, A-M. (2008) Elucidation of defence pathways activated in Betta near isogenic wheat lines upon infestation by *Diuraphis noxia*. Proceedings of the International congress of Entomology 2008. p202

Schultz, T., Swanevelder, Z.H., Botha-Oberholster, A-M. (2006) Isolation and characterization of Diuraphis noxia induced transcripts in wheat using cDNA-AFLP analysis. Proceedings of the South African Genetics Society 2006. p42

Conference presentations:

Schultz, T., Botha, A-M. (2008) Characterization of resistance pathways activated by *Dn1* and *Dn2* resistance genes in Betta wheat infested by *Diuraphis noxia*. Proceedings of the South African Genetics Society 2008. p48

Papers published:

Botha, A.M., Swanevelder, Z.H., Schultz, T., Van Eck, L., Lapitan, N.L.V. Deciphering defense strategies that are elucidated in wheat containing different *Dn* resistance genes. 11th Wheat Genetics Symposium, Brisbane, Australia, 24-29th August 2008.

Chapter 2

Literature Review

Chapter 2 LITERATURE REVIEW

2.1 Wheat

Wheat is an important staple food in many countries. It is a highly versatile grain used in the manufacture of many products such as flour for breads and baking, pasta, brewing, and feeds. In the US 20.6 Million hectares were harvested in 2007 (United States Department of Agriculture, USDA, 2007a), and Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) has reported that about 110 million acres of wheat are grown in over 70 different developing countries. In total, world wide wheat production in 2007 equaled 2.07 billion bushels (USDA 2007a).

The use of wheat and its products is widespread in South Africa and in many other countries, which rely on imports to supply a major portion of their needs. South Africa imported 44 % of the wheat needed for domestic consumption in 2008 (Department of Agriculture, South Africa, 2008). Thus, any factors affecting wheat production can have a global effect. There are biotic and abiotic factors that negatively affect yield. In recent years, with instability in climatic conditions as well as the addition of new uses for this grain, the balance of wheat production and demand have become skewed. In 2008, it was reported that wheat stores worldwide became depleted, which caused a rise in the price of wheat and its products internationally. Countries such as Australia and Canada's production figures dropped for 2007 due to low rainfall and heat, while Northern Europe suffered losses due to excessive rainfall. Collectively that added up to a forecast of 4.2 Million tons lower production for the 2007/2008 season (USDA 2007b). World wide consumption was raised by 0.8 million tons in 2008/2009 (USDA 2009).

2.1.1 Genomics

Modern wheat is an allopolyploid, meaning that two or more different genomes were brought together in the same nucleus followed by a duplication event (Allaby *et al.* 1999). Wheat started as a diploid, called einkorn (AA). Emmer wheat, a tetraploid (AABB), was then bred and farmed in the Middle East. A modern day tetraploid wheat used for pasta is Durum wheat, and the wheat most used today,

Triticum aestivum (dinkel) is a hexaploid (AABBDD) with an estimated 16,000 Mbp of DNA (Arumuganathan and Earle 1991).

Because of its hexaploid genome, *Triticum* wheat is a challenge to study. The genome consists of three sets of 7 chromosomes, each set named AA, BB and DD respectively (2n=6x=42). The origins of the chromosomes are of great interest to some research groups, who have traced the chromosomal contents back to their possible progenitors. AA is thought to be from *Triticum urartu*, BB has it's closest though not ideal progenitor in the form of *Aegilops speltoides*. They hypothesize that AA and BB formed *Triticum turgidum* (BBAA) about 500 000 years ago, which then, after incorporation of DD from *Aegilops tauschii* (about 9,500 YA) formed *Triticum aestivum* (AABBDD) (Feldman and Levy 2005).

The model organism for monocotyledons is rice (*Oryza sativa*) and the first draft sequence of this 430 Mbp genome was released in 2002 (Yu *et al.* 2002). The 430 Mbp of rice vs. the approximately 16,000 Mbp of wheat is a considerable difference and it has been found that when comparing rice and wheat it would be difficult to compare non-conserved regions (La Rota *et al.* 2004). However, as so little sequence information is available for wheat genes, the sequencing of rice has made it possible to make putative identification of sequences obtained which is a big step forward.

2.2 Resistance systems in plants

2.2.1 Gene-for-gene hypothesis: Plant innate immunity

Plants have a wide range of defence mechanisms to defend themselves against a pathogen or pest attack. Flor H.H. (1971) proposed the 'gene-for-gene' model for plant resistance to pathogens. It states that plant resistance will only occur if there is a dominant 'Resistance gene' (*R*-gene) product present in the plant, which interacts with a corresponding dominant 'Avirulence' gene (*Avr* gene) product present in the pathogen. Therefore, for a disease response to a specific pathogen or pest to occur, recognition of the pathogen's or pest's attack is required which will then induce a response. If

the pathogen or pest is not recognized, there is no response from the plant and disease, infection or infestation can continue unhindered and the plant is rendered susceptible. There are many interactions, pathways and organelles involved in a defence response, and a few of the major ones will be covered in this section.

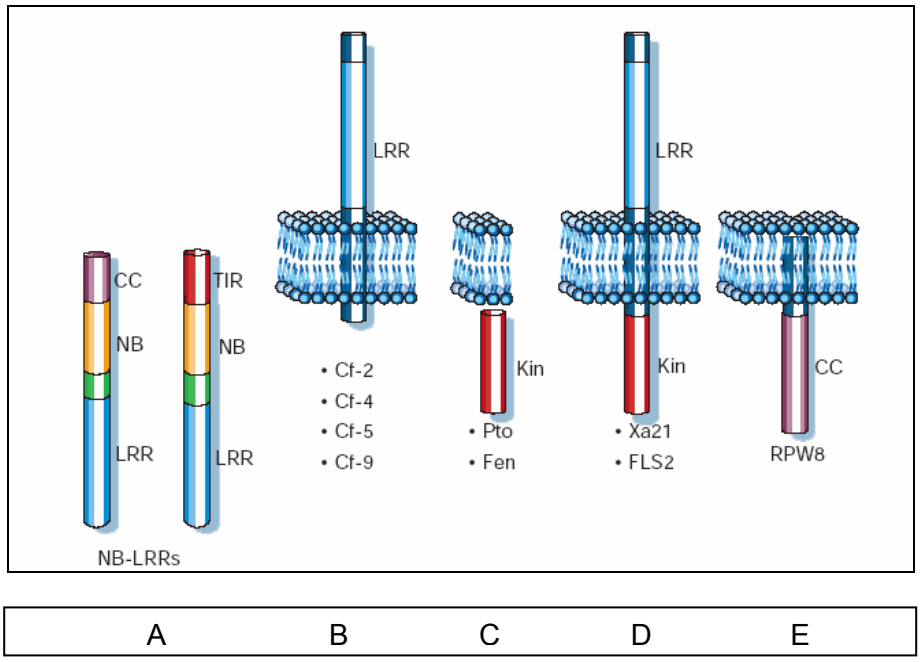


Figure 2.1: Figure representing the five classes of R proteins and their possible placement within the cell (Dangl and Jones 2001).

Resistance (*R*) genes have been found in many different plants such as tomato (Scofield *et al.* 1996; Tang *et al.* 1996), rice (Silué *et al*. 1992; Song *et al*. 1995; Wang *et al*. 1999) and *Arabidopsis* (Bisgrove *et al*. 1994; Century *et al*. 1997). The genes are specific, and react to a specific type of pathogen; from fungi (Silué *et al*. 1992), bacterial (Scofield *et al.* 1996; Tang *et al.* 1996) and viral (Cockerham 1970) infections to insects and nematodes (Rossi *et al*. 1998). There are five main plant R protein structural categories (Dangl and Jones 2001; Martin *et al*. 2003) (Figure 2.1). Each LRR repeat motif is about 24 amino acids long and contains hydrophobic amino acids with the main contingent being leucine with a consensus sequence of PxxLLxxxxxLxxLxLxxNxLxxL (Takahashi *et al*.

1985), and is thought to be one of the key regions in determining the specificity of a R protein to its target pathogen (Wang *et al*. 1998).

Other consensus sequences commonly found in R proteins (Figure 2.2) are nucleotide binding sites (NBS) which could bind ATP or GTP. The LRR motif is also found in conjunction with serine/threonine protein kinases, e.g. in Xa21 (Figure 2.1.D) (Song *et al.* 1995). A leucine zipper (LZ) has been found at the N-terminus before the NBS domains e.g. *Prf* from the tomato Prf-AvrPto interaction encodes a LZ-NBS-LRR (Figure 2.1 C) (Salmeron *et al*. 1996). A toll/interleukin-1/resistance (TIR) or a coiled coil (CC) domain can also be found at the N-terminus (Figure 2.1 E). An example of TIR domain containing proteins is that of the *RPP1* genes in *Arabidopsis* which convey resistance to *Peronospora parasitica* (downy mildew) (Botella *et al*. 1998). The TIR domain, which is not found in monocotyledons (Pan *et al.* 2000), is thought to be involved in plant recognition of some Avr proteins (Ellis *et al*. 1999; Burch-Smith *et al*. 2007). The mammalian homologues recognize diverse molecules from lipopolysaccharides (Poltorak *et al*. 1998) and flaggelin (Hayashi *et al*. 2001) to dsRNA (Alexopoulou *et al*. 2001).

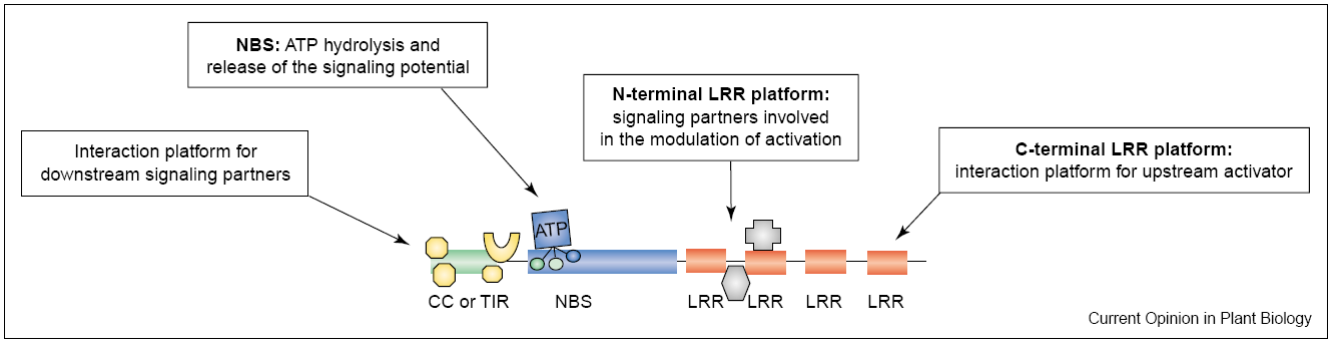


Figure 2.2: Figure representing an R protein in linear form (Belkadir *et al.* 2004).

One of the first well documented examples of a direct Avr-R protein interaction was the AvrPto-Pto interaction between *Pseudomonas syringae* avirulence protein avrPto and the tomato Pto R protein (Scofield *et al.* 1996; Tang *et al.* 1996). *Pto* encodes the first class of R proteins and does not have the

LRR motif (Figure 2.1.A) and encodes for a member of the serine/threonine kinase family of proteins that does not have a LRR domain. It was shown that the amino acids between residues 129 and 224 (Tang *et al.* 1996) or 190 and 213 were responsible for accurate binding of avrPto (Scofield *et al.* 1996). These Avr-R protein interactions are race and pathogen specific (Scofield *et al.* 1996; Aarts *et al.* 1998), and the binding of an Avr protein results in a defensive plant response that halts the spread of the pathogen.

The Avr-proteins that the R proteins recognize are usually required for the pathogens fitness or pathogenicity (Leach and White 1996), e.g. *avrBs2* which is conserved among all *Xanthomonas campestris* strains (Kearney and Staskawicz 1996). The different Avr virulence proteins have been found to be targeted to different parts of the plant cell, depending on the pathogens mode of attack and their respective R proteins are also found in corresponding areas of the cell from the nucleus (Deslandes *et al.* 2003) to the cytoplasm (Figure 2.1 C) (Scofield *et al.* 1996) and cell membrane (Figure 2.1 B) (Nimchuk *et al.* 2000).

The ‘arms race’ between pathogens and plants is a theory in which change in one e.g. plant, will put selection pressure on the pathogen. This, in turn, may cause the pathogen to adapt to the plants new defence, putting the pressure back on the plant to adapt (Dawkins and Krebbs 1979). R proteins and Avr-molecules may interact in this manner, as a reinstatement of pathogenicity has been shown to occur on previously resistant plants by a single nucleotide change in *Avr* sequence (Joosten *et al.* 1994). However, in a review by Bergelson *et al*. (2001) they are of the opinion that the arms race is a poor fit for the R-Avr protein interaction as the *R* gene alleles are not young and the loci are not monomorphic. The actual interaction between different R and Avr-proteins is not fully understood. It was originally thought that the R-Avr interaction is direct, but there are few examples of this (Dodds *et al.* 2006; Jia *et al.* 2000; Scofield *et al.* 1996; Tang *et al.* 1996; Ueda *et al.* 2006). This has lead to the development of other theories, such as the ‘guard hypothesis’ (van Der Biezen and Jones 1998).

In the guard hypothesis, it is stated that there is an interaction between proteins belonging to the pathogen and the plant, which is sensed by the plant causing a resistance response (Axtell *et al*. 2003; Burch-Smith *et al*. 2007; Shao *et al*. 2003) e.g. an Avr protein may try to hi-jack a plant protein for use by the pathogen, changing it's confirmation, and thereby setting off the R protein defence cascade (van Der Biezen and Jones 1998). An example of this type of interaction is the interaction between the *N*-gene (Whitham *et al.* 1996) from tobacco and the tobacco mosaic virus (TMV). N is a TIR-NBS-LRR type R protein (Whitham *et al.* 1996). The TMV Avr protein was shown to be a replicase protein, containing a helicase, and was named p50 (Erickson *et al.* 1999). The presence of p50 is necessary and sufficient for a HR response to occur. N and p50 were shown to be soluble and localized to both the cytoplasm and nucleus (Burch-Smith *et al.* 2007). The requirements for a resistance response from the plant is the presence of N in the cytoplasm and nucleus, and that all three domains of N (TIR, NBS and LRR) must be functional, as a mutation in any one of the three negates the response. The domain responsible for association with p50 is the TIR domain though a direct interaction could not be proven (Burch-Smith *et al.* 2007).

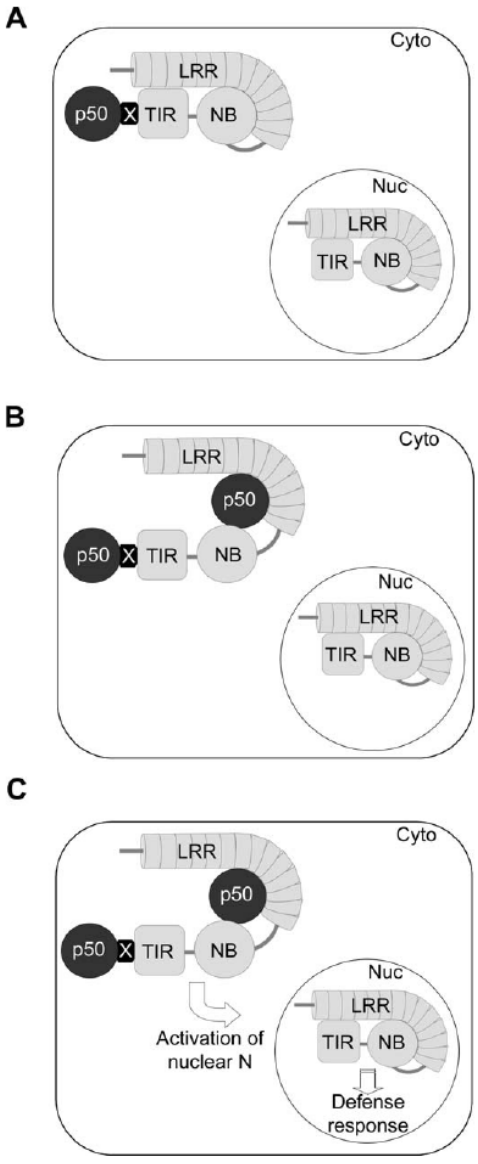


Figure 2.3: Model for N-protein mediated resistance response in tobacco (Burch-Smith *et al*. 2007).

A new model was proposed (Figure 2.3) in which p50 interacts with a host protein X (Figure 2.3A), and then this complex interacts with the TIR domain. This

leads to a structural reorganization of N, allowing p50 to bind directly to the NB and LRR domains (Figure 2.3B). This then starts the signalling cascade which moves into the nucleus and begins the defence response. The presence of N in the nucleus is necessary for a response to occur, however the last step, localized in the nucleus has not yet been clarified (Figure 2.3C) (Burch-Smith *et al*. 2007).

2.2.2 The wound response

Plants are sessile organisms which have had to evolve complex molecular defence mechanisms in order to protect themselves. Wounding caused by mechanical means, pathogens or pests results in either a direct defence via transcription and translation or an indirect defence (Nelson and Ryan 1980; Parsons *et al*. 1989) and accumulation of defence related proteins and signalling molecules (Casaretto *et al*. 2004; Green and Ryan 1974; Parsons *et al*. 1989). There is a difference between mechanical and herbivore induced wound defence (Howe and Jander 2008). The major molecules involved in the wounding response are jasmonic acid (Farmer and Ryan 1990), ethylene (O'Donnell *et al*. 1996), polysaccharides (Walker-Simmons *et al*. 1983), systemin (Pearce *et al*. 1991) and absisic acid (Peña-Cortéz *et al*. 1989).

Jasmonic acid (JA) is a lipid based signalling molecule known to initiate transcription (Weidhase *et al*. 1987; Kazan and Manners 2008), and it's volatile derivative methyl jasmonate (MeJA) is thought to spread a 'panic' signal between different plants (Farmer and Ryan 1990; Seo *et al*. 2001) in order to start a resistance cascade in neighboring plants before the pathogen or herbivore moves onto its next host. The wounding response may be separate and independent to *R*-gene mediated resistance, but systemic acquired resistance (SAR) may be activated by JA in some cases (Cohen *et al*. 1993; Schweizer *et al*. 1998). Senescence has been found to be caused by the application of methyl jasmonate (Ueda and Kato 1980). JA is produced from α-linolenic acid by a chloroplast based lipase, with the key enzymes of the pathway being lipoxygenase (LOX) (Vick and Zimmerman 1983), allene oxide synthase, and allene oxide cyclase. LOX2, found in the chloroplast, was found to be a prerequisite for production of JA in response to wounding in leaves of *Arabidopsis* (Bell *et al.* 1995).

An increase in LOX activity has been shown to increase resistance to insects such as aphids (Deng *et al*. 1992). LOX activity also increased in the presence of methyl jasmonate in the vacuoles of soybean seedlings (Franceschi *et al*. 1991). Franceschi *et al*. (1991) suggests that methyl jasmonate may affect source-sink relationships within a plant, by increasing the rate of nitrogen assimilation and amino acid storage.

Wounding induces the production of the proteinase inhibitor (PI) proteins which are produced locally (Green and Ryan 1972) and systemically (Green and Ryan 1972; Parsons *et al*. 1989). Trypsin protease inhibitor activity is induced by application of JA exogenously (Casaretto *et al*. 2004) and proteinaceous inhibitors of serine endopeptidase activity are released after wounding in potato (Pearce *et al*. 1982) and tomato (Nelson and Ryan 1980). There are two main proteinase inhibitors that were concentrated on in early studies, proteinase inhibitor I and II. Proteinase inhibitor I is an inhibitor of the endopeptidase chymotrypsin while proteinase inhibitor II inhibits both chymotripsin and trypsin (Bryant *et al*. 1976). Fatty acid based signalling molecules such as jasmonic acid (JA) and its ocadecanoid pathway precursors (Farmer and Ryan 1992) as well as polysaccharides (Walker-Simmons *et al*. 1983) also initiate PI production. PI proteins are thought to accumulate in order to inhibit gut proteases of the insect at the location of attack or wounding (Green and Ryan 1972). This may act as a deterrent to insect attack as digestibility of the leaf food is a factor in host selection (Saxena 1969).

Ethylene is not a causal agent of PI protein production in tomato plants (Ryan 1974) however it is required for the wound response (O'Donnell *et al*. 1996). JA and ethylene are needed in conjunction to activate transcription of *pin* PI genes (O'Donnell *et al*. 1996) and defensin (Penninckx *et al*. 1998). They can work independently from salicylic acid (SA) and the SA pathway to induce systemic resistance (Penninckx *et al*. 1996). JA induces the production of ethylene and ethylene action is thought to regulate JA levels in the plant to some extent. However, JA levels are only partially lowered

by the absence of ethylene, therefore it was postulated that there are two pathways that activate JA production, i.e. ethylene is dependant on the ethylene response pathway (O'Donnell *et al*. 1996).

Systemin, a polypeptide found in tomato (Pearce *et al.* 1991) induced the production of both proteinase inhibitor I and II. Systemin is 18 amino acids long, and was first isolated using high-performance liquid chromatography (HPLC), and was shown to move through the plant as a systemic signal in response to wounding.

A new term encompassing herbivore derived elicitors is 'herbivore associated molecular patterns' or HAMPs. These HAMPs include all herbivore derived causal agents that may come into contact with and/or interact with a plant. Examples of HAMPs are oral secretions and oviposition fluid (Mithofer and Boland 2008).

As in pathogen-plant interactions there are both direct and indirect defence responses to HAMPs. An example of an indirect defence is one where plant volatiles, released from cabbage plants when fed on by caterpillars (*Pieris brassicae*) were found to be an attractant to specific parasitic wasps (*Cotesia glomerata*). It was found that the larval regurgitant contained β-glucosidase, which elicited the release of the volatiles (Mattiacci *et al*. 1995).

A more direct defence response would be one where the plants defence systems are activated. In an experiment by Shmelz *et al*. (2006), the oral secretions of fall armyworm (*Spodoptera frugiperda*) feeding on cowpea (*Vigna unguiculata*) were examined, and a disulphide bonded peptide was isolated and named inceptin. At low levels inceptins were found to induce the production of ethylene, JA and SA. Inceptins were found to be proteolyzed fragments of plant chloroplast ATP synthase, ingested by the armyworm and then regurgitated. The plant recognized the altered form of the chloroplast ATP synthase in a guard-like manner and then induced a defence response.

A caterpillar pest, *Helicoverpa zea,* was been found to have a large percentage of glucose oxidase in it's saliva during feeding. It was suggested that the enzyme may function by; altering host plant defence responses, altering midgut O_2 and also has possible antimicrobial activity (Eichenseer *et al*. 1999).

In an experiment by Nelson and Ryan (1980) PI proteins released in wounded tomato leaves were studied. The PI proteins accumulate in the vacuole. It was suggested that wounding induces the release of a hypothetical hormone proteinase inhibitor-inducing factor (PIIF) that initiates the transcription of the PI proteins. It was further suggested that the function of PIIF is either to activate a signalling cascade that initiated transcription of the PI proteins, or that PIIF is directly involved in post-transcriptional activation of the PI transcripts. In a paper by Bishop *et al*. (1981), they found that a fungal endopolygalacturonase purified from *Rhizopus stolonifer* was able to degrade a tomato PIIF fraction into oligosaccharides. When these oligosaccharides were applied to tomato leaves, they induced the production of proteinase inhibitor I. They also showed that active polygalacturonase enzymes present in the tomato plant are able to digest tomato cell walls and tomato PIIF into PIIF-active oligosaccharides. This led to the hypothesis that either these hydrolytic enzymes are activated by cell wall damage, or introduced by the attacking pathogen or pest, which produces the olgosaccharides which are then able to act as intercellular or vascular messengers in order to activate the production of PI proteins. The active oligosaccharides named oligogalacturonide acids (OGAs) are fragments of plant cell wall pectin, and the size of the fragments determines the specificity of activation, e.g. a mixture of small OGAs was found to activate the synthesis of ethylene in tomato (O'Donnell *et al*. 1996).

2.2.3 Hypersensitive response

The hypersensitive response (HR) is a resistance response that requires recognition of an attack whether through wounding, or specific recognition of a pathogen elicitor (Maclean *et al*. 1974; Huang *et al*. 1988). It is thought of as the plants first induced line of defence (Apostol *et al*. 1989). It is

controlled by the plant and is a form of programmed cell death (PCD) (Dietrich *et al.* 1994; Greenberg *et al.* 1994; Levine *et al*. 1994). HR is one of the earliest responses which aim to limit the spread of a pathogen (Apostol *et al*. 1989; Brisson *et al*. 1994), and the rapid deployment of reactive oxygen species (ROS) associated with this is a highly controlled process, both spatially and temporally (Dietrich *et al.* 1994; Greenberg *et al.* 1994). The main effect of HR is death of the cells around the point of attack which is known as a necrotic lesion. HR attempts to limit pathogen establishment by killing off all cells, surrounding the area of attack (Maclean *et al*. 1974).

The HR is programmed by the plant, and initiated after a recognition event. Evidence for this is as follows; firstly, both transcription and translation are required for HR (Levine *et al*. 1994; Marineau *et al*. 1987). Secondly, purified preparations of a pathogen can elicit a HR event when applied to a resistant plant (Huang *et al*. 1988), but is not observed if the same preparations are applied to a susceptible plant (Cockerham 1970). And lastly, it has been found that mutations in a single gene can induce HR resulting in a lesion (Dietrich *et al*. 1994; Greenberg *et al*. 1993; Greenberg *et al*. 1994).

The production of reactive oxygen species (ROS) accompanying the HR was first described by Apostol *et al*. (1989). They described the rapid release of hydrogen peroxide (H_2O_2) which is also necessary and sufficient to cause cell death although it is threshold dependant (Tenhaken *et al*. 1995). One of the first steps in the production of ROS is an accumulation of superoxide ($O_2^{\cdot-}$) which is necessary but not sufficient for HR dependant cell death (Jabs *et al*. 1997).

Superoxide's characteristics include being unstable, on the whole non-toxic and cannot easily cross cell membranes. Superoxide dismutase [EC: 1.15.1.1] can convert superoxide to the more toxic hydrogen peroxide (H_2O_2) (Figure 2.4) which can easily cross membranes (Tenhaken *et al*. 1995). Oxidative cross-linking of proline-rich cell membrane proteins is activated by the presence of H_2O_2 (Brisson *et al*. 1994). Cross-liking in the cell walls is thought to be a key step in the hypersensitive

response as it toughens the cell walls to attack by hydrolytic enzymes (Apostol *et al*. 1989; Brisson *et al*. 1994; Levine *et al*. 1994; Tenhaken *et al*. 1995).

Roles for reactive oxygen intermediates (ROIs) such as H_2O_2 may include killing host or pathogen cells, as well as acting as signalling molecules (Apostol *et al*. 1989; Levine *et al.* 1994). Glutathion-S-transferase (GST) is produced in quantity by H_2O_2 treatment and it was found that H_2O_2 mediates induction of GST (Apostol *et al*. 1989; Levine *et al.* 1994). Superoxide has been found to be monitored by *LSD1*, a gene which is involved in initiating and limiting the spread of cell death (Jabs *et al.* 1996). And superoxide has also been found to be necessary and sufficient for phytoalexin synthesis (Jabs *et al.* 1997).

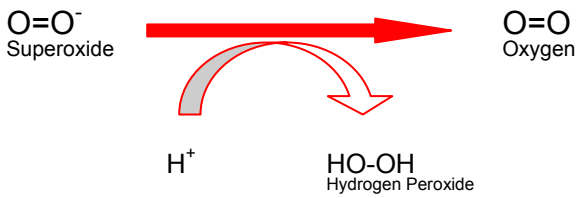


Figure 2.4: Mode of action of the enzyme superoxide dismutase [EC:1.15.1.1].

Another of the fast response molecules released with ROIs is nitric oxide (NO) (Delledonne *et al*. 1998). There are two waves of NO production recorded in an experiment by Floryszak-Wieczorek *et al*. (2007) involving ivy-leaved pelargonium and necrotrophic fungi. The first induction occurred within five minutes of inoculation with three times more NO being produced by the resistant cultivar in comparison with a non-resistant cultivar. This is followed by a second wave of induction which flows outwards from the initial point of necrosis in resistant cultivars only. H_2O_2 was detected only in the point of necrosis in resistant cultivars. NO was found to stimulate the production of antioxidants in resistant cultivars assisting in keeping cellular homeostasis. In the susceptible cultivars a spread of disease occurred, there was an increased H_2O_2 synthesis but only at a later time point of 48 hours,

which was followed by uncontrolled NO production in colonized tissue, adding to the damage caused by the necrotrophic pathogen.

In soybean, NO and H_2O_2 are both needed for HR related necrosis to occur as NO is not sufficient to cause cell death alone (Delledonne *et al*. 2001) and it was shown in *Arabidopsis* that NO production does not occur early enough to be an HR trigger (Zhang *et al*. 2003). Levine et al. (1996) shows that H_2O_2 stimulates calcium (Ca^{2+}) flux which in turn activates the production of GST. And they propose that Ca^{2+} signalling plays an important role in the activation of PCD.

The HR is not always present during an incompatible interaction. In a paper by Yu *et al*. (1998), a *dhd1* mutant Arabidopsis plant was found that produces SA constitutively. This plant showed gene-for-gene resistance against *Pseudomonas syringae* but did not show extensive cell death associated with the HR.

2.2.4 Systemic acquired resistance and induced systemic resistance

Systemic acquired resistance (SAR) has been known of and studied since the early 1900s with the first review written by Chester (1933). After the HR response or recognition event, SAR is activated. SAR is not a localized event, but a broad spectrum defence response that is activated throughout the plant. One of the main signalling molecules for SAR is salicylic acid (SA) (Malamy *et al*. 1990), however; it is not an intercellular or mobile messenger (Vernooij *et al*. 1994). The build-up of SA levels is necessary for SAR to occur (Gaffney *et al*. 1993), and the change in SA concentrations in the cell is known to affect the redox state of the cell (Chen *et al*. 1993; Mou *et al*. 2003).

The SAR phloem mobile signal is methyl salicylate (MeSA) (Park *et al.* 2007). MeSA is derived from salicylic acid and activates transcription factors involved in the production of numerous defence genes (Park *et al.* 2007). It is also possible that a lipid based molecule i.e. methyl jasmonate (MeJA) (Ueda and Kato 1980) may contribute to the initiation of the SAR cascade (Park *et al*. 2007; Truman *et al.*

2007). Moreover, both MeSA and MeJA may be responsible for the spread of the SAR response in nearby plants (Farmer and Ryan 1990; Shulaev *et al.* 1997).

Table 2.1: Table showing pathogenesis related protein families (Van Loon and van Strien 1999).

Family	Type member	Properties	Gene symbols
PR-1	Tobacco PR-1a	unknown	*ypr1*
PR-2	Tobacco PR-2	β-1,3-glucanase	*ypr2,* [Gns2 (Gib')]
PR-3	Tobacco P,Q	chitinase type I, II, IV, V, VI, VII	*ypr3, Chia*
PR-4	Tobacco R	chitinase type I, II	*ypr4, Chid*
PR-5	Tobacco S	thaumatin-like	*ypr5*
PR-6	Tomato inhibitor I	proteinase inhibitor	*ypr6, Pis* ('*Pin*')
PR-7	Tomato P69	endoproteinase	*ypr7*
PR-8	Cucumber chitinase	chitinase type III	*ypr8, Chib*
PR-9	Tobacco lignin forming peroxidase	peroxidase	*ypr9, Prx*
PR-10	Parsley 'PR1'	ribonuclease-like	*ypr10*
PR-11	Tobacco class V chitinase	chitinase type I	*ypr11, Chic*
PR-12	Radish Rs-AFP3	defensin	*ypr12*
PR-13	Arabidopsis THI2.1	thionin	*ypr13, Thi*
PR-14	Barley LTP4	lipid transfer protein	*ypr14, Ltp*
PR-15	Barley oxalate oxidase	oxalate oxidase	*Ypr15, pHvOxOa,. pHvOxOb*
PR-16	Barley oxalate oxidase-like	oxalate oxidase-like protein	*Ypr16, pHvOxOLP*
PR-17	Tobacco PRp27	unknown	*Ypr17, HvPR-17a, HvPR-17b, NtPRp27, WCI-5*

Many defence related proteins are transcribed during a defence reaction. Pathogenesis related (PR) proteins, defensins and antimicrobial peptides, making up the bulk, occur very soon after attack (Van Loon *et al*. 2006). PR proteins (Antoniw *et al*. 1980) are part of several families of proteins, such as the β-1,3-glucanases and chitinases. By 2002, seventeen different PR protein families had been identified (Table 2.1) (Christensen *et al*. 2002; Görlach *et al*. 1996; Okushima *et al*. 2000; van Loon and van Strien 1999; Wei *et al*. 1998; Zhou *et al*. 1998). Although PR proteins are associated with SAR they are not produced with every type of resistance response. Induced systemic resistance (ISR) is associated with infection by non-pathogenic rhyzobacteria and is SA independent (Pieterse *et al*. 1996). Rhizobacterial infections show none of the symptoms of HR or SAR. However, functioning JA and ethylene pathways are necessary for ISR (Pieterse *et al*. 1998).

SAR and ISR are both dependant on *NPR1* gene function (Pieterse *et al*. 1998; Spoel *et al*. 2003) as a mutation in *NPR1* blocks SA sensitivity (Cao *et al*. 1994). Mou *et al*. (2003) found that the NPR1 protein had different confirmations before and after treatment with SAR inducers. A NPR1 containing oligomer held together by disulphide bonds was found in uninduced tissue. After reduction, the oligomer released NPR1 monomers. These monomers then moved into the nucleus. It was thus their findings that the accumulation of SA induced a change in redox potential effecting a reducing environment. The reducing environment acted on the NPR1 oligomer, releasing NPR1 monomers, which could then move into the nucleus. The monomers could then interact with transcription factors to induce the production of PR proteins.

2.3 The Russian wheat aphid

Several fungal infections and herbivore pests can lead to major losses in wheat crops (Du Toit 1987; Pritsch *et al*. 2000). One of the sap-sucking insects responsible for the loss of millions of US Dollars annually in wheat yield is the Russian wheat aphid (*Diuraphis noxia:* Kurdjomov). After introduction in the U.S., the damage done to crops in just two years, from 1987-1989, was estimated to be $600 Million U.S. Dollars (U.S. Congress Office of Technology Assessment, OTA, 1993).

This tiny insect was first recorded as a pest of wheat and barley in Southern Russian and the Meditertanean (Walters 1984), and has spread to all cereal producing countries around the world except for Australlia (Quisenberry and Peairs 1998). The RWA has been named as one of the highest impacting non-indigenous species by the office of technology assessment (U.S. congress OTA, 1993). The Russian wheat aphid (RWA) was first reported as a pest of wheat in South Africa in the late 1970's (Du Toit 1978). Since then, 8 new biotypes have developed in the USA, the first appearing in 2003 in Colorado (Haley *et al.* 2004) and one other biotype has developed in South Africa (Boshoff and Du Toit 2006). New biotypes have also been recorded in other parts of Africa (Malinga *et al*. 2007), Asia (Dolatti *et al*. 2005), Europe (Basky 2003) and South America (Smith *et al*. 2004). Biotypes were determined by screening the RWA populations on known RWA resistant cultivars, as the new biotypes were able to feed and cause chlorosis damage on previously resistant cultivars (Burd *et al*. 2006). The ability of the RWA to evolve new biotypes due to the resistance genes bred into wheat lines (Basky 2003), suggests that breeding wheat with only one antibiotic type resistance e.g. *Dn4*, may accelerate aphid diversification in the arms race.

2.3.1 Physiology and life cycle

The Russian wheat aphid (*Diuraphis noxia*) is a small, pale yellow-green, spindle-shaped insect from the Aphidoidea family (Walters *et al*. 1980). The RWA occurs in two female forms, wingless or apterous, and winged or alate. These insects during most of the year reproduce as wingless females using parthenogenic cycles; giving birth to live, clonal young without male fertilization. If conditions become unfavourable to the RWA survival, winged females will develop to allow the aphid to find a better environ or host. RWA females give birth to live nymphs, which in turn are able to produce nymphs within two weeks. After becoming mature, these females can produce up to four nymphs per day (Walters *et al*. 1980). In ideal conditions, an aphid may be the start of an exponentially expanding population of sucking insects which will devastate a crop (Prescot *et al*. 1986).

2.3.2 Aphid feeding and saliva

The RWA is host species specificand primarily resides on wheat (Figure 2.5) and barley (Blackman and Eastop 2000) food sources where it is uniquely adapted to be able to subsist on the high sugar and low amino-acid containing phloem. One of the factors involved in this specialization may be the aphid's saliva. Different aphid species are specialized in their host choice and the effects of infestation on a plant will differ between aphid species e.g. one plant may be resistant to RWA but not to greenbug (*Schizaphis graminum*; Rondani). Different chromosomal origins of resistance apply to the RWA and greenbug respectively, though some are shared (Castro *et al.* 2001). This means that the severity of the symptoms of infestation may be determined by the toxicity of the aphid saliva in that plant, and will also determine if a host is suitable for a specific aphid (Miles 1999).

Figure 2.5: RWA feeding on susceptible wheat (Courtesy of Swanevelder, 2008).

An aphid will preferentially target an area on the wheat leaves where they can intercept nutrients either being imported, as is the case with younger leaves, or exported (Dixon 1976). The first type of saliva that is produced, described as a 'solid proteinaceous deposit', is used to create a hard coating around a stylet, forming a protective sheath (Miles 1967) for probing through-out the apoplast (Matsilisa an Botha 2002) towards the phloem contained in the sieve tubes. Matsilisa and Botha (2002) found evidence to the fact that the aphid (*Sitobion yakini*) preferentially target thin walled sieve tubes

in barley (Figure 2.6) which they thought to perhaps exert a higher osmotic potential due to the high concentration of sucrose present in the tubes. The second type of saliva is a watery, or non-gelling, and is used during the probing process and while feeding (Miles 1965).

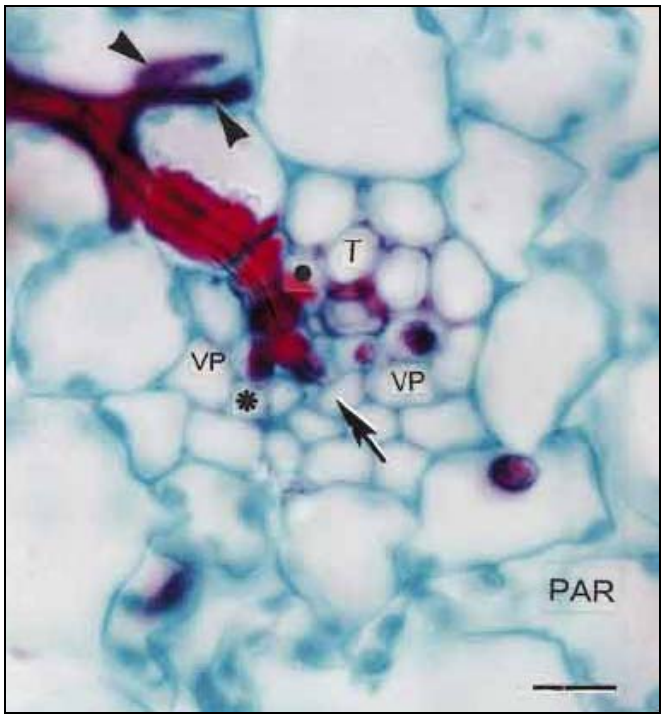


Figure 2.6: From Matsila and Botha (2002) in barley, showing multiple probing sites of *Sitobion yakini* stylet (arrow heads) before coming into contact with thin walled sieve elements (arrow). VP: vascular parenchyma, T: tracheary element, PAR: mesophyll parenchyma.

The insoluble stylet sheath and non-gelling saliva of aphids have been found to be made from different components excreted by different salivary glands. The employment of each salivary gland was assigned as follows: the lateral lobe salivary gland is responsible for the sheath material, the posterior lobe produces the digestive enzymes of the watery saliva and the accessory lobe produces a polyphenol oxidase which is possibly responsible for 'rapid discharges of water in the saliva' (Miles 1967).

2.3.3 Aphid saliva and the defence response

RWA infestation initiates a hypersensitive response in resistant wheat lines (Ni *et al.* 2001; Van der Westhuizen *et al*. 1998). When comparing the response of wheat plants, to injections of RWA extracts and buffer, a distinct reaction is seen which is similar to that seen when the RWA feeds (Laptitan *et al*. 2007a) which has lead researchers to believe that there is a salivary factor that is initiating a gene-for-gene resistance response (Botha *et al*. 2006). There are two main stages of salivary exposure; the first being during probing and the second during feeding (Miles 1999).

The spread of RWA salivary compounds into the plant phloem and thus into the rest of the plant has not been measured. In a study on greenbugs, (*Schizaphis graminum*: Rondani), by Burd (2002) the insects were fed on indole-3-acetic acid-1-^{14}C and ^{14}C-sucrose. The salivary components of the greenbug could thus be traced through the plant and were found to translocate, to both shoots and roots, from the origin of feeding. A similar study on RWA is necessary, as symptoms of infestation differ greatly between greenbug and RWA (Burd 2002).

A range of enzymes have been characterized from the aphid gut, saliva and sheath. A catechol oxidase and a peroxidase have been identified in the gut of the rose aphid. These gut enzymes are thought to convert ingested phenolics, effectively detoxifying them and perhaps other allelochemicals as well (Peng and Miles 1991). Peroxidase (Px) [E.C. 1.11.1.7] activity has also been identified from aphid saliva, along with polyphenol oxidase (PPO) [E.C. 1.10.3.1]. Both oxidoreductases were found in the stylet sheath of *Sitobion avenae* (Fabricius), while only the PPO, was found in the watery saliva. The PPO was found to be exuded during probing, and to react with all phenolics tested, from catechol to chlorogenic acid. The oxidoreductases may therefore have a protective role in detoxifying allelochemicals that the plant may produce in response to aphid probing (Urbanska *et al.* 1998). In sheath material, protein, phospholipids, amino acids, free sulphydryl groups and polyphenol oxidase have been found (Miles 1965; Miles 1967). In the watery saliva, enzymes such as amylase, cellulose, pectin polyglacturonase, proteinase and peroxidase along with metabolites such as amino acids have

been found (Miles 1965). The protein component from RWA is what interacts with the plant defence systems, in order for an incompatible reaction to occur (Lapitan *et al.* 2007a).

Aphid saliva induces a SAR response specific to the RWA. Plants previously infested by bird cherry-oat aphid (*Rhopalosiphum padi*) were avoided by *D. noxia* and *R. padi* in preference to control plants. While plants previously infested by *D. noxia* did not affect *R. padi* settling. It was also noted that the growth rate of RWA was not affected by previous *R. padi* infestation. Effects of the aphids infestation was therefore species specific, and it could be assumed that different species specific defence responses were initiated, for e.g. *R. padi* is more sensitive to hydroxamic acids than *D. noxia* (Messina *et al.* 2002).

In a study by Kruger and Hewitt (1984), the rate of photosynthesis was measured after injection with RWA extract. They found an initial increase in oxygen evolution, followed by a decrease over time. They ascribed the increase to partial destruction of the thylakoid membrane which resulted in an initial uncoupling of photophosphorylation, which stimulated electron flow. The decrease that followed was attributed to further deterioration of the membrane and photosystems. They then suggested that aphid extract can act as an uncoupler of photophosphorylation. This in turn causes the thylakoid membranes to become more permeable, upsetting the proton gradient which is responsible for phosphorylation, and the cycle ends with the destruction of the chloroplasts and chlorophyll.

A hypothesis was formed by Miles and Oertli (1993) called the ‘redox hypothesis’ which states: “ The oxidative processes in healthy plants are subject to control by reducing systems and that the insect enzymes served to change the natural redox equilibria in the immediate food source to the insects advantage.”

It is known that allelochemicals such as polyphenols are transported by the phloem (Peng and Miles, 1991). Phenolics that are pre-made and stored in various plant tissues, e.g. plant vacuoles and cell

walls (Hutzler *et al*. 1998), and may play an important role in plant-defence strategies (Beckman 2000). Many of the functions of phenolics, and their by-products, are related to defence e.g. the production of volatiles such as jasmonic acid (JA). The release of phenolics during wounding or attack acts as a caustic feeding deterrent, and lastly the phenolics can act in order to convey resistance against pathogens, i.e. during lignification. Polyphenoloxidase (PPO) [EC 1.10.3.1] in plants is a chloroplast thylakoid membrane embedded enzyme that has the ability to convert *O*-diphenols to *O*-diquinones. PPO also hydroxylates monophenols into diphenols. Oxidation of phenolics causes the 'browning' seen in insect infested tissues. After grain aphid (*Sitobion avenae* F.) infestation on various wheat lines, Chrzanowski et al. (2003) found that PPO activity was decreased across all cultivars, in a cultivar specific manner.

Phenolics need to be in their oxidated form to be toxic as they are able to cross link with other molecules and initiate free-radical chain reactions (Appel 1993). In order for plants to keep them in a reduced form, antioxidants i.e. ascorbate (Vitamin C) or glutathione-S-transferase (GST), are oxidized in their place and recycled by plant reductases, e.g. in the presence of hydrogen peroxide (Takahama and Oniki 1997). Without the presence of the antioxidants or after the store of antioxidants is depleted, the polyphenols would oxidize (Takahama and Oniki 1997), as is the case when the phloem is removed from the plant via insect feeding. When polyphenols are oxidized, several products can be formed. The first products formed are quinines which have the ability to copolymerize with proteins (van Fleet 1954), which would be toxic in the gut of an insect. Peng and Miles (1988) found that aphids fed on an artificial diet could oxidize toxic phenol monomers to non-toxic polymers while feeding. As the plant may produce more polyphenols as part of the defence mechanism (Peng and Miles 1988), the salivary oxidases are needed to 'oxidize potentially toxic phenolics to non-toxic end products en route to the midgut' (Miles and Oertli 1993). RWA was also found to be little affected by hydroxamic acids (Messina *et al*. 2002) and this may be due to the presence of peroxidase in the aphids' saliva (Miles 1999). The oxidases present in the aphid saliva and gut are therefore an

important and necessary component of aphid saliva, and may be one of the eliciting factors of plant recognition.

It has been found that a drop in sieve tube pressure, such as would occur during sieve tube occlusion by PR proteins, alters aphid feeding behavior. The drop in pressure results in the halt in aphid feeding, and the start of salivation (Will *et al.* 2008). This supports the hypothesis that some of the watery saliva components may be involved in keeping sieve elements unblocked (Walters 1980).

2.4 Wheat and the Russian wheat aphid

2.4.1 Symptoms of infestation

Symptoms of RWA feeding are unique when compared to some of the other types of aphids i.e. greenbug, *Schizaphis graminum*, (Rondani). Feeding on susceptible wheat, causes necrotic spots surrounded by chlorosis which radiate from their feeding sites whereas the RWA causes streaked chlorosis (Walters *et al*. 1980). Infestation on susceptible varieties stops new leaves from unrolling, which helps protect the aphids from predators and chemical sprays (Figure 2.7 B) and interaction with its host also increases the aphids' cold tolerance (Butts *et al.* 1997). In susceptible varieties of wheat, RWA feeding leads to the breakdown of chloroplasts which causes white and yellow longitudinal streaks to appear. This symptom is called chlorotic streaking (Figure 2.7 B) (Walters 1980). Grain heads can become trapped, interfering with grain formation. Plants also start becoming weak and flaccid, with few leaves keeping upright (Figure 2.7 A) due to loss of turgor (Walters *et al*. 1980). Some aphids have been accused of spreading viruses such as the barley yellow dwarf virus (A'Brook and Dewar 1980), but after surveying the RWA in South Africa, no viral particles have been found (Walters 1986).

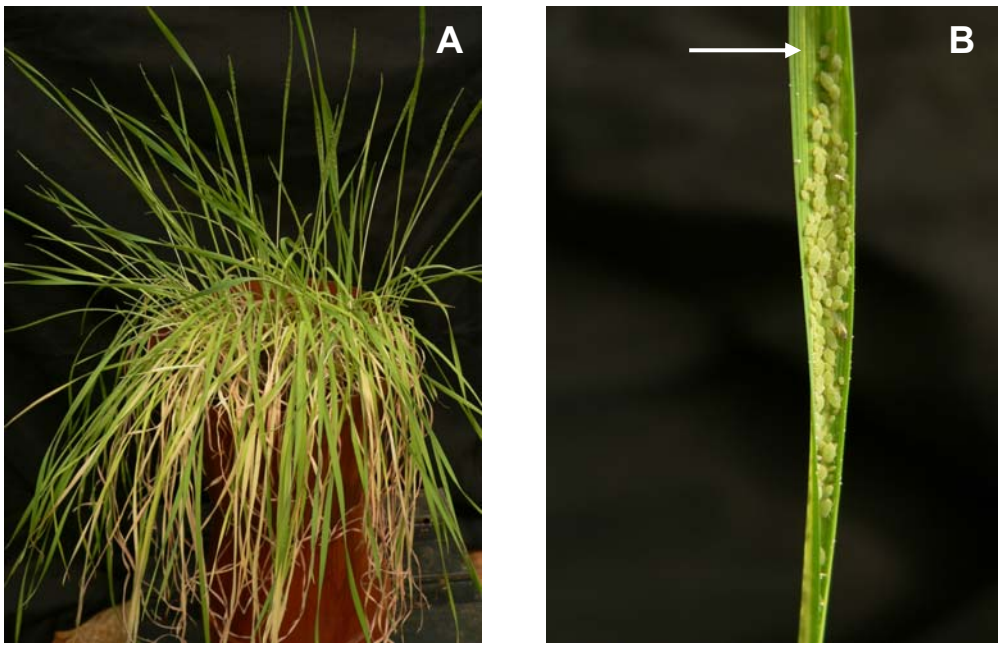


Figure 2.7: A) Weak, flaccid wheat leaves with heavy aphid infestation, **B)** Wheat leaf with heavy infestation showing chlorotic streaking (arrow) at the top of the figure and leaf rolling at the bottom

2.4.2 Existing genetic resistance

The RWA resistance response seen in wheat plants is specific (Botha *et al*. 1998), and can be compared to the responses seen against pathogens such as fungal and bacterial infections (Baker *et al*. 1997; Van der Biezen and Jones 1998). In 1987, Du Toit found two RWA resistant wheat accessions, PI 137739 and PI 262660. Using a backcrossing program, it was found that the both resistant lines had a single dominant gene which conveyed resistance, and named these genes *Dn1* and *Dn2* (Table 2.2) respectively (Du Toit 1989). Since then, resistance to RWA has been found in many wheat lines (Table 2.2), but none of the *Dn* genes have been fully characterized on a molecular level.

Three main types of resistance are conveyed by the *Dn*-genes; antibiosis, antixenosis and tolerance (Painter 1958). Tolerance is the ability of the plant to thrive even though there is an aphid infestation

(Painter 1958). Highly associated with tolerance were substitutions on chromosomes 1A, 1D and 6D (Castro *et al.* 2001). In an experiment by Quisenberry and Schotzko (1994) a *Dn2* containing wheat line was found to be tolerant because, while the plants showed leaf rolling and chlorosis, they still had high plant growth and dry weight and moisture measurements. The *Dn1* containing wheat line, after a time period of infestation, showed fewer aphids (dead or alive) indicative of a slower reproduction and therefore of antibiosis. Antixenosis (*Dn5*) is associated with non-settling or non-preference of the host by the insect. Antixenosis has been found to be linked to the group 7 chromosomes and in substitution lines with chromosomes 2B, 6A and 7D (Castro *et al.* 2001). And there is an indication that antixenosis is dependant on multiple genes (Castro *et al.* 2001). Antibiosis will affect aphid fecundity and longevity. The substitution line 1B was found to have the greatest effect on these characteristics (Castro *et al.* 2001).

Table 2.2: A summary of the *Dn* genes and their mode of action.

Accession number	Gene	Inheritance	Resistance type	References
PI 137739	*Dn*1	Single Dominant gene	Antibiosis	Du Toit (1989) Budak *et al.* (1999) Quisenberry and Schotzko (1994)
PI 262660	*Dn*2	Single Dominant gene	Tolerance	Du Toit (1989) Budak *et al.* (1999) Quisenberry and Schotzko (1994)
Triticum tauchii	*dn*3	Single recessive resistance gene		Nkongolo *et al.* (1991)
PI 372129	*Dn*4	Single dominant gene		Quick *et al.* (1991) Liu *et al*. (2002)
PI 294994	*Dn*5	Not known for definite. Multiple gene resistance Varying combinations of Dominant and recessive alleles have been found.	Moderately antibiotic	Marais and du Toit 1993 Zang *et al.* (1998) Budak *et al.* (1999)

PI 243781	*Dn*6	Single dominant gene	Tolerance	Harvey and Martin 1990 Saidi & Quick (1996) Liu *et al*. (2002) Lazzari *et al*. (2009)
1RS Translocation from Rye	*Dn7*	Single rye translocation	Antixenotic Moderately antibiotic	Marais *et al.* (1998) Anderson *et al*. (2003)
PI 294994	*Dn8*			Liu *et al*. (2001)
PI 294994	*Dn9*			Liu *et al*. (2001)
PI220127	*Dnx*	Putative serine/threonine kinase	Tolerance Antibiosis	Harvey and Martin (1990) Boyko *et al*. (2006)

2.5 Newest findings

In a study by Botha *et al*. (2006) it was suggested that RWA resistance is a gene-for-gene reaction, but also that the maintenance of photosynthetic function is one of the main factors determining survival of a resistant wheat line attacked by RWA. Macedo *et al*. (2003) found that RWA damage on susceptible wheat was a light activated process as no damage was reported in constant dark conditions.

Chlorophyll and cartenoid concentrations have been found to be affected by RWA feeding (Heng-Moss *et al*. 2003). Near isogenic lines (NILs) containing the *Dn1* and *Dn2* resistance genes were tested for chlorophyll and cartenoid concentrations along with aphid fecundity. *Dn2* was found to induce higher levels of chlorophyll and cartenoids under infestation conditions in contrast to *Dn1* which showed no differences (Heng-Moss *et al*. 2003).

Magnesium-dechelatase (Mg- dechelatase) is involved in the catabolism of chlorophyll *a*. Wang *et al*. (2004b) found that RWA feeding causes an imbalance in chlorophyll synthesis as RWA feeding induces increased expression of Mg-dechelatase in *Dn* containing wheat lines. This is in agreement with a previous paper by Ni *et al*. (2001b) who also found that the levels of magnesium dechelatase

were higher after RWA feeding. It was suggested that chlorophyll biosynthesis was inhibited in the susceptible lines, leading to severe chlorosis (Wang et al. 2004b).

RWA feeding induces oxidative stress in plants (Ni *et al*. 2001). Lipoxygenase (LOX) was found to be induced within 24 hours of infestation by RWA in a resistant plant "TugelaDN" and not in the susceptible control (Berner and van der Westhuizen 2006). H_2O_2 and NADPH oxidase levels were measured in "TugelaDN" and were found to accumulate early and increase to levels much higher than what was seen in the susceptible control plants. It was suggested that H_2O_2 may be an important signalling molecule during RWA resistance response in this *Dn1* containing line due to defence enzymes such as peroxidase and β-1,3-glucanase being activated downstream (Moloi and van der Westhuizen 2006). β-1,3-glucanase has been found to accumulate in the chloroplasts of resistant infested wheat (van der Westhuizen *et al*. 2002).

Sieve elements are hypothesized to utilize calcium dependant defence mechanisms against damage, which induce protein clogging and callose deposition (Will and Van Bel 2006). Supporting this theory is firstly, sieve elements that have been found to be enriched with Ca^{2+} channels which would explain the responsiveness of the phloem (Volk and Franceschi 2000), as well as a Ca^{2+} ATPase which was found in the sieve element reticulum (SER) (Arsanto 1986). In order for aphids to feed successfully on phloem sap, they need to prevent clogging of the sieve elements by proteins and callose. They do so actively; as a glass capillary of the same diameter as an aphid stylet causes immediate blockage of sieve tubes (Knoblauch and van Bel 1998). It is thought that the stylet sheath material is used to seal the wounds that the stylet causes in cell walls, also effectively sealing the stylet off from toxic contents of the cells (Miles 1987). Watery saliva has been shown to specifically unblock sieve elements by binding Ca^{2+} ions (Will *et al*. 2007). Therefore the aphid either reduces Ca^{2+} influx into the SE or sequesters Ca^{2+} ions inside the SE (Will and van Bel 2006).

In a study by Zaayman *et al*. (2009) notable differences were seen in expression profiles of a *Dn7* containing wheat line, when challenged with US biotypes and SA biotypes. Therefore the *Dn7* interacts specifically with the elicitor of each biotype. It could thus be said that the wheat-RWA resistance response is specific for the type of resistance present in the plant as well as for the aphid biotype.

2.6 References

Aarts, N., Metz, M., Holub., E., Staskawicz, B.J., Daniels, M.J. (1998) Different requirements rof *EDS1* and *NDR1* by disease resistance genes define at least two *R*-gene-mediated signalling pathways in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA*. 95: 10306-10311

A'Brook, J., Dewar, A.M. (1980) Barley yellow dwarf virus infectivity of alate aphid vectors in west Wales. *Annals of Applied Biology*. 96(1): 51-58

Alexopoulou, L., Holt, A.C., Medzhitov, R., Flavell, R.A. (2001) Recognition of double-stranded RNA and activation of NF-kB by Toll-like receptor 3. *Nature*. 413: 732-738

Alleby, R.G., Banerjee, M., Brown, T.A. (1999) Evolution of the high molecular weight glutenin loci of the A, B, D, and G genomes of wheat. *Genome*. 42: 296-307

Anderson, G.R., Papa, D., Peng, J., Tahir, M., Lapitan, N.L.V. (2003) Genetic mapping of *Dn7*, a rye gene conferring resistance to the Russian wheat aphid in wheat. *Theory of Applied Genetics*. 107: 1297-1303

Antoniw, J.F., Ritter, C.E., Pierpoint, W.S., Van Loon, L.C. (1980) Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. *Journal of General Virology*. 47: 79-87

Apostol, I., Heinstein, P.F., Low, P.S. (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells1: role in defense and signal transduction. *Plant physiology*. 90: 109-116

Appel, H.M. (1993) Phenolics in ecological interactions. *Journal of Chemical Ecology*. 19(7): 1521-1552

Arsanto, J.P. (1986) Ca2+-binding sites and phosphatase activities in sieve element reticulum and P-protein of chick-pea phloem. A cytochemical and X-ray microanalysis survey. *Protoplasma*. 132: 160-171

Arumuganathan, K., Earle, E.D. (1991) Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter*. 9(3): 208-218

Axtell, M.J., Staskawicz, B.J. (2003) Initiation of *RPS2*-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. *Cell*. 112: 369–377

Baker, B., Zambryski, P., Staskawicsz, B., Dinesh-Kumar, S.P. (1997) Signalling in plant-microbe interactions. *Science*. 276: 726- 733

Basky, Z. (2003) Biotypic and pest status differences between Hungarian and South African populations of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae). *Pest Management Science*. 59:1152–1158

Beckman, C.H. (2000) Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiological and Molecular Plant Pathology*. 57: 101-110

Belkhadir, Y., Subramaniam, R., Dangl, J.L. (2004) Plant disease resistance protein signalling: NBS–LRR proteins and their partners. *Current Opinion in Plant Biology*. 7:391–399

Bell, E., Creelman, R.A., Mullet, J.E. (1995) A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in Arabidopsis. *Proceedings of the National Academy of Sciences, USA*. 92: 8675-8679

Bergelson, J., Kreitman, M., Stahl, E.A., Tian, D. (2001) Evolutionary Dynamics of Plant *R*-Genes. *Science*. 292: 2281-2285

Berner, J., van der Westhuizen, A.J. (2006) Biochemistry of Russian wheat aphid resistance in wheat: Involvement of lipid-like products. *Philosophiae Doctor* . University of the Free State, Bloemfontein, South Africa.

Bisgrove, S.R., Simonich, M.T., Smith, N.M., Sattler, A., Innes, R.W. (1994) A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes. *The Plant Cell*. 6: 927-933

Bishop, P.D., Makus, D.J., Pearce, G., Ryan, C.A. (1981) Proteinase inhibitor-inducing factor activity in tomato leaves resides in oligosaccharides enzymically released from cell walls. *Proceedings of the National Academy of Sciences, USA*. 78(6): 3536-3540

Blackman, R.L., Eastop, V.F. (2000) Aphids on the world's crops: An identification and information guide, 2nd Edition. By the Natural History Museum, London.

Boshoff, W., du Toit, F. (2006) n' Deurbraak in weerstand. *Pitkos*. 3(3)

Botella, M.A., Parker, J.E., Frost, L.N., Bittner-Eddy, P.D., Beynon, J.L., Daniels, M.J., Holub, E.B., Jones, J.D.G. (1998) Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. *The Plant Cell*. 10: 1847-1860

Botha, A-M., Nagel, M.A.C., Van der Westhuizen, A.J., Botha, F.C. (1998) Chitinase isoenzymes in near-isogenic wheat lines challenged with Russian wheat aphid, exogenous ethylene, and mechanical wounding. *Botanical Bulletin of Academia Sinica*. 39: 99-106

Botha, A-M., Lacock, L., van Niekerk, C., Matsioloko, M.T., du Preez, F.B., Loots, S., Venter, E., Kunert, K.J., Cullis, C.A. (2006) Is photosynthetic transcriptional regulation in *Triticum aestivum* L. cv. 'TugelaDN' a contributing factor for tolerance to *Diuraphis noxia*(Homoptera: Aphididae)? *Plant Cell Reports*. 25: 41-54

Brisson, L.F., Tenhaken, R., Lamb, C. (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *The Plant Cell.* 6: 1703-1712

Bryant, J., Green, T., Gurusaddaiah, T., Ryan, C.A. (1976) Proteinase inhibitor II from potatoes: Isolation and characterization of the isoinhibitor subunits. *Biochemistry*. 15: 3418-3423

Budak, S., Quisenberry, S.S., Ni, X. (1999) Comparison of *Diuraphis noxia* resistance in wheat isolines and plant introduction lines. *Entomologia Experimentalis et Applicata.* 92(2): 157-164

Burch-Smith, T.M., Schiff, M., Caplan, J.L., Tsao, J., Czymmek, K., Dinesh-Kumar, S.P. (2007) A novel role for the TIR domain in association with pathogen derived elicitors. *PLOS Biology*. 5(3): 501-514

Burd, J.D. (2002) Physiological Modification of the Host Feeding Site by Cereal Aphids (Homoptera: Aphididae). *Journal of Economic Entomology*. 95(2): 463-468

Burd, J.D., Porter, D.R., Puterka, G.J., Haley, S.D., Peairs, F.B. (2006) Biotypic variation among North American Russian wheat aphid (Homoptera: Aphididae) populations. *Journal of Economic Entomology*. 99(5): 1862-1866

Butts, R.A., Howling, G.G., Bone, W., Bale, J.S., Harrington, R. (1997) Contact with the host plant enhances aphid survival at low temperatures. *Ecological Entomology*. 22: 26-31

Cao, H., Bowling, S.A., Gordon, A.S., Dong, X. (1994) Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell*. 6: 1583-1592

Casaretto, J.A., Zuniga, G.E., Corcuera, L.J. (2004) Abscisic acid and jasmonic acid affect proteinase inhibitor activities in barley leaves. *Journal of Plant Physiology*. 161(4): 389-396

Castro, A.M., Ramos, S., Vasicek, A., Worland, A., Giménez, D., Clúa1, A.A., Suárez, E. (2001) Identification of wheat chromosomes involved with different types of resistance against greenbug (*Schizaphis graminum*, Rond.) and the Russian wheat aphid (*Diuraphis noxia*, Mordvilko) *Euphytica*. 118: 321–330

Century, K.S., Shapiro, A.D., Repetti, P.P., Dahlbeck, D., Holub, E., Staskawicz, B.J. (1997) *NDR1*, a pathogen-induced component required for *Arabidopsis* disease resistance. *Science*. 278(5345): 1963 – 1965

Chen, Z., Silva, H., Klessig, D.F. (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science*. 262: 1883-1886

Chester, K.S. (1933) The problem of acquired physiological immunity in plants. *The Quartily Review of Biology*. 8: 275-324

Christensen, A.B., Cho, B.H., Næsby, M., Gregersen, P.L., Brandt, J., Madriz-Oedenana, K., Collinge, D.B., Thordal-Christensen, H. (2002) The molecular characterization of two barley proteins establishes the novel PR-17 family of pathogenesis-related proteins. *Molecular plant pathology*. 3(3): 135-144

Chrzanowski, G., Ciepiela, A.P., Sprawka, I., Sempruch, C., Sytykiewicz, H., Czerniewicz, P. (2003) Activity of polyphenoloxidase in the ears of spring wheat and triticale infested by grain aphid (*Sitobion avenae* /F./). *Electronic Journal of Polish Agricultural Universities, Biology*. 6(2): 1-5

Cockerham, G. (1970) Genetical studies on resistance to potato viruses X and Y. *Heredity*. 25(3): 309-348

Cohen, Y., Gisi, U., Niderman, T. (1993) Local and systemic protectin against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology*. 83: 1054-1062

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

Dawkins, R., Krebs, J.R. (1979) Arms races between and within species. *Proceedings of the Royal Society of London*. B205: 489-511

Delledonne, M., Xia, Y., Dixon, R.A., Lamb, C. (1998) Nitric oxide functions as a signal in plant disease resistance. *Nature*. 394: 585-588

Delledonne, M., Zeier, J., Marocco, A., Lamb, C. (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proceedings of the National Academy of Sciences USA*. 98(23): 13454–13459

Deng, W., Brown, G., Colins, G.B., Hildebrand, D.F. (1992) Plant lipid peroxidation manipulation and effects on aphid resistance. (Abst.). *Plant Physiology Suppliments*. 99: 109

Department of Agriculture (2008) Trends in the agricultural sector 2008. Economic review for the 12 months that ended 30 June. 1-66

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S., Marco, Y. (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proceedings of the National Academy of Sciences USA*. 100: 8024-8029

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A., Dangl, J.L. (1994) Arabidopsis mutants simulating disease resistance response. *Cell*. 77: 565-577

Dixon, A.F.G. (1976) Aphids and translocation. *In* Transport in Plants (ed. M.H. Zimmermenn and J.A. Milburn) Springer, Berlin. 154-170

Dodds, P.N., Lawrence, G.J., Catanzariti, A-M., Teh, T., Wang, C-I.A., Ayliffe, M.A., Kobe, B., Ellis, J.G. (2006) direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proceedings of the National Academy of Sciences USA*. 103(23): 8888-8893

Dolatti, L., Ghareyazie, B., Moharramipour, S., Noori-Daloii, M.R. (2005) Evidence for regional diversity and host adaption in Iranian populations of the Russian wheat aphid. *Entomologia Experimentalis et Applicata*. 144(3): 171-180

Du Toit, F. (1987) Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Hemiptera: Aphididae). *Cereal Research Communications*. 15 (2-3): 175-179

Du Toit, F. (1989) Inheritance of resistance in two *Triticum aestivum* lines to Russian wheat aphid (Homoptera: Aphididae). *Journal of Economic Entomology*. 82(4): 1251-1253

Eichenseer, H., Mathews, M.C., Bi, J.L., Murphy, J.B., Felton, G.W. (1999) Salivary glucose oxidase: multifunctional roles for helicoverpa zea? *Archives of Insect Biochemistry and Physiology*. 42: 99-109

Ellis, J.G., Lawrence, G.J., Luck, J.E., Dodds, P.N. (1999) Identification of regions in alleles of the flax rust resistance gene *L* that determine differences in gene-for-gene specificity. *The Plant Cell*. 11: 495-506

Erickson, F.L., Holzberg, S., Calderon-Urrea, A., Handley, V., Axtell, M., Corr, C., Baker, B. (1999) The helicase domain of the TMV replicase proteins induces the N-mediated defence response in tobacco. *The Plant Journal*. 18(1): 67-75

Farmer, E.E., Ryan, C.A. (1990) Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings by the National Academy of Sciences of the USA*. 87: 7713- 7716

Farmer, E.E., Ryan, C.A. (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *The Plant Cell*. 4: 129-134

Feldman, M., Levy, A.A. (2005) Allopolyploidy- a shaping force in the evolution of wheat genomes. *Cytogenetic Genome Resolution*. 109: 250-258

Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annual Review of Phytopathology*. 9: 275-296

Franceschi VR, Grimes HD (1991) Low levels of atmospheric methyl jasmonate induce the accumulation of soybean vegetative storage proteins and anthocyanins. *Proceedings of the National Academy of Sciences USA*. 88: 6745-6749

Floryszak-Wieczorek, J., Arasimowicz, M., Milczarek, G., Jelen, H., Jackowiak, H. (2007) Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defence responses of pelargonium to a necrotrophic pathogen. *New Phytologist*. 175(4): 718-730

Food and Agriculture organization (FAO) (2008) Crop Prospects and Food Situation Report. February 1.

Franceschi, V.R., Grimes, H.D. (1991) Induction of soybean vegetative storage proteins and anthocyanins by low-level atmospheric methyl jasmonate. *Proceedings of the National Academy of Science, USA*. 88: 6745-6749

Funk, C.J. (2001) Alkaline phosphatase activity in whitefly salivary glands and saliva. *Archives of Insect Biochemistry and Physiology*. 46: 165-174

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessman, H., Ryals, J. (1993) Requirement of salicylic acid for the induction of sustemic aquired resistance. *Science*. 261: 754-756

Görlach, J., Volrath, S., Knauf-Beite, G., Hengy, G., Beckhove, U., Kogel, K-H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., Ryals, J. (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *The Plant Cell*. 8: 629-643

Green, T.R., Ryan, C.A. (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science*. 175(4023): 776-777

Greenburg, J.T., Ausubel, F.M. (1993) *Arabidopsis* mutants compromised for the control of cellular damage during pathogenesis and aging. *The Plant Journal*. 4(2): 327-341

Greenberg, J.T., Ailan, G., Klessig, D.F., and Ausubel, F.M. (1994) Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. *Cell*. 77*:* 551-563

Haley, S.D., Peairs, F.B., Walker, C.B., Rudolph, J.B., Randolph, T.L. (2004) Occurrence of a new Russian wheat aphid biotype in Colorado. *Crop Science*. 44: 1589-1592

Harvey, T.L., Martin, T.J. (1990) Resistance to Russian wheat aphid, *Diuraphis noxia*, in wheat (*Triticum aestivum*). *Cereal Research Communication*. 18(1-2): 127-129

Hayashi, F., Smith, K.D., Ozinsky, A., Hawn, T.R., Yi, E.C., Goodlett, D.R., Eng, J.K., Akirak, S., Underhill, D.M., Aderem, A. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*. 410: 1099-1103

Heng-Moss, T.M., Ni, X., Macedo, T., Markwell, P.J., Baxendale, F.P., Quiesnberry, S.S., Tolmay, V. (2003) Comparison of chlorophyll and carotenoid Concentrations among Russian wheat aphid (Homoptera: Aphididae)-infested wheat isolines. *Journal of Economic Entomology*. 96(2): 475-481

Howe, G.A., Jander, G. (2008) Plant immunity to insect herbivores. *Annual Review of Plant Biology*. 59: 41-66

Huang, H-C., Schuurink, R., Denny, T.P., Atkinson, M.M., Baker, C.J., Yucel, I., Hutcheson, S.W., Collmer, A. (1988) Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco plants. *Journal of Bacteriology*. 170(10): 4748-4756

Hutzler, P., Fischbach, R., Heller, W., Jungblut, T.P, Reuber, S., Schmitz, R., Veit, M., Weissenböck, G., Schnitzler, J-P. (1998) Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. *Journal of Experimental Botany*. 49(323): 953–965

Jabs, T., Dietrich, R.A., Dangl, J.L. (1996) Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. *Science*. 273: 1853-1856

Jabs, T., Tscope, M., Colling, C., Hahlbrock, K., Scheel, D. (1997) Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. *Proceedings of the National Academy of Sciences USA*. 94: 4800-4805

Jia, Y., McAdams, S.A., Bryan, G.T., Hershey, H.P., Valent, B. (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *The European Molecular Biology Organization Journal*. 19(15): 4004-4014

Joosten, M.H., Cozijnsen, T.J., De Wit, P.J. (1994) Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. *Nature*. 367(6461):384-386

Kazan, K., Manners, J.M. (2008) Jasmonate signaling: Toward an integrated view. *Plant Physiology*. 146: 1459-1468

Kearney, B., Staskawicz, B.J. (1996) Widespread distribution and fitnesscontribution of *Xanthomonas campestris* avrBs2. *Nature*. 346: 385-386

Keenan, S.P., Burgener, P.A. (2008) Social and economic aspects. *In:* Areawide pest management: Theory and implementation. Wallingford, UK; Cambridge, MA.

Knoblauch, M., van Bel, A.J.E. (1998) Sieve tubes in action. *The Plant Cell*. 10: 35-50

Kruger, G.H.J., Hewitt, P.H. (1984) The effect of Russian wheat aphid (*Diuraphis noxia*) extract on photosynthesis of isolated chloroplasts: Prelimanary studies. *Technical Communication, Department of Agriculture, Republic of South Africa*. 191: 34-37

Lapitan, N.L.V., Li, Y-C., Peng, J., Botha, A-M. (2007a) Fractionated extracts of Russian wheat aphid eliciting defence responses in wheat. *Journal of Economic Entomology*. 100(3): 990-999

Lapitan, N.L.V., Li, Y., Walters, R.S.G., Peng, J., Peairs, F.B., Botha, A-M. (2007b) Limited nuclear and mitochondrial DNA variation among Russian wheat aphid (*Diuraphis noxia*) biotypes from the United States and South Africa. *Proceeds of the Annual Meeting of the Entomological Society of America*. DO0236

La Rota, M., Sorrells, M.E. (2004) Comparative DNA sequence analysis of mapped wheat ESTs reveals the complexity of genome relationships between rice and wheat. *Functional and Integrated Genomics.* 4: 34-46

Lazzari, S., Starkey, S., Reese, J., Ray-Chandler, A., McCubrey, R., Smith, M. (2009) Feeding behaviour of Russian wheat aphid (Hemiptera: Aphididae) biotype 2 in response to wheat genotypes exhibiting antibiosis and tolerance resistance. *Journal of Economic Entomology*. 102(3): 1291-1300

Leach, J.E., White, F.F. (1996) Bacterial avirulence genes. *Annual Review of Phytopathology*. 34: 153-179

Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell*. 79: 583-593

Levine, A., Pennell, R.I., Alvarez, M.E., Palmer, R., Lamb, C. (1996) Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Current biology. 6(4): 427-437

Liu, X.M., Smith, C.M., Gill, B.S., Tolmay, V. (2001) Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. *Theory of Applied Genetics*. 102: 504-510

Liu, X.M., Smith, C.M., Gill, B.S. (2002) Identification of microsatellite markers linked to Russian wheat aphid resistance genes *Dn4* and *Dn6*. *Theory of Applied Genetics*. 104: 1042-1048

Macedo, T.B., Higley, L.G., Ni, X., Quisenberry, S.S. (2003) Light activation of Russian wheat aphid-elicited physiological responses in susceptible wheat. *Journal of Economic Entomology*. 96(1): 194-201

Maclean, D.J., Sargent, J.A., Tommerup, I.C., Ingram, D.S. (1974) Hypersensitivity as the primary event in resistance to fungal parasites. *Nature*. 249: 186-187

Malamy, J., Carr, J.P., Klessig, D.F., Raskin, I. (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*. 250(4983): 1002-1003

Malinga, J.N., Kinyua, M.G., Kamau, A.W., Wanjama, J.K., Awalla, J.O., Pathak, R.S. (2007) Biotypic and genetic variation within tropical populations of Russian wheat aphid, Diuraphis noxia (Kurdjumov) (Homoptera: Aphididae) in Kenya. *Journal of Entomology*. 4(5): 350-361

Marais, G.F., du Toit, F. (1993) A monosomic analysis of Russian wheat aphid resistance in the common wheat PI 294994. *Plant Breeding*. 111: 246-248

Marais, G.F., Wessels, W.G., Horn, M., du Toit, F. (1998) Association of a stem rust gene (Sr45) and two Russian wheat aphid resistance genes (*Dn5* and *Dn7*) with mapped structural loci in common wheat. *South African Journal of Plant and Soil*. 15(2): 67-71

Marineau, C., Matton, D.P., Brisson, N. (1987) Differential accumulation of potato tuber mRNAs during the hypersensitive response induced by arachidonic acid elicitor. *Plant Molecular Biology*. 9: 335-342

Martin, G.B., Bogdanove, A.J., Sessa, G. (2003) Understanding the functions of plant disease resistance proteins. *Annual Review of Plant Biology*. 54: 23-61

Matsiliza, B., Botha, C.E.J. (2002) Aphid (*Sitobion yakini*) investigation suggests thin-walled sieve tubes in barley (*Hordeum vulgare*) to be more functional than thick-walled sieve tubes *Physiologia Plantarum*. 115: 137–143

Mehdy, M.C. (1994) Active Oxygen Species in Plant Defense against Pathogens. *Plant Physiology.* 105: 467-472

Messina, F.J., Taylor, R., Karren, M.E. (2002) Divergent responses of two cereal aphids to previous infestation of their host plant. *Entomologia Experimentalis et Applicata*. 103(1): 43 - 50

Miles, P.W. (1965) Studies on the salivary physiology of plant bugs: The salivary secretions of aphids. *Journal of Insect Physiology*. 11: 1261-1268

Miles, P.W. (1967) The physiological division of labour in the salivary glands of *Oncopeltus fasciatus* (Dall.) (Heteroptera: lygaeidae). *Australian Journal of the Biological Sciences*. 20: 785-795

Miles, P.W. (1987) Feeding process of *Aphidoidea* in relation to effects on their food plants. In: Minks AK, Herrewijn P, eds. Aphids: their biology, natural enemies and control, Vol. 2A. Amsterdam: Elsevier. 321-339

Miles, P.W. (1999) Aphid saliva. *Biologial Reviews*. 74: 41-85

Miles, P.W., Oertli, J.J. (1993) The significance of antioxidants in the aphid-plant interation: the redox hypothesis. *Entomologia experimentalis et applicata.* 67: 275-283

Mithofer, A., Boland, W. (2008) Recognition of herbivory-associated molecular patterns. *Plant Physiology*. 146: 825-831

Moloi, M.J., van der Westhuizen, A.J. (2006) The reactive oxygen species are involved in resistance responses of wheat to the Russian wheat aphid. *Journal of Plant Physiology*. 163: 1118-1125

Mou, Z., Fan, W., Dong, X. (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell*. 113: 935-944

Nelson, C.E., Ryan, C.A. (1980) In vitro synthesis of pre-proteins of vacuolar compartmented proteinase inhibitors that accumulate in leaves of wounded tomato plants. *Proceedings of the National Academy of Sciences USA*. 77(4): 1975-1979

Ni, X., Quisenberry, S.S., Heng-Moss, T., Markwell, J., Sarath, G., Klucas, R., Baxendale, F. (2001) Oxidative responses of resistant and susceptible cereal leaves to symptomatic cereal aphid (Hemiptera: Aphididae) feeding. *Journal of Economic Entomology*. 94(3): 743-751

Ni, X., Quisenberry, S.S., Markwell, J., Heng-Moss, T., Higley, L., Baxendale, F., Sarath, G., Klucas, R. (2001b) *In vitro* enzymatic chlorophyll catabolism in wheat elicited by cereal aphid feeding. *Entomologia Experimentalis et Applicata*. 101: 159-166

Nimchuk, Z., Marois, E., Kjemtrup, S., Leister, R.T., Katagiri, F., Dangl, J.L. (2000) Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell.* 101: 353-363

Nkongolo, K.K., Quick, J.S., Limin, A.E., Fowler, D.B. (1991) Source and inheritance of resistance to the Russian wheat aphid in *Triticum* species and *Triticum tauschii*. Canadian Journal of Plant Science. 71: 703–708

O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O., Bowles, D.J. (1996) Ethylene as a signal mediating the wound response of tomato plants. *Science*. 247: 1914-1917

Okushima, Y., Koizumi, N., Kusano, T., Sano, H. (2000) Secreted proteins of tobacco cultured BY2 cells: identification of a new member of pathogenesis-related proteins. *Plant Molecular Biology*. 42: 479-488

Painter, R.H. (1958) Resistance in plants to insects. *Annual Review of Entomology*. 3: 267-290

Pan, Q., Wendel, J., Fluhr, R. (2000) Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. *Journal of Molecular Evolution*. 50: 203–213

Park, S.W., Kaimoyo, E., Kumar, D., Mosher, S., Klessig, D.F. (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science*. 318: 113-116

Parsons, T.J., Bradshaw, H.D., Gordon, M.P. (1989) Systemic accumulation of specific mRNAs in response to wounding in poplar trees. *Proceedings by the National Academy of Sciences of the USA*. 86: 7895-7899

Pearce, G., Sy, L., Russell, C., Ryan, C.A., Hass, G.M. (1982) Isolation and characterization from potato tubers of two polypeptide inhibitors of serine proteinases. *Archives of Biochemistry and Biophysics*. 213(2): 456-462

Pearce, G., Strydom, D., Johnson, S., Ryan, C.A. (1991) A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science*. 253: 895-898

Peña-Cortéz, H., Sanchez-Serrano, J.J., Mertens, R., Willmitzer, L., Prat, S. (1989) Abscisic acid is involved in the wound-induced expression of the proteinase inhibitor II gene in potato and tomato. *Proceedings by the National Academy of Sciences of the USA*. 86: 9851-9855

Peng, Z., Miles, P.W. (1988) Studies on the salivary physiology of plant bugs: function of the catechol oxidase of the rose aphid. *Journal of Insect Physiology*. 34(11): 1027-1033

Peng, Z., Miles, P.W. (1991) Oxidases in the gut of an aphid, *Macrosiphum rosae* (L.) and their relation to dietary phenolics. *Journal of Insect Physiology*. 37(10): 779-787

Penninckx, I.A.M.A., Eggermont, K., Terras, F.R.G., Thomma, B.P.H.J., De Samblanx, G.W., Buchala, A., Métraux, J-P., Manneqa, J.M., Broekaert, W.F. (1996) Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. *The Plant Cell.* 8: 2309-2323

Penninckx, I.A.M.A., Thomma, B.P.H.J., Buchala, A., Métraux, J-P., Broekaert, W.F. (1998) Concomitant activation of jasmonate and ethylene response pathways Is required for induction of a plant defensin gene in *Arabidopsis*. *The Plant Cell*. 10: 2103-2113

Pieterse, C.M.J., van Wees, S.C.N., Hoffland, E., van Pelt, J.A., van Loon, L.C. (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *The Plant Cell*. 8: 1225-1237

Pieterse, C.M.J., van Wees, S.C.M., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J., van Loon, L.C. (1998) A Novel Signaling Pathway Controlling Induced Systemic Resistance in Arabidopsis. *The Plant Cell*. 10: 1571-1580

Pimental, D. (2005) Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability*. 7: 229-252

Poltorak, A., He, X., Smirnova, I., Liu, M-Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., Beutler, B. (1998) Defective LPS Signalling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in *Tlr4* Gene. *Science*. 282: 2085-2088

Prescot, J.M., Burnett, J.P.A., Saari, E.E., Ransom, J., Bowman, J., de Millano, W., Singh, R.P., Bekele, G. (1986) Wheat diseases and pests: a guide for field identification. *Centro Internacional de Mejoramiento de Maiz Y Trigo* (CIMMYT). 1-143

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A., Vance, C.P. (2000) Fungal development and induction of defence response genes during early infection of wheat spikes by *Fusarium graminearum*. *Molecular Plant Microbe Interaction*s. 13(2): 159-169

Quick, J.S., Nkongolo, K.K., Meyer, W., Peairs, F.B., Weaver, B. (1991) Russian wheat aphid reaction and agronomic and quality traits of a resistant wheat. *Crop Science*. 31: 50-53

Quiseberry, S.S., Peairs,

Quisenberry, S.S., Schtzko D.J. (1994) Russian wheat aphid (Homenoptera: Aphidae) population developement and plant damage on resistant and susceptible wheat. *Journal of Economic Entomology*. 87(6): 1761-1768

Rossi, M., Goggin, F.L., Milligan, S.B., Kaloshian, I., Ullman, D.E., Williamson, V.M. (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proceedings by the National Academy of Sciences of the USA.* 95: 9750-9754

Ryan, C.A. (1974) Assay and biochemical properties of the proteinase inhibitorinducing factor, a wound hormone. *Plant Physiology*. 54: 328-332

Ryan, C.A. (1992) The search for the proteinase inhibitor-inducing factor, PIIF. *Plant Molecular Biology.* 19: 123-133

Saidi, A., Quick, J.S. (1996) Inheritance and allelic relationships among Russian wheat aphid resistance genes in winter wheat. *Crop Science.* 36(2): 256-258

Salmeron, J.M., Oldroyd, G.E.D., Rommens, C.M.T., Scofield, S.R., Kim, H-S., Lavelle, D.T., Dahlbeck, D., Staskawicz, B.J. (1996) Tomato *Prf* Is a Member of the Leucine-Rich Repeat Class of Plant Disease Resistance Genes and Lies Embedded within the *Pto* Kinase Gene Cluster. *Cell.* 86: 123-133

Saxena, K.N. (1969) Patterns of insect-plant relationships determining susceptinility or resistance of different plants to an insect. *Entomologia Experimentalis et Applicata*. 12(5): 751-766

Schmelz, E.A., Carroll, M.J., LeClere, S., Phipps, S.M., Meredith, J., Chourey, P.S., Alborn, H.T., Teal, P.E.A. (2006) Fragments of ATP synthase mediate plant perception of insect attack. *Proceedings by the National Academy of Sciences of the USA*. 103(23): 8894-8899

Schweizer, P., Buchala, A., Dudler, R., Métraux, J-P. (1998) Induced systemic resistance in wounded rice plants. *The Plant Journal*. 14(4): 475-481

Scofield, S.R., Tobias, C.M., Rathien, J.P., Chang, J.H., Lavelle, D.T., Michelmore, R.W., Staskawicz, B.J. (1996) Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science*. 274: 2063-2065

Seo, H.S., Song, J.T., Cheong, J-J., Lee, Y-H., Hwang, I., Lee, J.S. (2001) Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. *Proceedings by the National Academy of Sciences of the USA.* 98(8): 4788-4793

Shao, F., Golstein, C., Ade, J., Stoutemyer, M., Dixon, J.E., Innes, R.W. (2003) Cleavage of *Arabidopsis* PBS1 by a Bacterial Type III Effector. *Science*. 301: 1230- 1233

Shulaev, V., Silverman, P., Raskin, I. (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature*. 385: 718-721

Silué, D., Notteghem, J.L., Tharreau, D. (1992) Evidence of a gene-for-gene relationship in the *Oryza sativa-Magnaporthe grisea* pathosystem. *Phytopathology*. 82: 577-580

Smith, M.C., Belay, T., Stauffer, C., Kubeckova, I., Starkey, S. (2004) Identification of Russian wheat aphid (Homoptera: Aphididae) populations virulent to the Dn4 resistance gene. *Journal of Economic Entomology*. 97(3): 1112-1117

Song, W-Y., Wang, G-L., Chen, L-L., Kim, H-S., Pi, L-Y., Holsten, T., Gardner, j., Wang, B., Zhai, W-X., Zhu, L-H., Fauquet, C., Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*. 270: 1804-1806

Spoel, S.H., Koornneef, A., Claessens, S.M.C., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J. et al. (2003) NPR1 modulates cross-talk between salicylate-and jasmonate-dependant defence pathways through a novel function in the cytosol. *The Plant Cell*. 15: 760-770

Takahama, U., Oniki, T. (1997) A peroxidase/phenolics/ascorbate system can scavenge hydrogen peroxide in plant cells. *Physiologia Plantarum*. 101: 845-852

Takahashi, N., Takahashi, Y., Putnam, F.W. (1985) Periodicity of leucine and tandem repetition of a 24-amino acid segment in the primary structure of leucine-rich α_2-glycoprotein of human serum. *Proceedings by the National Academy of Sciences of the USA*. 82: 1906-1910

Tang, X., Frederick, R.D., Zhou, J., Halterman, D.A., Jia, Y., Martin, G.B. (1996) Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science*. 274: 2060-2063

Tenhaken, R., Levine, A., Brisson, L.F., Dixon, R.A., Lamb, C. (1995) Function of the oxidative burst in hypersensitive disease resistance. *Proceedings by the National Academy of Sciences, USA*. 92: 4158-4163

Truman, W., Bennett, M.H., Kubigsteltig, I., Turnbull, C., Grant, M. (2007) Arabidopsis systemic immunity uses conserved defence signalling pathways and is mediated by jasmonates. *Proceedings by the National Academy of Sciences, USA*. 104(3): 1075-180

Ueda, K., Kato, J. (1980) Isolation and Identification of a Senescence-promoting Substance from Wormwood (*Artemisia absinthium* L.). *Plant Physiology*. 66: 246-249

Ueda, H., Yamaguchi, Y., Sano, H. (2006) Direct interaction between the tobacco mosaic virus helicase domain and the ATP-bound resistance protein, N factor during the hypersensitive response in tobacco plants. *Plant Molecular Biology*. 61: 31-45

Urbanska, A., Tjallingii, W.F., Dixon, A.F.G., Leszczynski, B. (1998) Phenol oxidising enzymes in the grain aphid's saliva. *Entomologia Experimentalis et Applicata*. 86: 197–203

U. S. Congress Office of Technology Assessment (OTA) (1993) Harmful non-indigenous species in the United States, OTA-F-565 (U.S. Government Printing Office, Washington, D.C., 1993)

USDA (2007a) Annual statistical bulletin 2007. Table 4 World and U.S. wheat production, exports and ending stocks. http://www.nass.usda.gov/**Statistics**_by_State/Michigan/Publications/Annual_Statistical_Bulletin/**stats**08/**wheat**.pdf

USDA (2007b) World agricultural supply and demand estimates. WASDE-450. 1-39. http://www.cottonusa.org/files/economicData/September-2007.pdf

USDA (2009) World agricultural supply and demand estimates. WASDE-472. 1-39. http://www.usda.gov/oce/commodity/wasde/latest.pdf

Van der Biezen, E.A., Jones, J.D.G. (1998) Plant disease-resistance proteins and the gene-for-gene concept. *Trends in Biochemical Sciences*. 23: 454-456

Van der Westhuizen, A.J., Qian, X.-M., Botha, A.-M. (1998) Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Reports*. 18: 132-137

Van der Westhuizen, A.J., Qian, X-M., Wilding, M., Botha, A-M. (2002) Purification and immunocytochemical localization of a wheat β-1,3-glucanase induced by Russain wheat aphid infestation. *South African Journal of Science*. 98: 197-202

Van Fleet, D.S. (1954) The significance of the histochemical localization of quinones in the differentiation of plant tissues**.** *Phytomorphology*. 4. 300-310

Van Loon, L.C., van Strien, E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology*. 55: 85-97

Van Loon, L.C., Rep, M., Pieterse, C.M.J. (2006) Significance of inducible defense-related proteins in infected plants. *Annual Reveiw of Phytopathology*. 44:135-62

Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H., Ryals, J. (1994) Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *The Plant Cell*. 6: 959-965

Vick, B.A., Zimmerman, D.C. (1983) The biosynthesis of jasmonic acid: A physiological role for plant lipoxygenase. *Biochemical and Biophysical Research Communications*. 111(2): 470-477

Volk, G.M., Franceschi, V.R. (2000) Localization of a calcium channel-like protein in the sieve element plasma membrane. *Australian Journal of Plant Physiology*. 27: 779-786

Walker-Simmons, M., Hadwiger, L., Ryan, C.A. (1983) Chitosans and pectic polysaccharides both induce the accumulation of the antifungal phytoalexin pisatin in pea pods and antinutrient proteinase inhibiters in tomato leaves. *Biochemical and Biophysical Research Communications*. 110(1): 194-199

Walters, M.C., Penn, F., Du Toit, F., Botha, T.C., Aalbersberg, K. (1980) G.3 The Russian wheat aphid. *Farming in South Africa*. G.3: 1-6

Wang, G-L., Ruan, D-L., Song, W-Y., Sideris, S., Chen, L., Pi, L-Y., Zhang, S., Zhang, Z., Fauquet, C., Gaut, B.S., Whalen, M.C., Ronald, P.C. (1998) *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. *The Plant Cell*. 10: 765–779

Wang, Z.X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., Katayose, Y., Sasaki, T. (1999) The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *The Plant Journal*. 19(1): 55-64

Wang, T., Quisenberry S.S., Ni, X., Tolmay, V. (2004) Aphid (Hemiptera: Aphididae) resistance in wheat near-isogenic lines. *Journal of Economic Entomology*. 97(2): 646-653

Wang, T., Quisenberry S.S., Ni, X., Tolmay, V. (2004b) Enzymatic chlorophyll degradation in wheat near-isogenic lines elicited by cereal aphid (Homoptera: Aphididae) feeding. *Journal of Economic Entomology*. 97(2): 661-667

Wei, P., Zhang, Z., Andersen, C.H., Schmelzer, E., Gregersen, P.L., Collinge, D.B., Smedegaard-Petersen, V., Thordal-Christensen, H. (1998) An epidermis/papilla-specific oxalate oxidase-like protein in the defence response of barley attacked by the powdery mildew fungus. *Plant Molecular Biology*. 36: 101-112

Weidhase, R.A., Kramell, H-M., Lehmann, J., Liebisch, H-W., Lerbs, W., Parthier, B. (1987) Methyl jasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. *Plant Science*. 51: 177-186

Whitham, S., McCormick, S., Baker, B. (1996) The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. *Proceedings of the National Academy of Sciences USA*. 93: 8776-8781

Will, T., Hewer, A., van Bel, A.J.E. (2008) A novel perfusion system shows that aphid feeding behavior is altered by decrease of sieve-tube pressure. *Entomologia Experimentalis et Applicata.* 127: 237-245

Will, T., Tjalling, W.F., Thönnessen, A., van Bel, A.J.E. (2007) Molecular sabotage of plant defense by aphid saliva. *Proceedings of the National Academy of Sciences USA*. 104(25): 10536-10541

Will, T., van Bel, A.J.E. (2006) Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany*. 57(4): 729-737

Yencho, G.C., Cohen, M.B., Byrne, P.F. (2000) Applications of tagging and mapping insect resistance loci in plants. *Annual Review of Entomology*. 45: 393-422

Yu, I-C., Parker, J., Bent, A.F. (1998) Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *of the National Academy of Sciences USA*. 95: 7819-7824

Yu, J., Hu, S., Wang, J., Wong, G.K-S., Li, S., Liu, B., *et al*. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science*. 296: 79- 92

Zaayman, D., Lapitan, N.L.V., Botha, A-M. (2009) Dissimilar molecular defense responses are elicited in *Triticum aestivum* after infestation by different *Diuraphis noxia* biotypes. *Physiologia Plantarum*. 136: 209-222

Zhang, Y., Quick, J. S., Liu, S. (1998) Genetic variation in PI 294994 wheat for resistance to Russian wheat aphid. *Crop Science*. 38: 527–530

Zhang, C., Czymmek, K.J., Shapiro, A.D. (2003) Nitric oxide does not trigger early programmed cell death events but may contribute to cell-to-cell signaling governing progression of the *Arabidopsis* hypersensitive response. *Molecular Plant-Microbe Interactions*. 16(11): 962–972

Zhou, F., Zhang, Z., Gregersen, P.L., Mikkelsen, J.D., de Neergaard, E., Collinge, D.B., Thordal-Christensen, H. (1998) Molecular characterization of the oxalate oxidase involved in the response of barley to the powdery mildew fungus. *Plant Physiology*. 117: 33-41

Web resources

CIMMYT (International Maize and Wheat Improvement center):
http://www.cimmyt.org/english/docs/ann_report/recent_ar/Amapof.htm

International grains council: http://www.igc.org.uk

National Association of British and Irish Millers: http://www.nabim.org.uk/ecoBrief07_4.asp

The economist: http://www.economist.com/finance/displaystory.cfm?story_id=9769373

United States Department of Agriculture: http://www.invasivespeciesinfo.gov/animals/rwa.shtml

Chapter 3

Transcript profiling of wounded and Russian wheat aphid-infested

Betta wheat near isogenic lines

Chapter 3.1 ABSTRACT

As a pest that has a major impact on yield and production of wheat, the Russian Wheat Aphid (*Diuraphis noxia*, Kurdjomov) is of particular interest to the research and commercial fields alike. Various *Diuraphis noxia* (*Dn*) resistance genes have been identified in wheat but none have been fully characterized or cloned. *Dn1* confers an antibiotic resistance, similar to plant defense against pathogen attack, while *Dn2* confers a tolerance for aphid infestation. We aimed to investigate the systemic changes and biological pathways activated due to systemic acquired resistance (SAR) conferred by *Dn1* and *Dn2*. We used material from susceptible 'Betta' and two of it's resistant near isogenic lines 'BettaDN' and Betta *Dn2*. 146 transcript derived fragments (TDFs) from cDNA-AFLPs were cloned for putative identification with BLASTn and BLASTx. Wounding, *Dn1* and *Dn2* related transcripts were grouped into clusters using TreeView and putative functions assigned within the wounding, antibiosis and tolerance clusters respectively. Wounding was found to involve cellular processes such as Ca^{2+} signalling, photosynthesis, energy production, protein anabolism and catabolism as well as a cell wall protein. Putative clone identities included a serine/threonine phosphatase, a calcium-dependent protein kinase, NADH dehydrogenase, ribulose-1,5-bisphosphate carboxylase/oxygenase, ubiquitin carboxy-terminal hydrolase and a Shaggy-like kinase. Interestingly, the wounding response seems to be altered by the presence of the *Dn* genes suggesting cross talk between the pathways. TDFs involved in *Dn1* mediated resistance implicated elicitor recognition, ion flux, chloroplast proteins involved in detoxification during oxidative stress, toxin producers and exporters and ubiquitin mediated protein degradation. Putative TDF identities assigned included a *Xa21* homologue, H^+-ATPase, 10kD PSII protein, defence related F-box protein and an ATP-synthase. *Dn2* mediated tolerance was found to regulate TDFs involved in homeostasis, chloroplast proteins and energy conservation. Therefore the *Dn1* mode of resistance seems associated with a hypersensitive mediated defensive response i.e. systemic response. In the *Dn2* type of resistance, results suggest that the tolerance response is a function of maintenance of chloroplast function and cellular homeostasis that may be as a result of a timely recognition event. Putative clone identities included a lipoic acid synthetase, chloroplast 23S rRNA and a selenium binding protein.

Chapter 3.2 INTRODUCTION

Wheat is a primary source of food for many countries, and the demand for wheat and bread products is increasing in the Far East and in Africa. With a substantial amount of cereal crops being demanded for animal feed and for bio-fuel production in combination with stores being depleted, the international price of wheat was up 74.5 % in January 2008 compared to July 2007 (Food and Agriculture Organization, FAO, Crop Prospects and Food Situation Report, 2008). The Russian wheat aphid, *Diuraphis noxia* (Kurdjomov: Homoptera), (RWA), is a major pest of wheat and barley. The economic impact of RWA infestation is estimated at $1.2 billion since 1986 (Keenan and Burgener 2008), and although treating crops with pesticides is an answer, it is a costly one. Some of the major drawbacks of pesticides are the environmental impact, the development of pesticide resistance and the increasing costs (Pimental 2005).

There have been numerous germplasms found which contain genes that convey resistance to the RWA, named the *Diuraphis noxia* (*Dn*) genes. The first of the resistant accessions (PI 137739 and PI 262660) screened by Du Toit (1987) that provided resistance against RWA were named *Dn1* and *Dn2* (Du Toit 1989). These genes could be used to characterize two of the main types of resistance, antibiosis and tolerance respectively. Neither *Dn1* nor *Dn2* have been cloned or sequence characterized, nor have the pathways that contribute to the resistance response of either been fully characterized. Placement of the genes is putatively on wheat chromosome 7D (Liu *et al*. 2001).

In the first protein studies done on the RWA-wheat interaction in *Dn1* containing wheat, it was found that RWA infestation initiated a specific response in the resistant cultivars (Van der Westhuizen and Botha 1993; Van der Weshuizen and Pretorius 1996; Van der Westhuizen *et al*. 1998). It was also found that the response seen in RWA infested plants is different to that seen in mechanically wounded or ethylene treated plants (Botha *et al*. 1998).

Pathogenesis response (PR) proteins are implicated in plant-pathogen interactions and are a known by-product of systemic acquired resistance (SAR) (Van Loon and Van Strien 1999). A 1,3-β-glucanase PR protein was purified from RWA infested wheat by Van der Westhuizen *et al*. (2002). They also found that the protein was localized to the cell walls, vascular bundle cells and chloroplasts.

As chlorotic streaking is one of the main symptoms of RWA infestation on susceptible plants, it has been suggested that chloroplast function may be adversely affected by a component of RWA saliva (Heng-Moss *et al*. 2003; Macedo *et al*. 2003). The capacity and efficiency of photosystem II was found to be affected by RWA infestation, and one hypothesis is the RWA saliva affects protein synthesis (Burd and Eliot 1996). In photosystem II, protein D1 is regularly turned over due to light damage, and thus a halt to D1 synthesis would impair photosystem II function (Burd and Eliot 1996). Heng-Moss *et al*. (2003) speculated that by feeding on the phloem, the RWA changes the pH, affecting the cartenoids responsible for nonphotochemical quenching of excitation energy.

Resistance conveyed by the *Dn1* and *Dn2* genes is known to be conveyed by a single dominant gene respectively (Du Toit 1989) and the two genes may be allelic (Saidi and Quick 1996). Resistance to RWA attack is understood to act on the gene-for-gene resistance model (Botha *et al*. 2006). The gene-for-gene model stating that for a resistance response to present, an *R*-gene must be present in the plant that encodes for a protein that recognizes an elicitor or avirulence (Avr)-protein from the pathogen (Flor 1971). Resistance (R)-proteins are receptor type signalling proteins, with leucine rich repeat (LRR) motifs, known for providing resistance to infections in many plant-pathogen interactions (Bisgrove *et al*. 1994; Scofield *et al.* 1996; Silué *et al*. 1992; Song *et al*. 1995; Tang *et al.* 1996). Many NBS-LRR sequences have been isolated from RWA-resistant wheat lines (Lacock *et al*. 2003).

Dn1 containing wheat seems to follow the classic pathogen defence response showing necrotic lesions at the point of attack and the expression of SAR associated proteins (Botha et al. 1998; Mohase and Van der Westhuizen 2002; Van der Westhuizen *et al*. 1998). Peroxidase activity has been found to increase under infestation conditions indicating the activity of hydrogen peroxide which is mediated by SA (Mohase and Van der Westhuizen 2002; Van der Westhuizen *et al*. 1998). Lines containing *Dn1* show an antibiosis type resistance and RWA feeding on this line show decreased fecundity. Specific proteins that may be involved in *Dn1* conveyed resistance is the NBS-LRRs and chloroplast ATP synthases (Botha *et al* 2006).

Wheat lines containing *Dn2* show a tolerance type resistance in that they can withstand RWA infestation without suffering detrimental effects (Quisenberry and Schotzko 1994). Very little information is available on *Dn2* as most studies have centered on *Dn1* (Botha et al. 1998; Lacock *et al*. 2003; Botha *et al*. 2006), *Dn5* (Botha et al. 2006), *Dn7* (Zaayman *et al*. 2009) and *Dnx* (Boyko *et al*. 2006). In a study by Heng-Moss *et al*. (2003) chlorophyll and cartenoid concentrations were measured, comparing *Dn1* and *Dn2* plants. Minimal loss of chloroplasts and cartenoids was observed in *Dn2* plants, showing that a compensatory mechanism for chloroplast damage is active.

Previously, wheat lines bred with RWA resistance mainly used *Dn1* and *Dn2*, and due to this selection pressure, a new South African biotype has developed RWASA2 (Boshoff and Du Toit 2006a). Previously resistant cultivars such as the Pannar lines PAN 3364, PAN 3235, Caledon and Gariep are all susceptible to the new biotype (Boshoff and Du Toit 2006b). New sources of RWA resistance have had to be incorporated into breeding programs and new RWA resistant lines have been developed in order to counter the affects of RWASA2 (Boshoff and Du Toit 2006b). However, as is stated in the article, the ability of the organization to release the new line in 2008 was as of a result of development strategies implemented in the 1990's. This is a long development time, and only molecular information such as markers for resistance or the actual resistance genes will be able to speed up the process of plant breeding.

As using a combined approach towards aphid population control is necessary, the pyramiding of different genes conveying the different types of resistance is needed along with the biological and pesticide control (Yencho *et al*. 2000). And in order to do that there is a need to find recognition or initiation proteins involved in the RWA resistance systems. Markers will need to be developed and implemented within breeding programs.

The objectives of this study was to compare the RWA-wheat interaction at the transcript level using susceptible Betta wheat and it's near isgenic lines (NILs) BettaDN and Betta *Dn2*. Also included was a wounded treatment, in order to further the information available on the differences and similarities between the wounded and resistance responses. The putative involvement of organelles and possible down-stream biological pathways were also inferred.

3.3 MATERIALS AND METHODS

3.3.1 Material

RWA susceptible commercial wheat line 'Betta' and its' near isogenic lines (NILs) 'BettaDN' (Betta/SA1684*5) and Betta *Dn2* (Betta/SA2199*5) seeds were obtained from Small Grain Institute (SGI) Bethlehem. All repeats were done as follows: 12 plants of each wheat line were grown in the greenhouse in natural light until the 3 leaf stage (Tottman *et al*. 1979; Botha *et al*. 1998). Seedlings were then moved to the laboratory and allowed to acclimatise for 48 hours. Aphids raised in the laboratory were starved for ±4 hours prior to use. For the infestation treatment ±20 RWA were used per plant; the wounding treatment was meted out by pricking the leaves of each plant 15 times, and the control set was left untreated, as described by Botha *et al.* (1998). Sampling was carried out 48h after treatment. All leaves excluding the flag leaf were harvested. Liquid nitrogen was used to freeze samples as soon as they were harvested to preserve the samples integrity. All samples were kept at -80ºC until RNA was extracted.

3.3.2 Treatment of items used during the experiment

All equipment and solutions were treated with 0.1% diethyl pyrocarbonate (DEPC) for 24h and then autoclaved (Sambrook *et al.* 1989). Ceramics and metal ware were kept RNase free by baking them at 165^{o}C while all surfaces, gloves, etc. were treated with RNAse Away (Scientific group, SA). Sambrook *et al.* (1989)'s general laboratory procedures for RNA work were followed during the execution of the experiment.

3.3.3 Total RNA isolation, mRNA purification and cDNA synthesis

Mortars and pestles were used to grind plant material in liquid nitrogen. Total RNA was extracted from material using 4 M GITC buffer (4 M guanidine thiocynate; 100 mM Tris-HCl pH8; 25 mM sodium citrate pH8; 0.5% N-laryl sarcosine (w/v)), following an adapted Chomczynski and Sacchi (1987) protocol. Modifications include an additional 1:1 phenol and chloroform cleaning step and an increase in the isopropanol precipitation time to 2 hours. The RNeasy cleanup kit (Qiagen, Southern Cross

Biotechnologies, USA) was used following manufacturers guidelines and the optional DNase treatment was included. For the mRNA isolation, the Oligotex mRNA Midi kit (Qiagen, Southern Cross Biotechnologies) was used according to manufacturers protocol and mRNA was eluted in 50 µl elution buffer. RNA was quantified by means of the 'NanoDrop 1000' spectrophotometer using 1 µl sample volume measured at wavelengths of 230 nm, 260 nm and 280 nm. The 260/280 and 230/260 ratios were used to calculate the purity of the sample (NanoDrop, Wilmington, DE).

Double stranded cDNA was synthesised using the Roche cDNA Synthesis system (Roche, Germany) following manufacturers guidelines. First and second strand synthesis was completed using the MyCycler thermal cycler (Bio-Rad, South Africa) for the incubation steps. First strand synthesis included two incubation steps, the first at 70ºC for 10 min, and the second at 42ºC for 1 hour. Second strand synthesis had two incubations as well, the first at 16ºC for 2 hours and the second at 16ºC for 5 min.

3.3.4 cDNA-AFLP and analysis

cDNA-AFLPs (Bachem *et al.* 1996) were performed using synthesized cDNA of concentrations between 6.21 and 10.5 ng/µl and the cDNA-AFLP kit (LI-COR Biosciences, Lincoln, NB) according to the manufacturers protocol. The MyCycler thermal cycler (Bio-Rad, South Africa) was used for incubation steps. During restriction enzyme digestion, the mixture was incubated at 65ºC for 2 hours and then at 37ºC for 2 hours followed by inactivation at 80ºC for 20 minutes. During adapter ligation with Taq1 and Mse1 adapters (LI-COR Biosciences, Lincoln, NB) the mixture was incubated at 20ºC for 2 hours. During pre-amplification, a cycle of 94ºC for 30 sec, 56ºC for 1 min and 72ºC for 1 min for 20 cycles followed by a soak at 4ºC was used. For confirmation of pre-amplification, samples were run on 1 % agarose (w/v) (Figure 3.4.1C). Concentrations of samples were quantified spectrophotometrically using the 'Nano-drop 1000 (NanoDrop, Wilmington, DE) (Table 3.4.1), and sample concentrations were adjusted using ddH_2O to within a range of 300-400 ng/µl. IR700-labeled Taq1 primers and Mse1 primers supplied by the AFLP^R Expression analysis kit (LI-COR Biosciences,

Lincoln, NB) were used for selective amplification PCR: one cycle of 94ºC for 30 sec, 65ºC for 30 sec, 72ºC for 1 min which was followed by 12 cycles of successively lowering annealing temperature (65ºC), by 0.7ºC per cycle, while the denaturing (94ºC) and amplification (72ºC) temperatures remained the same. This was followed by 23 cycles of 94ºC for 30 sec, 56ºC for 30 sec and 72ºC for 1 min and a soak at 4ºC. Before each gel was loaded, the samples, including loading buffer, were denatured at 94ºC for 3 min.

Ten selective primer pairs, containing one MseI and one IR-labeled (700-labeled) TaqI primer were screened. Each MseI and TaqI primer has two extra nucleotide bases added at 3' end to make them more specific. Each primer was abbreviated using the first letter of either MseI (M) or TaqI (T) and the two extra nucleotides. Using this abbreviation, the following primer pairs: Ttg/ Mag; Tca/Mag; Tag/ Mac; Tca/ Mac; Tgt/ Mac; Tac/ Mga; Tac/Mgt; Tac/ Mag; Ttg/ Mct; Tgt/ Mct were screened. Polyacrylamide gels consisting of 20 ml 8 % (v/w) Long Ranger acrylamide gel solution (Cambrex Corp, East Rutherford, NJ), 150 µl 10x ammonium persulphate and 15 µl Temed (Myburg *et al.* 2001) were run on the LI-COR IR^2 4200S automated system.

3.3.5 Image analysis

After gel electrophoresis, the image was stored in (.tiff) format for further analysis. The AFLP Quantar-Pro program (LI-COR Biosciences) was used for analysis of the (.tiff) output file. Bands of interest were plotted as data points, their intensity measured and each was given an M- score (weight of DNA fragment). Data from this program was exported in a tab delimited format into Excel (Office package, Microsoft). As there were two lanes loaded for each sample, the data was edited to show the average of each sample and all ambiguous data was omitted. All headings were removed except for sample names. This file was then saved in Tab delimited format to be used in cluster analysis (Section 3.3.6).

3.3.6 Cluster analysis

Cluster 3.0 (Eisen *et al.* 1998) was used to filter, normalize and apply hierarchical clustering algorithms to the data. The following settings were used: a filter, with >100% present, a standard deviation of 0.5, at least 1 observation, absolute value ≥ 2 and a MaxVal-MinVal of 2.0. Datasets were adjusted by centering them on a mean, followed by normalizing; and hierarchical clustering was done with Spearman's rank correlation (Spearman 1904) and average linkage was used (Eisen *et al.* 1998). Cluster 3.0 produced a (.cdt) file output. Java TreeView 1.1.1 (Saldanha 2004) was used to visualize the clustered Transcript Derived Fragments (TDFs). Each TDF was clustered into groups containing other TDFs with similar expression patterns. Treatments that are up-regulated are shown in red, while comparative down-regulation is shown in green.

3.3.7 cDNA-AFLP selection and excision of TDFs

Primer combinations Tca/Mag, Tca/Mac, Tgt/Mac, Tac/Mag, Ttg/Mct, Tgt/Mct, were selected based on percentage polymorphism and re-run using 0.4 mm polyacrylamide gels (40 ml 8 % Long Ranger, 300 µl 10x APS, 30 µl Temed). The movement of the bands was monitored until the 400 bp band marker was visualized. The LI-COR machine was then stopped, and the gel was scanned using the Odyssey infrared scanner (LI-COR Biosciences, USA) and printed in actual size using the Quantity One program (LI-COR Biosciences, USA). The Odyssey scanner produced an image of the AFLP gel (Figure 3.4.3A) which was then printed in real actual size. The print out was aligned under the gel and used to excize the TDFs of interest previously identified by analysis with Quantar-Pro (LI-COR Biosciences). After excision, a second scan was taken to confirm absence of excised band. Gel fragments were kept in dH_2O, and the cDNA fragments were eluted *via* freeze-thaw cycles as suggested by the AFLP^R Expression Analysis Kit (LI-COR Biosciences, Lincoln, NB) manual. The elute was used as template for re-amplification using the AFLP kit's selective primers. PCR fragment sizes were compared to the original fragment sizes on the cDNA-AFLP gels. If more than one band was seen on the agarose gel, the band corresponding to the original fragment size was cut from the agarose. These fragments were used as template for re-amplification and later cloning.

3.3.8 Cloning and sequencing

The vector from InsTAclone™ PCR Cloning Kit #K1214 (Fermentas, Inqaba, South Africa) was used to clone the re-amplified fragments. *E. coli* strain JM109 was used to prepare competent cells (Inoue *et al*. 1990). Blue/white screening was used to identify colonies with inserts. Four positive transformed colonies from each plate were picked, grown up overnight in LB (Luria-Bertani) medium (per liter: 10 g Tryptone, 5 g Yeast extract, 8.56 mM NaCl) with 100 µg/ml Ampicilin and frozen away as 15 % (v/v) glycerol stocks. Glycerol stocks were then used for colony PCR using 0.2 µM M13 Forward (5'-TGTAAAACGACGGCCAGT-3') and M13 Reverse (5'-AGGAAACAGCTATGACC-3') primers, 2.5 mM $MgCl_2$, 1X PCR buffer, 100 µM of each dNTP and 1 U Supertherm Taq polymerase (Southern Cross Biotechnology). Thermocycling consisted of an initial 5 min denaturing step at 94°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C, before terminating with a 7 min 72°C step. One of each clone was sent to Macrogen Incorporated (Rockville, USA) for dideoxy-dye terminator sequencing.

3.3.9 Assigning putative identity

Sequences obtained were edited using the 'BioEdit' software (Hall 1999). VecScreen (NCBI, www.ncbi.nlm.nih.gov, 2007/11/02) was used to screen the edited sequences for vector contamination. To circumvent primer sequence contamination, the first and last 50 bp was removed from each sequence. The sequences were then screened against the Genbank database using the BLAST (Basic Local Alignment Search Tool algorithms) function found at the NCBI website (www.ncbi.nlm.nih.gov, 2007/11/02), tBlastX and Blastn (by Altschul *et al.* 1990; Altschul *et al.* 1997) to attain putative sequence identities. A cut off point of $E=1e^{-05}$ was used. An E-value is dependant on the lengths of the query sequence and the database searched and is the 'number of distinct alignments with equivalent or superior score that might have been expected to have occurred purely by chance. Therefore an E value of four is not statistically significant, but an E value of 10^{-5} is (Altschul *et al*. 1998). If not identified or not present in Genbank, the sequence was classified as unknown or unidentified and submitted to Genbank (Appendix B).

3.3.10 Pathway Analysis

Putative enzymes identified were submitted to the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000; http://www.genome.jp/kegg/) to obtain information on their putative functional roles in biochemical pathways (Appendix C).

3.3.11 qRT-PCR

To confirm transcriptional differences observed during the cDNA-AFLP analysis, quantitative reverse transcriptase PCR (qRT-PCR) was performed. RNA was extracted in a repeat experiment with sampling was done at 5 hours, 12 hours, 24 hours and 72 hours post infestation. RNA was extracted as described in section 3.3.3. RNA was treated with DNase I (Roche, Mannheim, Germany), and cleaned with RNeasy mini kit from Qiagen (SouthernCross, South Africa). RNA was tested for genomic DNA contamination by screening all the RNA samples with primer 92 (Table 3.4.6). First strand cDNA was then synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). Primers were designed using Primer3 v0.4.0 (Rozen and Skaletski 2000), based on sequence information from sequenced TDFs (Section 3.3.6). Primers were obtained from IDT (SouthernCross Biotechnologies, South Africa). qRT-PCRs were done using the LightCycler®480 system from Roche (Mannheim, Germany). The LightCycler 480 SYBR Green I master kit was used as prescribed by the users manual. Cycling parameters were set with an initial denaturing step of 95°C for 10 min; followed by 45 cycles of 95°C for 10 sec, Tm:°C for 15 sec, 72°C for 20 sec; followed by a melting curve of 95°C for 5 sec, 65 for 1 min and 95°C continuous. A 16S rRNA primer set (Table 3.4.6) was used as a reference gene, and all samples were tested in replicas of three. Analysis was performed following the recommendations for relative quantification by Pfaffl (2004) with the following formula:

$$\text{ratio} = \frac{(E\ \text{target})^{\Delta CP_{\text{target}}(\text{control-sample})}}{(E\ \text{ref})^{\Delta CP_{\text{ref}}(\text{control-sample})}}$$

3.4 RESULTS

3.4.1 RNA extraction

After extraction of RNA, the concentration and integrity of the samples were verified spectrophotometrically (Table 3.4.1) and by running them on a 1 % Agarose gel (Figure 3.1A). The lowest concentration of total RNA was found for the 'Betta' control sample (1228.2 ng/µl) and the highest measurement was the Betta *Dn2* control sample (1790.8 ng/µl) (Table 3.4.1).

Table 3.4.1: Total RNA, mRNA, cDNA and cDNA-AFLP pre-amplification (pre-amp) product concentrations. The purity of the total RNA was evaluated using the 260/280 absorbance ratio, where <1.8 is an indicator of protein contamination.

	Total RNA		mRNA	cDNA	cDNA dilution	Pre-amp
Treatment	**ng/µl**	**260/280**	**ng/µl**	**ng/µl**	**ng/µl**	**ng/µl**
Betta Control	1228.2	2.20	4.91	15.26	6.60	361.22
Betta Wounded	1608.8	2.20	8.55	15.85	7.30	293.41
Betta Infested	1280.3	2.20	8.96	20.35	7.34	319.39
BettaDN Control	1446.1	2.21	6.33	14.20	6.21	318.43
BettaDN Wounded	1457.0	2.20	8.87	15.79	6.40	364.17
BettaDN Infested	1555.3	2.19	7.49	14.90	6.10	291.55
Betta *Dn2* Control	1790.8	2.20	6.53	13.97	10.50/ 7.18	371.01
Betta *Dn2* Wounded	1660.4	2.20	9.99	17.92	6.77	307.08
Betta *Dn2* Infested	1450.7	2.19	7.56	12.81	6.32	429.49

Sample quality was assessed using the 260/280 absorbance ratio. All samples were of good quality as measurements obtained were between 2.19 and 2.21 (NanoDrop, Wilmington, DE). Messenger RNA (mRNA) was purified from the total RNA samples (Table 3.4.1) and the concentrations obtained after purification were all between 6.33 ng/µl and 9.99 ng/µl except for the 'Betta' control sample which had

a concentration of 4.91 ng/µl. The highest mRNA concentration was obtained with the Betta *Dn2* wounded sample, 9.99 ng/µl.

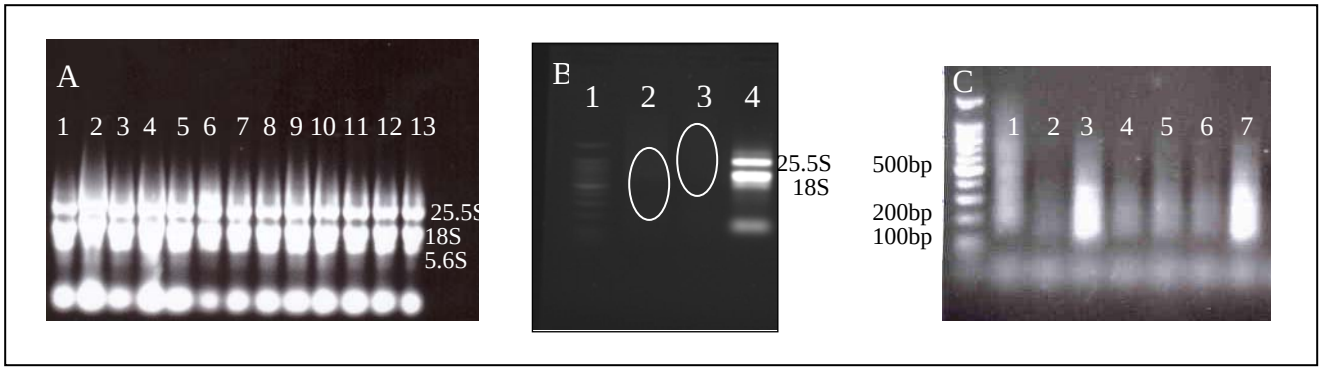


Figure 3.4.1: (A) Total RNA extraction, 1 = 'BettaDN' wounded; 2,3,4 = Betta *Dn2* wounded; 5,6,7 = 'Betta' aphid infested; 8,9,10 = 'BettaDN' aphid infested; 11,12,13 = Betta *Dn2* aphid infested; 14 lambda III marker. (**B)**: cDNA synthesis, 1 = Lambda marker III; 2 = ssDNA; 3 = cDNA; 4 = total RNA. (**C):** Pre-amplified cDNA run on a 1 % gel. 1 = lambda III marker. 4 = Betta Wounded; 8 = 'BettaDN' Infested.

First and second strand cDNA synthesis were evaluated by running first strand and double stranded cDNA on a 2% agarose gel (Figure 3.4.1B). The cDNA concentrations are given in Table (3.4.1). All the measurements obtained fell between 12.81 ng/µl (Betta *Dn2* infested) and 20.35 ng/µl Betta infested. The cDNA dilution to 100 ng/µl for the pre-amplification step (Table 3.4.1), showed all samples to be within a close range of between 6.10 ng/µl (BettaDN infested sample) and 7.34 ng/µl (Betta infested), with the exception of Betta *Dn2* control (10.50 ng/µl). This was re-diluted to a concentration of 7.18 ng/µl. Pre-amplification results (Figure 3.4.1C) indicated that the samples that amplified the best were 'Betta' aphid infested and Betta *Dn2* control (lanes four and eight). The pre-amplification material concentrations (Table 3.4.1) were measured. The lowest concentration was found for 'BettaDN' aphid-infested sample and the highest concentration was Betta *Dn2* control sample.

3.4.2 cDNA-AFLP primer screening

Selective amplification makes use of primers that amplify a subset of the pre-amplification material. The selective primers contain additional nucleotides at the 3' end of each primer. Primer pairs were screened for the number of polymorphisms observed between the different samples. Of the screened primer sets that gave unambiguous results, primer pair Tgt/Mct showed the most polymorphic TDFs (40.9 %), while Tac/Mag gave the least polymorphic fragments (30 %) (Table 3.4.4). Percentage polymorphism was worked out by dividing the number of scored polymorphic bands by the number of constituent bands (Equation 2.1).

Equation 3.1: $$\text{Percentage polymorphism} = \frac{\text{number of scored polymorphic bands}}{\text{number of constituent bands}}$$

Table 3.4.2: Number of monomorphic and polymorphic fragments after cDNA-AFLP image analysis.

***Taq*I primer (T)**	***Mse*I primer (M)**	**Monomorphic fragments**	**Polymorphic fragments**	**Total fragments**	**Percentage Polymorphism**
Tca	Mag	80	40	120	33 %
Tca	Mac	85	50	135	37 %
Tag	Mac	93	57	150	38 %
Tgt	Mac	90	50	140	35.7 %
Tac	Mag	70	30	100	30 %
Ttg	Mct	110	55	165	33 %
Tgt	Mct	65	45	110	40.9 %

After the selective amplifications were completed, they were run on a polyacrylamide gel using the Li-Cor gel system in order to screen for the levels of polymorphism. Three primer combinations were run

at a time, with each sample loaded twice to ensure consistency and to remove all ambiguous TDFs (Figure 3.4.2).

3.4.3 cDNA-AFLP data analysis

In order to assess the differences in banding patterns of the TDFs between the sample treatments and genotypes are visualized by importing the image created by the LICOR machine (Figure 3.4.2) into the Quantar pro software (LI-COR Biosciences). Differences, or changes in expression pattern, are of interest as they signal differences in transcription profile between the samples. Bands of interest were chosen with characteristics such as up- or down-regulation or if they were present or absent (polymorphic) in comparison to other samples (Figure 3.4.2).

The chosen primer combinations (Tca/Mag, Tca/Mac, Tgt/Mac, Tac/Mag, Ttg/Mct, Tgt/Mct) were then re-run on a 0.4mm gel. 235 TDFs were identified and scored using the QuantarPro software, giving them an AFLP marker number (M* where the star is a software generated number based on the weight of the band). These bands of interest were then excised (Figure 3.4.3). The TDFs were re-amplified (Figure 3.4.4A) to produce PCR products. The PCR products were then cloned, in order to assign putative identities to them (Figure 3.4.4B).

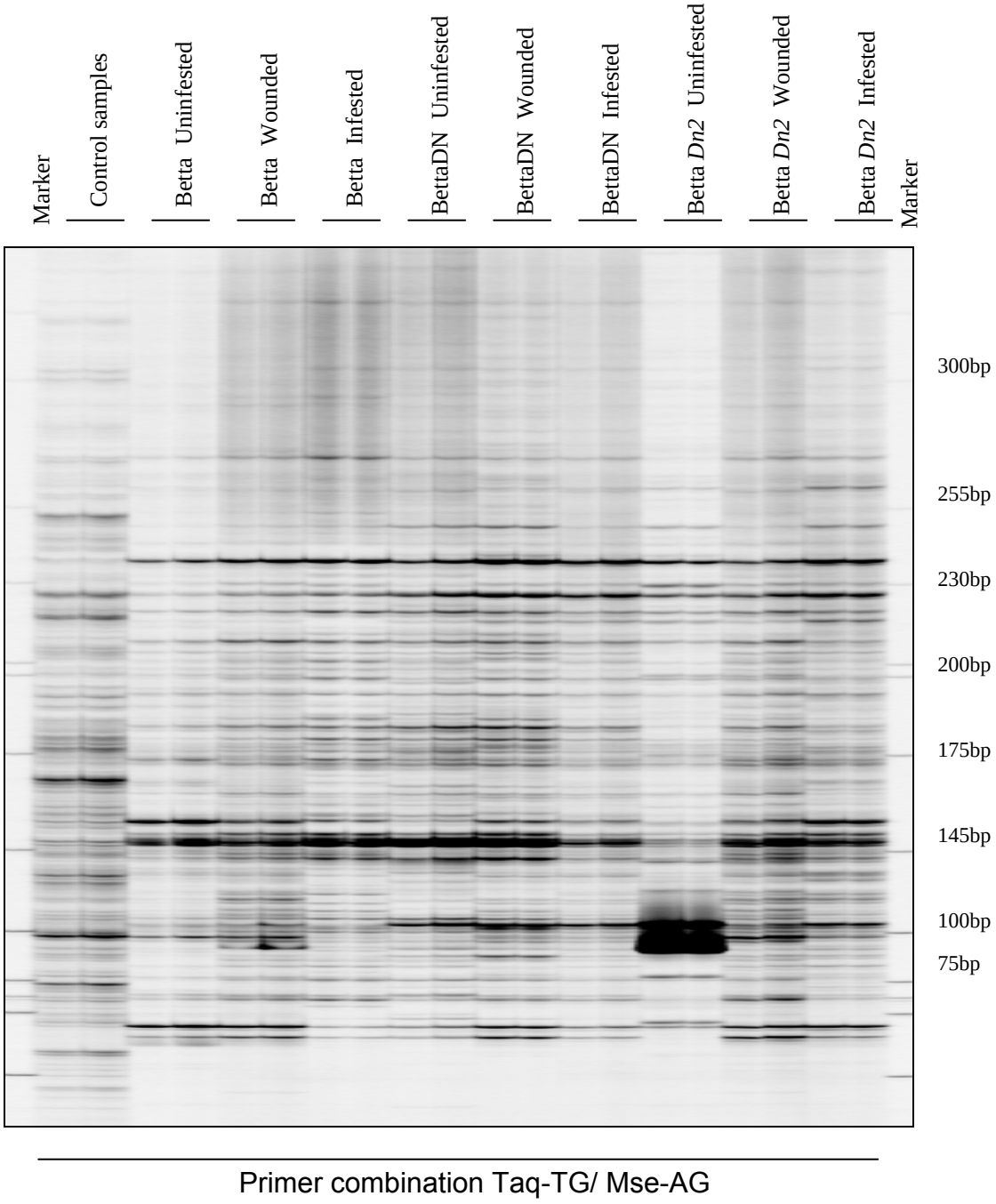


Figure 3.4.2: cDNA-AFLP selective amplification with Primer combination Ttg/ Mag. 700 marker-dye was used, with treatment conditions indicated above their respective lanes.

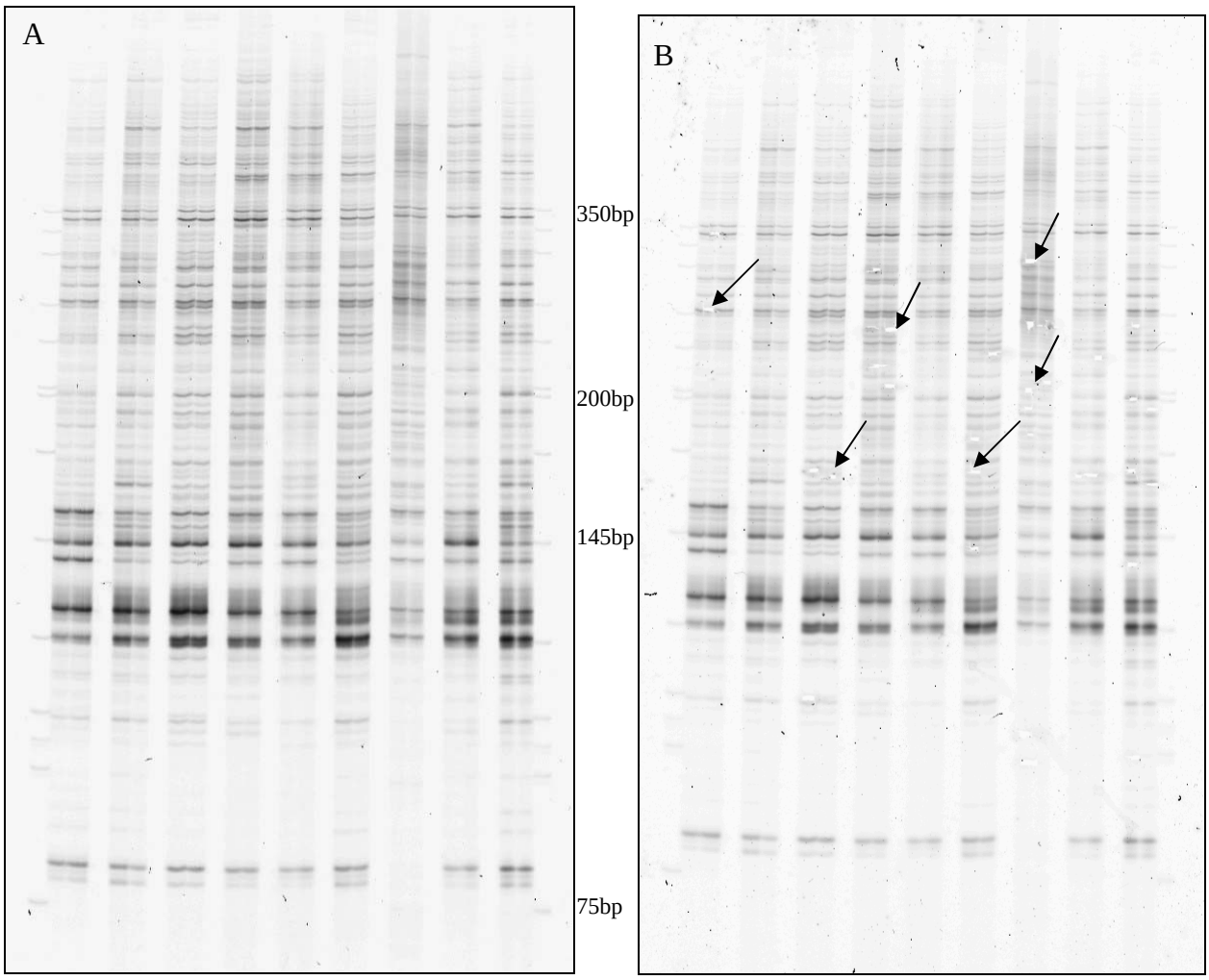


Figure 3.4.3: (A) cDNA-AFLP gel image produced by the Odyssey scanner, (B) figure scanned after bands had been cut out. Primer combination Tca/Mag was used. Arrows point out a few of the bands that were excised.

Of the 235 TDFs excised from the gels, 150 were cloned and sequenced for analysis (Table 3.4.3). The BLAST program (National Center for Biotechnology Information, NCBI, 2008) takes query sequences and compares it to a database of sequences to find homology. The statistical weight or probability of TDF's putative identity is important and is measured with the e-value (statistical expectancy value). The size of the fragment and level of homology is used to work out the e-value.

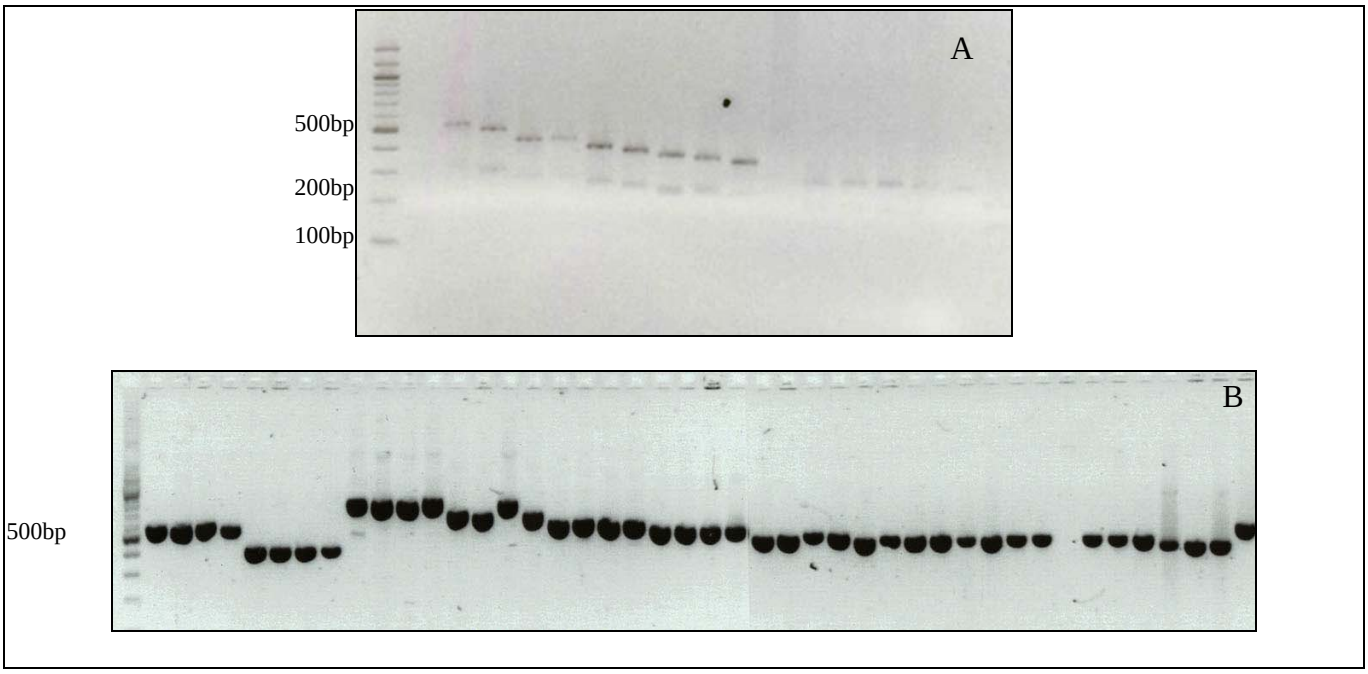


Figure 3.4.4: (A) Re-amplified bands from cDNA-AFLP gel were run on a 2% agarose gel. Lane 1 contains 100bp ladder from New England Biolabs (B) Colony PCR products run on a 1.5% agarose gel.

Of the 150 cloned sequences, 132 had putative identities, 10 had homology to proteins of unknown function and 18 had no homology to known sequences (Table 3.4.3). The 18 sequences with no known identity or homology were submitted to GenBank (Appendix B).

Table 3.4.3: Putative identity was assigned to cloned TDFs. Accession numbers of putative homologues, whether the result is from BLASTn or BLASTx, the length of the fragment and the E-value of the blast results are indicated.

TDF	Putative Identity	Accession	BLASTn/ BLASTx	Length	E-value
	Amino Acid Metabolism				
TgtMac115	2-isopropylmalate synthase B [*Zea mays*]	EU957146.1	BLASTn	295	3e-90
TgtMac116	WAP2 aspartic proteinase [*Triticum aestivum*]	AB219969.1	BLASTn	240	2e-105
TgtMac123	2-isopropylmalate synthase B [*Zea mays*]	EU957146.1	BLASTn	258	3e-90
TtgMct177	Propionyl-CoA carboxylase beta chain [*Zea mays*]	EU957259.1	BLASTn	330	3e-23
TtgMct189	Hydroxymethylglutaryl coenzyme A synthase [*Oryza sativa* (japonica cultivar-group)]	NM_001068945.1	BLASTn	472	6e-88
	Nucleic Acid Processing				
TcaMag50	Polynucleotide adenylyltransferase [*Oryza sativa* (japonica cultivar-group)]	AY785762.1	BLASTn	409	2e-118
TcaMag73	Os04g0566500 PAZ_argonaute_like domain [*Oryza sativa* (Japonica Group)]	NM_001060116.1	BLASTn	399	3e-09
TcaMag75	Transcription factor Tfb4 [*Zea mays*]	NM_001137714.1	BLASTn	232	4e-146
TcaMac89	Similar to topoisomerase II alpha [*Pinus koraiensis*]	NP_817269.1	BLASTx	305	3e-17
TcaMac99	Splicing factor, CC1-like family protein [*Oryza sativa* (japonica cultivar-group)]	NM_001055532.1	BLASTn	225	2e-36
TgtMac112	Repressor protein Dr1 [*Triticum aestivum*]	AF464903.1	BLASTn	406	0.0
TgtMac120	α-1,3-mannosyl-glycoprotein β-1,2-	AP008208.1	BLASTn	361	6e-11

	Nacetylglucosamine transferase [*Oryza sativa* (japonica cultivar-group)]				
TtgMct181	Macoilin; Transmembrane protein [*Arabidopsis thaliana*]	NM_122275.3	BLASTn	179	7e-18
TgtMct206	RNA-binding region RNP-1 [*Oryza sativa* (japonica cultivar-group)]	NM_001058955.1	BLASTn	342	4e-52
	Fatty Acid and Lipid Metabolism				
TcaMag53	Myristoyl-ACP thioesterase [*Zea Mays*]	EU956909.1	BLASTn	296	4e-42
TcaMag57	Putative lipoic acid synthetase [*Zea Mays*]	EU966377.1	BLASTn	150	6e-30
TcaMac93	Acyl-ACP thioesterase [*Oryza sativa* (japonica cultivar-group)]	AP008212.1	BLASTn	254	5e-44
TcaMac95	rRNA-45S [*Oryza sativa* (japonica cultivar-group)]	AP008216.1	BLASTn	265	9e-136
TtgMct173	Putative zinc finger DHHC domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_001051454.1	BLASTn	294	3e-29
	Starch and Sucrose Metabolism				
TcaMac92	Putative beta-glucosidase [*Oryza sativa* (japonica cultivar-group)]	NM_001051826.1	BLASTn	275	5e-37
TacMag145	Fructose-1,6-bisphosphatase (cytosolic isoform) [*Oryza sativa* (japonica cultivar-group)]	DQ865190.1	BLASTn	250	1e-71
TacMag163	Spliceosome RNA helicase BAT1 [*Zea mays*]	EU963228.1	BLASTn	714	0.0
TtgMct186	NADP-dependant Malate deydrogenase chloroplast precursor [*Paspalum paniculatum*]	AJ512372.1	BLASTn	238	6e-50
TgtMct235	Ribulose-1,5-bisphosphate carboxylase oxygenase small subunit [*Triticum aestivum*]	AB042069.1	BLASTn	250	7e-112

Protein Production

TcaMac87	Chaperone protein dnaJ-like [*Oryza sativa* (japonica cultivar-group)]	NM_0010541 28.1	BLASTn	351	2e-117
TcaMac91	Elongation factor 1 alpha-subunit (TEF1) [*Triticum aestivum*]	M90077.1	BLASTn	300	2e-78
TgtMac129	Putative poly(A)-binding protein (PABPs) [*Oryza sativa* (japonica cultivar-group)]	NM_0010680 50.1	BLASTn	211	4e-19
TacMag135	eIF4-gamma/eIF5/eIF2-epsilon domain-containing Protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010742 92.1	BLASTn	366	2e-130
TacMag136	eIF4-gamma/eIF5/eIF2-epsilon domain-containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010742 92.1	BLASTn	364	3e-134
TacMag142	NADH dehydrogenase [*Zea mays*]	DQ490951.2	BLASTn	262	6e-78
TacMag143	Elongation factor 1 gamma [*Oryza sativa* (japonica cultivar-group)]	NM_0010644 51.1	BLASTn	283	3e-91
TacMag160	NADH dehydrogenase subunit 1 NADH dehydrogenase subunit 5 [*Zea mays* subsp. mays]	DQ490951.2	BLASTn	100	1e-42
TtgMct196	Peptidase M48, Ste24p family protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010545 79.1	BLASTn	379	6e-18
TgtMct213	rRNA-45S ribosomal RNA [*Oryza sativa* (japonica cultivar-group)]	AP008216.1	BLASTn	203	1e-78
TgtMct229	Splicing factor 1 KH-domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010515 64.1	BLASTn	236	3e-20

Cell Cycle

TgtMac132	Putative VHS1 protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010490 38.1	BLASTn	421	3e-66
TacMag133	DNA mismatch repair protein family protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010538 27.1	BLASTn	458	5e-56
TacMag134	RabGAP/TBC domain-containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010744 26.1	BLASTn	411	9e-98
TtgMct187	Putative Kinesin heavy chain [*Oryza sativa* (japonica cultivar-group)]	NM_0010612 19.1	BLASTn	183	6e-25

Cell Maintanance

TtgMct166	Lissencephaly type-1-like homology motif domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_001050946.1	BLASTn	524	9e-162
TtgMct174	Myosin heavy chain [*Oryza sativa* (japonica cultivar-group)]	NM_001054826.1	BLASTn	376	6e-103
TgtMct201	DNA-directed RNA polymerase III largest subunit [*Oryza sativa* (japonica cultivar-group)]	NM_001059710.1	BLASTn	528	3e-148
TgtMct209	Protein H2A [*Triticum aestivum*]	D38091.1	BLASTn	250	1e-96

Membrane Proteins

TcaMag54	Plasma membrane P-type proton pump ATPase (Ha1) [*Hordeum vulgare* subsp. vulgare]	AY136627.1	BLASTn	200	9e-22
TcaMac85	Putative zinc transporter zupT [*Oryza sativa* (japonica cultivar-group)]	NM_001052725.1	BLASTx	400	2e-67
TcaMac103	Vacuolar ATP synthase subunit H [*Triticum aestivum*]	DQ681104.1	BLASTn	188	4e-70
TcaMac104	Vacuolar ATP synthase subunit H [*Triticum aestivum*]	DQ681104.1	BLASTn	154	3e-66
TgtMac117	UNC50 [*Oryza sativa* (japonica cultivar-group)]	NM_001069101.1	BLASTn	219	5e-75
TgtMac119	Integral membrane protein NRAMP [*Hordeum vulgare* subsp. vulgare]	AJ514946.1	BLASTn	454	1e-157
TgtMac125	Ca2+/H+-exchanging protein (CAX) [*Hordeum vulgare* subsp. vulgare]	EF446604.1	BLASTn	200	4e-77
TgtMac127	UNC-50 family protein [*Oryza sativa* (japonica cultivar-group)]	NM_001069101.1	BLASTn	221	2e-75
TgtMac127b	V ATPase H, regulatory vacuolar ATP synthase subunit H [*Zea mays*]	EU952681.1	BLASTn	197	8e-16
TacMag137	α-adaptin C [*Oryza sativa* (japonica cultivar-group)]	NM_001055264.1	BLASTn	320	6e-49

TacMag140	Putative COX VIIa-like protein [*Zea mays*]	EU975680.1	BLASTn	314	3e-40
TtgMct190	Na+/H+ antiporter (NHX1) [*Triticum aestivum*]	AY040245.1	BLASTn	159	1e-52
TtgMct191	PDR11 ABC transporter [*Oryza sativa* (japonica cultivar-group)]	NM_001074706.1	BLASTn	508	5e-172
TtgMct192	PDR11 ABC transporter [*Oryza sativa* (japonica cultivar-group)]	NM_001074706.1	BLASTn	531	1e-179
TgtMct225	Plasma membrane H+-ATPase (Ha1) [*Triticum aestivum*]	AY543630.1	BLASTn	350	7e-93
	Chloroplast related/Photosynthesis				
TcaMag51	10kDa PSII protein [*Hordeum vulgare*]	X97771.1	BLASTn	169	2e-29
TcaMag74	Transcription factor Tfb4 [*Zea mays*]	NM_001137714.1	BLASTn	323	4e-146
TcaMag76	Transcription factor Tfb4 [*Zea mays*]	NM_001137714.1	BLASTx	324	8e-19
TcaMac90	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	BAA10929.1	BLASTx	301	3e-21
TcaMac94	Similar to topoisomerase II alpha [*Pinus koraiensis*]	NP_817269.1	BLASTx	300	8e-13
TgtMac111	Aldose reductase (NAD(P)(H) oxidoreductases family) [*Oryza sativa* (japonica cultivar-group)]	NM_001062361.1	BLASTn	455	3e-128
TgtMac114	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	BAA10929.1	BLASTx	351	2e-27
TgtMac121	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	BAA10929.1	BLASTx	305	5e-24
TgtMac122	Similar to topoisomerase II alpha [*Pinus koraiensis*]	NP_817269.1	BLASTx	305	4e-15
TgtMac126	Ubiquinol-cytochrome C reductase complex [*Oryza sativa* (japonica cultivar-group)]	NM_001067179	BLASTn	215	5e-18
TtgMct129b	Acyl-carrier protein, mitochondrial precursor NADH-ubiquitinone oxidoreductase [*Oryza sativa* (japonica cultivar-group)]	NM_001065739.1	BLASTn	204	1e-07
TacMag148	rRNA-45S [*Oryza sativa* (japonica cultivar-group)]	AP008216.1	BLASTn	355	4e-178

TtgMct171	23S ribosomal RNA gene; Chloroplast [*Festuca arundinacea*]	DQ490949.1	BLASTn	450	0.0
TtgMct172	10kD PSII protein [*Hordeum vulgare*]	X97771.1	BLASTn	343	2e-114
TtgMct181b	ATP-dependent Clp protease ATP-binding subunit clpA chloroplast precursor [*Oryza sativa* (japonica cultivar-group)]	NM_001072974.1	BLASTn	448	2e-68
TtgMct198	Chloroplast carbonic anhydrase [*Hordeum vulgare*]	L36959.1	BLASTn	210	4e-64
TgtMct207	Nonphototrophic hypocotyl 1 (Phototropin) [*Oryza sativa* (japonica cultivar-group)]	NM_001058950.1	BLASTn	331	5e-108
TgtMct224	Putative ribosomal protein 23S [*Festuca arundinacea*]	DQ490949.1	BLASTn	436	0.0
	Resistance and Stress-Related Proteins				
TcaMac86	Alpha-1,3-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase [*Oryza sativa* (japonica cultivar-group)]	NM_001055166.1	BLASTn	368	7e-08
TcaMac88	Putative protein kinase [*Oryza sativa* (japonica cultivar-group)]	AP008208.1	BLASTn	358	1e-20
TcaMac97	Serine/threonine phosphatase type 5 [*Oryza sativa* (japonica cultivar-group)]	AP008211	BLASTn	251	4e-26
TcaMac98	Calcium-dependent protein kinase 2 [*Oryza sativa* (japonica cultivar-group)]	AP008209	BLASTn	250	2e-17
TcaMac100	Ubiquitin [*Oryza sativa* (japonica cultivar-group)]	NM_001059183.1	BLASTn	242	1e-19
TcaMac101	PKc-like protein kinase [*Oryza sativa* (japonica cultivar-group)]	NM_001059776.1	BLASTx	208	1e-51
TgtMac124	Single-stranded nucleic acid binding protein(whGRP1) [*Triticum aestivum*]	U32310.1	BLASTn	377	2e-49
TgtMac128	Isochorismatase hydrolase family protein/ Cystein hydrolase-like protein [*Oryza sativa* (japonica cultivar-group)]	NM_001053916.1	BLASTn	209	3e-15/ 4e-07
TgtMac130	Putative Zn-finger, RING protein [*Oryza sativa* (japonica cultivar-group)]	AP008217.1	BLASTx	263	6e-24
TacMag138	Os06g0647600 [*Oryza sativa* (japonica cultivar-group)]	NM_001064743.1	BLASTn	286	1e-37

TacMag139	E3 ubiquitin protein ligase URE-B1 [*Oryza sativa* (japonica cultivar-group)]	NM_001073203.1	BLASTn	286	5e-69
TacMag141	Isoflavone reductase [*Hordeum vulgare*]	EU977178.1	BLASTx	300	1e-109
TacMag144	GTP1/OBG domain containing protein; Ribosomal biogenesis GTPase [*Oryza sativa* (japonica cultivar-group)]	NM_001062159.1	BLASTn	243	5e-76
TacMag146	Potential cadmium/Zn transporting ATPase HMA1 [*Oryza sativa* (japonica cultivar-group)]	NM_001064952.1	BLASTn	217	1e-70
TacMag147	Disease resistance protein; P-loop containing Nucleoside Triphosphate Hydrolases [*Oryza sativa* (japonica cultivar-group)]	NM_001053097.1	BLASTn	263	3e-47
TacMag149	F1F0- ATPase inhibitor protein [*Oryza sativa* (japonica cultivar-group)]	NM_001069033.1	BLASTn	497	4e-18
TacMag152	Putative immunophilin [*Hordeum vulgare* subsp. vulgare]	AJ495769.1	BLASTn	478	0.0
TacMag153	Putative dioxygenase PcbC [*Oryza sativa* (japonica cultivar-group)]	NM_001062812.1	BLASTn	197	5e-63
TacMag155	Putative disease resistance protein family [*Oryza sativa* (japonica cultivar-group)]	AP008212.1	BLASTn	384	9e-79
TacMag156	NADH dehydrogenase (Ubiquinone oxidoreductase) [*Oryza sativa* (japonica cultivar-group)]	NM_001066990.1	BLASTn	224	3e-19
TacMag157	Peroxisomal ascorbate peroxidase (APX) [*Triticum aestivum*]	EF555121.1	BLASTn	187	2e-24
TacMag158	Peroxisomal ascorbate peroxidase (APX) [*Triticum aestivum*]	EF555121.1	BLASTn	204	3e-25
TacMag159	SKIP interacting protein 2 (SIP2) [*Oryza sativa* (japonica cultivar-group)]	EU368692.1	BLASTn	186	5e-59
TacMag162	Zn finger containing protein [*Oryza sativa* (japonica cultivar-group)]	AP008208.1	BLASTn	121	2e-35
TtgMct164	LIM-domain binding protein [*Oryza sativa* (japonica cultivar-group)]	NM_001073994.1	BLASTx	565	4e-96
TtgMct165	ARM repeat fold domain containing protein [*Oryza sativa* (japonica cultivar-group)]	AP008218.1	BLASTn	539	6e-20
TtgMct167	Defense-related F-box protein [*Oryza sativa* (indica cultivar-group)]	DQ237916.1	BLASTn	485	9e-162

TtgMct168	ARIADNE-like protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010597 09.1	BLASTn	416	8e-169
TtgMct183	SKIP interacting protein 2 [*Oryza sativa* (japonica cultivar-group)]	ACA64827.1	BLASTx	159	7e-23
TtgMct183b	Short chain alcohol dehydrogenase [*Oryza sativa* (japonica cultivar-group)]	NM_0010562 11.1	BLASTn	159	3e-47
TtgMct184	WD40 domain containing TOR family protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010724 93.1	BLASTn	300	2e-37
TtgMct184b	SEU3A protein [*Oryza sativa* (japonica cultivar-group)]	ABA91996.2	BLASTx	552	4e-10
TtgMct188	TASK5 mRNA [*Triticum aestivum*]	AB281487.1	BLASTn	100	6e-25
TtgMct193	Annexin like protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010696 31.1	BLASTn	504	5e-77
TtgMct195	TPR-like domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010580 28.1	BLASTn	409	5e-19
TtgMct197	TPR-like domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010691 35.1	BLASTn	210	3e-78
TtgMct199	TOR family protein, WD40 domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010724 93.1	BLASTn	302	1e-32
TgtMct202	ARM-repeat containing protein [*Oryza sativa* (japonica cultivar-group)]	AP008218	BLASTn	536	1e-57
TgtMct204	ADP-ribosylation factor [*Oryza sativa* (japonica cultivar-group)]	NM_0010657 42.1	BLASTn	434	1e-147
TgtMct210	TPR-like domain containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010499 92.1	BLASTn	198	2e-43
TgtMct211	Shaggy-like kinase [*Triticum aestivum*]	AB281487.1	BLASTn	108	2e-37
TgtMct223	Selenium binding protein (SBP) [*Oryza sativa* (japonica cultivar-group)]	NM_0010517 29.1	BLASTn	632	0.0
TgtMct226	LRR ribonuclease inhibitor subtype containing protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010729 73.1	BLASTn	217	1e-14
TgtMct230	Peptidase (ubiquitin carboxy-terminal hydrolase 2 family) [*Oryza sativa* (japonica cultivar-group)]	NM_0010635 27.1	BLASTn	161	4e-56
TgtMct232	ADP-ribosylation factor [*Oryza sativa* (japonica cultivar-group)]	NM_0010657 42.1	BLASTx	433	4e-103

TgtMct234	Protein phosphatase 2C [*Oryza sativa* (japonica cultivar-group)]	NM_0010717 38.1	BLASTn	312	6e-94
	Homology with Proteins of Unknown Function				
TcaMag52	Complete chloroplast genome [*Lolium perenne*]	AM777385.2	BLASTn	426	7e-23
TcaMag55	J080318E23 cDNA clone [*Oryza sativa* (japonica cultivar-group)]	AK242543.1	BLASTn	78	2e-17
TcaMac84	rRNA-45S [*Oryza sativa* (japonica cultivar-group)]	AP008216.1	BLASTn	536	1e-133
TgtMac113	OsI_20857 [*Oryza sativa* (japonica cultivar-group)]	EAY98902.1	BLASTx	389	2e-16
TacMag150	Flbaf76103 [*Hordium vulgare*]	AK250404.1	BLASTn	285	1e-17
TacMag151	Retrotransposon protein [*Oryza sativa* (japonica cultivar-group)]	EF576506.1	BLASTn	350	6e-163
TtgMct169	Os05g0546800 [*Oryza sativa* (japonica cultivar-group)]	NP_0010562 20.1	BLASTx	401	2e-17
TtgMct170	Flbaf79p20 [*Hordeum vulgare* subsp. Vulgare]	AK250451.1	BLASTn	354	8e-11
TtgMct178	p8MTCP1 family protein [*Oryza sativa* (japonica cultivar-group)]	NM_0010607 09.1	BLASTn	318	2e-09
TgtMct191b	clone wlk1.pk0028.e1:fis, [*Triticum aestivum*]	BT009249.1	BLASTn	162	2e-36
	No Homology/Unknown				
TcaMag56	No Homology		BLASTn	164	
TcaMag58	No Homology		BLASTn	150	
TcaMac96	No Homology		BLASTn	262	
TacMag154	No Homology		BLASTn	213	
TacMag161	No homology		BLASTn	337	
TtgMct175	No Homology		BLASTn	228	
TtgMct176	No Homology		BLASTn	368	
TtgMct179	No Homology		BLASTn	250	
TtgMct180	No Homology		BLASTn	236	
TtgMct182	No Homology		BLASTn	176	

TtgMct185	No Homology	BLASTn	351
TtgMct194	No Homology	BLASTn	472
TtgMct200	No Homology	BLASTn	177
TgtMct203	No Homology	BLASTn	550
TgtMct208	No Homology	BLASTn	272
TgtMct212	No Homology	BLASTn	246
TgtMct215	No Homology	BLASTn	200
TgtMct217	No Homology	BLASTn	200
TgtMct226b	No Homology	BLASTn	152

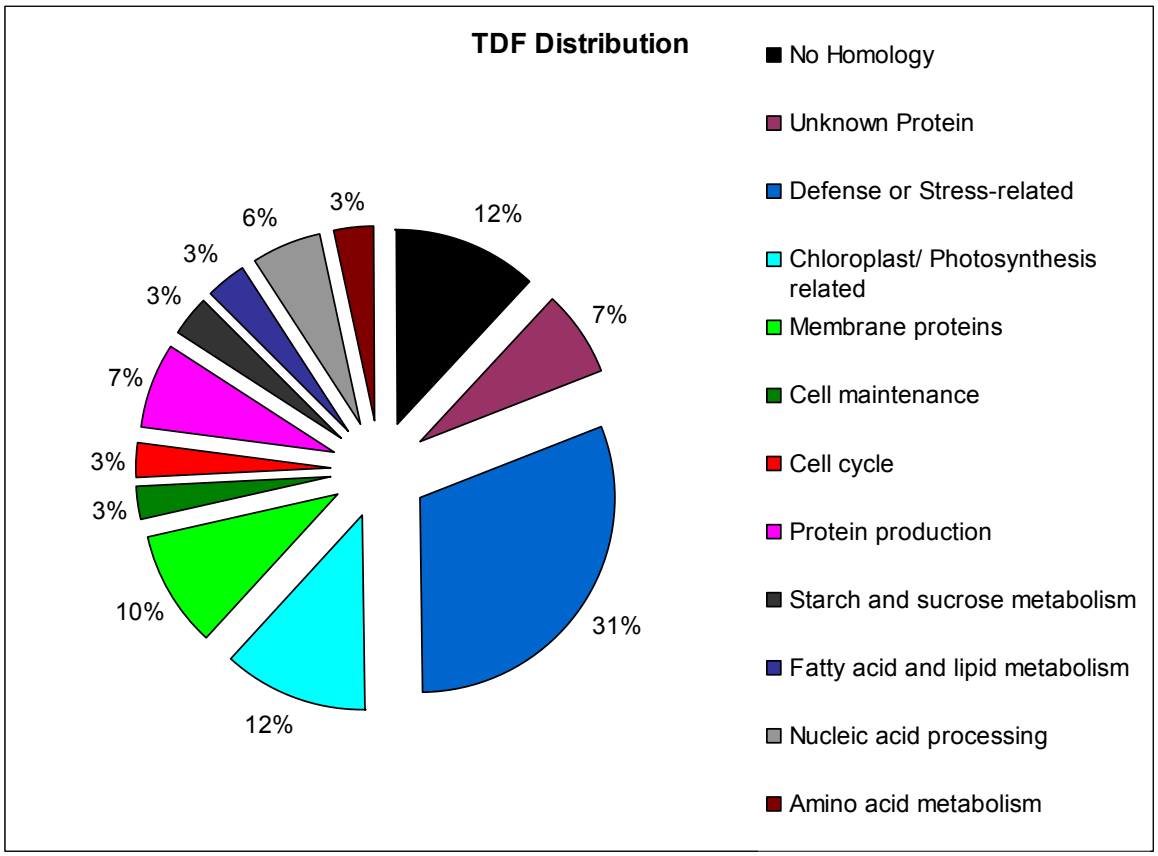


Figure 3.4.5: Pie chart showing percentage of TDFs which fall into each cellular process.

After appointing all TDFs to functional categories; the defense or stress-related category contains the largest percentage of TDFs, with 31 % of TDFs falling into this category. The second largest category (12 %) contains TDFs involved in the chloroplast or photosynthesis. The categories containing the

least TDFs are amino acid metabolism, starch and sucrose metabolism, fatty acid and lipid metabolism, cell cycle and cell maintenance were all 3 % respectively (Figure 3.4.5).

3.4.4 Cluster Analysis

Cloned TDFs were clustered using TreeView, after scoring on the Quantar Pro software (LI-COR Biosciences) (Figure 3.4.7). Two clusters were obtained with the largest difference between Cluster A and B, is that in Cluster A, there is higher up-regulation in *Dn* and *Dn2* treatments, whereas, in Cluster B, there is mostly up-regulation in the Betta treatments in comparison to the BettaDN and Betta *Dn2* treatments (Figure 3.4.6).

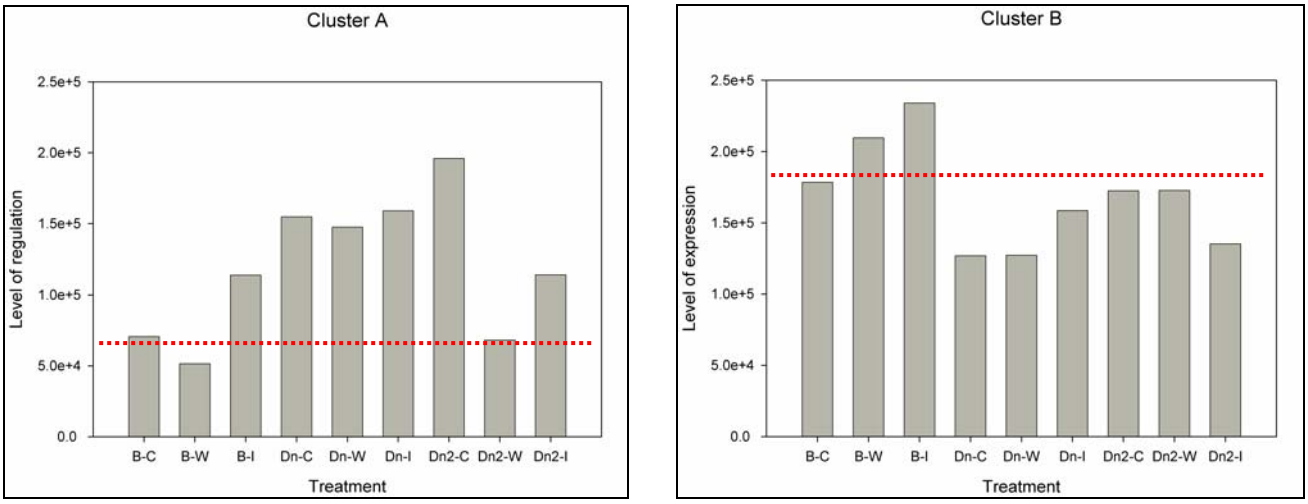


Figure 3.4.6: Graphs representing the average trend of treatments within each major cluster A and B of the TDF TreeView cluster tree (Figure 3.4.7), where B-C= Betta control; B-W= Betta wounded, B-I= Betta infested; Dn-C= BettaDN control; Dn-W= BettaDN wounded; Dn-I= BettaDN infested; Dn2-C= Betta *Dn2* control; Dn2-W= Betta *Dn2* wounded; Dn2-I= Betta *Dn2* infested.

Graphs representing the average trend for each cluster were also calculated (Figure 3.4.8 and 3.4.9). In Cluster A1, (Figure 3.4.8) there is a large up-regulation in *Dn2* control treatments in comparison to the Betta control treatment. Relative to the control, Betta wounded and *Dn2* wounded and infested treatments are down-regulated. Cluster A2, (Figure 3.4.8) is characterized by up-regulation in the *Dn2* control treatment. Cluster A3 (Figure 3.4.8) shows up-regulation in relation to the Betta control

treatment in all of the BettaDN treatments, as well as in Betta *Dn2* infested, with Betta *Dn2* control down-regulated.

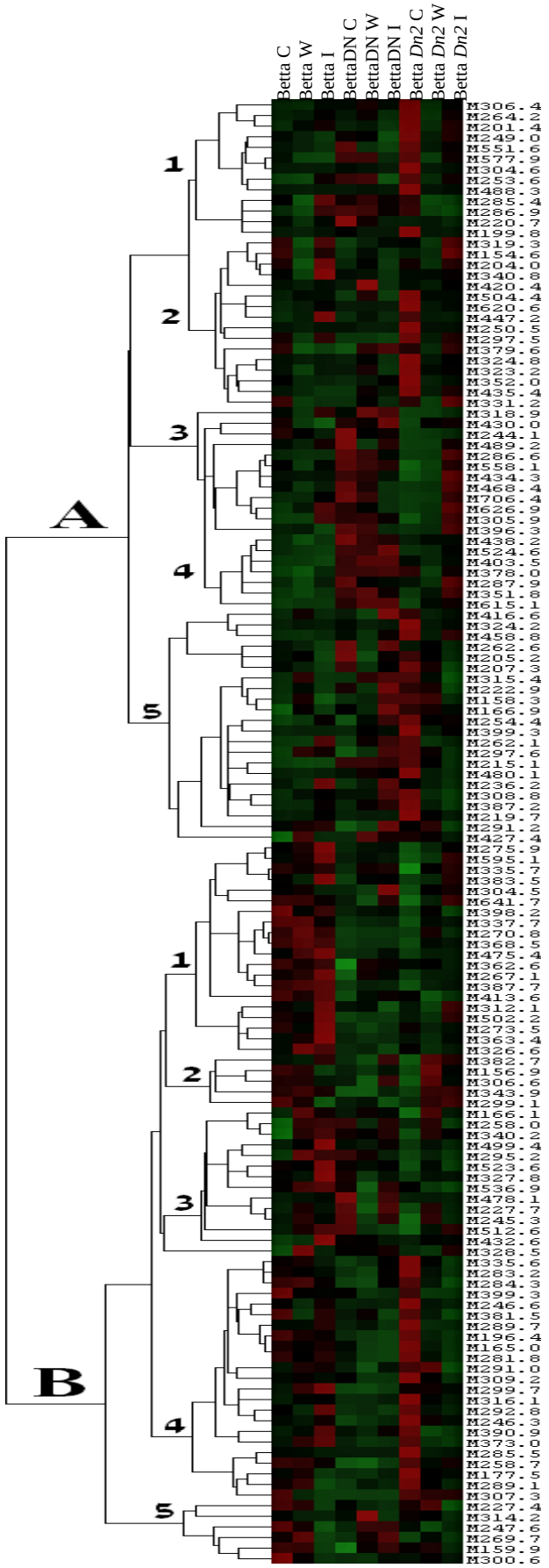


Figure 3.4.7: TreeView image of cloned TDFs.

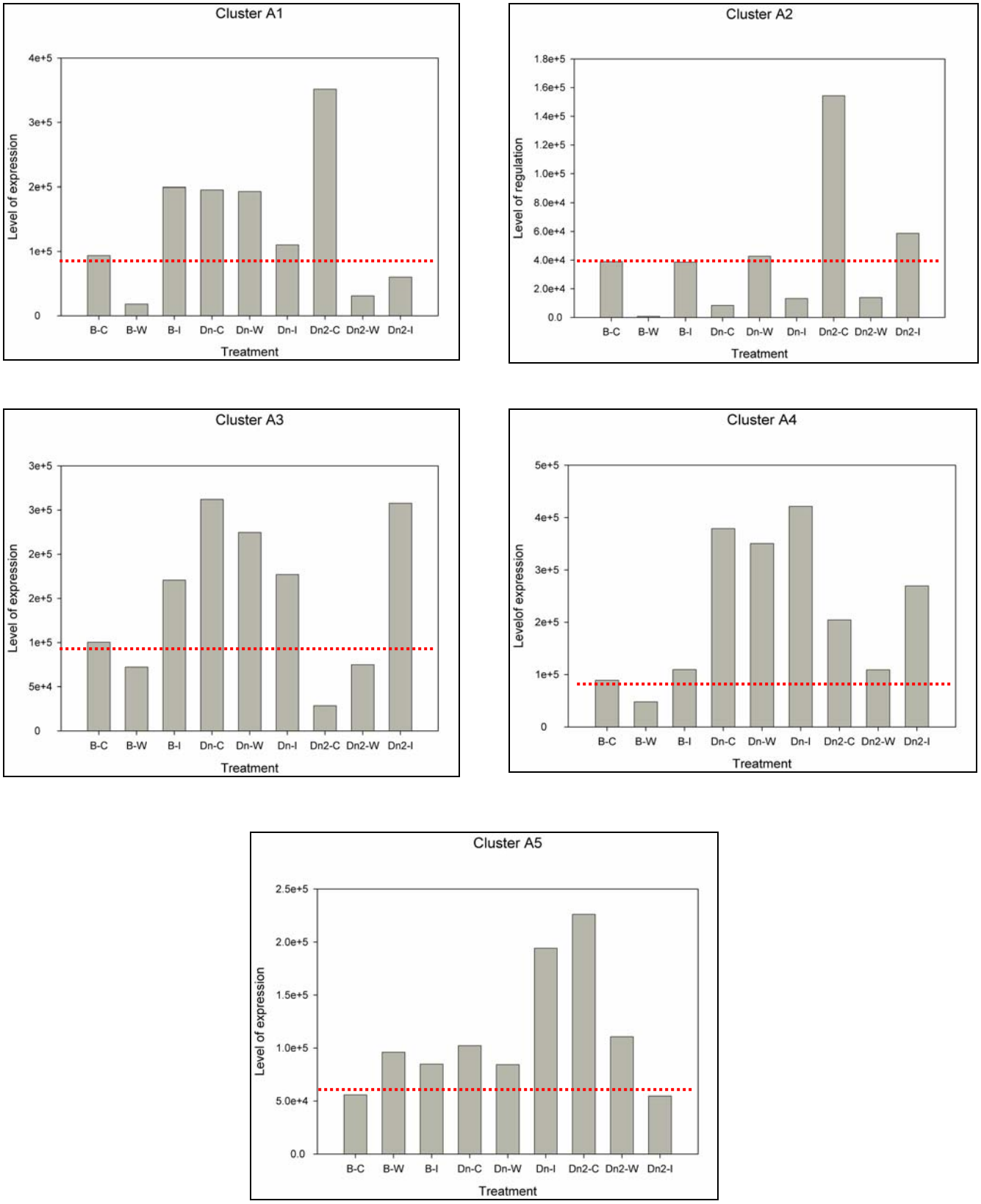
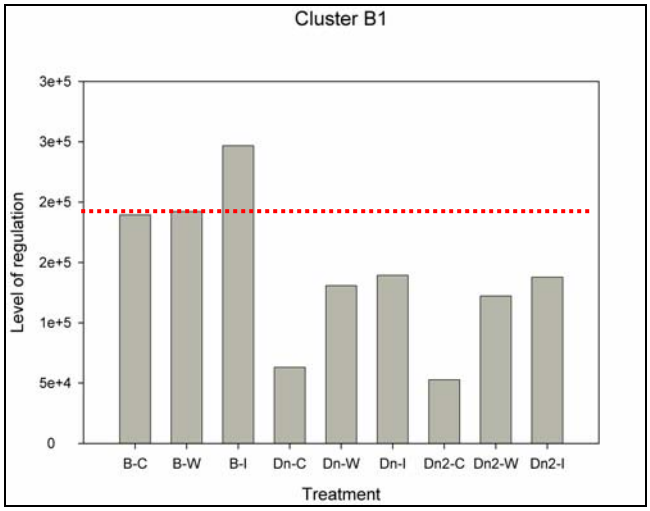
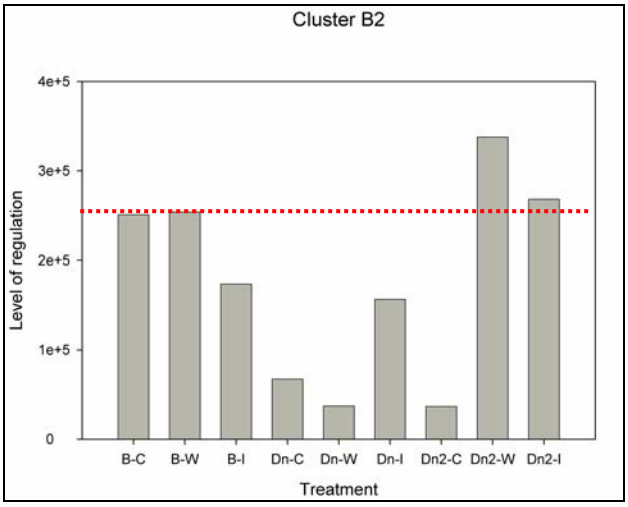
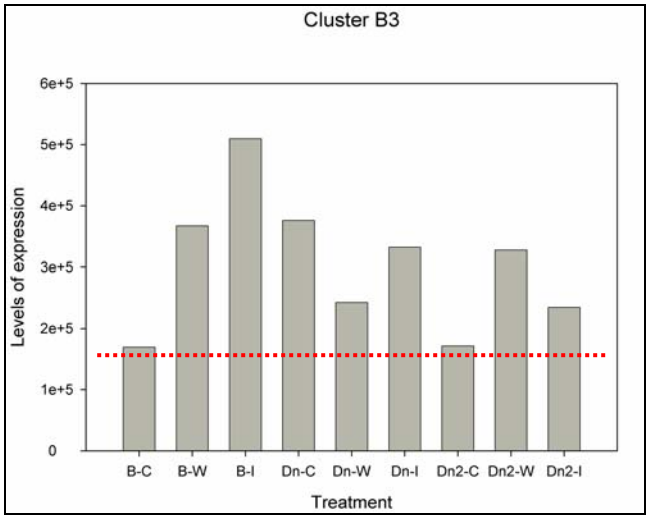
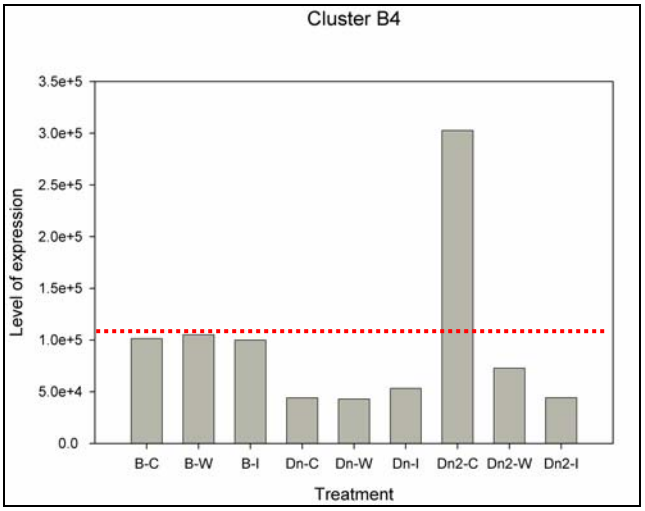


Figure 3.4.8: Graphs showing the averaged trends for each cluster in cluster A (Figure 3.4.7).









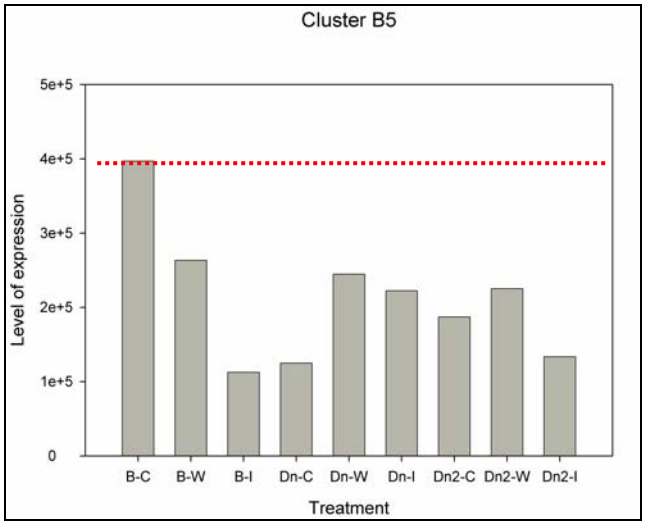


Figure 3.4.9: Graphs showing the averaged trends for each cluster in cluster B (Figure 2.4.7).

Cluster A4 (Figure 3.4.8) shows TDFs with up-regulation in the BettaDN treatments and in Betta *Dn2* infested in relation to Betta control. In cluster A5, (Figure 3.4.8) TDFs are up-regulated in BettaDN infested and *Dn2* control treatments in relative to the control.

Cluster B1 (Figure 3.4.9) has down regulation in all BettaDN and Betta *Dn2* treatments in relation to Betta control. Cluster B2 (Figure 3.4.9) is characterized by TDFs up-regulated in comparison to Betta control in the Betta *Dn2* wounded treatment. Betta infested, all of the BettaDN treatments and Betta *Dn2* control are down-regulated. Cluster B3 (Figure 3.4.9) shows Betta wounded and infested treatments up-regulated in comparison to the control, as well as BettaDN control and infested, and Betta *Dn2* wounded. Cluster B4 (Figure 3.4.9) is characterized by high up-regulation in *Dn2* control in comparison to the Betta control treatment. The BettaDN treatments are all down-regulated as well as Betta *Dn2* wounded and infested. Cluster B5 (Figure 3.4.9) has down-regulation of all samples in comparison to the Betta control treatment with the largest down-regulation recorded in the Betta infested treatment.

In order to identify possible biochemical relationships via similar expression pattern, identities were assigned to each TDF in the tree, and the TDFs were split into four categories; for Betta, BettaDN, Betta *Dn2* and wounded, depending on whether regulation was present (Figures 3.4.10; 3.4.11; 3.4.12; 3.4.13 respectively). Regulation was described as up- or down- regulation dependant on the band intensities. The tree for Betta related TDFs (Figure 3.4.10) shows a high up-regulation in TDFs from Betta infested treatments in cluster 3 with down-regulation in most other treatments. This cluster contains many ubiquitin and other stress related TDFs, such as peroxisomal ascorbate peroxidase and heat repeat family protein (Appendix C: Table 5.2).

The cluster tree for BettaDN related transcripts (Figure 3.4.11) shows clusters with up- and down-regulation in the BettaDN treatments. Cluster 5 and 6 of this tree shows TDFs with up-regulation in

mostly BettaDN infested treatments only, and contains fragments with identities such as H+ATPase, ABC transporter and ATP synthase (Appendix C: Table 5.3).

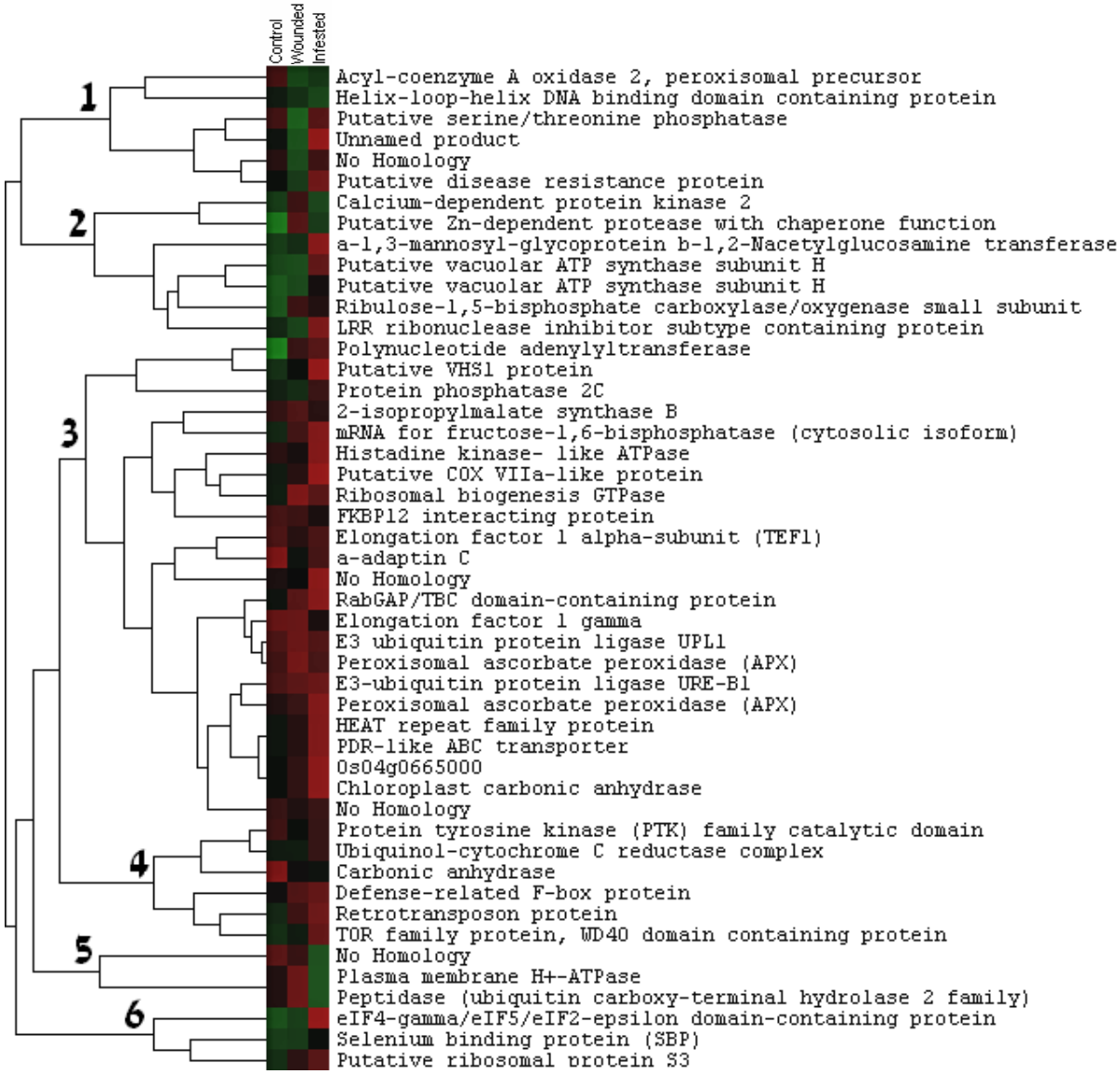


Figure 3.4.10: TreeView image of cloned TDFs showing regulation in Betta samples.

The cluster tree for regulated TDFs from Betta *Dn2* treatments (Figure 3.4.12) has two main trends. The first trend is up-regulation in Betta *Dn2* control treatments and includes clusters 1-4. And the second, trend, including clusters 5-8, has up-regulation of Betta *Dn2* infested treatments. In cluster 2 there are TDFs with unknown identity or function form the mitochondrion, and chloroplast. In cluster 3

there are TDFs such as ubiquinol-cytochrome C reductase complex and β-glucosidase. Cluster 4 contains TDFs involved in the vacuole and zinc transport (Appendix C: Table 5.4).

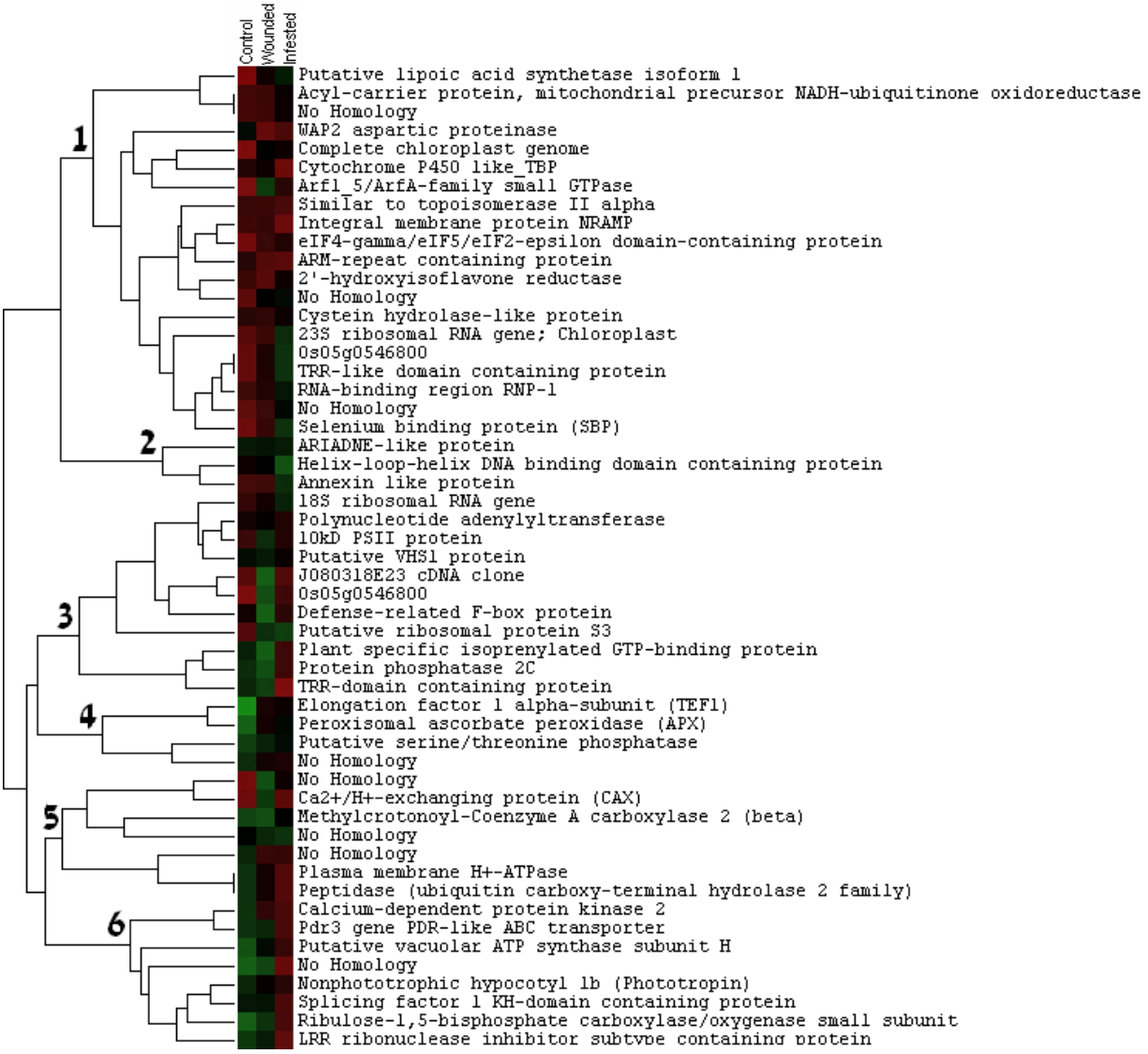


Figure 3.4.11: TreeView image of cloned TDFs showing regulation in *Dn1* samples.

In the tree for TDFs involved in wounding (Figure 3.4.13) there are 4 clusters. The TDFs in Cluster 1 involved in wounding are in the BettaDN wounded treatment and include a TDF for chaperone protein dnaJ-like. Cluster 2 has wounding related up-regulation mostly in the Betta wounded treatments and includes TDFs such as peptidase and hydroxymethylglutharyl- CoA-synthase. Cluster 4 includes up-regulation in some Betta *Dn2* wounded and Betta wounded treatments, including TDFs for malate dehydrogenase and methylcrotonoyl coenzyme A carboxylase 2 (Appendix C: Table 5.5).

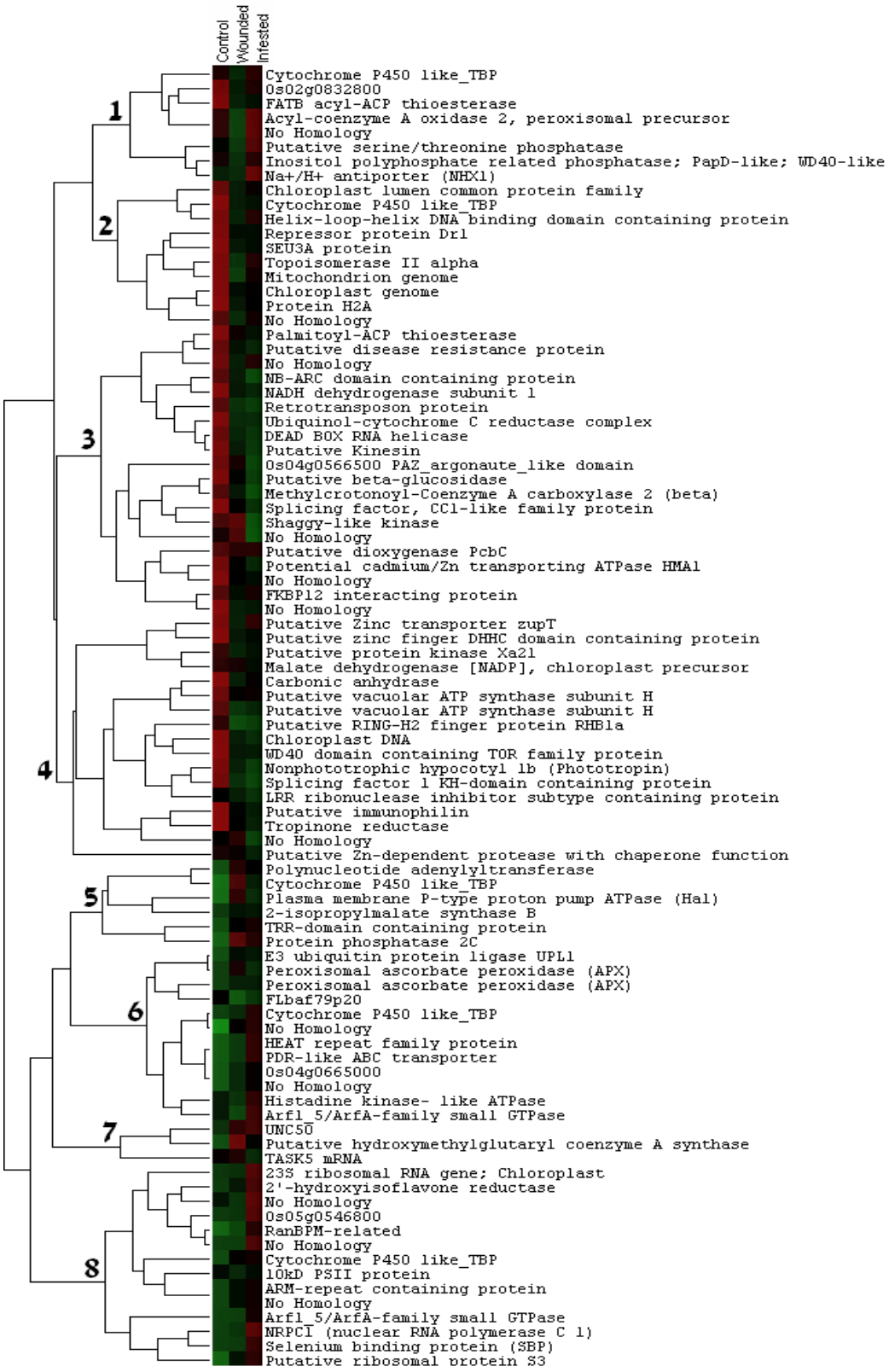


Figure 3.4.12: TreeView Image of Cloned TDFs showing regulation in *Dn2* samples.

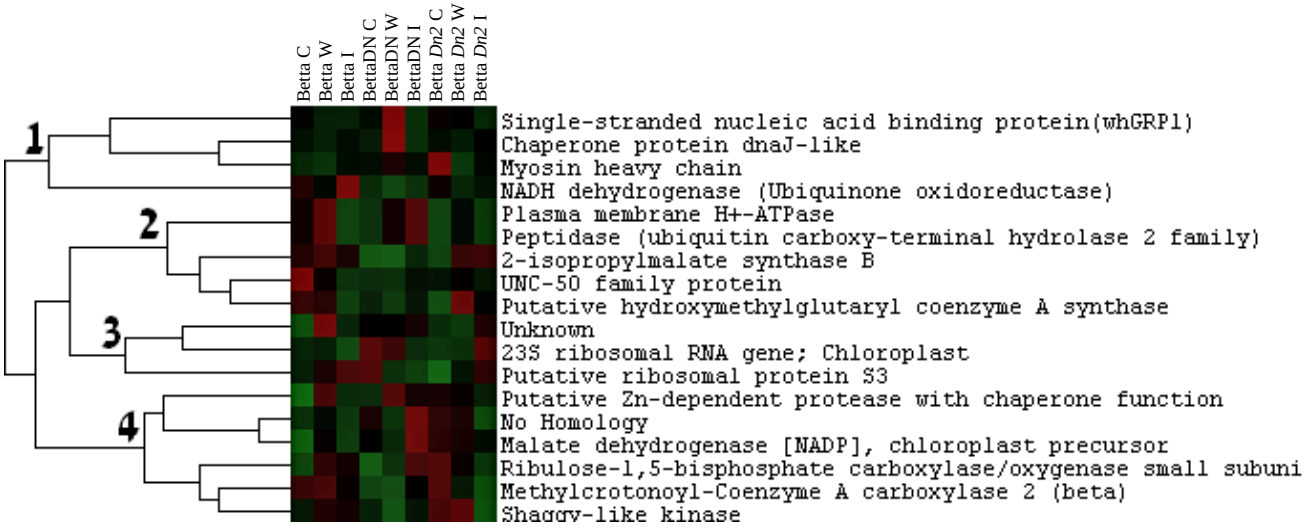


Figure 3.4.13: TreeView Image of Cloned TDFs showing regulation in wounded samples.

3.4.5 Identification of biological pathways

Separation of the TDFs into the different resistance categories allows for the study of the different pathways that may be involved in each of the susceptible, *Dn1* type antibiosis, *Dn2* type tolerance and the wounding responses. After analysis of the putative identities (Table 3.4.4) with their functions (Appendix C: Tables 5.2; 5.3, 5.4 and 5.5), and cross reference that to the band intensities recorded (Figures 3.4.10; 3.4.1; 3.4.12 and 3.4.13), general trends were identified. Involvement in biochemical pathways and putative functional data was mined from the Kyoto encyclopedia of genes and genomes (KEGG) (Kanehisa and Goto 2000; http://www.genome.jp/kegg/) internet resource.

Of the TDFs involved in the susceptible Betta treatments, there are two protein kinases (cluster 1 and one in cluster 3, Figure 3.4.8) putatively involved in mitogen activated MAPK signaling pathways (Appendix C: Table 3.4.4) and they are both up-regulated in the Betta infested treatment (Figure 3.4.8). There are also three TDFs involved in oxidative phosphorylation including H^+ ATPase which is up-regulated in Betta infested cluster 2, and up-regulated in the wounded treatment and down-regulated in the infested treatment in cluster 5 (Figure 3.4.8), as well as ubiquinol-cytochrome C reductase in

cluster 4 which is up-regulated in the Betta infested treatment. Two TDFs are involved in energy metabolism, both are carbonic anhydrases, the first in cluster 3 is a chloroplast based carbonic anhydrase and is up-regulated in Betta wounded, but has higher up-regulation in the Betta infested treatment. The second carbonic anhydrase is in cluster 5 and is up-regulated in the Betta control treatment only (Figure 3.4.8).

In the BettaDN treatments, there are three TDFs involved in oxidative phosphorylation, with another involved in mitochondrial homeostasis. The first TDF involved in oxidative phosphorylation is a mitochondrial precursor to NADH-ubiquinone oxidoreductase that is up-regulated in BettaDN control and wounded treatments and is present in cluster 1 (Figure 3.4.9). The second TDF is a plasma membrane H^+-ATPase in cluster 5 that is up-regulated in BettaDN control and infested treatments and down-regulated in BettaDN wounded (Figure 3.4.9). The TDF involved in mitochondrial homeostasis is an atypical Rho GTPase, Arf1_5/ArfA-family small GTPase, present in cluster 1 and is up-regulated in BettaDN control and infested, with BettaDN wounded down-regulated (Figure 3.4.9). There are also two TDFs involved MAPK and calcium signaling pathways (Appendix C: Table 5.2). The first is a protein phosphatase 2C in cluster 3 that is up-regulated in BettaDN infested only (Figure 3.4.9), and the second is a putative serine/threonine phosphatase in cluster 4 that is down regulated in BettaDN control, less down-regulated in BettaDN wounded, and in BettaDN infested the TDF is not-regulated or up-regulated in comparison to the control and wounded treatments (Figure 3.4.9).

In the Betta *Dn2* treatments, there is a markedly higher number of TDFs involved in the organel function. There are four TDFs involved in the chloroplast or photosynthesis, and four in the mitochondria or oxidative phosphorylation (Appendix C: Table 5.2). In the chloroplast or photosynthesis cellular processes, the first TDF is a lumen protein in cluster 2 that is up-regulated in the Betta *Dn2* control treatment and down-regulated in the Betta *Dn2* wounded treatment (Figure 3.4.10). The second is malate dehydrogenase [NADP] chloroplast precursor in cluster 4 and it is down-regulated in Betta *Dn2* infested in comparison to the control and wounded treatments. The third

is a 10kD PS II antenna protein in cluster 8 that is down-regulated in the Betta wounded treatment. The last is a PapD-like ATPase synthase that is found in both photosynthesis and oxidative phosphorylation. The PapD TDF is in cluster 1 and is up-regulated in Betta *Dn2* infested, and down-regulated in Betta *Dn2* wounded. The other TDFs involved in oxidative phosphorylation are NADH dehydrogenase subunit 1 and ubiquinol-cytochrome C reductase complex in cluster 3 which are both up-regulated in Betta *Dn2* control treatments. Arf1_5/ArfA-family small GTPase in cluster 6 is possibly involved in mitochondrial homeostasis and is up-regulated in Betta *Dn2* infested and down-regulated in the wounded treatment.

3.4.6 qRT-PRC analysis on biological repeat material

Absence of genomic DNA contamination was confirmed using a primer pair for β-glucosidase which spanned an intron region: primer pair 92 (Table 3.4.6). A band of approximately 250 bp was found when primer 92 was screened on genomic DNA, and a band of 120 bp when screened on cDNA clean of DNA contamination (Figure 3.4.14 A). All RNA samples were screened using primer pair 92 (Figure 3.4.14).

Figure 3.4.14: A) Test using primer pair 92 run on a 2 % agarose gel. Lane 1=100 bp ladder; Lane 2= RNA; Lane 3= cDNA; Lane 4= DNA; (B and C) Screen of all RNA samples using primer pair 92, run on a 2 % agarose gel. C) Lane 7= positive control; Lane 8= cDNA control; Lane 9=RNA.

Samples Betta control 5 h; Betta infested 5 h; Betta infested 72 h; BettaDN control 5 h; BettaDN wounded 48 h; BettaDN infested 5 h; BettaDN infested 12 h; Betta *Dn2* infested 5 h; Betta *Dn2* infested 24 h and the positive control all tested positive for a band at 200 bp (Figure 3.4.14 B and C).

Samples found to have residue DNA contamination were again DNase treated, and then cleaned, re-tested with primer 92 and tested spectrophotometrically for RNA concentration and integrity (Table 3.4.4). Clean RNA was used for single stranded cDNA synthesis that was tested spectrophotometrically for ssDNA concentration and reliability (Table 3.4.5).

Table 3.4.4: Table showing total RNA concentrations in ng/µl and purity using the 260/280 ratio.

Treatment	ng/µl	260/280	Treatment	ng/µl	260/280
Betta Control 5 h	40.30	2.11	BettaDN Infested 48 h	53.75	2.08
Betta Wounded 5 h	37.41	2.17	Betta *Dn2* Control 48 h	41.11	2.00
Betta Infested 5 h	74.41	2.08	Betta *Dn2* Wounded 48 h	37.11	2.03
BettaDN Control 5 h	33.6	1.88	Betta *Dn2* Infested 48 h	38.66	2.05
BettaDN Wounded 5 h	201.4	2.12	Betta Infested 12 h	35.49	2.17
BettaDN Infested 5 h	159.51	2.14	Betta Infested 24 h	35.21	2.15
Betta *Dn2* Control 5 h	106.57	2.14	Betta Infested 72 h	36.76	2.14
Betta *Dn2* Wounded 5 h	38.61	2.18	BettaDN Infested 12 h	359.13	2.11
Betta *Dn2* Infested 5 h	207.36	2.23	BettaDN Infested 24 h	105.32	2.11
Betta Control 48 h	65.91	1.88	BettaDN Infested 72 h	237.65	2.12
Betta Wounded 48 h	46.91	2.16	Betta *Dn2* Infested 12 h	126.04	2.12
Betta Infested 48 h	36.14	2.17	Betta *Dn2* Infested24 h	234.46	2.12
BettaDN Control 48 h	47.47	2.09	Betta *Dn2* Infested 72 h	191.87	2.12
BettaDN Wounded 48 h	242.59	2.14			

Table 3.4.5: Table showing ssDNA concentrations in ng/µl and purity using the 260/280 ratio.

Treatment	ng/µl	260/280	Treatment	ng/µl	260/280
Betta Control 5 h	1461.75	1.83	BettaDN Infested 48 h	1445.01	1.83
Betta Wounded 5 h	1352.96	1.83	Betta *Dn2* Control 48 h	1369.55	1.83
Betta Infested 5 h	1430.96	1.84	Betta *Dn2* Wounded 48 h	1355.13	1.83
BettaDN Control 5 h	1538.65	1.83	Betta *Dn2* Infested 48 h	1425.91	1.83
BettaDN Wounded 5 h	1468.34	1.84	Betta Infested 12 h	1666.07	1.82
BettaDN Infested 5 h	1427.14	1.84	Betta Infested 24 h	1384.36	1.82
Betta *Dn2* Control 5 h	1367.19	1.84	Betta Infested 72 h	1426.34	1.82
Betta *Dn2* Wounded 5 h	1397.53	1.84	BettaDN Infested 12 h	1439.82	1.84
Betta *Dn2* Infested 5 h	1320.09	1.83	BettaDN Infested 24 h	1438.11	1.83
Betta Control 48 h	1371.35	1.83	BettaDN Infested 72 h	1432.29	1.83
Betta Wounded 48 h	1590.07	1.82	Betta *Dn2* Infested 12 h	1386.07	1.82
Betta Infested 48 h	1428.88	1.82	Betta *Dn2* Infested24 h	1441.25	1.82
BettaDN Control 48 h	1356.52	1.83	Betta *Dn2* Infested 72 h	1425.56	1.83
BettaDN Wounded 48 h	1460.44	1.84			

Primers were designed for representative TDFs. However, many TDF sequences were not useful, as they were too short to design reliable primers from. Five primer pairs were chosen to continue with (Table 3.4.6). Clones chosen to do qRT-PCRs on were as follows:

Clone AmoTS-M335.6 (primer 92; Table 3.4.6) is a putative β-glucosidase homologue. In the cDNA-AFLP and cluster results the TDF showed up-regulation in the Betta *Dn2* control treatment (Figure 3.4.15).

Table 3.4.6: Primers used for qRT-PCRs based on TDF sequence.

Primer	TDF	Cluster	Sequence 5'-3'	Product size (bp)	Annealing temperature
16S Forward	16S rRNA		TCAAGTCCGCCGTCAAATC		56°C
16S Reverse			TCGCCGTTGGTGTTCTTTC		56 °C
92 L	AmoTSM335.6	B4	CTGCAAAGAACTCGAAGACG	93 bp	56°C
92 R			CATGGGTAGCACACTGAAGG		56 °C
104 L	AmoTSM254.4	A5	CAGAGCAGCGAGTTCTTCC	114 bp	56°C
104 R			CCTTCAGTACCACCCTTCTGG		56 °C
126 L	AmoTSM289.7	B4	GGATGGGAAATCAAACAAGG	136 bp	56°C
126 R			AGAAGCCCGAGCCAGAGG		56 °C
129 L	AmoTSM286.9	A1	CAGCAGCAAACCAATGTTCC	124 bp	56°C
129 R			CAGGTCTGAAGCACCAAGC		56 °C
235 L	AmoTSM297.6	A5	GGTGGAGGAGGTCAAGAAGG	97 bp	56°C
235 R			GAATGCGATGAAGCTGACG		56 °C

Clone AmoTSM254.4 (primer 104; Table 3.4.6) is a putative vacuolar ATP synthase that was up-regulated in the Betta *Dn2* control treatment, but also showed a larger intensity in the BettaDN infested treatment in comparison to it's control (Figure 3.4.16).

Clone AmoTSM289.7 (primer 126; Table 3.4.6) is a putative ubiquinol-cytochrome C-reductase and the most intense band for this transcript was found in the Betta *Dn2* control treatment (Figure 3.4.17).

Clone AmoTSM286.9 (primer 129; Table 3.4.6) is a putative NADH-ubiquinone oxidoreductase. In the Betta *Dn2* treatments, the wounded treatment is down-regulated in contrast to the control, with the infested treatment even further down-regulated. The same is true for the BettaDN treatments, though

they are not as strongly regulated. While in the Betta treatment there is up-regulation of this transcript in the infested treatment (Figure 3.4.18).

Clone AmoTSM297.6 (primer 235; Table 3.4.6) is a putative ribulose-1,5-bisphosphate carboxylase. This TDF shows up-regulation in the BettaDN infested treatment, with down-regulation in the Betta *Dn2* infested treatment (Figure 3.4.19).

Primers were tested by screening them on cDNA for a single product. They were then screened using the same serial dilution as was used to test the housekeeping gene: chloroplast 16S rRNA. Melting curves were checked for primer-dimers and multiple products.

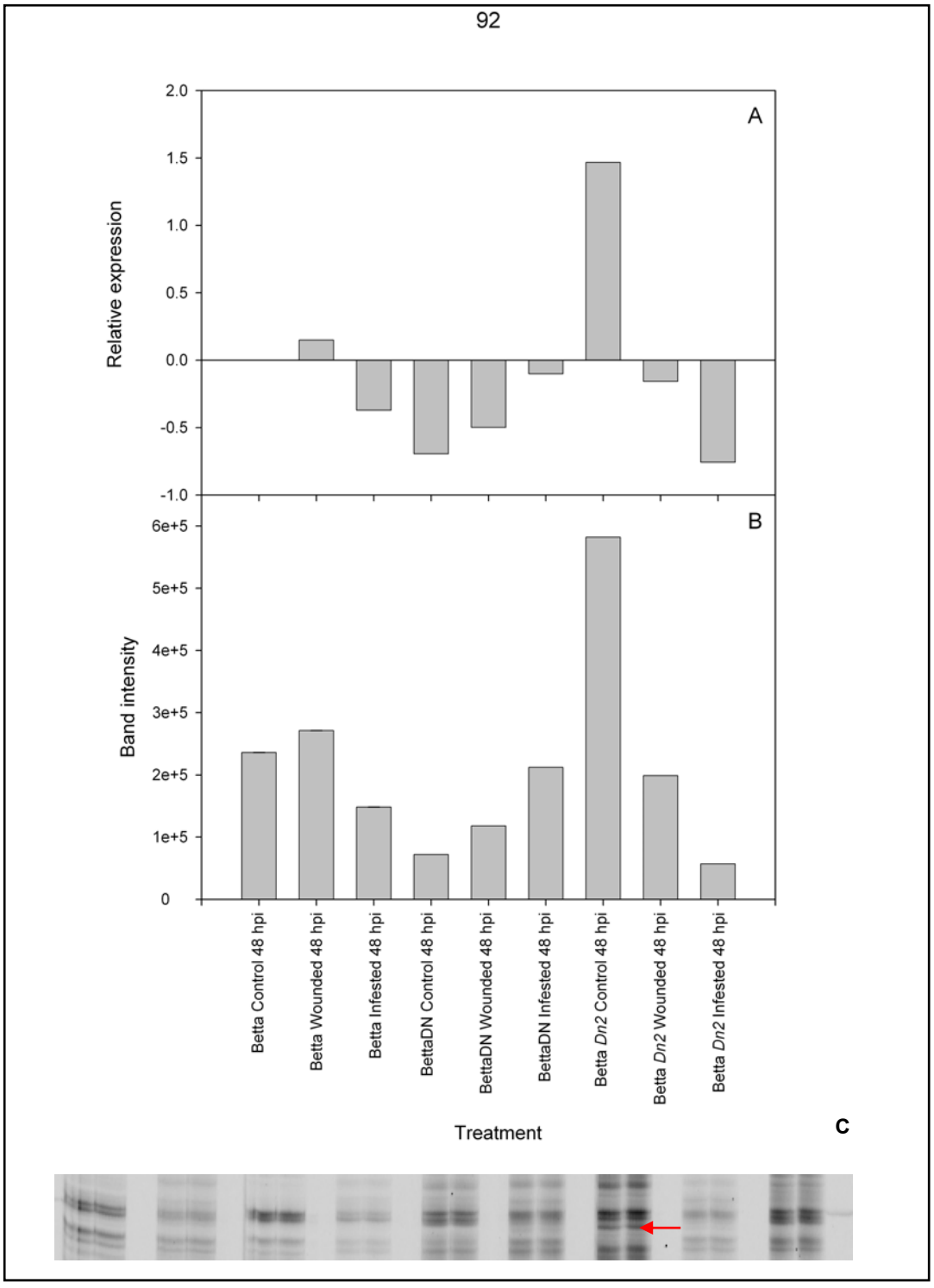


Figure 3.4.15: Graph (A) shows relative expression of the TDF 92 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine. Image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M335.6) that was excised.

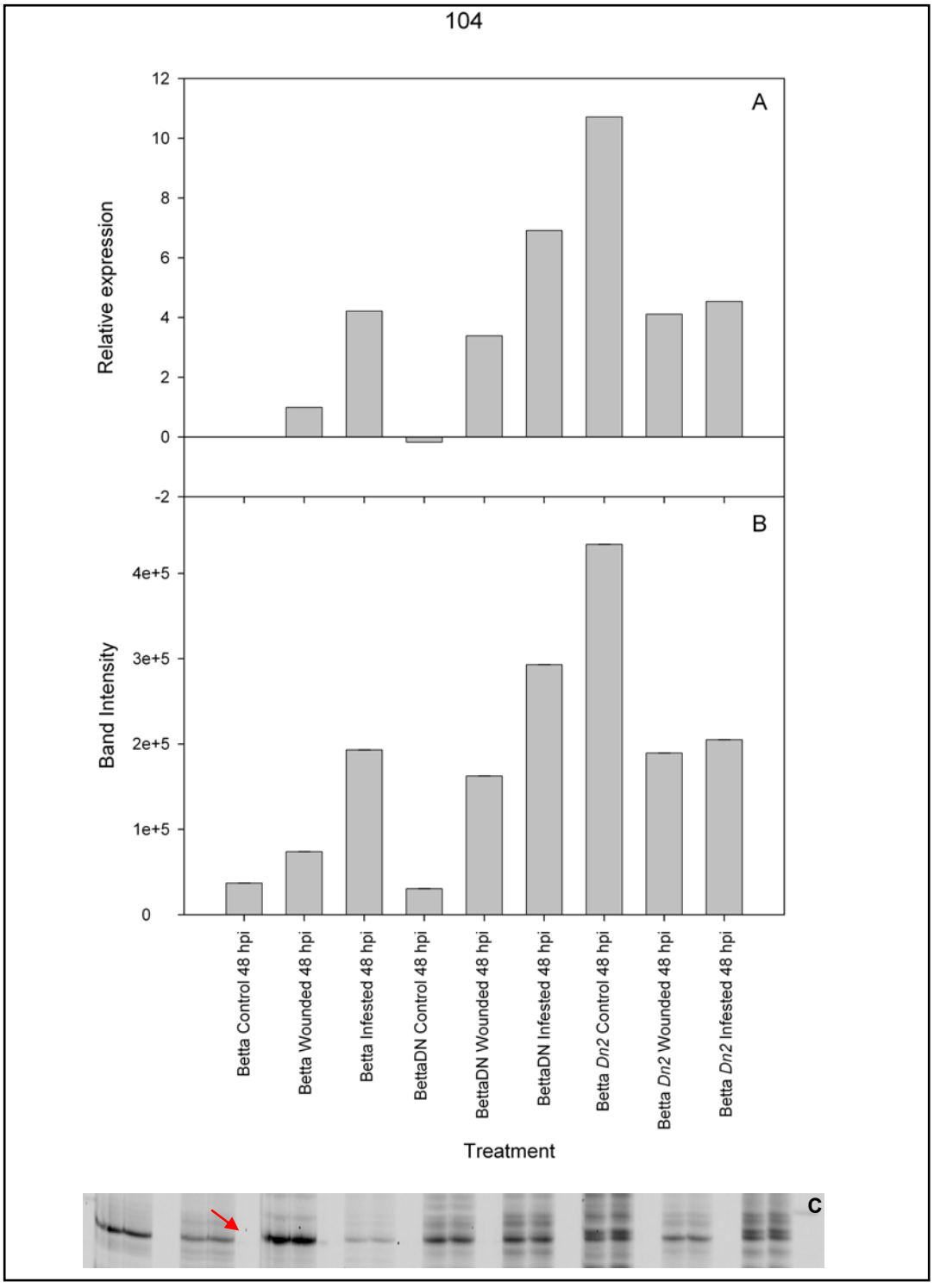


Figure 3.4.16: Graph (A) shows relative expression of the TDF 104 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine. Image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M254.4) that was excised.

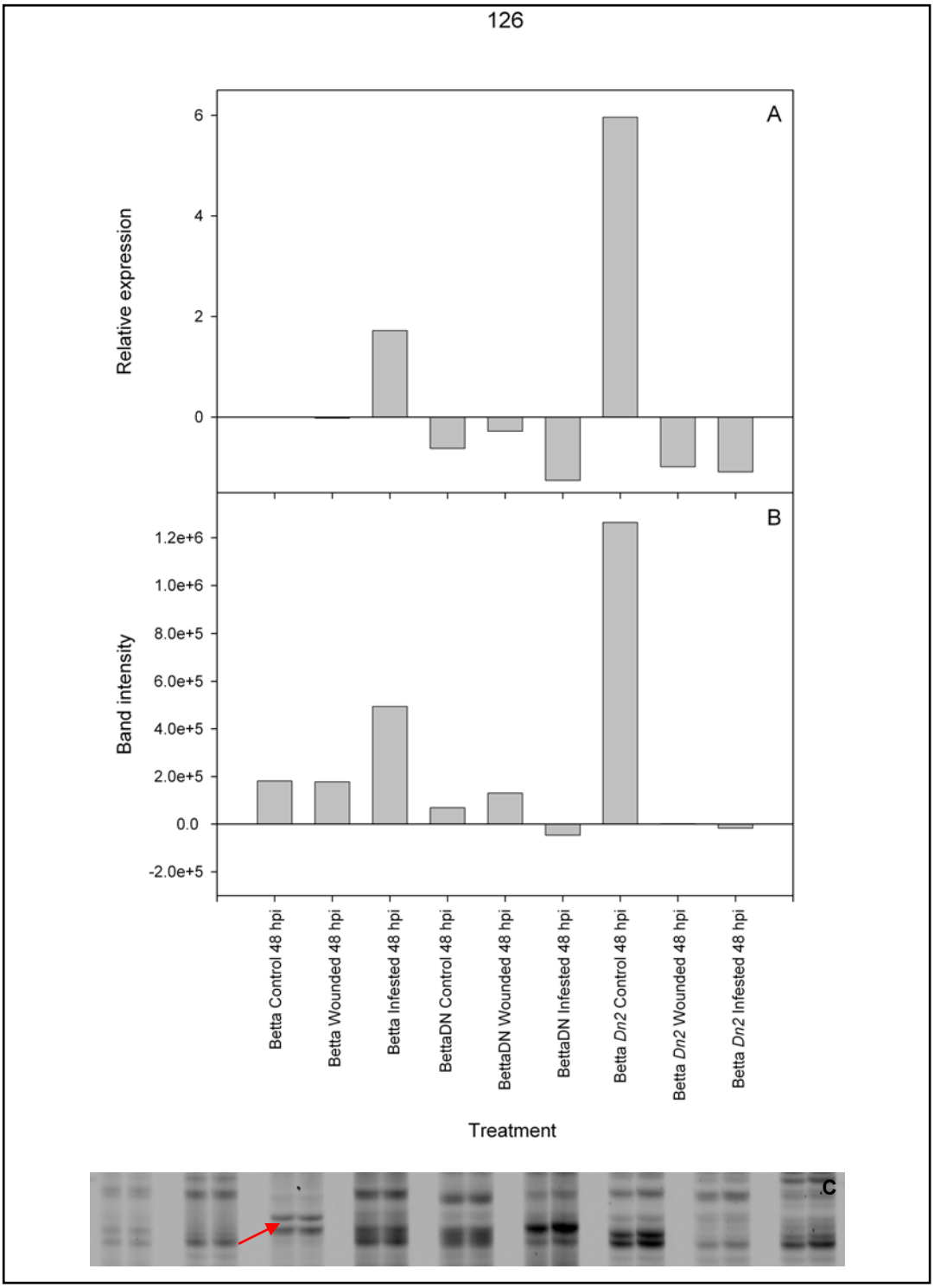


Figure 3.4.17: Graph (A) shows relative expression of the TDF 126 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine. Image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M289.7) that was excised.

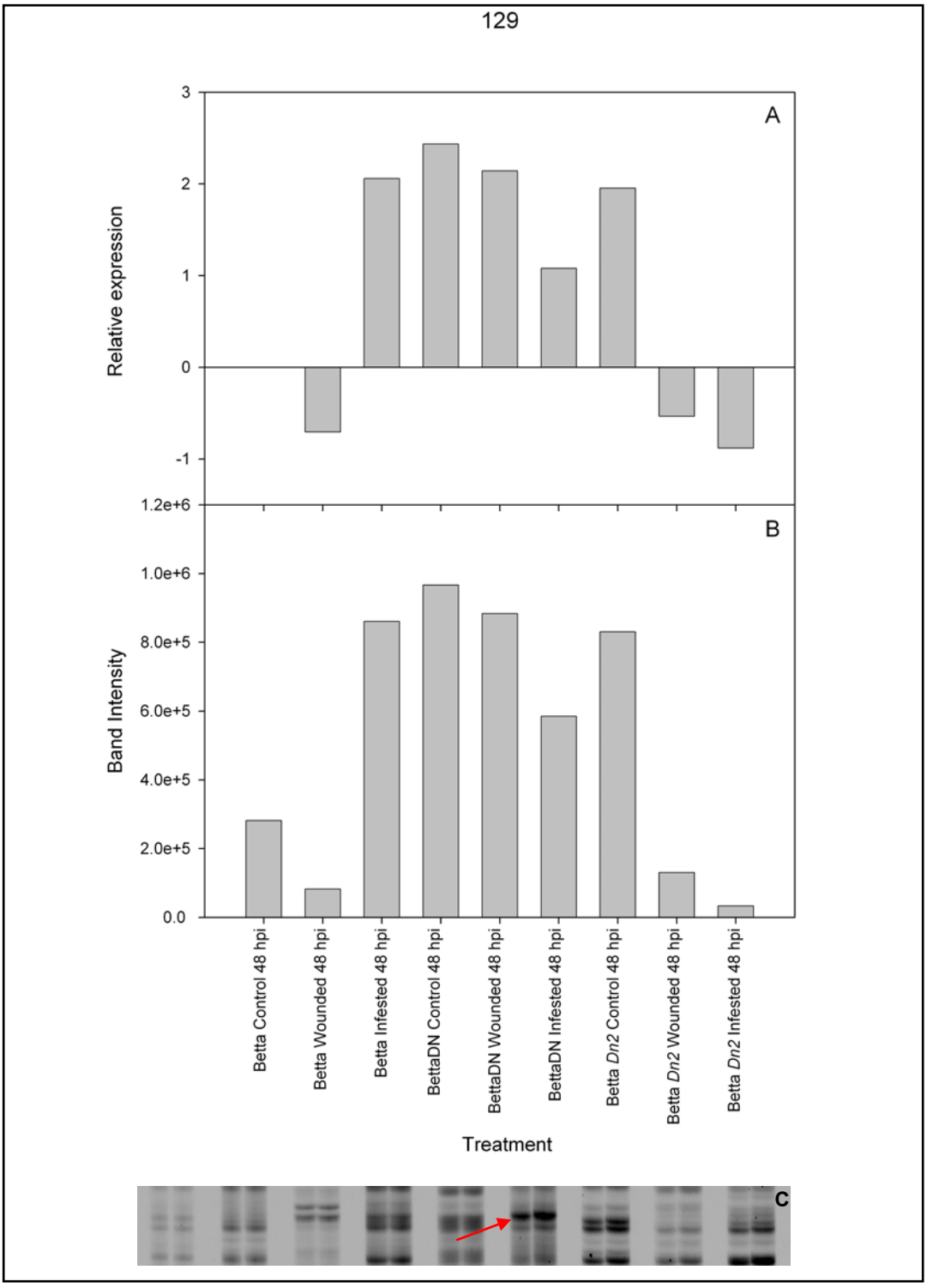


Figure 3.4.18: Graph (A) shows relative expression of the TDF 129 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine. Image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M286.9) that was excised.

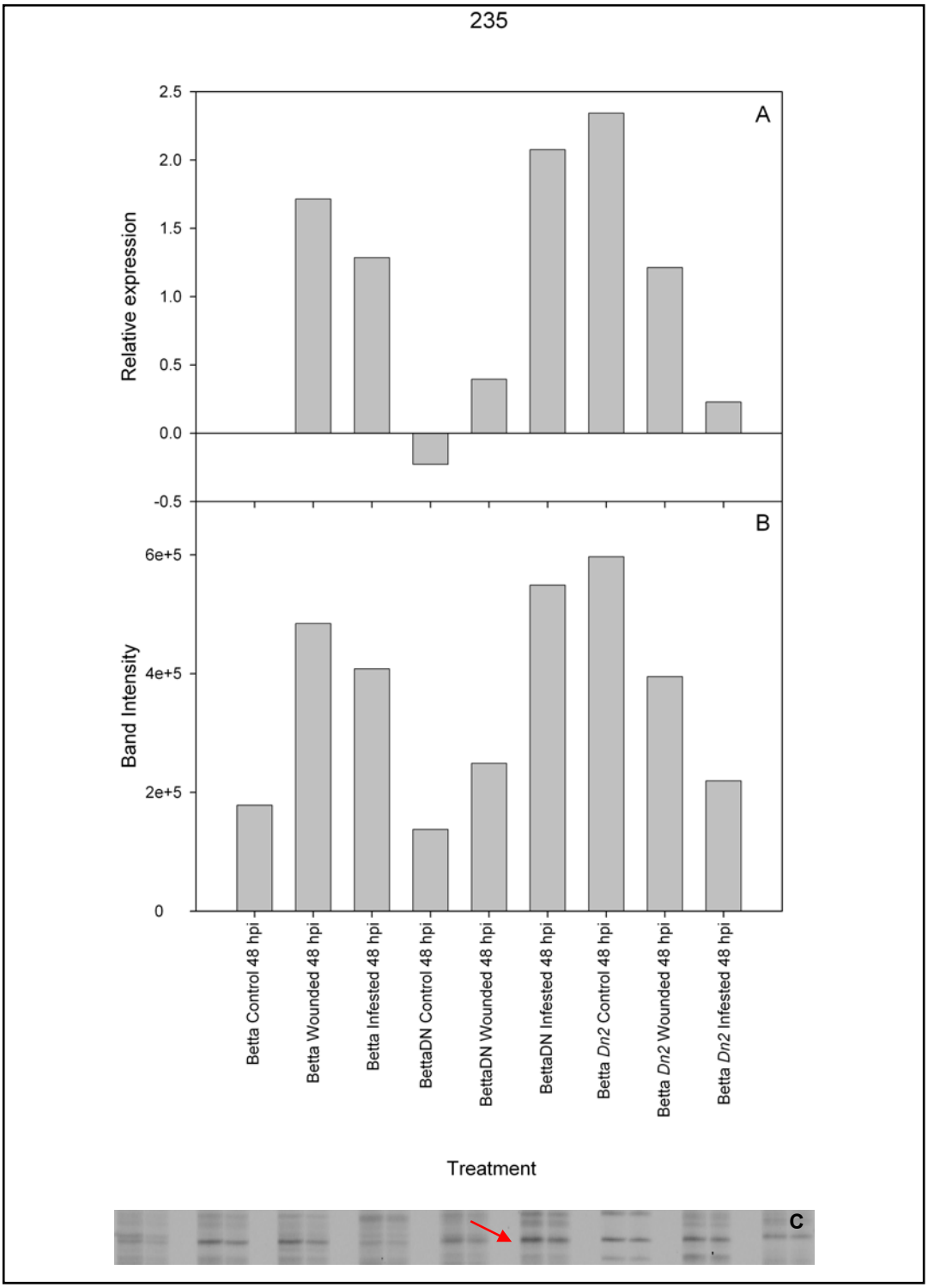


Figure 3.4.19: Graph (A) shows relative expression of the TDF 235 using normalized cluster data. Graph (B) shows the band intensity as recorded by the LICOR machine. Image (C) is a cDNA-AFLP gel with a red arrow indicating the band of interest (M287.6) that was excised.

In order to verify the trends of regulation of the respective genes, qRT-PCR analysis was performed on biological repeat material sampled at: 5 hours and 48 hours as control, 5 hours and 48 hours post wounding, and 5 hours, 12 hours, 24 hours, 48 hours and 72 hours post infestation. The levels of transcript are described as fold change in relation to the controls.

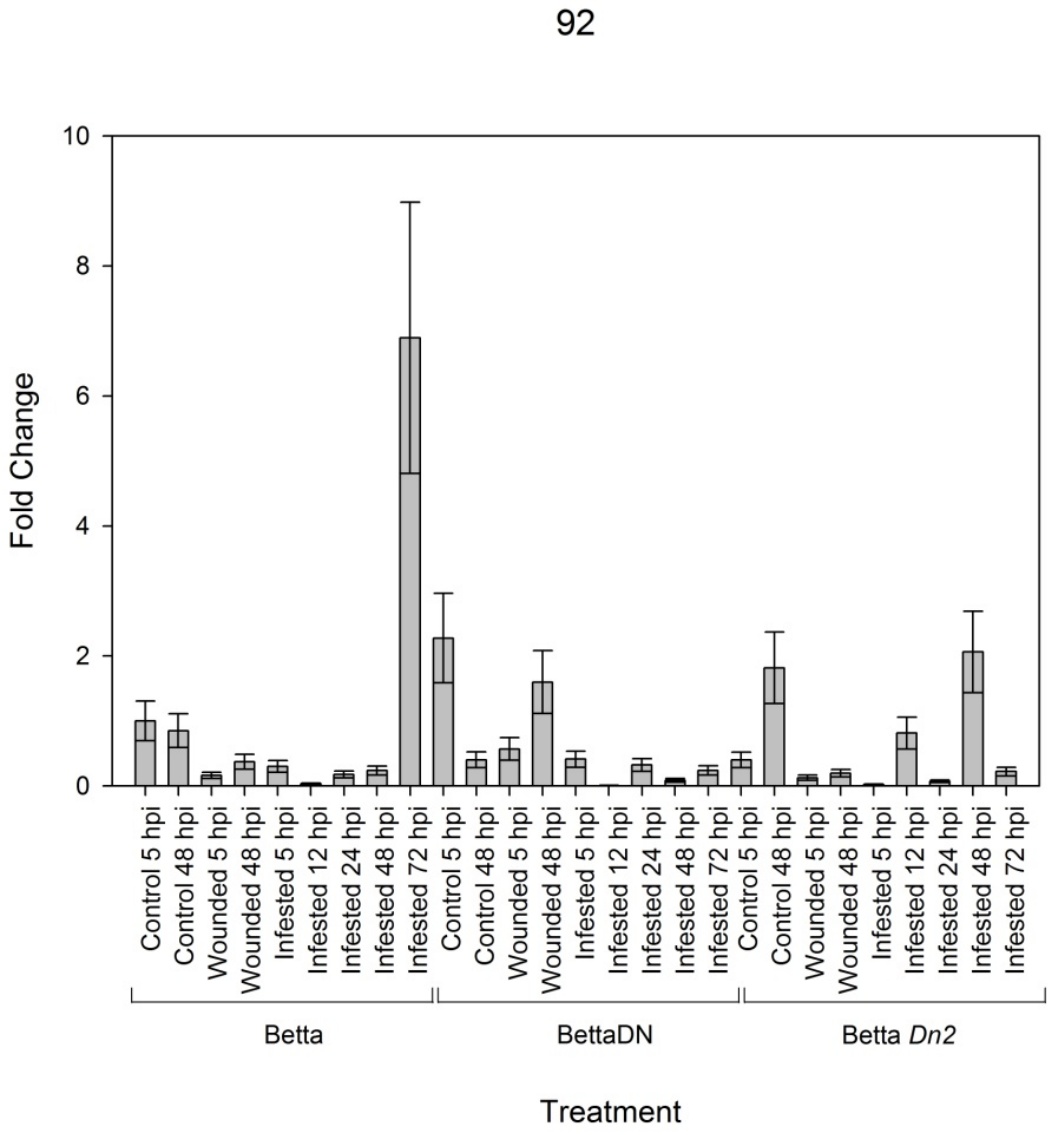


Figure 3.4.20: Graph of the qRT-PCR results showing fold change of the TDF 92.

TcaMac92, a putative β-glucosidase has a seven fold change in the Betta infested treatment at 72 hours post infestation. However, there is a two fold up-regulation of the TDF in BettaDN contol 5 hours

and wounded 48 hours, and in Betta *Dn2* control 48 hours and infested 48 hours in comparison to Betta (Figure 3.4.20).

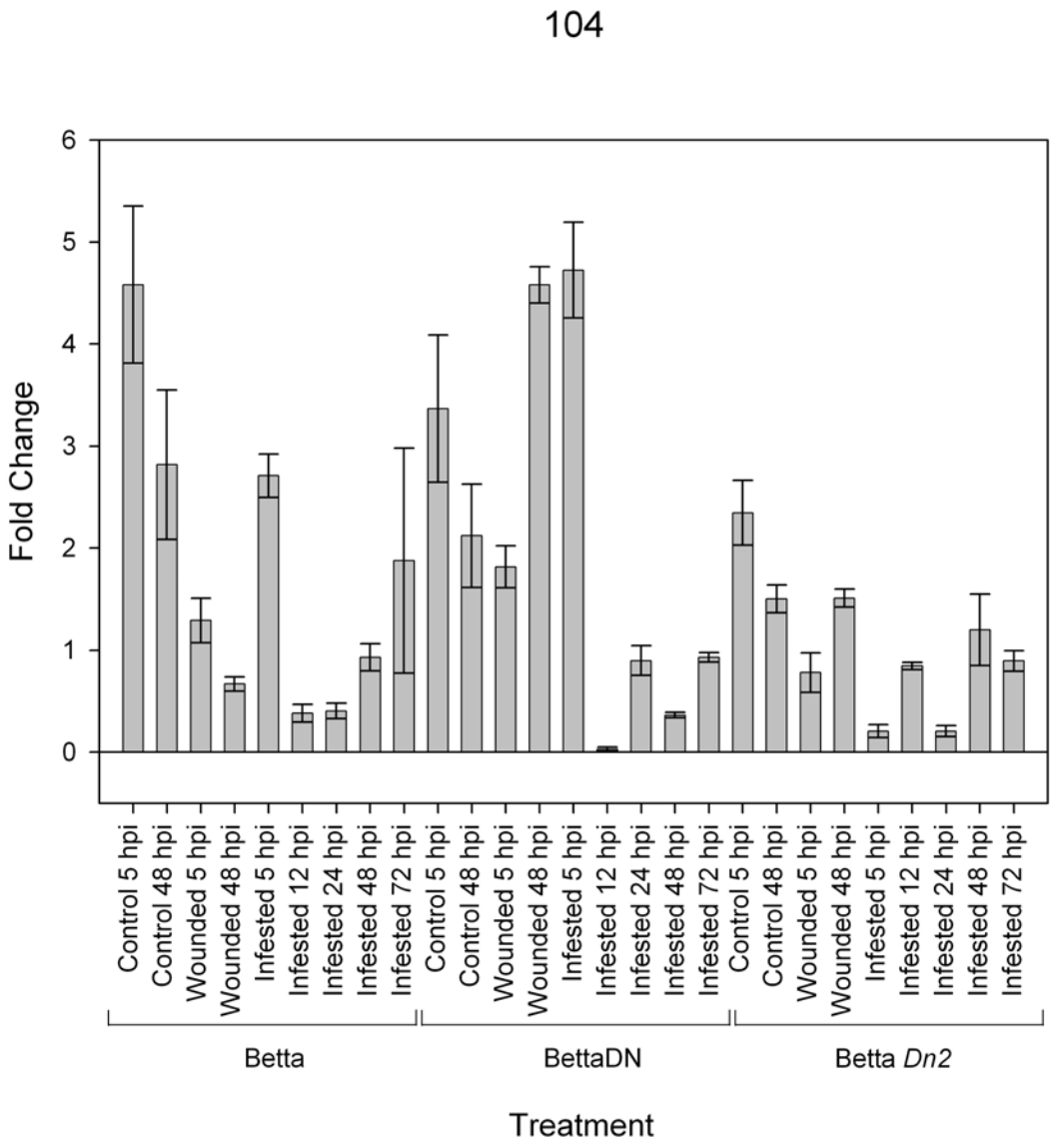


Figure 3.4.21: Graph of the qRT-PCR results showing fold change of the TDF 104.

TcaMac104, a putative vacuolar ATP-synthase has high numbers of transcript in the Betta control and BettaDN control treatments, but less is seen in the Betta *Dn2* control treatments respectively. In all the susceptible Betta wounded and the infested treatments the transcript levels are down-regulated in comparison to the controls except the 5 hours post infestation treatment which has an equal or lesser

amount. In the BettaDN treatments, the wounded 48 hours and infested 5 hours treatments are up-regulated in comparison to the controls. In Betta *Dn2* none of the treatments are up-regulated in comparison to the controls (Figure 3.4.21).

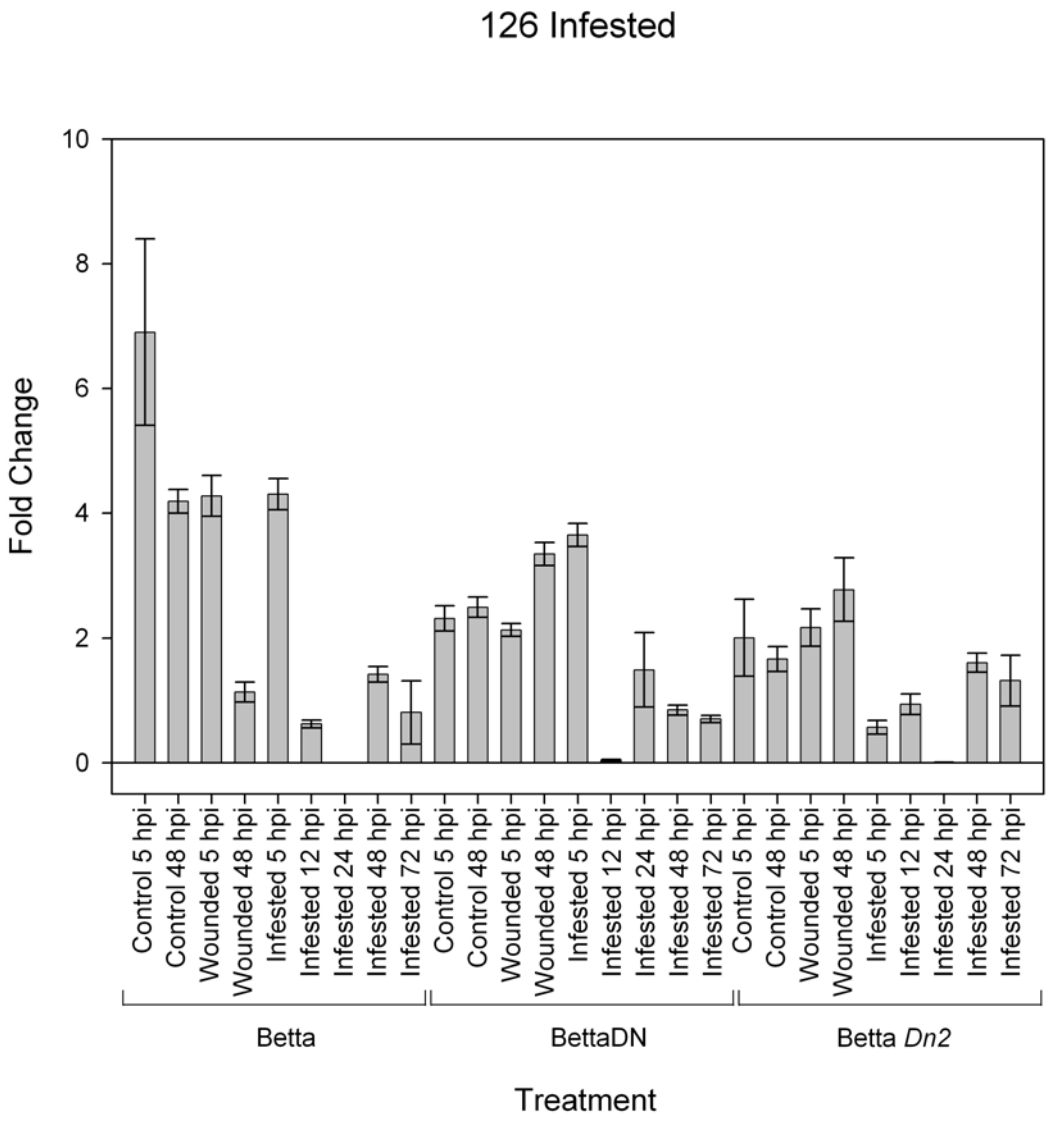


Figure 3.4.22: Graph of the qRT-PCR results showing fold change of the TDF 126.

TgtMac126, a putative ubiquinol-cytochrome C reductase complex is present in high numbers in the Betta control treatments as well as in the wounded and infested 5 hours treatments which are on par with the control 48 hour treatment; all other treatments are down-regulated. In the BettaDN treatments the wounded 48 and infested 5 hour treatments are up-regulated in comparison to the controls. The

Betta *Dn2* treatments show a small increase in levels in the wounded 48 hours treatment, but down-regulation in the 5 hours post infestation treatment (Figure 3.4.22).

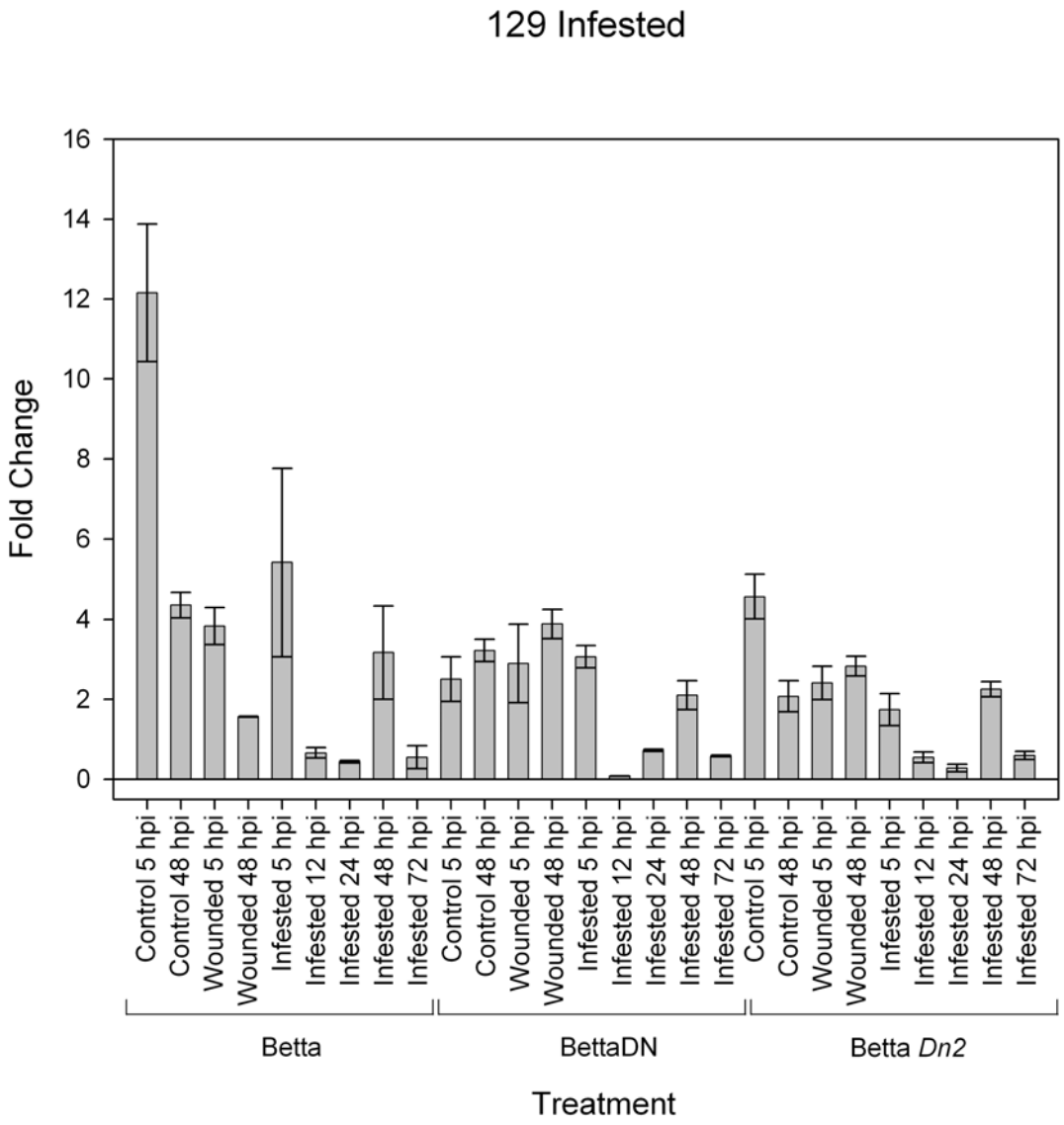


Figure 3.4.23: Graph of the qRT-PCR results showing fold change of the TDF 129.

TgtMac129 a putative acyl-carrier protein or mitochondrial precursor NADH-ubiquitinone oxidoreductase is present in very high copy numbers in the Betta control 5 hours treatment. However, the interesting trend is in the infested treatments. All three NILs have higher amounts of transcript at 5 hours and 48 hours post infestation showing similar regulation at these time points: the Betta

treatments having the highest levels, followed by BettaDN and Betta *Dn2* having the lowest levels of the three (Figure 3.4.23).

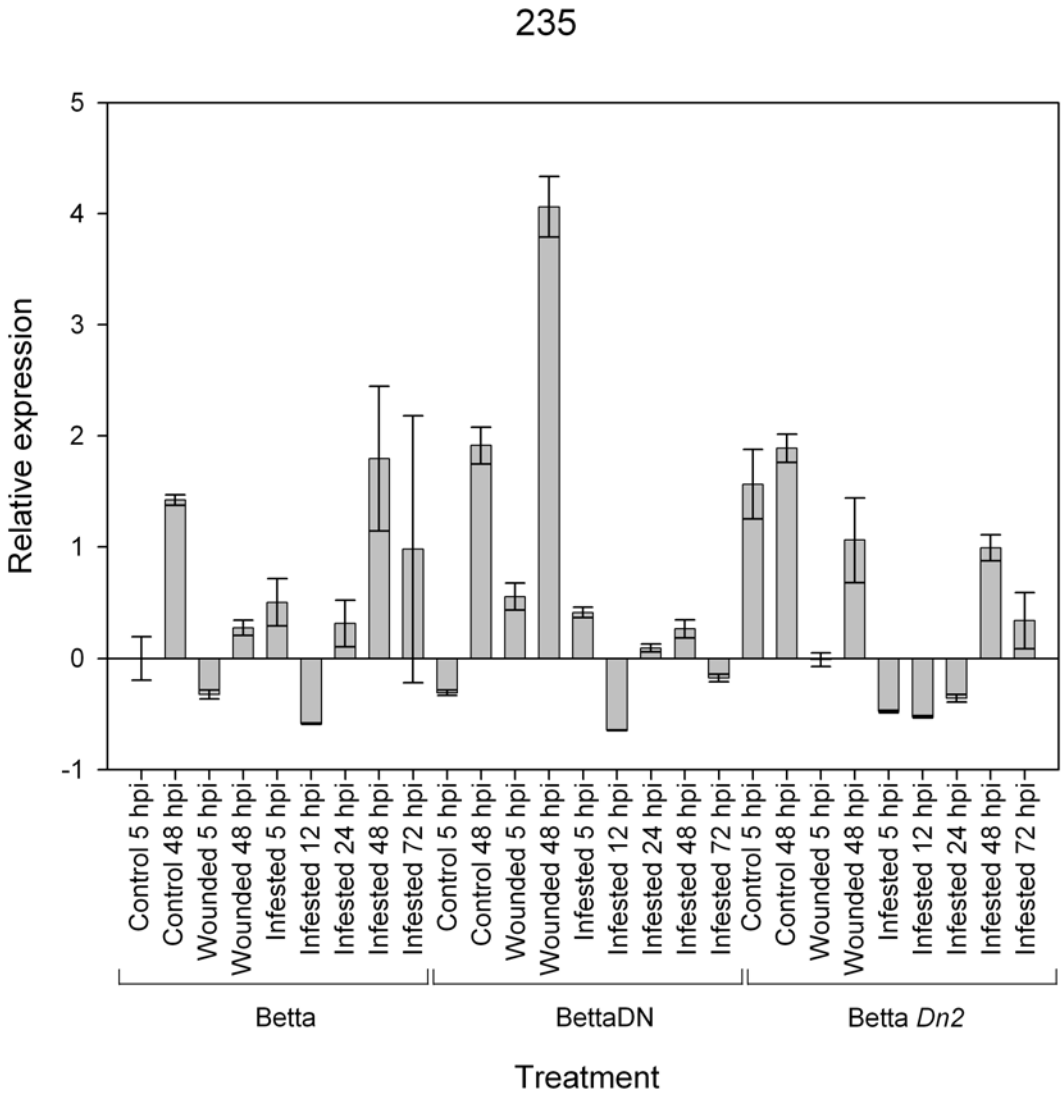


Figure 3.4.24: Graph of the qRT-PCR results showing fold change of the TDF 235.

TgtMct235 is a putative ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit that has the highest expression in the BettaDN wounded treatment at 48 hours post wounding with all infested treatments down-regulated. In the Betta treatments expression is increased in the 48 and 72 hours post infestation. The Betta *Dn2* shows low levels of up-regulation in the 48 hours and 72 hours post infestation treatments (Figure 3.4.24).

3.5 Discussion

3.5.1 cDNA-AFLPs as a relevant technique

The different resistance responses conveyed to wheat plants by the *Diuraphis noxia* resistance genes (Table 2.2) have been phenotypically characterized, but little has been done to study the molecular interactions that take place between the aphid and the wheat host. If long term sustainable resistance is to be bred into wheat lines, the basis of the resistance response, as well as the key genes involved, need to be elucidated. In this study, we aimed to further characterize the systemic resistance response in wheat induced by Russian wheat aphid (RWA) feeding using cDNA-AFLPs. 235 transcript derived fragments (TDFs) (Figure 3.4.3) were excised and identified as putatively involved in RWA resistance from near isogenic line (NILs) wheat plants. 146 of the TDFs were cloned, sequenced and had a putative identity assigned using BLAST searches (National Center for Biotechnology Information, NCBI, 2008) (Table 3.4.3). The TDFs were separated into 10 clusters with similar expression patterns (Figure 3.4.7) and then into their respective cellular processes (Figure 3.4.5). Approximately 17 % of the TDFs were unknown or had no homology, which is less reported than in previous studies (Van Eck 2007; Zaayman *et al*. 2009), probably due to the increase in sequence information obtained from the rice and wheat genome projects. The TDFs were then further analyzed for functionality within each mode of resistance and a further four cluster trees were drawn up showing TDFs with involvement in susceptibility (Figure 3.4.10), antibiosis (*Dn1*) (Figure 3.4.11), tolerance (*Dn2*) (Figure 3.4.12) and the wounding response (Figure 3.4.13).

The wheat genome is very large, approximately 16 Mbp with little sequence information available. In a study by Sorrells *et al*. (2003), wheat expressed sequence tags (ESTs) were mapped onto the sequenced rice genome. They found that wheat had numerous rearrangements and low similarity of biological organization which would complicate transfer of information or markers from one species to the next. Previous studies using the cDNA-AFLP technology have been useful for studying plant responses to pathogen or pest attack (De Paepe *et al*. 2004; Durrant *et al*. 2004; Eckey *et al*. 2004; Van Eck 2007; Zaayman *et al*. 2009). Thirty percent of the TDFs cloned in this study were found to

have a defence or stress related transcript identity (Figure 3.4.5), therefore the cDNA-AFLP analysis was useful for the identification and isolation of this type of fragment. Seventeen percent of the TDFs identified had either no homology or were unknowns. These proteins would not have been identified using cDNA chip technology, as only previously identified clones or sequence information are included on the chip (Lemieux *et al*. 1998).

Near isogenic lines (NILs) are useful for studying specific traits bred into different lines of wheat. In a previous study using NILs, Zaayman *et al*. (2009) found differentially expressed transcripts between wheat (*Dn7*) infested with different RWA biotypes. Also, in a study by Van Eck (2007) "*Tugela*" NILs were used containing different defence genes *Dn1*, *Dn2* and *Dn5* in a parallel study to this one. In this study, the Betta, BettaDN and Betta *Dn2* NILs were compared for transcriptional differences after a wounding and RWA infestation treatments.

3.5.2 The wounding response

Definite differences were noted between the wounded and infested treatments as can be seen in the cluster tree figures (Figures 3.4.10, 3.4.11, 3.4.12). It has been previously found that wounding produces a different response to that of Russian wheat aphid infestation on a protein level (Botha *et al*. 1998). Eighteen TDFs were found to be specifically related to wounding (Figure 3.4.13) in this experiment.

3.5.2.1 Wounding response and calcium signalling:

In the RWA susceptible line Betta, a serine/threonine phosphatase (AmoTS-M319.3) is down-regulated in the wounded treatment in comparison to the infested and control samples and a calcium-dependent protein kinase 2 (AmoTS-M315.4) is up-regulated in the wounded treatment in relation to the infested and control samples. Kinases and phosphatases form a circuit whereby the kinase adds a phosphate group to a serine or threonine residue – inducing a signal, and the phosphatase removes the phosphate group in order to stop the reaction or cascade, therefore if the phosphatase is

suppressed, then the signal is allowed to continue (Cohen 1989). It can be speculated that the Amo-TS319.3 serine/threonine phosphatase is involved in continuation of a defence response related to the wounding treatment, while the Ca^{2+} dependant kinase protein levels are increased to facilitate a higher sensitivity to the cells integrity.

M269.7, a putative plasma membrane H^+-ATPase, is up-regulated in Betta and in the BettaDN wounded treatments. A link has been established between induced proton flux and induction of the wounded signal (Moyen and Johannes 1996; Schaller 1998; Vera-Estrella *et al*. 1994). A model has been proposed by Schaller and Oeckingh (1999) in which systemin activates a Ca^{2+} channel. They suggest that the increase in cytosolic Ca^{2+} flux may activate a Ca^{2+} dependent protein kinase. The plasma membrane H^+-ATPase is known to be de-activated by phosphorylation in a Ca^{2+} dependant manner (Schaller *et al*. 1992). This would lead to electrical signalling via depolarization of the plasma membrane. In an experiment by Moyen and Johannes (1996), they found that systemin, a wound induced polypeptide, induced changes in ion transport which therefore plays an important role in early systemin induced wound signalling. Interestingly, this TDF is not up-regulated in the *Dn2* treatment, possibly the same proton flux is not used in the *Dn2* containing plants after wounding.

M262.6 a putative Ca^{2+}/H^+-exchanging protein (calcium exchanger: CAX) is down-regulated in the BettaDN wounded treatment. Ca^{2+} is an important intracellular signalling molecule (Bush 1995). The vacuole is a primary repository of Ca^{2+}, and Ca^{2+} transporters in the vacuole membrane (tonoplast) are involved in regulating cytosolic Ca^{2+} levels (Johannes *et al*. 1992). Down regulation of CAX may be a way of controlling the levels of Ca^{2+} influx into the cytosol in BettaDN.

M306.4 is a putative myosin heavy chain which is up-regulated in the BettaDN wounded treatment and down-regulated in the *Dn2* wounded treatment, while up-regulated in the *Dn2* control treatment. Myosin is “a mechanochemical enzyme that hydrolyses ATP and supports many important motile activities” (Yamamoto *et al*. 1999). During wounding, chloroplasts detach with their actin filament bundles creating ‘windows’ (Kamitsubo 1972). Organelles such as vesicles are moved during the wound reaction in response to increased Ca^{2+} concentrations towards the wounded region where the contents are spread in order to heal the gap (Foissner *et al*. 1996). As seen in M269.7, this shows

that perhaps the ATP hydrolysis plays more of an important role in *Dn1* containing plants than in *Dn2* containing plants.

In accordance with these results, we can conclude that Ca^{2+} signalling plays an important role in the wounding response in wheat and that the presence of the *Dn* genes may affect the wound response.

3.5.2.2 Wounding response and the mitochondria:

M427.4 is a putative Zn-dependent protease with chaperone function which is up-regulated in the Betta wounded treatment. The N-presequence of imported proteins about to be folded are cleaved off by the Zn-dependent protease. In plants this protease is an inner mitochondrial membrane bound protein that is part of the redox chain (Eriksson *et al*. 1996). The proteases of the mitochondria are important especially for the import and assembly of peptides from the cytosol (Glaser *et al*. 1998). The up-regulation of this transcript in the susceptible wounded treatment may indicate an up-regulation of protein production for use in the mitochondria or energy production. This may indicate a higher amount of stress in the Betta plants at 48 hours, as this chaperone is almost absent from the *Dn1* and *Dn2* containing NILs.

M273.5 is a putative NADH dehydrogenase (Ubiquinone oxidoreductase) which is down-regulated in Betta, BettaDN and Betta *Dn2* wounded treatments in comparison to their control and infested treatments. NADH: ubiquinone oxidoreductase is a mitochondrial proton pump involved in the synthesis of a proton motive force used for the production of ATP (Mitchell 1961). It could be speculated that the down-regulation of this transcript 48 hours after wounding in all treatments shows that it is an important regulator of proton flux in the wound response.

M284.3 a putative methylcrotonoyl-Coenzyme A carboxylase 2 (beta) mitochondrial enzyme is involved in the valine, leucine and isoleucine degradation pathway (Anderson *et al*. 1998; Faivre-Nitschke *et al*. 2001). It is up-regulated in the Betta wounded treatment, down-regulated in BettaDN wounded treatment and in Betta *Dn2* it is up-regulated in the control and down-regulated in the infested transcripts in comparison to the wounded treatment. This enzyme is involved in the

conversion of 3-methylbut-2-enoy-CoA into 3-methyl-glutaconyl-CoA, which is then either used in sterol synthesis or the production of Acetyl-CoA. Acetyl-CoA is used in the citrate cycle, as well as in the production of oxylipins (KEGG; Kanehisa and Goto 2000; http://www.genome.jp/kegg/). If the pathway follows the production of oxylipins, the path ends with the production of jasmonic acid (JA) which is a pivotal molecule in the wound response (Farmer and Ryan 1990). The TDF is up-regulated in the susceptible Betta wounded treatment, which may indicate that JA is being produced, but at a late time point as sampling was done 48 hours post wounding. It may also imply that the *Dn1* and *Dn2* containing plants have already finished with early signalling and thus the transcript is down-regulated. Reports by Van der Westhuizen *et al*. (1998) indicated that host defence is also time dependant since they observed that PR proteins were up-regulated in susceptible treatments, but long after the induction in resistant plants.

Mitochondrial ion flux may thus play an important role in the wounding response. It is possible that the presence of the *Dn* genes, by some means, improves signalling in the pathways, minimalizing the detrimental effects of elongated signalling via ion flux potentials and the process may be time dependant.

3.5.2.3 Wounding response and the chloroplast:

M297.6 a putative ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit better known as RuBisco, is involved in two biological processes. RuBisco, during carboxylation of ribulose-1,5-bisphosphate (RuBP), gives two molecules of 3-phosphoglycerate (3-PGA) which is used in C3 photosynthesis. Oxygenation of RuBP gives one molecule of 3-PGA and one of 2-phosphoglycolate. The phosphoglycolate is converted to 3-PGA in photorespiration. RuBisco is up-regulated in the Betta wounded treatment, probably due to a need for energy to drive the wounding response.

Only the Betta susceptible wounded treatment is up-regulated at 48 hours post wounding which may be another indicator of delayed signalling that is not present in the *Dn* containing plants.

3.5.2.4 Wounding response and protein anabolism or catabolism:

M489.2 a putative Arf1_5/ArfA-family small GTPase was down regulated in the Betta*DN* wounded treatment in comparison with BettaDN control and infested treatments. ARF1 or adenosine diphosphate ribosylation factor-1, in its GTP-bound form regulates the interaction of non-clatherin coat protein (coatomer) with Golgi stacks (Donaldson *et al*. 1992). Hydrolosis of the GTP dislocates the protein from the Golgi membranes and vesicles (Tanigawa *et al*. 1993). An ARF1-directed GTPase protein (GAP) is needed for GTP hydrolosis (Cukierman *et al*. 1995). As the GTPase is up-regulated, in only the Betta*DN* control and infested treatments, it could be assumed that at 48 hours post wounding, a negative feedback loop is in position to control levels of the GTPase or perhaps the down-regulation allows the plant to heal the wound before valuable proteins are exocytosed into the cytoplasm.

M420.4 is a putative DnaJ-like protein which is up-regulated in the BettaDN wounded treatment. DnaJ is a co-chaperone that interacts with the Hsp70 (Heat shock protein 70) chaperone protein. Hsp70 proteins bind to the hydrophobic areas of unfolded proteins, and their activity is regulated by ATP hydrolosis. The J-domain of DnaJ binds the Hsp70 ATPase domain causing conformational changes (Greene *et al*. 1998; Suh *et al*. 1998). DnaJ stimulates the ATP hydolysis (Liberek *et al*. 1991; Minami *et al*. 1996), and basically determines the activity of Hsp70 by stabilizing the interaction between Hsp70 and the unfolded peptides. DnaJ has been found in association with wounding in plants as well as in animals (Schafleitner and Wilhelm 2002; Tawk *et al*. 2000). We can speculate that this TDF in up-regulated in BettaDN wounded in order to fold proteins more effectively under stress.

M269.7 is a putative peptidase (ubiquitin carboxy-terminal hydrolase 2) which is up-regulated in the Betta and BettaDN wounded treatments. Ubiquitin is a signal peptide that covalently bonds to a protein and acts as a signal for ATP-dependant protein degradation via the 26S proteasome (Matthews *et al*. 1989; Eytan *et al*. 1989; Driscoll *et al*. 1990; Orino *et al*. 1991; Richter-Ruoff *et al*. 1992). The ubiquitination pathway has been found to be responsive to ethylene treatment (De Paepe

et al. 2004). Ethylene is a well documented signal for the wounding response (Saltveit *et al*. 1978; O'Donnell *et al*. 1996).

The production and breakdown of proteins and enzymes is an important part of the wounding response and shows signal transduction to be active.

3.5.2.5 Wounding response and signalling proteins:

M291.0 is a putative Shaggy-like kinase which is down-regulated in BettaDN wounded and up-regulated in the *Dn2* wounded treatments. Shaggy is a non-receptor serine/threonine kinase and a homologue of glycogen synthase kinase 3 (GSK-3). A previous study has identified a GSK-3-like protein (WIG) that may be involved in early wound signalling (Jonak *et al*. 2000). BIN2 (Li *et al*. 2002), a GSK-3 homologue (BIN2) has been found to be involved in a feedback loop involving a nuclear protein BZR1 and the brassinosteroid (BR) signalling pathway in *Arabidopsis* (He *et al*. 2002). BIN2 was found to be a negative regulator of the BR pathway (Li *et al*. 2002) by targeting nuclear protein BZR1 for degradation by phosphorylation, while BR signalling causes BZR dephosphorylation and accumulation by inhibiting BIN2 (He *et al*. 2002). In a study by Souter *et al*. (2002), they found that sterols are required for correct auxin and ethylene signalling. A leucine-rich repeat receptor-like kinase tBri1/SR160 possibly has a dual role in systemin (Sheer and Ryan 2002) and steroid hormone signalling (Montoya *et al*. 2002). BR therefore plays an important role in the wound signal. Many functions have been assigned to BRs including being assigned as phytohormones and have functions in plant growth and development (Rao *et al*. 2002). Other speculation points to possible roles in cell wall growth and the regulation of factors involved in cell wall strength (Altmann 1998). The down-regulation of the shaggy-like kinase in the *Dn1* containing plants could thus suggest that BR signalling does not play an important role in wounding in BettaDN. The transcript is however up-regulated in the *Dn2* containing plants, which could suggest a role for BRs in homeostasis or plant growth in Betta *Dn2* plants.

3.5.2.6 Wounding response and the cell wall:

M314.2 is a putative single-stranded nucleic acid binding protein (whGRP1) which is up-regulated in BettaDN wounded treatment. whGRP is a wheat glycine rich protein (GRP) homologue. Functions of GRPs include the development of the vascular system and have been found to be expressed in the cell walls of wounded tissue (Keller *et al*. 1988). It has been suggested that GRPs associate with cells about to be lignified; their tyrosine residues acting as a catalyst for oxidative polymerization (Keller *et al*. 1989). It is possible that increased numbers of this transcript in BettaDN promotes cell wall lignification. Increase in cell wall thickness has been reported for *Dn1* containing plants (Van der Westhuizen *et al*.1998) under infestation conditions.

3.5.3 RWA infestation and the defence response

Existing resistance to RWA infestation is thought to act in a gene-for-gene manner (Botha *et al*. 2006). A suppressive subtractive hybridization (SSH) study was done using a wheat line containing *Dnx* in which they found *Pti* and *Pto*-like sequences to be involved in the RWA-wheat interaction (Boyko *et al*. 2006). *Dnx* confers antibiosis and tolerance (Boyko *et al*. 2006) and thus the results from the study are useful for comparison. They hypothesize that the antibiosis characteristic involves “defensive chemical production, cellular transport and exocytosis, while tolerance involves self defence against reactive oxygen species and toxins and proteolysis, DNA, RNA and protein synthesis, chloroplast and mitochondrial function, carbohydrate metabolism and maintenance of cellular homeostasis”. A study comparing the responses of *Dn1*, *Dn2* and *Dn5* NILs the major systems implicated in the RWA-wheat response were regulatory proteins, photosynthesis and stress and signal transduction (Van Eck 2007). In this study, the majority of putative genes fell into defence or stress related cellular processes, with the rest in descending order of photosynthesis, membrane proteins, nucleic acid processing and protein production, starch and sucrose metabolism and fatty acid and lipid metabolism.

Several parallels can be drawn between these three studies, and the TDF results presented in the present study. The most prominent similarity between the studies is the regulation of the chloroplast

and photosynthesis genes (Boyko *et al*. 2006; Van Eck 2007). Transcripts such as those encoding for RuBisco (AmoLve-66.306, AmoLve-66.319, AmoLve-86.322) and fructose-1,6-bisphosphate (AmoLve-88.303) (Van Eck 2007) are echoed by TDFs AmoTS-M297.6 (Table 5.1; cluster A5) and AmoTS-M299.7 (Table 5.1; cluster B4) respectively. Transcripts involved in cell maintenance via the chloroplast like photosystem II pTADnx158 (Boyko *et al*. 2006) was also found in this study in AmoTS-M403.5 (Table 5.1; cluster A4). Specific transcripts involved in the response to infestation and the similarities to previous studies will be discussed in the following sections.

3.5.3.1 Infestation response and ion flux:

Redox potentials have been well tied in to defence responses to pathogens, especially when considering the hypersensitive response (HR) and oxidative stress (Foyer and Noctor 2005).

Several ATPases were found to be regulated in this study (Table 5.1; cluster B5). M269.7 is a putative plasma membrane H^+ -ATPase which is significantly up-regulated in the BettaDN infested treatment. This protein is a proton pump that moves protons out of the cell creating a pH and electrical gradient across the cell membrane. Schaller and Oecking (1999) found a difference in the activation of wound and pathogen elicited defence corresponds to a difference in pH, due to differential activation of a plasma membrane H^+ -ATPase. BettaDN has a significant increase in the levels of ATPase at 48 hours suggesting involvement in the defence reaction against the RWA as reported previously (Botha *et al*. 2006)

AmoTS M368.5b TacMag149 is a putative F_1F_0- ATPase inhibitor protein (IF_1) involved in ATP synthase inhibition in the mitochondria, (Pullman and Manroy 1963) and is pH dependant (Panchenko and Vinogradov 1985). In mammalian mitochondria the F_1F_0 ATPase is most active under ischemic conditions (oxygen deprivation) and it has been postulated that IF helps conserve ATP under conditions of oxygen deprivation (Green and Grover 2000). This TDF was present in high numbers in all the Betta treatments with highest band intensity in the wounded treatment and equal expression in the control and infested treatments. While present in the BettaDN and Betta *Dn2* treatments, they are

at a lower expression level. The induction of this inhibitor in the susceptible Betta plants could be an indicator of oxygen stress and a need for ATP.

M220.7 is a putative lipoic acid synthetase isoform 1 that is highly up-regulated in the BettaDN control treatments however, the level of expression is lower in the wounded treatment, and down-regulated in the infested treatment. Lipoic acid synthetase is involved in the production of Protein N6 (Lipoyl) lycine which in turn is utilized by the glycine cleavage system (H Protein) or oxo-acid dehydrogenase complexes. The oxo-acid dehydrogenase complex plays an important role in redox regulation as it is directly involved in the NAD^+/NADH ratio in the mitochondria (Massey *et al*. 1960; Sanadi *et al*. 1958). A second TDF was also found to be involved in redox regulation. M468.4b is a TRR-like domain containing protein that is down-regulated in the BettaDN infested treatment in comparison to the wounded and control treatments. In Betta *Dn2* this TDF was highly up-regulated in the infested treatment in comparison to the wounded and control treatments. Thioredoxin is a redox protein which falls into the family of flavoenzymes that includes glutathione-S-transferase. The thioredoxin reductase (TrR) domain catalyzes the reduction of enzyme bound FAD by NADPH (Williams 1995). Thioredoxin has been found to be regulated by Botha *et al*. (2006) in a *Dn1* containing wheat line. The generation of reactive oxygen species (ROS) is dependant on the NADPH oxidase system (Doke 1985; Murphy and Auh 1996) and the pH dependant peroxidase system (Pedreño *et al*. 1989). It could thus be postulated that the BettaDN lines down-regulate these molecules in infested lines because the antibiotic NILs uses a redox potential to combat the RWA, were as the Betta *Dn2* plants up-regulate this transcript in order to keep the redox potential balanced.

3.5.3.2 Infestation response and the chloroplast:

There are two putative 10kD PSII protein TDFs, M258.0 and M403.5. M258.0 is differently regulated in each of the NILs with the TDF being up-regulated in Betta plants. In BettaDN, there is an elevated amount present in the control and infested treatments, but it is down-regulated in the wounded treatment. In Betta *Dn2*, there is a lower amount of the transcript in the control and infested treatments, with an up-regulation seen in the wounded treatment. With M403.5 all the BettaDN treatments are up-

regulated. The 10kD polypeptide is associated with the water-splitting complex located in the thylakoid lumen. In an experiment by Stockhause *et al*. (1990) the lack of the polypeptide is seen to introduce a 'general disorder' which may slightly modify PSII reaction sequence. Flourescence measurements taken, show that the silencing of the 10kD polypeptide retards reoxidation of Q_A- (first quinine acceptor) by Q_B (Second quinine acceptor) and Q_B-. In a study by Heng-Moss *et al*. (2003) they suggested that the RWA causes damage to photosystem II. It could be that an increase in this polypeptide may help protect against oxidative stress.

Three TDFs are involved light-activated processes. M297.6 is a putative fructose-1,6-*bis*phosphate that is part of the Calvin cycle for cabon fixation. The TDF is up-regulated in the susceptible Betta wounded and infested treatments and is down-regulated in all the BettaDN treatments. M166.9, a putative malate dehydrogenase [NADP] chloroplast precursor is up-regulated in BettaDN infested treatment, as well as the Betta *Dn2* control and wounded treatments. M297.6 is a putative ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (RuBisco) that is covered in section (3.5.4.5). RWA damage in susceptible plants is a light-activated process and it has been suggested that the RWA salivary component is a light "sensitizer" (Macedo *et al*. 2003). Other light sensitive reactions include the synthesis of ATP by ATPase, the Calvin cycle (Malkin and Niyogi 2000), and activation of RuBisco activase (Zhang and Portis 1999). Potentially one of the light activated processes is directly involved in prevention of light activated damage during RWA feeding.

One previous studies involving a fungal pathogen causing photosensitization involves cercosporin (Daub 1982a). The light activated cercosporin generates singlet oxygen and superoxide, which peroxidizes host lipid membranes (Daub 1982b; Daub and Hangarter 1983). In a study by Daub *et al*. (2000) using a resistant fungi line, *Cercospora*, they found resistance to be as a result of the fungi's ability to keep the photosensitizer in a reduced state and that cellular localization within an aqueous environment such as the cytosol may also decrease the quantity of damaging oxygen species produced.

In light conditions, ferroredoxin and ferroredoxin reductase move electrons from photosystem I (PSI) to thioredoxins which in turn transfer them to target enzymes were they reduce disulphide bonds,

changing their catalytic activity thus iron is an important component of the system. Ferritin is an iron sequestering protein found in the chloroplast. In a study using a transgenic plant over expressing ferriton (Déak *et al*. 1999), it was found that plants undergoing attack by virus or fungal pathogens exhibited normal photosynthetic function. They proposed that by sequestering intracellular iron involved in the generation of hydroxyl radicals produced during ROS, ferritin protects the plant from oxidative damage. Iron homeostasis was proposed by Van Eck (2007) as an avenue of investigation after study of the wheat-RWA interaction.

There is a putative chloroplast 23S ribosomal RNA gene M396.3, up-regulated in the BettaDN control and wounded treatments, and up-regulated in the Betta *Dn2* infested treatment. The *Dn2* plants show up-regulation at only 48 hours post infestation (h.p.i.), indicating a large induction of translation in the chloroplast, possibly involved in countering the affects of the RWA salivary toxin over time.

3.5.3.3 Infestation response and transporters:

M158.3 is a putative *Pdr3* an ATP-binding cassette (ABC) transporter with a pleiotropic drug resistance (PDR) domain which is up-regulated in the BettaDN infested treatment, but down-regulated in the Betta *Dn2* infested treatment. PDR-like ABC transporters are well described in yeast and fungal systems, and function as efflux transporters for cytotoxic compounds and are well known for their ability to convey drug resistance (Balzi *et al*. 1994). In plants they are known to have a detoxifying role as vacuolar enzymes (Martinoa et al 1993; Klein *et al*. 1996), as regulators of ion flux (Inagaki *et al*. 1995) and in plant defence (Jasiński *et al*. 2001). In an experiment by Jasiński *et al*. (2001) an ABC transporter NpABC1 (PDR5-like family) in tobacco was found to be up-regulated after treatment with antifungal diterpene sclareolide. Their data suggests that NpABC1 may be involved in the secretion of antimicrobial terpenoids that may play a role in plant defence. This transcript was also found to be regulated in the antixenotic *Dn7* wheat lines after RWA infestation (Zaayman *et al*. 2009). There are many roles for ABC transporters, however, this specific TDF had homology similar to that of *Pdr3*. *Pdr3* encodes for a zinc finger transcription factor, which is known to affect the transcription levels of

other ABC transporters i.e. *PDR5* and *Snq2* (Katzmann *et al*. 1994; Mahé *et al*. 1996). This transcript may therefore be up-regulated in order to up-regulate transcription of a transporter involved in *PDR5* export of a toxic molecule that may be involved in plant defence.

M524.6 is a putative integral membrane protein NRAMP (natural resistance-associated macrophage protein) that is up-regulated in all BettaDN treatments in comparison to the Betta and Betta *Dn2* treatments. This TDF is also up-regulated in the BettaDN infested treatment in comparison to the wounded and control treatments. This metal-transporting protein (Belouchi *et al*. 1997) has been found to have homologues in plants with up to 40 % sequence identity (Cellier *et al*. 1995). *OsNramp* genes have been associated with the plant-pathogen interaction in rice and are thought to mediate the resistance response by increasing iron up-take (Zhou and Yang 2004). OsNramp1 specifically was down-regulated in a susceptible reaction, which may indicate that it is necessary for defence (Zhou and Yang 2004). The presence of high numbers of transcript in all BettaDN treatments with high up-regulation (1e+6) in the BettaDN infested treatment (Table 5.1; cluster A4) suggests that this transporter may be very important in *Dn1* mediated resistance.

3.5.3.4 Infestation response and ubiquitin:

The ubiquitination of proteins marks them for degradation by the 26S proteasome (Fu *et al*. 1999). The ubiquitin/proteasome system (UPS) has been implicated in plant defence (Peart *et al*. 2002). SGT1 was identified in yeast by Kitigawa *et al*. (1999) as a component of the SCF ubiquitin-ligase complex. SGT1 has been confirmed as necessary for *R*-gene mediated resistance response, as well as in non-host resistance (Peart *et al*. 2002). From the results on BettaDN there are three TDFs associated with the UPS that are all up-regulated in the infested treatments. M536.9 is a putative defence related F-box protein that is up-regulated in BettaDN infested. F-boxes are receptor domains that recruit phosphorylated substrates to the ubiquitin-ligase complex (Skowrya *et al*. 1997). M318.9 is a putative aspartic proteinase (WAP2) that is up-regulated in BettaDN wounded and infested treatments. In a paper by Tamura *et al*. (2007), using germinating wheat seeds, they found that WAP2 may function as a vacuolar enzyme, acting to hydrolyze proteins that have been transported into the vacuole. M269.7b

is a putative peptidase or ubiquitin carboxy-terminal hydrolase 2 protein that is up-regulated in BettaDN infested. Peptidases function by further hydrolyzing small peptides released by the 26S proteasome. This shows that the UPS has an important function in *Dn1* mediated resistance.

3.5.3.5 Infestation response and toxin production:

M706.4 is a putative selenium binding protein (SBP) that is down regulated in the BettaDN infested treatment but up-regulated in the Betta *Dn2* infested treatment. Selenium binding proteins have been found to be induced in response to plant attack in a fungus- rice system (Sawada *et al*. 2003). *OsSBP* was found to be up-regulated in response to treatment with both JA and SA (Sadawa *et al*. 2004). Sadawa *et al*. (2004) used a transgenic system to show that over-expression of *OsSBP* enhanced plant defence against the rice blast pathogen. *OsSBP* was involved in PR protein induced protection as both PR protein and phytoalexin synthesis was accelerated. The TDF was down-regulated in BettaDN at 48 hours, however, levels of the *OsSBP* transcript looks to be most active at an earlier infection time of 24 hours. Therefore, it could be said that there is a possibility that this molecule is up-regulated at a 24 hour time point and then down-regulated at the later time point. This will have to be tested using a qRT-PCR strategy and time trialed samples.

3.5.3.6 Infestation response and elicitor recognition:

TDF M416.6 is a putative protein kinase *Xa21* homologue that is highly up-regulated in BettaDN infested at 48 hours. There is up-regulation of the transcript in Betta *Dn2* control, but it is 50 % lower than was measured in the BettaDN infested treatment. Xa21 is a receptor kinase-like protein from rice that conveys resistance to *Xanthomonas oryzae* (Figure 2.1.D) (Song *et al*. 1995). The sequence of *Xa21* contains an NBS-LRR motif and a serine/threonine kinase domain, and the protein is implicated in cell surface recognition (Song *et al*. 1995). NBS-LRRs are well known as R-proteins involved in pathogen elicitor (Avr-protein) recognition (Bisgrove *et al*. 1994; Scofield *et al.* 1996; Silué *et al*. 1992; Song *et al*. 1995; Tang *et al.* 1996), starting the signalling cascade that leads to SAR (Section 2.3.1; Figure 2.2). Interestingly, *Xa21* has been found to contain transposable elements

(Song *et al*. 1998). In the study by Botha *et al*. (2006) transposable elements were found to be regulated during RWA infestation and hypothesize that they may be regulated under stress conditions. It is thought that the transposable elements may contribute to sequence diversity of the Xa21 family members (Song *et al*. 1998). This TDF was highly up-regulated in BettaDN infested, and, as a possible RWA elicitor sensor would thus be a good target for further study.

3.5.4 qRT-PCR

qRT-PCR was performed on representative TDFs (Table 3.4.4) using biological repeat material sampled at five time points after infestation. Possible functions of the proteins are hypothesized using the patterns of transcription elucidated by the qRT-PCRs.

3.5.4.1 qRT-PCR fragment 92:

TcaMac92 or M335.6 is a putative β-glucosidase. The plant β-glucosidase has been found to be induced by herbivore feeding, and has been found to attract predators to the site of attack in a lettuce-caterpillar system (Mattiacci *et al*. 1995). β-glucosidase has been found to accumulate in endoplasmic reticulum of damaged *Arabidopsis* rosettes (Matsushima *et al*. 2003). β -glucosidase in wheat has been found to interact with glucosides during wounding or damage from insect feeding, which then enables the enzyme to produce toxic aglycones or volatiles (Boland *et al*. 1992; Sue *et al*. 2000). In the phenylpropanoid pathway, it is involved in the production of coumarine (KEGG; Kanehisa and Goto 2000; http://www.genome.jp/kegg/), and methyl jasmonate is a known inducer of the phenylpropanoid pathway (Graham and Graham 1996). In the study by Boyko *et al*. (2006) a β-glucosidase homologue, pTaDnx680, was found to be regulated in *Dnx* containing wheat. According to the qRT-PCR results for this TDF, in Betta infested at 72 hours, there is a 7 fold change of β-glucosidase (Figure 3.4.20) which is a very late induction. There is a larger amount of the transcript already present in treatments BettaDN control 5 hours and Betta *Dn2* control 48 hours and in BettaDN wounded 48 hours and Betta *Dn2* infested 48 hours in comparison to Betta (Figure 3.4.20). Prehaps the presence of a certain level of β-glucosidase already present in the plants may play an important

role in early indirect defence and the spikes at 48 hours may be involved in the attraction of aphid predators or toxin production. It is interesting that in the *Dn1* containing plants, the two fold induction is seen in the wounded treatment, while in the *Dn2* containing plant the induction is seen in the infested treatment.

3.5.4.2 qRT-PCR fragment 104:

TcaMac104 M254.4 is a putative vacuolar ATP-synthase subunit H that is up-regulated in BettaDN infested. This TDF is up-regulated in the Betta *Dn2* control treatment in comparison with the wounded and infested treatments (Figure 3.4.21). From data obtained after qRT-PCR analysis (Figure 3.4.21), this TDF is shown to be important in the BettaDN infested treatments 5 h.p.i as well as at 48 hours post wounding (h.p.w.). This is in contrast to the Betta *Dn2* treatments which show much less transcript present in comparison with the BettaDN and Betta treatments. The vacuolar ATP-synthase is involved in a few cellular functions. One is the acidification of intercellular compartments and another, the removal of protons after the 'respiratory burst' in neutrophils (Nanda *et al*. 1992). It can be postulated that the transcript is up-regulated in the BettaDN infested treatments, putatively as a detoxifier after ROS induction.

3.5.4.3 qRT-PCR fragment 126:

TgtMac126 M289.7 a putative Ubiquinol-cytochrome C reductase complex is a mitochondrial membrane bound flavoprotein that functions by transferring protons from NADPH to cytochrome P450 and have a detoxifying role in the mitochondria (Turrens 2003). According to the qRT-PCR results, this TDF is present in larger amounts in the Betta control treatment than the BettaDN and Betta *Dn2* control treatments. It also seems to be more important during wounding than infestation, as there is up-regulation in the levels of transcript in Betta, BettaDN and Betta *Dn2* wounded treatments, while the infested treatments are down-regulated in comparison. However, in the Betta and BettaDN infested 5 hours treatments, there is a comparable amount of the transcript present in relation to the wounded treatments, whereas the Betta *Dn2* 5 hours post infestation treatment shows down-

regulation (Figure 3.4.22). This is an indicator of ROS in Betta and BettaDN plants, which maybe less prominent in infested Betta *Dn2* plants. ROS has previously been shown to be up-regulated in BettaDN plants through the measurement of peroxidase (Van der Westhuizen *et al*. 1998).

3.5.4.4 qRT-PCR fragment 129:

TgtMac129 M286.9 a putative acyl-carrier protein, mitochondrial precursor NADH-ubiquitinone oxidoreductase is the first complex in the mitochondrial electron transfer chain. Transcripts are present in high numbers in the Betta treatments, especially in the control treatments as well as the 5 h.p.i and 5 h.p.w. treatments. In all three NILs the transcript is up-regulated in the wounded treatments, and the other infestation treatments are all down regulated except in 5 h.p.i and 48 h.p.i (Figure 3.4.23). As this gene is directly involved in energy production it can be assumed, looking at the levels of regulation, that the Betta plants are under pressure to produce large amounts of energy for its responses, followed by BettaDN, and Betta *Dn2* being the most conservative of energy expenditure. Also, this TDF seems to be up-regulated in the wounding treatments in comparison to infestation treatments. This is indicative that the two responses have different energy needs.

3.5.4.5 qRT-PCR fragment 235:

M297.6 is a putative ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (RuBisco) that has varied expression levels across the NILs plants. RuBisco is involved in the Calvin cycle of photosynthesis and is responsible for CO_2 fixation (Malkin and Niyogi 2000). RuBisco has been found to be regulated after potato challenged by fungus, and may be up-regulated to compensate for lost chloroplasts in infected or damaged tissue (Lee *et al*. 2006). The most noticeable trait is a large up-regulation in the BettaDN wounded 48 hour treatment, while all the infested treatments are down-regulated (Figure 3.4.24). RuBisco has previously been reported to be induced in *Dn1* containing plants at a protein level (Van der Westhuizen and Botha 1993). The increase in expression in the Betta infested 72 h.p.i. treatments may show a late attempt by the plant to replace carbohydrates removed by the aphid. This would increase the expenditure of energy and loss of newly formed

carbohydrates, putting the plant at a disadvantage. While the Betta *Dn2* plants show some regulation in the infested treatments at 48 and 72 hours, it is minimal showing a small expenditure of carbohydrates to balance out the effects of the aphid infestation.

3.5.5 Wounding *vs.* infestation

In a study by Romeis *et al*. (1999), they found that the R-Avr interaction may be mediated via parallel signalling cascades and that the cascades may be linked at the MAPK stage of signalling. This includes abiotic stressors such as wounding and mechanical stress. Results from this study indicate some similarities between the wounded and the infested responses at the 48 hours time point as many TDFs are regulated similarly in the wounded and infested samples in comparison to the controls (Figure 3.4.7).

There are four main defence pathways that can be activated during attack or infestation. 1) The ROS dependant pathway that leads to the production of salicylic acid (SA). This path is sparked by direct recognition and produces the hypersensitive response (HR) in an attempt to limit pathogen spread. 2) The SA dependant pathway utilizes methyl salicylate (MeSA) as its mobile messenger, and promotes the expression of plant wide defence involving PR proteins. 3) Jasmonic acid (JA) regulated pathway that is initiated after wounding or herbivore attack and initiates the production of ethylene (O'Donnell *et al*. 1996). 4) Ethylene dependant pathway that is also initiated after wounding or herbivore attack. JA and Ethylene act together to induce the production of PI proteins (O'Donnell *et al*. 1996) and defensin (Penninckx *et al*. 1998) and can induce a broad spectrum tolerance to attack called induced systemic resistance (ISR) (Pieterse *et al*. 1996).

It is now well known that there is cross-talk between the SA and JA/ethylene-dependant pathways, which is thought to be mediated by *NPR1* (Pieterse *et al*. 1998). It is believed that an increase in SA negatively regulates JA production through NPR1 (Spoel *et al*. 2003). However, there are several more convergence points that may come into play such as the MAP kinases (Romeis *et al*. 1999), and so the complex signalling networks have not been fully elucidated at this time.

It is the current consensus that the octadecanoid pathway is the most important in the wound response as it produces hormones such as JA (Farmer and Ryan 1992). It has been demonstrated in *Arabidopsis* that signalling downstream of JA and SA differs between herbivores which induce an indirect defence and pathogens which induce a direct defence (Van Poecke and Dicke 2003). In previous studies on RWA, it has been found that the response to RWA infestation is utilizes different pathways to that of the wounding response (Botha *et al*. 1998; Messina *et al*. 2002; Zaayman *et al*. 2009). In this study, there are some parallels between the wounding and infestation responses down-stream at a molecular level.

The TDFs similarly expressed in wounding and infestation treatments are numerous (Table 5.1), and a few are will be covered here. AmoTSM315.4 is a calcium dependant protein kinase activated in both wounded and infested treatments of BettaDN. Ca^{2+} signalling is an important signalling molecule for wounding as shown in section (3.5.2.1), and has been found to be involved in the infestation response (Will and van Bel 2006). Both wounding and SAR responses make use of H^{+}ATPase (Sections 3.5.2.1 and 3.5.3.1) showing that both processes utilize ion flux. These results may indicate that a JA or ethylene pathway is activated in parallel with the SA one as suggested previously by Botha *et al*. (2006), which runs against the known cross-talk model. However, it may be that a resistance pathway specific to the RWA interaction meets up with the JA/ethylene pathway down-stream and this leads to similar expression of transcripts in the wounded and infested treatments.

Many of the TDFs are down-regulated in the wounded and infested treatments of Betta *Dn2*, showing a different pattern of expression to the Betta and BettaDN plants. Results from the cDNA-AFLPs and the qRT-PCRs indicate marked differences in response to wounding between the NILs, e.g. there is a high amount of TDF 235 in the wounded treatment of BettaDN (Figure 3.4.24) but in none of the other treatments. This may mean that the presence of the *Dn* genes may alter, or affect the wound response downstream, initiating a better or different defence.

3.5.6: The susceptible response

It has been suggested that the susceptible plant does not recognize the threat in time and thus does not activate the appropriate machinery for cell maintenance, and so the breakdown in chlorophyll and photosynthesis leads to cell death (Botha *et al*. 2006). The late induction of high levels of chloroplast associated transcripts for proteins such as RuBisco (Section 3.4.5) and fructose-1,6-bisphosphate and carbonic anhydrase (Figure 3.4.10; cluster 3) in the susceptible Betta plants after infestation shows a possible attempt to balance out levels of carbohydrates that the plant senses are missing, due to the RWA feeding. Also, due to the damage that occurs in the susceptible plants, i.e. chlorotic streaking, the number of functioning chloroplasts are reduced, perhaps forcing the plant to increase production of carbohydrates, using the remaining organelles. Also, the up-regulation of mitochondrial energy production proteins (Section 3.5.4.4) as well as the attempt to conserve ATP by inhibition of the mitochondrial F_10_1 ATPase indicates a large expansion of energy and resources by the susceptible plant that does not occur in the resistant lines.

3.5.7 The resistance response: antibiosis *vs.* tolerance

In an antibiotic resistance model, aphids are less fecund and thus fewer aphids are born when fed on antibiotic resistance containing wheat (Quisenberry and Schotzko 1994). *Dn1* is a known antibiosis conveying resistance gene (Du Toit 1989). SA induction is the result of a recognition event (Ryals *et al*. 1996), and SA levels have been seen to increase in *Dn1* containing plants upon RWA infestation (Mohase and Van der Westhuizen 2002). In BettaDN, the responses to infestation seem to follow the gene-for-gene defence response model as proposed in Botha *et al*. (2006). The results show that there is a HR response as suggested by Van der Westhuizen *et al*. (1998), with many transcripts involved in ion flux (Section 3.5.3.1). There is also possible secretion or production of toxic/volatile compounds (Section 3.5.3.3) which suggests activity of a JA/ethylene response pathway. BettaDN also possibly down-regulates carbohydrate production, making the plant a less attractive target for infestation (Section 3.5.4.5). A putative NBS-LRR, highly up-regulated in the BettaDN infested treatment was also isolated and should be a target for further study.

Tolerance is the ability of the plant to withstand an aphid population without loosing moisture or compromising plant growth (Quisenberry and Schotzko 1994). In comparison to BettaDN, Betta *Dn2* does not show a HR response. Rather, the molecules that are involved seem to be involved in sustaining function of the organelles and their integrity (Sections 3.5.3.2; 3.5.4.4; 3.5.4.5). There is also possible involvement of an NBS-LRR protein present in the Betta *Dn2* control plants, indicating the ability to recognize elicitor proteins timeously.

These results add to the *Dn1*- antibiotic resistance and *Dn2*- tolerance models, increasing our understanding of the pathways involved in the different modes of resistance.

3.6 Reference list

Altmann, T. (1998) Molecular physiology of brassinosteroids revealed by the analysis of mutants. *Planta*. 208: 1-11

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*. 25(17): 3389- 3402

Altschul, S.F. (1998) Fundamentals of database searching. *Trends in Biotechnology*. 16: 7-9

Anderson, M.D., Che, P., Song, J., Nikolau, B.J., Wurtele, E.S. (1998) 3-Methylcrotonyl-coenzyme A carboxylase is a component of the mitochondrial leucine catabolic pathway in plants. *Plant Physiology*. 118: 1127-1138

Bachem, C.W.B., Oomen, R.J.F.J., Visser, G.F. (1998) Transcript imaging with cDNA-AFLP: A step-by-step protocol. *Plant Molecular Biology Reporter*. 16: 157-173

Balzi, E., Wang, M., Leterme, S., Van Dyck, L., Goffeau, A. (1994) PDR5, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator PDR1. *The Journal of Biological Chemistry*. 269(3): 2206-2214

Belouchi, A., Kwan, T., Gros, P. (1997) Cloning and characterization of the *OsNramp* family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions. *Plant Molecular Biology*. 33: 1085-1092

Bisgrove, S.R., Simonich, M.T., Smith, N.M., Sattler, A., Innes, R.W. (1994) A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes. *The Plant Cell*. 6: 927-933

Boland, W., Feng, Z., Donath, J., Gabler, A. (1992) Are acyclic C_{11} and C_{16} homoterpenes plant volatiles indicating herbivory? *Naturwissenschaften*. 79(8): 368-371

Boshoff, W., du Toit, F. (2006a) n' Deurbraak in weerstand. *Pitkos*. 3(3)

Bosshof, W., Du Toit, F. (2006b) Pannar cultivar has resistance to new Russian wheat aphid 04/09/2006. *Pannar News*. http://pannar.com/news.php?id=6&country_id=1

Botha, A-M., Nagel, M.A.C., Van der Westhuizen, A.J., Botha, F.C. (1998) Chitinase isoenzymes in near-isogenic wheat lines challenged with Russian wheat aphid, exogenous ethylene and mechanical wounding. *Botanical Bulletin of Academia Sinica*. 39: 99-106

Botha, A.-M., Lacock, L., Van Niekerk, C., Matsioloko, M.T., Du Preez, F.B., Myburg, A.A., Kunnert, K., Cullis, C.A. (2003) Gene expression profiling during *Diuraphis noxia* infestation Triticum aestivum cv.' TugelaDN' using microarrays. *In* Proceedings:Tenth International Wheat symposium. 334-338

Botha, A-M., Lacock, L., van Niekerk, C., Matsioloko, M.T., du Preez, F.B., Loots, S., Venter, E., Kunert, K.J., Cullis, C.A. (2006) Is photosynthetic transcriptional regulation in *Triticum aestivum* L. cv. 'TugelaDN' a contributing factor for tolerance to *Diuraphis noxia*(Homoptera: Aphididae)? *Plant Cell Reports*. 25: 41-54

Boyko, E.V., Smith, M., Thara, V.K., Bruno, J.M., Deng, Y., Starkey, S.R., Klaahsen, D.L. (2006) Molecular basis of plant gene expression during aphid invasion: wheat *Pto*- and *Pti*-like sequences are involved in interactions between wheat and Russian wheat aphid (Homoptera: Aphididae). *Journal of Econonomic Entomology*. 99(4): 1430-1445

Bunik, V.L. (2003) Minireview. 2-Oxo acid dehydrogenase complexes in redox regulation: Role of the lipoate residues and thioredoxin. *European Journal of Biochemistry*. 270: 1036-1042

Burd, J. D., Elliott, N.C. (1996) Changes in chlorophyll a ßuorescence induction kinetics in cereals infested with Russian wheat aphid (Homoptera: Aphididae). *Journal of Econonomic Entomology*. 89: 1332-1337

Bush, D.S. (1995) Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology and Plant Molecular Biology*. 46: 95-122

Cellier, M., Prive, G., Belouchi, A., Kwan, T., Rodrigues, V., Chia, W., Gros, P. (1995) Nramp defines a family of membrane proteins. *Proceedings of the National Academy of Science, USA*. 92: 10089-10093

Chomczynski, P., Sacchi, S. (1987) Single-step method of RNA isolation by acid guanidium thiocynate-phenol-chlorophorm extraction. *Annals of Biochemistry*. 162: 156-159

Cohen, P. (1989) The structure and regulation of protein phosphatases. *Annual Review in Biochemistry*. 58: 453-508

Cukierman, E., Huber, I., Rotman, M and Dan Cassel. (1995) The ARF1 GTPase-activating protein: zinc finger motif and Golgi complex localization. *Science*. 270(5244): 1999-2003

Daub, M.E. (1982a) Cercosporin, a photosensitizing toxin from *Cercospora* species. *Phytopathology*. 72(4): 370-374

Daub, M.E. (1982b) Peroxidation of tobacco membrane lipids by the photosensitizing toxin, cercosporin. *Plant Physiology*. 69: 1361-1364

Daub, M.E., Hangarter, R.P. (1983) Light-induced production of singlet oxygen and superoxide by the fungal toxin, cercosporin. *Plant Physiology*. 73: 855-857

Daub, M.E., Li, M., Bilski, P., Chignell, C.F. (2000) Dihydrocercosporin singlet oxygen production and subcellular localization: a possible defense against cercosporin phototoxicity in *Cercospora*. *Photochemistry and Photobiology*. 71(2): 135-140

Déak, M., Horváth, G.V., Davletova, S., Török, K., Sass, L., Vass, I., Barna, B., Király, Z., Dudits, D. (1999) Plants ectopically expressing the iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens. *Nature Biotechnology*. 17: 192-196

De Paepe, A., Vuylsteke, M., Van Hummelen, P., Zabeau, M., Van Der Straeten, D. (2004) Transcriptional profiling by cDNA-AFLP and microarray analysis reveals novel insights into the early response to ethylene in *Arabidopsis*. *The Plant Journal*. 39: 537-559

Dixon, R.A., Paiva, N.L. (1996) Stress-induced phenylpropanoid metabolism. *The Plant Cell*. 7: 1085-1097

Doke, N., (1985) NADPH-dependent O^{2-} generation in membrane fractions isolated from wounded potato tubers inoculated with Phytophthora infestans. *Physiological Plant Pathology*. 27: 311-322

Donaldson, J.G., Cassel, D., Kahn, R.A., Klausner, R.D. (1992) ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein β-COP to Golgi membranes. *Proceedings of the National Academy of Science, USA*. 89: 6408-6412

Driscoll, J., Goldberg, A.L. (1990) The proteasome (multicatalytic protease) is a component of the 1500-kDa proteolytic complex which degrades ubiquitin-conjugated proteins. *The Journal of Biological Chemistry*. 265(9): 4789-4792

Durrant, W.E., Rowland, O., Piedras, P., Hammond-Kosack, K.E., Jones, J.D.E. (2004) cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. *The Plant Cell*. 12: 963-977

Du Toit, F. (1987) Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Hemiptera: Aphididae). *Cereal Research Communications*. 15 (2-3): 175-179

Du Toit, F. (1989) Inheritance of resistance in two *Triticum aestivum* lines to Russian wheat aphid (Homoptera: Aphididae). *Journal of Economic Entomology*. 82(4): 1251-1253

Eckey, C., Korell, M., Leib, K., Biedenkopf, D., Jansen, C., Langen, G., Kogel, K-H. (2004) Identification of powdery mildew-induced barley genes by cDNA-AFLP: functional assessment of an early expressed MAP kinase. *Plant Molecular Biology*. 55: 1-15

Eisen, M.B., Spellman, P.T., Brown, P.O., Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proceedings of the National Acadamy of Science, USA*. 95(25): 14863-14868

Eriksson, A., Sjoling, S., Glaser, E. (1996) Characterization of the bifunctional mitochondrial processing peptidase (MPP)/bc_1 complex in *Spinacia oleracea*. *Journal of bioenergetics and biomembranes*. 28(3): 285-292

Eytan, E., Ganoth, D., Armon, T., Hershko, A. (1989) ATP-dependant incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin. *Proceedings of the National Acadamy of Science, USA*. 86(20): 7751-7755

Faivre-Nitschke, S.E., Couee, I., Vermel, M., Grienenberger, J.M., Gualberto, J.M. (2001) Purification, characterization and cloning of isovaleryl-CoA dehydrogenase from higher plant mitochondria. *European Journal of Biochemistry*. 268: 1332–1339

Farmer, E.E., Ryan, C.A. (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *The Plant Cell*. 4: 129-134

Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annual Review of Phytopathology*. 9: 275-296

Foissner, I., Lichtscheidl, I.K., Wasteneys, G.O. (1996) Actin-based vesicle dynamics and exocytosis during wound wall formation in characean internodal cells. *Cell Motility and the Cytoskeleton*. 35: 35-48

Food and Agriculture organization (2008) Crop Prospects and Food Situation Report. February 1.

Foyer, C.H., Noctor, G. (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell*. 17: 1866-1875

Fu, H., Girod, P-A., Doelling, J.H., van Nocker, S., Hochstrasser, M., Finley, D., Vierstra, R.D. (1999) Structure and functional analysis of the 26S proteasome subunits from plants: Plant 26S proteasome. *Molecular Biology Reports*. 26: 137-146

Gianoli, E., Niemeyer, H.M. (1997) Characteriztics of hydroxamic acid induction in wheat triggered by aphid infestation. *Journal of Chemical Ecology*. 23(12): 2695-2705

Glaser, E., Sjöling, S., Tanudji, M., Whelan, J. (1998) Mitochondrial protein import in plants: signals, sorting, targeting, processing and regulation. *Plant Molecular Biology.* 0: 311-338

Graham, T.L., Graham, M.Y. (1996) Signalling in soybean phenylpropanoid responses, *Plant physiology*. 110: 1123-1133

Green, D.W., Grover, G.J. (2000) The IF1 inhibitor protein of the mitochondrial F1F0-ATPase. *Biochimica et Biophysica Acta - Bioenergetics*. 1458: 343-355

Greene, M.K., Maskos, K., Landry, S.J. (1998) Role of the J-domain in the cooperation of Hsp40 with Hsp70. *Proceedings of the National Academy of Science, USA*. 95: 6108-6113

Hall, T.A. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleaic Acid Symposium Series*. 41: 95-98

He, J-X., Gendron, J.M., Yang, Y., Li, J., Wang, Z-Y. (2002) The GSK3-like linase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in *Arabidopsis*. *Proceedings of the National Acadamy of Science, USA*. 99(15): 10185-10190

Heng-Moss, T.M., Ni, X., Macedo, T., Markwell, P.J., Baxendale, F.P., Quiesnberry, S.S., Tolmay, V. (2003) Comparison of chlorophyll and carotenoid Concentrations among Russian wheat aphid (Homoptera: Aphididae)-infested wheat isolines. *Journal of Economic Entomology*. 96(2): 475-481

Illius, A.W., Jessop, N.S. (1995) Modeling metabolic costs of allelochemical ingestion by foraging herbivores. *Journal of Chemical Ecology*. 21(6): 693-719

Inagaki, N., Gonoi, T., Clement IV, J.P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., Bryan, J. (1995) Reconstitution of IKAPT: An inward rectifier subunit plus the sulfonylurea receptor. *Science*. 270: 1166-1170

Inoue, H., Nojima, H., Okayama, H. (1990) High efficiency transformation of *Escherichia coli* with plasmids. *Gene*. 96: 23-28

Jasiński, M., Stukkens, Y., Degand, H., Purnelle, B., Marchand-Brynaert, J., Boutry, M. (2001) A plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion. *The plant cell*. 13: 1095-1107

Johannes, E., Brosnan, J.M., Sanders, D. (1992) Calcium channels in the vacuolar membrane of plants: multiple pathways for intracellular calcium mobilization. *Philosophical Transactions: Biological Sciences.* 338: 105-112

Jonak, C., Beisteiner, D., Beyerly, J., Hirt, H. (2000) Wound-induced expression and activation of *WIG*, a novel glycogen synthase kinase 3. *The Plant Cell*. 12: 1467-1475

Kamitsubo, E. (1972) A 'window technique' for detailed observation of charcean cytoplasmic streaming. *Experimental Cell research*. 74(2): 613-616

Kanehisa, M., Goto, S. (2000) KEGG: Kyoto Encyclopedia of genes and genomes. *Nucleic Acids Research*. 28: 27-30

Katzmann, D.J., Burnett, P.E., Golin, J., Mahe, Y., Moye-Rowley, W.S. (1994) Transcriptional control of the yeast *PDR5* gene by the *PDR3* gene. *Molecular and Cellular Biology*. 14(7): 4653-4661

Keenan, S.P., Burgener, P.A. (2008) Social and economic aspects. *In:* Areawide pest management: Theory and implementation. Wallingford, UK; Cambridge, MA.

Keller, B., Sauer, N., Lamb, C.J. (1988) Glycine-rich cell wall proteins in bean: gene structure and association of the protein with the vascular system. *The EMBO Journal*. 7(12): 3625-3633

Keller, B., templeton, M.D., Lamb, C.J. (1989) Specific localization of a plant cell wall glycine-rich protein in protoxylem cells of the vascular system. *Proceedings of the National Acadamy of Science, USA*. 86: 1529-1533

Kitigawa, K., Skowyra, D., Elledge, S.J., Harper, J.W., Hieter, P. (1999) *SGT1* Encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. *Molecular Cell*. 4: 21-33

Klein, M., Weissenbock, G., Dufaud, A., Gaillard, C., Kreuz, K. ,Martinoia, E. (1996) Different energization mechanisms drive the vacuolar uptake of a flavonoid glucoside and a herbicide glucoside. *The Journal of Biological Chemistry*. 271(47): 29666-29671

Kreimer, G., Melkonian, M., Holtum, J.A.M., Latzko, E. (1987) Stromal free calcium concentration and light-mediated activation of chloroplast fructose-1,6-bisphosphatase. *Plant physiology*. 86: 423-428

Lacock, L., Van Niekerk, C., Loots, S., du Preez, F., Botha, A.-M. (2003) Functional and comparative analysis of expressed sequences from *Diuraphis noxia* infested wheat obtained utilizing the conserved Nucleotide Binding Site. *African Journal of Biotechnology*. 2(4): 75-81

Lee, J., Bricker, T.M., Lefevre, M., Pinson, S.R.M., Oard, J.H. (2006) Proteomic and genetic approaches to identifying defence-related proteins in rice challenged with the fungal pathogen *Rhizoctonia solani*. *Molecular Plant Pathology*. 7(5): 405-416

Lemieux, B., Aharoni, A., Schena, M. (1998) Overview of DNA chip technology. *Molecular Breeding*. 4: 277-289

León, J., Rojo, E., Titarenko, E., Sánchez-Serrano, J.J. (1998) Jasmonic acid-dependent and -independent wound signal transduction pathways are differentially regulated by Ca2+/calmodulin in *Arabidopsis thaliana*. Molecular and *General Genetics*. 258: 412-419

Li, X., Schuler, M.A., Berenbaum, M.R. (2002) Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature*. 419: 712-715

Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C., Zylicz, M. (1991) Escherichia coli DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. *Proceedings of the National Acadamy of Science, USA*. 88: 2874-2878

Liu, X.M., Smith, C.M., Gill, B.S., Tolmay, V. (2001) Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. *Theory of Applied Genetics*. 102: 504-510

Macedo, T.B., Higley, L.G., Ni, X., Quisenberry, S.S. (2003) Light activation of Russian wheat aphid-elicited physiological responses in susceptible wheat. *Journal of Economic Entomology*. 96(1): 194-201

Mahé, Y., Parle-McDermott, A., Nourani, A., Delahodde, A., Lamprecht, A., Kuchler, K. (1996) The ATP-binding cassette multidrug transporter Snq2 of *Saccharomyces cerevisiae*: a novel target for the transcription factors Pdr1 and Pdr2. *Molecular Microbiology*. 20(1): 109-117

Malkin, R., Niyogi, K. (2000) Chapter 12. Photosynthesis. *In*: Biochemistry and Molecular Biology of Plants. (Eds) Buchanan, B.B., Gruissem, W., Jones, R.L. 585-618

Martinoa, E., Grill, E., Tommasini, R., Kreuz, K., Amrhein, N. (1993) ATP-dependent glutathione *S*-conjugate 'export' pump in the vacuolar membrane of plants. *Nature*. 364: 247-249

Massey, V., Gibson, Q.H., Veeger, C. (1960) Intermediates in the catalytic action of lipoyl dehydrogenase (Diaphorase). *Biochemistry Journal*. 77: 341-351

Matthews, W., Tanaka, K., Driscoll, J., Ichihara, A., Goldberg, A.L. (1989) Involvement of the proteasome in various degradative processes in mammalian cells. *Proceedings of the National Acadamy of Science, USA*. 86(8): 2597-2601

Matsushima, R., Kondo, M., Nishimura, M., Hara-Nishimura, I. (2003) A novel ER-derived compartment, the ER body, selectively accumulates a β-glucosidase with an ER-retention signal in *Arabidopsis*. *The Plant Journal*. 33: 493-502

Mattiacci, L., Dicke, M., Posthumus, M.A. (1995) 18-Glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the National Acadamy of Science, USA*. 92: 2036-2040

Messina, F.J., Taylor, R., Karren, M.E. (2002) Divergent responses of two cereal aphids to previous infestation of their host plant. *Entomologia Experimentalis et Applicata*. 103(1): 43 - 50

Minami, Y., Höhfeld, J., Ohtsuka, K., Hartl, F.U. (1996) Regulation of the heat-shock protein 70 reaction cycle by the mammalian DnaJ homolog, Hsp40. *The Journal of Biological Chemistry*. 271(32): 19617-19624

Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*. 191: 144-148

Mohase, L., Van der Westhuizen, A.J. (2002) Salicylic acid is involved in resistance responses in the Russian wheat aphid-wheat interaction. *Journal of Plant Physiology*. 159: 585-590

Montoya, T., Nomura, T., Farrar, K., Kaneta, T., Yokota, T., Bishop, G.J. (2002) Cloning the tomato *Curl3* gene highlights the putative dual role of the leucine-rich repeat receptor kinase tBRI1/SR160 in plant steroid hormone and peptide hormone signaling. *The Plant Cell*. 14(12): 3163-3176

Moyen, C., Johannes, E. (1996) Systemin transiently depolarizes the tomato mesophyll cell membrane and antagonizes fusicoccin-induced extracellular acidification of mesophyll tissue. *Plant, Cell and Environment.* 19: 464-470

Murphy, T.M., Auh, C-K. (1996) The superoxide synthases of plasma membrane preparations from cultured rose cells. *Plant Physiology*. 110: 621-629

Myburg, A.A., Remington, D.L., O'Malley, D.M., Sederhoff, R.R., Whetten, R.W. (2001) High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. *Biotechniques*. 30(2): 384-357

Nanda, A., Gukovskaya, A., Tseng, J., Grinstein, S. (1992) Activation of vacuolar-type proton pumps by protein kinase C. Role in neutrophil pH regulation. *Journal of Biological Chemistry*. 267(32): 22740-22746

Newman, R.M., Kerfoot, W.C., Hanscom, Z. III (1996) Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology*. 77(8): 2312-2323

O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O., Bowles, D.J. (1996) Ethylene as a signal mediating the wound response in tomato plants. *Science*. 274(5294): 1914-1917

Orino, E., Tanaka, K., Tamura, T., Sone, S., Ogura, T., Ichihara, A. (1991) ATP-dependent reverable association of proteasomes with multiple components to form 26S complexes that degrade ubiquitinated proteins in human HL-60 cells. *Federation of European Biochemical Societies (FEBS) Letters*. 284(2): 206-210

Osier, T.L., Traugott, M.S., Stamp, N.E. (1996) Allelochemicals in tomato leaves affect a specialist insect herbivore *Manduca sexta* negatively but with no ill effects on a generalist insect predator, *Podisus maculiventris*. *OIKOS*. 77: 481-488

Panchenko, M.V., Vinogradov, A.D. (1985) Interaction between the mitochondrial ATP synthetase and ATPase inhibitor protein Active/inactive slow pH-dependent transitions of the inhibitor protein. *Federation of European Biochemical Societies (FEBS) Letters*. 184(2): 226-230

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C., Schauser, L., Jaggard, D.A.W., Xiao, S., Coleman, M.J., Dow, M., Jones, J.D.G., Shirasu, K., Baulcombe, D.C. (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proceedings of the National Acadamy of Science, USA*. 99(16): 10865-10869

Pedreño, M.A., Barceló, A.R., Sabater, F., Muñoz, R. (1989) Control by pH of cell wall peroxidase activity involved in lignification. *Plant and Cell Physiology*. 30(2): 237-241

Penninckx, I.A.M.A., Thomma, B.P.H.J., Buchala, A., Métraux, J-P., Broekaert, W.F. (1998) Concomitant activation of jasmonate and ethylene response pathways Is required for induction of a plant defensin gene in *Arabidopsis*. *The Plant Cell*. 10: 2103-2113

Pfaffl, M.W. (2004) A-Z of quantitative PCR, Chapter 3: Quantification strategies in quantitative PCR. Ed: Bustin, A.R. International University Line, La Jolla, CA, USA

Pieterse, C.M.J., van Wees, S.C.N., Hoffland, E., van Pelt, J.A., van Loon, L.C. (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *The Plant Cell*. 8: 1225-1237

Pieterse, C.M.J., van Wees, S.C.M., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J., van Loon, L.C. (1998) A Novel Signaling Pathway Controlling Induced Systemic Resistance in *Arabidopsis*. *The Plant Cell*. 10: 1571-1580

Pimental, D. (2005) Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability*. 7: 229-252

Pullman, M.E., Monroy, G.C. (1963) A naturally occurring inhibitor of mitochondrial adenosine triphosphatase. *The Journal of Biological Chemistry*. 238(11): 3762-3769

Qiu, X-B., Shao, Y-M., Miao, S., Wang, L. (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cellular and Molecular Life Sciences*. 63: 2560-2570

Quisenberry S.S., Schtzko D.J. (1994) Russian wheat aphid (Homenoptera: Aphidae) population developement and plant damage on resistant and susceptible wheat. *Journal of Economic Entomology*. 87(6): 1761-1768

Rao, S.S.R., Vardhini, B.V., Sujatha, E., Anuradha, S. (2002) Brassinosteroids - a new class of phytohormones. *Current Science*. 82(10): 1239-1245

Richter-Ruoff, B., Heinemeyer, W., Wolf, D.H. (1992) The proteasome/multicatalytic-multifunctional proteinase: In vivo function in the ubiquitin-dependent N-end rule pathway of protein degradation in eukaryotes. *Federation of European Biochemical Societies (FEBS) Letters*. 302(2): 192-196

Romeis, T., Piedras, P., Zhang, S., Klessig, D.F., Hirt, H., Jones, J.D.G. (1999) Rapid Avr9- and Cf-9–dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. *The Plant Cell*. 11: 273-287

Rozen, S., Skaletsky, H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics Methods and Protocols: Methods in Molecular Biology.* Humana Press, Totowa, NJ. 365-386

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., Hunt, M.D. (1996) Systemic acquired resistance. *The Plant Cell*. 8: 1809-1819

Sadawa, K., Tokuda, L., Shinmyo, A. (2003) Characterization of the rice blast fungal elicitor-responsive gene OsSBP encoding a homologue to the mammalian selenium-binding proteins. *Plant Biotechnology*. 20(2): 177-181

Sadawa, K.I., Hasegawa, M., Tokuda, L., Kametama, J., Kodama, O., Kohchi, T., Yoshida, K., Atsuhiko, S. (2004) Enhanced resistance to blast fungus and bacterial blight in transgenic rice constitutively expressing OsSBP, a rice homologue of mammalian selenium-binding proteins. *Bioscience, Biotechnology and Biochemistry*. 68(4): 873-880

Saidi, A., Quick, J.S. (1996) Inheritance and allelic relationships among Russian wheat aphid resistance genes in winter wheat. *Crop Science*. 36(2): 256-258

Saldanha, A.J. (2004) Java Treeview-extensible visualization of microarray data. *Bioinformatics applications note*. 20(17): 3246-3248

Saltveit, M.E., Dilley, D.R. (1978) Rapidly induced wound ethylene from excised segments of etiolate *Pisum sativum* L., cv. Alaska. *Plant Physiology*. 61: 447-450

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular cloning: a laboratory manua,2nd edition. Cold Spring Harbour Laboratory Press, NY.

Sanadi, D.R., Langley, M., Searls, R.L. (1958) a-Ketoglutaric dehydrogenase VI. Reversable oxidation of dihydtothioctamide by diphosphopyridine nucleotide. *The Journal of Biological Chemistry*. 234(1): 178-182

Schaller, G.E., Harmon, A.C., Sussman, M.R. (1992) Characterization of a calcium- and lipid- dependant protein kinase associated with the plasma membrane of oat. *Biochemistry*. 31: 1721-1727

Schaller, A., (1998) Action of proteolysis resistant systemin analogues in wound signalling. *Phytochemistry*. 47(4): 605-612

Schaller, A., Oecking, C. (1999) Modulation of plasma membrane H -ATPase activity differentially activates wound and pathogen defense responses in tomato plants. *The Plant Cell*. (11): 263-272

Schafleitner, R., Wilhelm, E. (2002) Isolation of wound inducible genes from *Castanea sativa* stems and expression analysis in the bark tissue. *Plant Physiology and Biochemistry*. 40: 235-245

Schmidt, D.D., Voelcke, C., Hart, M., Schmidt, S., Baldwin, I.T. (2005) Specificity in ecological interactions. Attack from the same Lepidopteran herbivore results in species-specific transcriptional responses in two Solanaceous host plants. *Plant Physiology*. 138: 1763-1773

Scofield, S.R., Tobias, C.M., Rathien, J.P., Chang, J.H., Lavelle, D.T., Michelmore, R.W., Staskawicz, B.J. (1996) Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science*. 274: 2063-2065

Sheer, J.M., Ryan, C.A. (2002) The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. *Proceedings of the National Acadamy of Science, USA*. 99(14): 9585-9590

Silué, D., Notteghem, J.L., Tharreau, D. (1992) Evidence of a gene-for-gene relationship in the *Oryza sativa-Magnaporthe grisea* pathosystem. *Phytopathology*. 82: 577-580

Skowrya, D., Craig, K.L., Tyers, M., Elledge, S.J., Harper, J.W. (1997) F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell*. 91: 209-219

Song, W-Y., Wang, G-L., Chen, L-L., Kim, H-S., Pi, L-Y., Holsten, T., Gardner, j., Wang, B., Zhai, W-X., Zhu, L-H., Fauquet, C., Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. *Science*. 270: 1804-1806

Song, W.-Y., Pi, L.-Y., Bureau, T.E., Ronald, P.C. (1998) Identification and characterization of 14 transposon-like elements in ht enon-coding regions of members of the Xa21 family of disease resistance genes in rice. *Molecular and General Genetics*. 258: 449-456

Sorrells, M.E., La Rota, M., Bermudez-Kandianis, C.E., Greene, R.A., Kantety, R., Munkvold, J.D., Mahmoud, M.A., Ma, X., *et al*. (2003) Comparative DNA sequence analysis of wheat and rice genomes. *Genome Research*. 13:1818-1827

Souter, M., Topping, J., Pullen, M., Palme, K., Hackett, R., Grierson, D., Lindsey, K. (2002) *hydra* Mutants of *Arabisopsis* are defenctive in sterol profiles and auxin and ethylene signalling. *The Plant Cell*. 14: 1017-1031

Spearman, C. (1904) The proff and measurement of association between two things. *American Journal of Psychology*. 15: 72-101

Spoel, S.H., Koornneef, A., Claessens, S.M.C., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J. et al. (2003) NPR1 modulates cross-talk between salicylate-and jasmonate-dependant defence pathways through a novel function in the cytosol. *The Plant Cell*. 15: 760-770

Stockhause, J., Hofer, M., Renger, G., Westhoff, P., Wydrzynski, T., Willmitzer, L. (1990) Anti-sense RNA efficiently inhibits formation of the 10kD polypeptide of photosystem II in transgenic potato plants: analysis of the role of te 10kD protein. *The EMBO Journal*. 9(9): 3013-3021

Sue, M., Ishihara, A., Iwamura, H. (2000) Purification and characterization of a hydroxamic acid glucoside β-glucosidase from wheat (*Triticum aestivum* L.) seedlings. *Planta*. 210: 432-438

Suh, W.-C., Burkholder, W.F., Lu, C.Z., Zhao, X., Gottesman, M.E., Gross, C.A. (1998) Interaction of the Hsp70 molecular chaperone, DnaK, with its cochaperone DnaJ. *Proceedings of the National Acadamy of Science, USA*. 95: 15223-15228

Swanepoel, E., Lacock, L., Myburg, A.A., Botha, A.-M. (2003) A leucine rich homolog to *Aegilops tauchii* from breadwheat line PI 137739 obtained by suppression subtractive hybridization show linkage to Russian wheat aphid resistance gene *Dn1*. *In*: Proceedings of the 10th International Wheat Genetics Symposium. 1263-1265

Tamura, T., Terauchi, K., Kiyosaki, T., Asakura, T., Funaki, J., Matsumoto, I., Misaka, T., Abe, K. (2007) Differential expression of wheat aspartic proteinases, WAP1 and WAP2, in germinating and maturing seeds. *Journal of Plant Physiology*. 164: 470-477

Tang, X., Frederick, R.D., Zhou, J., Halterman, D.A., Jia, Y., Martin, G.B. (1996) Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science*. 274: 2060-2063

Tanigawa, G., Orci, L., Amherdt, M., Ravazzola, M., Helms, J.B., Rothman, J.E. (1993) Hydrolosis of bound GTP by ARF protein triggers uncoating of Golgi-derived COP-coated vesicles. *The Journal of Cell Biology*. 123(6): 1365-1371

Tawk, M., Joulie, C., Vriz, S. (2000) Zebrafish Hsp40 and Hsc70 genes are both induced during caudal fin regeneration. *Mechanisms of Development*. 99: 183-186

Tottman, D.R., Makepeace, R.J., Broad, H. (1979) An explanation of the decimal code for the growth stages of cereals, with illustrations. *Annual applied Biology*. 93: 221-234

Turrens, J.F. (2003) Mitochondrial formation of reactive oxygen species. *The Journal of Physiology*. 552(2): 335-344

Van der Westhuizen, A.J., Botha, F.C. (1993) Effect of the Russian wheat aphid on the composition and synthesis of water soluble proteins in resistant and susceptible wheat. *Journal of Agronomy and Crop Science*. 170: 322-326

Van der Westhuizen, A.J., Pretorius, Z. (1996) Protein composition of wheat apoplastic fluid and resistance to the Russian wheat aphid. *Australian Journal of Plant Physiology*. 23: 645-648

Van der Westhuizen, A.J., Qian, X.-M., Botha, A.-M. (1998) Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Reports*. 18: 132-137

Van der Westhuizen, A.J., Qian, X-M., Wilding, M., Botha, A-M. (2002) Purification and immunocytochemical localization of a wheat β-1,3-glucanase induced by Russain wheat aphid infestation. *South African Journal of Science*. 98: 197-202

Van Eck, L., (2007) Aphid induced transcriptional regulation in near-isogenic wheat. Magister Scientae. Dissertation. University of Pretoria. p145

Van Loon, L.C., Van Strien, E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology*. 55: 85-97

Van Poecke, R.P.M., Dicke, M. (2003) Signal transduction downstream of salicylic acid and jasmonic acid in herbivory-induced parasitoid attraction by *Arabidopsis* is independent of JAR and NPR1. *Plant, Cell and Environment*. 26: 1541-1548

Vera-Estrella, R., Barkla, B.J., Higgins, V.J., Blumwald, E. (1994) Plant defense response to fungal pathogens: activation of host-plasma membrane H+-ATPase by elicitor-induced enzyme dephosphorylation. *Plant Physiology*. 104: 209-215

Werck-Reichhart, D., Feyereisen, R. (2000) Cytochromes P450: a success story. *Genome Biology*. 1(6): 1-9

Will, T., van Bel, A.J.E. (2006) Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany*. 57(4): 729-737

Williams, C.H. Jr, (1995) Mechanism and structure of thioredoxin reductase from *Escherichia coli. Flavoprotein Structure and Mechanism 6*. 9: 1267-1276

Yamamoto, K., Hamada, S., Kashiyama, T. (1999) Myosins from plants. *Cellular and Molecular Life Sciences*. 56: 227-232

Yencho, G.C., Cohen, M.B., Byrne, P.F. (2000) Applications of tagging and mapping insect resistance loci in plants. *Annual Review of Entomology*. 45: 393-422

Zaayman, D., Lapitan, N.L.V., Botha, A-M. (2009) Dissimilar molecular defense responses are elicited in *Triticum aestivum* after infestation by different *Diuraphis noxia* biotypes. *Physiologia Plantarum*. 136: 209-222

Zhang, N., Portis, A.R. Jr. (1999) Mechanism of light regulation of Rubisco: a specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-f. *Proceedings of the National Acadamy of Science, USA*. 96: 9438-9443

Zhou, X., Yang, Y. (2004) Differential expression of rice Nramp genes in response to pathogen infection, defense signal molecules and metal ions. *Physiological and Molecular Plant Pathology*. 65: 235-243

Chapter 4 Summary

Profiling of wounding and *Diuraphis noxia* induced transcripts in hexaploid wheat using cDNA-AFLP analysis

Schultz, T.S.[1], Botha, A.-M.[1]

[1]Cereal Genomics, University of Pretoria, Hillcrest, Pretoria, 0002, South Africa

As one of the three major grains produced, wheat is an economically important food source. With the increase in demand for this food source, pest and pathogen outbreaks cause serious damage to yield. The Russian wheat aphid (RWA) (*Diuraphis noxia:* Kurdjomov) is one such pest, known to suddenly break out in vast populations that can decimate a crop. Some resistance to this pest in the form of the *Diuraphis noxia* (*Dn*) resistance genes has been found, however little is known about the molecular basis of resistance. Two of the *Dn* genes were covered in this study. *Dn1*, which confers an antibiotic resistance and reduces fecundity of the aphid; *Dn2* confers a tolerance type resistance, allowing the plant to survive aphid infestation.

The putative enzymes and proteins were investigated for the possible biological processes that they may be involved in. The wounding response is a product of cell wall damage and is mediated by the jasmonic acid (JA) and ethylene pathways. TDFs involved in the downstream wound response were found to involve cellular processes such as kinase type signalling, Ca^{2+} signalling, chloroplast and mitochondrial enzymes, protein anabolism and catabolism as well as a cell wall protein.

An interesting addition to this study was the analysis of the wound response TDFs. The wounding response seems to be altered by the presence of the *Dn* genes, also suggesting cross talk between the pathways.

Dn1 mediated resistance is thought to be a gene-for-gene resistance response. When RWA is fed on antibiosis conveying *Dn1* wheat, the leaves show necrotic lesions, attributed to the hypersensitive response and the RWA is seen to produce less young. TDFs involved in *Dn1* mediated antibiosis involved an NBS-LRR elicitor recognition protein, ion flux and redox potentials, chloroplast proteins involved in detoxification during oxidative stress and ubiquitin mediated protein degradation.

TDFs representing proteins involved in ion flux and de-toxification of ROS were found. As well as a putative NBS-LRR protein that may be involved in signalling after RWA elicitor recognition. JA/ethylene pathway markers such as glutathione-S-transferase and PI proteins have been found under infestation conditions. This would indicate cross talk between the SA pathways and JA/ethylene pathways downstream.

Tolerance is described as the ability of a plant to thrive under infestation conditions. The only *Dn* gene that conveys only tolerance to wheat is *Dn2*. TDFs involved in *Dn2* mediated tolerance were involved in elicitor recognition, homeostasis, chloroplast proteins and energy conservation. The *Dn2* containing wheat plants appear to minimize carbohydrate and energy expenditure and use indirect defence strategies in comparison to the susceptible and BettaDN treatments. Also there is a marked lack of transcripts involved in the HR. The tolerance model could be explained by maintenance of chloroplast function and cellular homeostasis. A timeous recognition of RWA infestation with a receptor molecule like an NBS-LRR is likely.

Appendix A

Table 5.1: Normalized intensity values (expression) of TDFs used to construct the TreeView Cluster. Also shown are the assigned TDF number, cDNA-AFLP Marker number and size, Cluster number and putative identity (where RED = up regulated; GREEN =down regulated; WHITE = value doesn't differ significantly from control value at $p \leq 0.05$).

TDF	Marker	Clust	Putative ID	B-C	B-W	B-I	DN-C	DN-W	DN-I	Dn2-C	Dn2-W	Dn2-I
TtgMct174	AmoTS M306.4	A1	Myosin heavy chain[*Oryza sativa* (japonica cultivar-group)]	44284	31878	81475	78278	139350	80338	317675	24910	88776
TgtMct213	AmoTS M264.2	A1	Mitochondrion genome Features flanking this part of subject sequence:12032 bp at 5' side: rRNA-18S ribosomal RNA 574 bp at 3' side: NADH dehydrogenase subunit 4 [*Tripsacum dactyloides* cultivar Pete]	-2109	5022	27331	6983.5	32264	-8307	138808	-19651	37971
TacMag161	AmoTS M201.4	A1	Topoisomerase II alpha [*Pinus koraiensis*]	-3277	15256	36718	4690.5	10428	-7396	121678	-5660	47949
TtgMct181	AmoTS M249.0	A1	Helix-loop-helix DNA binding domain containing protein [*Oryza sativa* (japonica cultivar-group)]	86563	54447	22179	124731	105771	-10139	327128	54278	165997
TtgMct181b	AmoTS M249.0	A1	ATP-dependent Clp protease ATP-binding subunit clpA CD4B,chloroplast precursor [*Oryza sativa* (japonica cultivar-group)]	86563	54447	22179	124731	105771	-10139	327128	54278	165997
TgtMac111	AmoTS M551.6	A1	Chloroplast lumen common protein family [*Arabidopsis thaliana*]	9187.5	1557	-6249	81470	-4253	-18199	94279	6509	27999
TtgMct193	AmoTS M577.9	A1	Annexin like protein [*Medicago sativa*]	18998	1582.5	441	50239	47811	11952	61775	3297.5	33070
TcaMag74	AmoTS M304.6	A1	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	-16067	-18127	-22970	43058	9015.5	-17872	763736	-20623	10921
TgtMct215	AmoTS M253.6	A1	No Homology	10132	7066	80939	113586	123036	1765	147374	33406	82498

TtgMct168	AmoTS M488.3	A1	ARIADNE-like protein [*Oryza sativa* (japonica cultivar-group)]	-6026	-14895	-250.5	915	11978	-2600	411818	2807	10786
TgtMac130	AmoTS M285.4	A1	Putative RING-H2 finger protein RHB1a [*Oryza sativa*]	754200	66344	1E+06	867107	1E+06	787725	1E+06	158938	226209
TgtMac129	AmoTS M286.9	A1	Acyl-carrier protein, mitochondrial precursor NADH-ubiquitinone oxidoreductase [*Oryza sativa* (japonica cultivar-group)]	281363	82594	860589	966667	884219	585012	831013	130741	33565
TgtMac129b	AmoTS M286.9 b	A1	No Homology	281363	82594	860589	966667	884219	585012	831013	130741	33565
TcaMag57	AmoTS M220.7	A1	Putative lipoic acid synthetase isoform 1 [*Oryza sativa* (japonica cultivar-group)]	20153	-6764	22799	153214	44401	7592.5	5533	10889	-1639
TtgMct184	AmoTS M199.8	A1	WD40 domain containing TOR family protein [*Oryza sativa* (japonica cultivar-group)]	19004	11128	28069	49740	23101	23211	265591	21900	18191
TtgMct184b	AmoTS M199.8	A1	adh1-adh2 region [*Oryza sativa*]	19004	11128	28069	49740	23101	23211	265591	21900	18191
TcaMac97	AmoTS M319.3	A2	Putative serine/threonine phosphatase [*Oryza sativa* (japonica cultivar-group)]	63034	-10653	76023	4823	17771	28937	30482	4937.5	88300
TtgMct190	AmoTS M154.6	A2	Na+/H+ antiporter (NHX1) [*Triticum aestivum*]	162725	12439	124252	36943	71429	36571	56453	42253	240106
TacMag162	AmoTS M204.0	A2	Inositol polyphosphate related phosphatase; PapD-like; WD40-like [*Arabidopsis thaliana*]	36191	-19811	80977	5400.5	18863	-2319	29362	350	42285
TacMag142	AmoTS M340.8	A2	Unnamed product [*Vitis vinifera*]	-4186	-18192	35014	-6285	-6922	-12874	-5216	-5866	-616
TcaMac87	AmoTS M420.4	A2	Chaperone protein dnaJ-like [*Oryza sativa* (japonica cultivar-group)]	9832	-526.5	16498	3769	219346	-15994	16248	1559.5	30581
TgtMac112	AmoTS M504.4	A2	Repressor protein Dr1 [*Arabidopsis thaliana*]	-10719	-3744	-3584	-18628	13090	-20954	59771	-4494	-4179
TtgMct164	AmoTS M620.6	A2	SEU3A protein [*Oryza sativa* (japonica cultivar-group)]	3652	9441.5	20366	9310	10031	8931.5	117810	11002	16882
TgtMac120	AmoTS M447.2	A2	α-1,3-mannosyl-glycoprotein β-1,2-Nacetylglucosamine transferase [*Oryza sativa* (japonica cultivar-group)]	-442.5	7657	95141	1955	12909	3751	63028	8951	5288
TacMag154	AmoTS M297.5	A2	No Homology	7257.5	-25449	16728	-23161	-23284	-27772	50450	-14228	12709
TcaMac90	AmoTS M379.6	A2	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	70920	5893.5	19162	10456	69108	83979	65038	26895	75493
TgtMct209	AmoTS	A2	Protein H2A [*Triticum aestivum*]	42612	16502	39669	17951	72801	-12344	458865	31085	81885

	M324.8											
TacMag151	AmoTS M323.2	A2	Chloroplast genome [*Lolium perenne*]	-8343	-21576	-9755.5	-10288	2537	-16553	39222	-7701	-3153
TcaMac93	AmoTS M352.0	A2	FATB acyl-ACP thioesterase [*Populus tomentosa*]	52599	-621	4870.5	1406.5	17288	51661	262934	6182	23813
TcaMac86	AmoTS M435.4	A2	Os02g0832800 [*Oryza sativa* (japonica cultivar-group)]	50130	26959	8625.5	7245.5	103254	48296	327940	40429	134693
TcaMac95	AmoTS M331.2	A2	Acyl-coenzyme A oxidase 2, peroxisomal precursor [*Oryza sativa* (japonica cultivar-group)]	69083	-8587	2759.5	1767	29313	5530.5	56864	-12977	104775
TcaMac96	AmoTS M331.2 b	A2	No Homology	69083	-8587	2759.5	1767	29313	5530.5	56864	-12977	104775
TgtMac116	AmoTS M318.9	A3	WAP2 aspartic proteinase [*Triticum aestivum*]	181200	111829	274211	177557	368003	312681	92404	94515	119033
TgtMac114	AmoTS M430.0	A3	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	251505	72153	4031	264836	218207	449136	48483	200314	231403
TcaMag52	AmoTS M244.1	A3	Complete chloroplast genome [*Lolium perenne*]	109563	67001	37194	227933	69275	85871	452	8628.5	18858
TgtMct204	AmoTS M489.2	A3	Arf1_5/ArfA-family small GTPase [*Physcomitrella patens* subsp. patens]	21946	15607	34924	74666	9256.5	40512	5351.5	5338	34503
TgtMct212	AmoTS M286.6	A3	No Homology	41676	32771	32805	303990	224462	102891	5041	4049	297887
TtgMct166	AmoTS M558.1	A3	RanBPM-related [*Arabidopsis thaliana*]	24191	13656	22325	41810	34471	30671	4624.5	11069	31768
TgtMct206	AmoTS M434.3	A3	RNA-binding region RNP-1 [*Oryza sativa* (japonica cultivar-group)]	149228	141709	146928	285003	240686	145366	25287	71824	393088
TtgMct169	AmoTS M468.4	A3	Os05g0546800 [*Oryza sativa* (japonica cultivar-group)]	72427	-3406	52740	310596	154124	12979	42.5	7414	290185
TtgMct195	AmoTS M468.4 b	A3	TRR-like domain containing protein [*Oryza sativa* (japonica cultivar-group)]	72427	-3406	52740	310596	154124	12979	42.5	7414	290185
TgtMct223	AmoTS M706.4	A3	Selenium binding protein (SBP) [*Oryza sativa* (japonica cultivar-group)]	42393	49833	94138	258121	167109	43811	19982	25097	180913

TgtMct201	AmoTS M626.9	A3	NRPC1 (nuclear RNA polymerase C 1) [*Arabidopsis thaliana*]	14550	30672	77206	55918	37714	23770	15318	19222	89666
TgtMac128	AmoTS M305.9	A4	Isochorismatase hydrolase-like protein [*Oryza sativa* (japonica cultivar-group)]	286800	321658	1E+06	1E+06	1E+06	869132	123740	446014	1E+06
TtgMct171	AmoTS M396.3	A3	23S ribosomal RNA gene; Chloroplast [*Festuca arundinacea*]	9511.5	13074	-7956	72557	54625	8557	1744.5	5117.5	73966
TacMag135	AmoTS M438.2	A4	eIF4-gamma/eIF5/eIF2-epsilon domain-containing protein [*Arabidopsis thaliana*]	-693.5	-10740	-2545.5	49182	26651	21041	2904	7691	8764.5
TgtMac119	AmoTS M524.6	A4	Integral membrane protein NRAMP [*Hordeum vulgare* subsp. vulgare]	100920	25811	-16244	751261	670207	1E+06	73270	111175	315275
TtgMct172	AmoTS M403.5	A4	10kD PSII protein [*Hordeum vulgare*]	-2663	-1262	-5671	72002	97259	99595	30731	13212	28342
TgtMac122	AmoTS M378.0	A4	Similar to topoisomerase II alpha [*Pinus koraiensis*]	473136	317379	687465	1E+06	1E+06	1E+06	1E+06	521957	1E+06
TtgMct176	AmoTS M287.9	A4	No Homology	53737	-4983	85527	328569	130365	115709	99204	37950	357301
TacMag141	AmoTS M351.8	A4	2'-hydroxyisoflavone reductase [*Oryza sativa* (japonica cultivar-group)]	-9898	-15279	-5659	34808	47656	18555	-8304	4512	43718
TacMag150	AmoTS M351.8 b	A4	FLbaf76103 [*Hordium vulgare*]	-9898	-15279	-5659	34808	47656	18555	-8304	4512	43718
TcaMac202	AmoTS M615.1	A4	ARM-repeat containing protein [*Oryza sativa* (japonica cultivar-group)]	9051	25143	24662	124347	184424	191207	7201	68034	104813
TgtMct203	AmoTS - M615.1 b	A4	No Homology	9051	25143	24662	124347	184424	191207	7201	68034	104813
TcaMac88	AmoTS - M416.6	A5	Putative protein kinase Xa21 [*Oryza sativa* (japonica cultivar-group)]	6313.5	52363	8096.5	19336	-11857	149216	78747	14261	23951
TtgMct173	AmoTS	A5	Putative zinc finger DHHC domain containing	-4908	23844	11250	48888	7931	26893	455266	21713	50417

	- M324.2		protein [*Oryza sativa* (japonica cultivar-group)]								
TcaMac85	AmoTS - M458.8	A5	Putative Zinc transporter zupT [*Oryza sativa* (japonica cultivar-group)]	4605	1764.5	8925.5	22508	4390	36750	112457	12195	58651
TgtMac125	AmoTS M262.6	A5	Ca2+/H+-exchanging protein (CAX) [*Hordeum vulgare* subsp. vulgare]	104119	45917	-68705	622935	-15279	546208	75733	150392	1564
TcaMag58	AmoTS M205.2	A5	No Homology	68811	85464	20745	219257	17981	98790	151861	83390	42204
TcaMag73	AmoTS M207.3	A5	Os04g0566500 PAZ_argonaute_like domain [*Oryza sativa* (japonica cultivar-group)]	40686	30523	18995	80948	24925	46236	112270	60380	7926.5
TcaMac98	AmoTS M315.4	A5	Calcium-dependent protein kinase 2 [*Panax ginseng*]	54925	164461	49426	56214	161894	196443	197882	90753	-10756
TcaMag56	AmoTS M222.9	A5	No Homology	36943	60955	34193	85155	39099	153609	98665	87891	15763
TtgMct191	AmoTS M158.3	A5	*Pdr3* gene PDR-like ABC transporter [*Oryza sativa* (japonica cultivar-group)]	258577	457887	169342	276555	303733	577922	594028	554229	265516
TtgMct186	AmoTS M166.9	A5	Malate dehydrogenase [NADP], chloroplast precursor [*Zea mays*]	-10446	92117	35701	83184	53533	198271	124917	109415	72345
TcaMac104	AmoTS M254.4	A5	Putative vacuolar ATP synthase subunit H [*Oryza sativa* (japonica cultivar-group)]	37057	73805	193289	30582	162555	293077	433913	189302	205214
TtgMct197	AmoTS M399.3	B4	TRR-like domain containing protein [*Oryza sativa* (japonica cultivar-group)]	3676	15302	19579	12500	97324	59853	500665	29973	112839
TcaMac103	AmoTS M262.1	A5	Putative vacuolar ATP synthase subunit H [*Oryza sativa* (japonica cultivar-group)]	21133	19503	199292	20653	105117	160125	207716	26505	43821
TgtMct235	AmoTS M297.6	A5	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [*Triticum aestivum*]	178504	484329	408100	137779	248990	549123	596713	394870	219297
TcaMac84	AmoTS M480.1	A5	Chloroplast DNA [*Triticum aestivum*]	984.5	-125.5	7715.5	24601	69141	49554	263230	9428	-11651
TgtMct226	AmoTS	A5	LRR ribonuclease inhibitor subtype containing	24027	194	173440	-913.5	24421	146910	43003	19048	-5901

	M236.2		protein [*Oryza sativa* (japonica cultivar-group)]								
TgtMct226b	AmoTS M236.2	A5	No homology	24027	194	173440	-913.5	24421	146910	43003	19048	-5901
TgtMct229	AmoTS M308.8	A5	Splicing factor 1 KH-domain containing protein [*Oryza sativa* (japonica cultivar-group)]	51229	95507	120593	91633	94661	237917	326428	53677	9943.5
TgtMct207	AmoTS M387.2	A5	Nonphototrophic hypocotyl 1b (Phototropin) [*Oryza sativa* (japonica cultivar-group)]	36780	39278	85612	42947	84671	116518	229492	47896	10814
TtgMct183	AmoTS M219.7	A5	Tropinone reductase [*Oryza sativa* (japonica cultivar-group)]	-3159	1242.5	34049	-3634	2562	1020.5	225363	32861	-4800
TtgMct183b	AmoTS M219.7	A5	Short chain alcohol dehydrogenase [*Oryza sativa* (japonica cultivar-group)]	-3159	1242.5	34049	-3634	2562	1020.5	225363	32861	-4800
TtgMct179	AmoTS M291.2	A5	No Homology	275737	321701	336105	160371	200029	450944	289699	347290	183955
TtgMct196	AmoTS M427.4	A5	Putative Zn-dependent protease with chaperone function [*Oryza sativa* (japonica cultivar-group)]	-7668	81153	29530	31159	88594	57476	59286	51291	33206
TtgMct178	AmoTS M275.9	B1	Os04g0665000 [*Oryza sativa* (japonica cultivar-group)]	317653	397502	667414	252566	284462	292838	136476	244782	344371
TtgMct198	AmoTS M275.9 b	B1	Chloroplast carbonic anhydrase [*Oryza sativa*]	317653	397502	667414	252566	284462	292838	136476	244782	344371
TtgMct200	AmoTS M275.9 c	B1	No Homology	317653	397502	667414	252566	284462	292838	136476	244782	344371
TtgMct165	AmoTS M595.1	B1	HEAT repeat family protein [*Oryza sativa* (japonica cultivar-group)]	75396	102493	196642	58212	69584	63649	3450	34635	132356
TtgMct192	AmoTS M595.1 b	B1	PDR-like ABC transporter [*Oryza sativa* (japonica cultivar-group)]	75396	102493	196642	58212	69584	63649	3450	34635	132356
TgtMct208	AmoTS M335.7	B1	No Homology	964326	867715	977981	629737	875921	916060	100912	817618	986083
TgtMac121	AmoTS M383.5	B1	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	58868	12361	254233	-16768	47498	52563	-23713	6675	107264
TgtMct210	AmoTS M304.5	B1	TRR-domain containing protein [*Oryza sativa* (japonica cultivar-group)]	62965	85362	38517	35051	18702	201227	-618	63425	91524

TacMag137	AmoTS M398.2	B1	α-adaptin C [*Oryza sativa* (japonica cultivar-group)]	208899	109645	152544	91645	101265	106710	68285	115116	61257
TacMag143	AmoTS M337.7	B1	Elongation factor 1 gamma [*Oryza sativa* (japonica cultivar-group)]	198651	193034	93097	29473	40881	44542	-10698	61719	53078
TacMag157	AmoTS M270.8	B1	Peroxisomal ascorbate peroxidase (APX) [*Triticum aestivum*]	135175	243500	139326	-45828	-30168	-28162	-45702	89467	-25641
TacMag139	AmoTS M368.5	B1	E3 ubiquitin protein ligase UPL1 [*Oryza sativa* (japonica cultivar-group)]	168277	216547	169316	16139	29135	29328	-10097	65070	53669
TacMag149	AmoTS M368.5 b	B1	F1F0- ATPase inhibitor protein [*Hordeum vulgare* subsp. vulgare]	168277	216547	169316	16139	29135	29328	-10097	65070	53669
TacMag134	AmoTS M475.4	B1	RabGAP/TBC domain-containing protein [*Oryza sativa* (japonica cultivar-group)]	87893	214910	313119	34509	41697	72963	1822	51730	45911
TcaMac91	AmoTS M362.6	B1	Elongation factor 1 alpha-subunit (TEF1) [*Triticum aestivum*]	1E+06	836061	968376	128161	840430	785060	691460	724189	608777
TacMag158	AmoTS M267.1	B1	Peroxisomal ascorbate peroxidase (APX) [*Triticum aestivum*]	231092	272130	462230	-1503	230435	174235	68282	127909	128791
TacMag138	AmoTS M387.7	B1	E3-ubiquitin protein ligase URE-B1 [*Oryza sativa* (japonica cultivar-group)]	109460	127450	140453	21868	41029	37972	587	38577	45135
TtgMct170	AmoTS M413.6	B1	FLbaf79p20 [*Hordeum vulgare* subsp. vulgare]	50057	37932	55919	11003	26124	32987	30127	5852.5	14551
TgtMct232	AmoTS M312.1	B1	Arf1_5/ArfA-family small GTPase [*Physcomitrella patens* subsp. patens]	13678	4461	49103	4378.5	6525	-14330	2998.5	-11702	32149
TacMag133	AmoTS M502.2	B1	Histidine kinase- like ATPase [*Oryza sativa* (japonica cultivar-group)]	16858	13082	37662	-440	2607	1517	6804	2596.5	21330
TacMag156	AmoTS M273.5	B1	NADH dehydrogenase (Ubiquinone oxidoreductase) [*Oryza sativa* (japonica cultivar-group)]	17255	-5176	65827	-18860	-29830	-20486	4109	-16768	-9882.5
TacMag140	AmoTS M363.4	B1	Putative COX VIIa-like protein [*Pinguicula* sp. Jobson 240]	15424	30241	89304	6908	2248.5	5053.5	13522	2296	15221
TacMag144	AmoTS M326.6	B1	Ribosomal biogenesis GTPase [*Oryza sativa* (japonica cultivar-group)]	5981	44124	30027	618.5	1397.5	-6784	11172	-1822.5	2303.5
TgtMct234	AmoTS M382.7	B2	Protein phosphatase 2C [*Oryza sativa* (japonica cultivar-group)]	227006	204598	337039	188350	126119	366523	83659	433578	333335

TtgMct189	AmoTS M156.9	B2	Putative hydroxymethylglutaryl coenzyme A synthase [*Oryza sativa* (japonica cultivar-group)]	245408	207690	43564	88676	57111	107998	16882	350213	130873
TcaMac100	AmoTS M306.6	B2	Plant specific isoprenylated GTP-binding protein [*Hordeum vulgare* subsp. vulgare]	61165	59557	15359	12402	-39604	83723	-42971	116565	23973
TgtMac123	AmoTS M343.9	B2	2-isopropylmalate synthase B [*Oryza sativa* (japonica cultivar-group)]	585652	734768	527548	38153	22976	269642	133743	695137	730395
TgtMac117	AmoTS M299.1	B2	UNC50 [*Arabidopsis thaliana*]	134879	62682	-54684	8930	19286	-44885	-7786	92529	121733
TcaMag76	AmoTS M166.1	B3	Cytochrome P450 like_TBP [*Nicotiana tabacum*]	173417	468386	298922	219584	293569	244370	17850	438783	198436
TcaMag51	AmoTS M258.0	B3	10kD PSII protein [*Hordeum vulgare*]	786027	2E+06	2E+06	2E+06	1E+06	2E+06	1E+06	2E+06	1E+06
TcaMag50	AmoTS M340.2	B3	Polynucleotide adenylyltransferase [*Oryza sativa* (japonica cultivar-group)]	571214	2E+06	2E+06	2E+06	1E+06	2E+06	923888	2E+06	1E+06
TgtMac132	AmoTS M499.4	B3	Putative VHS1 protein [*Oryza sativa* (japonica cultivar-group)]	55392	142335	694324	116490	90630	183270	-6893	100772	19403
TcaMag75	AmoTS M295.2	B3	18S ribosomal RNA gene [*Triticum aestivum*]	71361	352320	262383	254364	201250	121714	62700	112186	68093
TtgMct194	AmoTS M523.6	B3	No Homology	23649	21287	50442	23193	18980	20702	6593.5	18201	7020
TgtMac115	AmoTS M327.8	B3	2-isopropylmalate synthase B [*Oryza sativa*]	409047	294210	2E+06	665777	56988	316554	30212	300726	276753
TtgMct167	AmoTS M536.9	B3	Defense-related F-box protein [*Oryza sativa* (indica cultivar-group)]	149076	210615	229697	164372	63634	185597	145120	92219	70587
TgtMac113	AmoTS M478.1	B3	Os05g0546800 [*Oryza sativa* (japonica cultivar-group)]	30500	41792	54247	257931	-24452	154061	25718	32705	21011
TcaMag55	AmoTS M227.7	B3	J080318E23 cDNA clone	34438	76988	46246	120753	-2256	113047	9044.5	83069	32475
TcaMag54	AmoTS M245.3	B3	Plasma membrane P-type proton pump ATPase (Ha1) [*Hordeum vulgare* subsp. vulgare]	37763	77001	46812	110597	18357	81815	-17272	82916	25298
TgtMct224	AmoTS M512.6	B3	Putative ribosomal protein S3 [*Vigna unguiculata*]	44528	83341	115304	121275	37669	28347	-2624	51668	97177
TacMag136	AmoTS M432.6	B3	eIF4-gamma/eIF5/eIF2-epsilon domain-containing protein [*Arabidopsis thaliana*]	-9020	-5001	41404	6827.5	6460.5	-112.5	-1610	3839	3667.5
TcaMac92	AmoTS	B4	Putative beta-glucosidase [*Oryza sativa*	236057	271201	148391	71934	118221	212091	582205	198833	57178

	M335.6		(japonica cultivar-group)]									
TtgMct180	AmoTS M283.2	B4	No Homology	82406	66609	46632	18785	-4850	65089	221044	42800	2087.5
TtgMct177	AmoTS M284.3	B4	Methylcrotonoyl-Coenzyme A carboxylase 2 (beta) [*Xenopus tropicalis*]	238278	263630	158056	55378	39840	152386	287998	110971	15879
TcaMac89	AmoTS M399.3 b	A5	Similar to topoisomerase II alpha [*Pinus koraiensis*]	79959	29563	26401	30184	10521	14544	53340	7046	6144
TacMag160	AmoTS M246.6	B4	Genotype CMS-S mitochondrion; Features NADH dehydrogenase subunit 1 NADH dehydrogenase subunit 5 [*Zea mays* subsp. mays]	8316	-18745	17984	-13371	-10083	-13988	110714	-7092	-15605
TacMag147	AmoTS M381.5	B4	NB-ARC domain containing protein [*Oryza sativa* (japonica cultivar-group)]	35232	4756	24349	11843	18718	2430	36362	10752	-644
TgtMac126	AmoTS M289.7	B4	Ubiquinol-cytochrome C reductase complex [*Hordeum vulgare* subsp. vulgare]	181677	177846	493901	68946	130785	-46673	1E+06	2147	-16090
TacMag163	AmoTS M196.4	B4	DEAD BOX RNA helicase [*Oryza sativa* (japonica cultivar-group)]	141595	95455	80598	35714	20662	11376	182283	37029	17680
TtgMct187	AmoTS M165.0	B4	Putative Kinesin [*Oryza sativa* (japonica cultivar-group)]	39983	27012	39165	20845	550.5	-4704	96412	14067	-1490.5
TcaMac101	AmoTS M281.8	B4	Protein tyrosine kinase (PTK) family catalytic domain [*Oryza sativa* (japonica cultivar-group)]	98845	28013	83706	10095	-40207	-50511	208134	9281.5	-47548
TgtMct211	AmoTS M291.0	B4	Shaggy-like kinase [*Triticum aestivum*]	94848	163114	147450	89022	27287	98635	216612	257192	10224
TcaMac99	AmoTS M309.2	B4	Splicing factor, CC1-like family protein [*Oryza sativa* (japonica cultivar-group)]	-6808	7541	-7318	1600	-33452	-31756	117062	3873.5	-35455
TacMag145	AmoTS M299.7	B4	mRNA for fructose-1,6-bisphosphatase (cytosolic isoform) [*Oryza sativa*]	4636	53336	106209	-22574	-24270	-15516	30076	31345	11686
TacMag152	AmoTS M316.1	B4	Putative immunophilin [*Hordeum vulgare* subsp. vulgare]	20501	36128	37517	14669	16143	21864	155587	36182	21711
TtgMct155	AmoTS	B4	Putative disease resistance protein [*Oryza*	-1178	-17618	51730	-18876	-17051	-24757	56500	-7563	-16266

	M292.8		*sativa* (japonica cultivar-group)]									
TcaMag53	AmoTS M246.3	B4	Palmitoyl-ACP thioesterase [*Elaeis guineensis*]	3113.5	432.5	11036	-10163	-5210	-2416	48156	10905	1926.5
TtgMct199	AmoTS M390.9	B4	TOR family protein, WD40 domain containing protein [*Oryza sativa* (japonica cultivar-group)]	164054	181027	280965	174793	160164	203373	308125	119022	136574
TacMag148	AmoTS M373.0	B4	Retrotransposon protein [*Oryza sativa* (japonica cultivar-group)]	12726	29445	41947	11076	6463.5	8196.5	40639	7027.5	2854.5
TtgMct175	AmoTS M285.5	B4	No Homology	96559	82014	5956.5	5702	-467	17578	998008	-183	28416
TacMag159	AmoTS M258.7	B4	FKBP12 interacting protein [*Oryza sativa* (japonica cultivar-group)]	111882	100772	60535	-44444	-57201	-11570	145776	32729	76687
TtgMct185	AmoTS M177.5	B4	No Homology	620302	719393	499019	539913	523104	569431	1E+06	630373	605641
TacMag146	AmoTS M289.1	B4	Potential cadmium/Zn transporting ATPase HMA1 [*Oryza sativa* (japonica cultivar-group)]	89581	138279	-30005	-1949	34074	21199	239825	71208	16621
TacMag153	AmoTS M307.3	B4	Putative dioxygenase PcbC [*Oryza sativa* (japonica cultivar-group)]	55521	-6973	-18349	-16375	-14494	-16454	53335	32739	32871
TtgMct182	AmoTS M227.4	B5	No Homology	145598	112992	30541	74437	51350	44241	100516	147696	584.5
TgtMac124	AmoTS M314.2	B5	Single-stranded nucleic acid binding protein(whGRP1) [*Triticum aestivum*]	86037	32780	29451	72376	443224	291	130000	95503	3987
TgtMct217	AmoTS M247.6	B5	No Homology	67003	24986	-33250	-7283	40914	39127	4779	-29162	-8793
TgtMct225	AmoTS M269.7	B5	Plasma membrane H+-ATPase [*Triticum aestivum*]	150940	301728	6570	36924	150045	266218	2823	98968	15556
TgtMct230	AmoTS M269.7 b	B5	Peptidase (ubiquitin carboxy-terminal hydrolase 2 family) [*Oryza sativa* (japonica cultivar-group)]	150940	301728	6570	36924	150045	266218	2823	98968	15556
TtgMct188	AmoTS M159.9	B5	TASK5 mRNA [*Triticum aestivum*]	924824	901274	720150	605286	838902	902533	826324	866463	748300
TgtMac127	AmoTS M300.6	B5	UNC-50 family protein [*Arabidopsis thaliana*]	1E+06	206047	-78091	-32212	-55612	80509	58412	172194	40991
TgtMac127b	AmoTS M300.6	B5	VATPase H, regulatory vacuolar ATP synthase subunit H [*Oryza sativa* (japonica cultivar-group)]	1E+06	206047	-78091	-32212	-55612	80509	58412	172194	40991

Appendix B

GenBank: GR881173.1

AmoTS058 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS058, mRNA sequence

IDENTIFIERS

dbEST Id: **66624648**
EST name: AmoTS058
GenBank Acc: GR881173
GenBank gi: 254546197

CLONE INFO
Clone Id: AmoTS058
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

GAGCAAAACGGAGGGCATGTGACACTTGTTGAACTCTGTGTTGCCAAAAAATATGACTAA
AAGGATTTCAATCGAATGCAAAACAGACAGTCCCCGAAACACATATCATCTTGGATTTAT
TGGTGGATTTACAGTGTCTTTTTAGTGGAT

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

COMMENTS

cDNA-AFLP fragment

LIBRARY
Lib Name: Wheat Betta NILs RWA induced cDNA library
Organism: Triticum aestivum
Cultivar: Betta NILs
Tissue type: Leaf
Develop. stage: 3-4 leaf stage

SUBMITTER
Name: Botha-Oberholster AM
Lab: Department of Genetics and Forestry and Agricultural Biotechnology Institute
Institution: Faculty of Agriculture and Natural Science, University of Pretoria
Address: 74 Lunnon Str. Hillcrest, Pretoria, Gauteng, ZA0002, South Africa
Tel: 27 12 420 3984
Fax: 27 12 420 3947
E-mail: anna.oberholster@up.ac.za

CITATIONS

Title: Elucidation of defence pathways activated in Betta near isogenic lines upon infestation by Diuraphis noxia

Authors: Schultz,T., Botha,A.M.

Year: 2009

Status: Unpublished

GenBank: GR881174.1

AmoTS154 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS154, mRNA sequence

IDENTIFIERS

dbEST Id: **66624649**
EST name: AmoTS154
GenBank Acc: GR881174
GenBank gi: 254546198

CLONE INFO
Clone Id: AmoTS154
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

ACAAGAACCATCCAATGAAACATGTGCTGATCAAAAGGACGTTATTCATCATATATAAAT
ATCAAGTAGAATGTCCGTTTGTCTAGGAGACAAAAGCTAGCACTGGGAGTGGGGAGTTTC
TTGTAACTTTGCAGAATCCAGGAAAACGAACTGAACTCAGAAGAGCTTCCAAAGAGGTGC
ACCCAAAAGAACTGTTGTATCCTTG

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881175.1

AmoTS175 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS175, mRNA sequence

IDENTIFIERS

dbEST Id:	**66624650**
EST name:	AmoTS175
GenBank Acc:	GR881175
GenBank gi:	254546199

CLONE INFO

Clone Id:	AmoTS175
DNA type:	cDNA

PRIMERS

PCR forward:	M13F
PCR backward:	M13R
Sequencing:	M13 Forward
PolyA Tail:	no

SEQUENCE

GATCATCTTGCTAAAGTACTTTATTGTAGTATTCCTCCATCCATTACAGAGAATGTTGCA
GTTGGTTGGCCTCCTTGACCTTCAATTTACCATGCGAAGTTCTGGACGAGTATTTTGCCT
TGATCCTCTGTGTTGTGAATACTAAATAGAATGTCAACAGCAAATCTGACCCTTGCGTTT
GCTACGTCGTTCATTGTGAACTGTCGCTGCGTAGGATTTC

Entry Created:	Jul 21 2009
Last Updated:	Jul 21 2009

GenBank: GR881176.1

AmoTS176 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS176, mRNA sequence

IDENTIFIERS

dbEST Id: **66624651**
EST name: AmoTS176
GenBank Acc: GR881176
GenBank gi: 254546200

CLONE INFO
Clone Id: AmoTS176
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

CCCCAGACTGAAAGCAAGAAACGCGATCGGGTCCTTGACAGCCGCCGCCCAGATTGAACC
TCGCCGTCGCCGGGGACGAACCACTTGGCACGACGAAATCGCCATGGAAGCATCACCTGC
TCGCCATAGCCTCCGAGCCGCCACTGGAACTGCCTCCCATCAGGCACGCAAGCCGGGAGG
AATATAGTCAGCCGCCATTCGGCTAGAAGAAACAGGGCACCTACGAAGACCGGCAGCCAT
CGGTACGCAGTCTACAGAGCTCCAGATACTATAACGGCGGCTGATTTATTCTCGCTTATT
TTTAGACAGGTTCCCGTTTATGTTTGGATTCCACCATCATCACTGGCTATTTCGGGG

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881177.1

AmoTS179 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS179, mRNA sequence

IDENTIFIERS

dbEST Id: **66624652**
EST name: AmoTS179
GenBank Acc: GR881177
GenBank gi: 254546201

CLONE INFO
Clone Id: AmoTS179
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

ATCCTCGAAGGCATAAAGACCACACAGACACTTACTTTTTTATGGTTTTTCTTCGCAGCT
GATGGCAAGCTCCTTTCTTGCTAATTGTGATTTCTTTTGTACATGGCCTCTCACCAGAGT
AAGTAAATAGACCTGTGATGTTTCTTTCTGATTTTCTTGGAGTTTTCAGGGGTATTTTGA
GCTTCTAGATTGATTGTTTCATGAAGCATTATAGATCAGACT

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881178.1

AmoTS180 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS180, mRNA sequence

IDENTIFIERS

dbEST Id: **66624653**
EST name: AmoTS180
GenBank Acc: GR881178
GenBank gi: 254546202

CLONE INFO
Clone Id: AmoTS180
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

CTACAACAGCAGCAAGAAAGCATCATGGTTTAGGCTTCGCAACTACCAAAATGTGATCTC
ATGCGAAAAAAAACGACAATAAATTGTTCGGCTTAGCATCATGTTTCAGCTTACACAACTA
CCAAAACATCAGGCTAAGCCATTGTCTTATAGTTTCAGGAAAAGGGACTACAAGGAAGTT
CACCCATCAATGTGATTCAGATTCA

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881179.1

AmoTS182 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS182, mRNA sequence

IDENTIFIERS

dbEST Id: **66624654**
EST name: AmoTS182
GenBank Acc: GR881179
GenBank gi: 254546203

CLONE INFO
Clone Id: AmoTS182
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

TAATGCATTATAACAATCGCTCTGTGCCTCTGCCTATTCTTACACTCTGACTTCGGAAGT
ATAAACCGATGCGTAGAAAGACAAAGAACGTGCAATTTATTAGAGCTGGAACTGGCAGAA
CCTATCATCAGGATATGGAGTTACAGTTGCCAAGAG

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881180.1

AmoTS185 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS185, mRNA sequence

IDENTIFIERS

dbEST Id: **66624655**
EST name: AmoTS185
GenBank Acc: GR881180
GenBank gi: 254546204

CLONE INFO
Clone Id: AmoTS185
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

TATACAGACTGGAAGTGTATGTTGTTCTTTTTCTTTTTTGAACTAAACTAGAAGTAGGAA
TTCATTTTATAGGGTTAGTGTTGTAAATTGTGGATGCTTCCTTTCTGTTATTGGCTTATT
GCTTGCTTGATTTGGGACATCTACAACCGAAATTATTATGATGCCATATTTTTTATATTA
CTCCATGCTCACCAAGTTATTGAGTTGAAGTTGTCGTTGTTTGCTGTCAAGATTTGCCTA
GCCTGAATACATGGTTATGTTTTGTGTACTGTTGTCCTGGTGTTGAGCTGTTCAATTTCA
CTGTTGTACTGCTATCAAGATCCTGCTGTACTG

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881181.1

AmoTS194 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS194, mRNA sequence

IDENTIFIERS

dbEST Id: **66624656**
EST name: AmoTS194
GenBank Acc: GR881181
GenBank gi: 254546205

CLONE INFO
Clone Id: AmoTS194
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

GTTGTTATTTCCTAATAATTTTGATGGCCTCTAAGCATCGTTGTTCATGTCTATTATTAT
TTATGAAAGCTACATGTCTTGGAGGGAAGTTGCGACTTGAGGGTGTGAGCTCCCATTCTC
TCAATTTCTTGGCATGGCCATGCTTGCAGTACTGATAAGACATCGTTGTTCATGTCTATT
ATTATTTATGAAAGCTACATGTCTTGGAGGGAAGTTGCGACTTGAGGGAGTGAGCTCCCA
TTCTCTCAATTTCTTGGCATGGCCATACTTGCAGTACTGATAAGACATGCTTCAAAAATG
GAGGAGGGAGGACGCGAGCTGCTGCCACAGTATGTAATCAGGCCTCCACTATCGTCCTCT
GAATTTTGGATTACATGCATCTACAAGAGCGTGAGAGCTTGAGAGGGTAGCACAGTGCTA
CGTAGAAGTTATTTTTCTCCATGAAGTTGATCA

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881182.1

AmoTS200 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS200, mRNA sequence

IDENTIFIERS

dbEST Id:	**66624657**
EST name:	AmoTS200
GenBank Acc:	GR881182
GenBank gi:	254546206

CLONE INFO

Clone Id:	AmoTS200
DNA type:	cDNA

PRIMERS

PCR forward:	M13F
PCR backward:	M13R
Sequencing:	M13 Forward
PolyA Tail:	no

SEQUENCE

ACCAGCGACAGCTGCGGTGACATGATCAGCAGTGGGTTGCCTGCCTCACTTTATATTCTG
AGGTTCTTCTGCGAAGCTGGCATCACGGAATTGCCTGACACCTCGGGGCTTGAAAGGATG
TGCTATTAGGGTGTGAACCCCTTATGTTTTCCCTACCGTTCTCAAGAGAAGAACTGC

Entry Created:	Jul 21 2009
Last Updated:	Jul 21 2009

GenBank: GR881183.1

AmoTS203 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS203, mRNA sequence

IDENTIFIERS

dbEST Id: **66624658**
EST name: AmoTS203
GenBank Acc: GR881183
GenBank gi: 254546207

CLONE INFO
Clone Id: AmoTS203
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

GATGATAGCATGCAGGTCTTCATGTGTTTGCTGATGGTGTAACTTGCACAAGTAACTCCA
CGCTTAGTCTGACAAAATGATGACAGGTTCCATGTCTCTATGGAGCTTCCAGATCCGAGA
AGCACAGAAAAAAGAACGGGAGTAAACCGGAGATGTCATGAGAATATAAATTTGTCAAAA
GTGCAGGGATCAATGCACGGTATTTATCAAACCGAAATGCGCTAATTGGTTTGGGAGTAC
AACATTCACTTGATTTTATTTGGAAAAAAGGGAGTGTCTAACTGATTGATGGTGGATGTA
AGTGTGAAGGAGGCAATGTAACTACAATCACACATGATGATGCCTTCAGAACAATAAAAC
AACCCAGCAAGGCCTGAAAACTTGCATAAGGGCATGGTCTCCATAAATACTTGATTCAAG
CACCAAATCTCATATCAAATCATAATATAGTAATAGTGGTTCAGCATAAAGCAACAATTT
AGGGGCAAGCATGTGCATTTTTTCGGTTTGATTTGCACTGTGCTAA

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881184.1

AmoTS208 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS208, mRNA sequence

IDENTIFIERS

dbEST Id: **66624659**
EST name: AmoTS208
GenBank Acc: GR881184
GenBank gi: 254546208

CLONE INFO
Clone Id: AmoTS208
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

CTAAGCTAGCCATGTTCCCAGTAAGCTCAGAAATTTCCTGGCATACACTTGACACCAGTT
ATCTCTCATATTTCCCTGATTTCCATAGCTGTGTTGTATCCCTTACATGTTGTTTTAGTC
GGTCTTTGCGATTGGTTCAGTTAGAGGCTTATTTAGTTGTGATTTAGTCATTGGCTATGG
TCATTGGTATGATAGAAGCATTACACAGTGCATCAATGATAAAGCTACTGTTCTCTCAAA
CTGGTAATATTGTTCTA

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881185.1

AmoTS212 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS212, mRNA sequence

IDENTIFIERS

dbEST Id: **66624660**
EST name: AmoTS212
GenBank Acc: GR881185
GenBank gi: 254546209

CLONE INFO
Clone Id: AmoTS212
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

GGCATCGCGAAATCCGGAGCAGGGACAGTTCACAATGCACGACTTATGGAAGATGAGGGT
CATATTTGCTGTTGACATTCTAGTTAGCATTCTGATCACAGAAGATCGAGGCAAAGTACG
CTCCATCAACTTCGCATGGTCAAGTTAAGGTCACGGAGCCCGGCCAACTGCAATATTCTC
TGAAATGGATGGCGGACTACCACTGCTGAGGATTTTACCAGTCTGATCAAGATCCATCCC
TACCCTGATT

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881186.1

AmoTS215 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS215, mRNA sequence

IDENTIFIERS

dbEST Id: **66624661**
EST name: AmoTS215
GenBank Acc: GR881186
GenBank gi: 254546210

CLONE INFO
Clone Id: AmoTS215
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

GATGGCCAGGCCCTCAATCGACACCCAGCAAGAATGTAACATCTCCTATTCGATTGAACA
ATGGTGATCATGCTAAGGGAAGTGTATGGTTATAGAGAGACATGTGGTTTGCCTGGATGC
AGTCAGACCGGCCTAGTCTGTGATAGGATTAGTTGGTTGGCCTTGA

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881187.1

AmoTS217 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS217, mRNA sequence

IDENTIFIERS

dbEST Id: **66624662**
EST name: AmoTS217
GenBank Acc: GR881187
GenBank gi: 254546211

CLONE INFO
Clone Id: AmoTS217
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

GAATGAACCAGCGACAGCTGCGGTGACATGATCAGCAGTGGGTTGCCTGCCTCACTTTAT
ATTCTGAGGTTCTTCTGCGAAGCTGGCATCACGGAATTGCCTGACACCTCGGGGCTTGAA
AGGATGTGCTATAGGGTGTGAACCCCTTATGTTTTCCCTAC

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

GenBank: GR881188.1

AmoTS226b Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS226b, mRNA sequence

IDENTIFIERS

dbEST Id:	**66624663**
EST name:	AmoTS226b
GenBank Acc:	GR881188
GenBank gi:	254546212

CLONE INFO

Clone Id:	AmoTS226b
DNA type:	cDNA

PRIMERS

PCR forward:	M13F
PCR backward:	M13R
Sequencing:	M13 Forward
PolyA Tail:	no

SEQUENCE

GATCTTTGACGATTTGAGGACCTGGGCTACTGCAGGAGCATTTGGAGGATGATCGGTACC
TGAGTAGTAGAGTAGCATTAGGAGTGGGGTGGAGGCTTGGTTGAGGTTGATTTGTGATCT
CCAACTAGCGCATTTGTAAAATTCTTGTACAT

Entry Created:	Jul 21 2009
Last Updated:	Jul 21 2009

GenBank: GR881189.1

AmoTS096 Wheat Betta NILs RWA induced cDNA library *Triticum aestivum* cDNA clone AmoTS096, mRNA sequence

IDENTIFIERS

dbEST Id: **66624664**
EST name: AmoTS096
GenBank Acc: GR881189
GenBank gi: 254546213

CLONE INFO
Clone Id: AmoTS096
DNA type: cDNA

PRIMERS
PCR forward: M13F
PCR backward: M13R
Sequencing: M13 Forward
PolyA Tail: no

SEQUENCE

TAACCCTTAGAAATAAGTCCCAAGAGCCTAGTATTTGGAAACCTATAAGTCTGGGTAAAC
ATAAGATCATTCATACACTTGATCCGCTAATAACTAAGCAACACATAGTAGTTATCTTCA
TGCTGGTAAAGAGAGCCAACCAAACAACCAATATGCCTATGCTTCAGGGAAGAAAAAATT
GCCTGAAGGAATAACCTTGGCCGATAATTTATAAATACACC

Entry Created: Jul 21 2009
Last Updated: Jul 21 2009

Appendix C

Table 5.2: Table of TDFs from Betta cluster tree, including biochemical pathway and other functional data from KEGG (Kanehisa and Goto 2000; http://www.genome.jp/kegg/).

Clone	Marker	Cluster	Identity	KEGG Putative Pathways	KEGG Putative Function
TcaMac95	AmoTS-M331.2b	1	Acyl-coenzyme A oxidase 2, peroxisomal precursor [EC:1.3.3.6]	Fatty acid metabolism alpha-Linolenic acid metabolism Biosynthesis of unsaturated fatty acids	A flavoprotein (FAD). Acts on CoA derivatives of fatty acids with chain lengths from 8 to 18.
TtgMct181	AmoTS-M249.0	1	Helix-loop-helix DNA binding domain containing protein		Possible transcription factor
TcaMac97	AmoTS-M319.3	1	Putative serine/threonine phosphatase [EC:3.1.3.16]	MAPK signaling pathway Calcium signaling pathway	Removes serine- or threonine- bound phosphate group from a range of phosphoproteins. Also acts of phenolic phosphates and phosphamides.
TcaMac98	AmoTS-M315.4	2	Calcium-dependent protein kinase 2 [EC:2.7.1.-]	Inositol phosphate metabolism Benzoate degradation via CoA ligation Phosphatidyinositol signaling system	
TtgMct196	AmoTS-M427.4	2	Putative Zn-dependent protease with chaperone function [EC:3.4.24.-]		Genetic information processing; folding, sorting and degradation.
TgtMac120	AmoTS-M447.2	2	alpha-1,3-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase [EC:2.4.1.101]	N-Glycan biosynthesis Glycan structures - biosynthesis 1	
TcaMac104	AmoTS-M254.4	2	Putative vacuolar ATP synthase subunit H [EC:3.6.3.14]	Oxidative phosphorylation	Hydrogen ion transporting ATP synthase with rotational mechanism
TgtMct235	AmoTS-	2	Ribulose-1,6-	Glyoxylate and dicarboxylate	

	M297.6		bisphosphate carboxylase/oxygenase small subunit [EC:4.1.1.39]	metabolism Carbon fixation	
TacMag145	AmoTS-M299.7	2	mRNA for fructose-1,6-bisphosphatase (cytosolic isoform) [EC:3.1.3.11]	Glycolysis / Gluconeogenesis Pentose phosphate pathway Fructose and mannose metabolism Carbon fixation	
TgtMct226	AmoTS-M236.2	2	LRR ribonuclease inhibitor subtype containing protein [EC:3.1.-.-]		Binds proteins; protein degradation tagging activity; putative translation initiation inhibitor
TgtMac132	AmoTS-M499.4	3	Putative VHS1 protein		Cytoplasmic serine/threonine protein kinase; suggested role in G1/S phase progression
TgtMct234	AmoTS-M382.7	3	Protein phosphatase 2C [EC:3.1.3.16]	MAPK signaling pathway Calcium signaling pathway	Removes serine- or threonine- bound phosphate group from a range of phosphoproteins. Also acts of phenolic phosphates and phosphamides.
TgtMac123	AmoTS-M343.9	3	2-isopropylmalate synthase B [EC:2.3.3.13]	Valine, leucine and isoleucine Metabolism	
TacMag133	AmoTS-M502.2	3	HATPase_c [EC:2.7.13.3]		HATPase_c; Histidine kinase-like ATPases; This family includes several ATP-binding proteins for example: histidine kinase, DNA gyrase B, topoisomerases, heat shock protein HSP90, phytochrome-like ATPases and DNA mismatch repair proteins
TcaMac91	AmoTS-M362.6	3	Elongation factor 1 alpha-subunit (TEF1) [EC:3.6.5.3]		Protein synthesizing GTPase
TacMag137	AmoTS-M398.2	3	a-adaptin C		A heterotetramer Which regulates clatherin-bud formation.
TacMag134	AmoTS-	3	RabGAP/TBC domain-		A shared domain between a spindle assembly

	M475.4		containing protein		checkpoint protein and Ypt/Rab-specific GTPase activators.
TacMag140	AmoTS-M363.4	3	Putative COX VIIa-like protein [EC:1.9.3.1]		Chaperone that specifically facilitates the assembly of cytochrome c oxidase, integral to the mitochondrial inner membrane; interacts with a subcomplex of subunits VII, VIIa, and VIII (Cox7p, Cox9p, and Cox8p) but not with the holoenzyme
TacMag144	AmoTS-M326.6	3	Ribosomal biogenesis GTPase		GTP binding protein
TacMag159	AmoTS-M258.7	3	FKBP12 interacting protein		Contains taxilin domain: Taxilin contains an extraordinarily long coiled-coil domain in its C-terminal half and is ubiquitously expressed. It is a novel binding partner of several syntaxin family members and is possibly involved in Ca2+-dependent exocytosis in neuroendocrine cells. Gamma-taxilin, described as leucine zipper protein Factor Inhibiting ATF4-mediated Transcription (FIAT), localizes to the nucleus in osteoblasts and dimerises with ATF4 to form inactive dimers, thus inhibiting ATF4-mediated transcription
TacMag143	AmoTS-M337.7	3	Elongation factor 1 gamma [EC:3.6.1.48]		
TacMag139	AmoTS-M368.5	3	E3 ubiquitin protein ligase UPL1 [EC:6.3.2.19]		
TacMag157	AmoTS-M270.8	3	Peroxisomal ascorbate peroxidase (APX) [EC:1.11.1.11]	Ascorbate and aldarate metabolism Glutathione metabolism	Carbohydrate Metabolism; Ascorbate and aldarate metabolism. Metabolism of Other Amino Acids; Glutathione metabolism.
TacMag138	AmoTS-M387.7	3	E3-ubiquitin protein ligase URE-B1		Upstream regulatory element-binding protein 1

			[EC:6.3.2.19]		
TtgMct165	AmoTS-M595.1	3	HEAT repeat family protein		Receptor tail, linking cargo to vesicle
TtgMct191	AmoTS-M158.3	3	PDR-like ABC transporter [EC:3.6.3.-]		Involved in environmental information processing as well as membrane transport.
TtgMct198	AmoTS-M275.9b	3	Chloroplast carbonic anhydrase [EC:4.2.1.1]	Energy Metabolism; Nitrogen metabolism:; reduction and fixation	
TcaMac101	AmoTS-M281.8	4	Protein tyrosine kinase (PTK) family catalytic domain [EC:2.7.10.2]		Transferring phosphorus-containing groups; Protein-tyrosine kinases
TgtMac126	AmoTS-M289.7	4	Ubiquinol-cytochrome C reductase complex [EC:1.10.2.2]	Oxidative phosphorylation	Acting on diphenols and related substances as donors; With a cytochrome as acceptor
TtgMct197	AmoTS-M399.3	4	Carbonic anhydrase [EC:4.2.1.1]	Energy Metabolism; Nitrogen metabolism:reduction and fixation	
TtgMct167	AmoTS-M536.9	4	Defense-related F-box protein		Receptor that recruits phosphorylated substrates to the SCF ubiquitin ligase complex.
TtgMct199	AmoTS-M390.9	4	TOR family protein, WD40 domain containing protein		WD40 repeat protein similar to S. cerevisiae LST8 (YNL006W) component of both TOR1 and TOR2 protein/ phosphatidylinositol kinase complexes
TgtMct225	AmoTS-M269.7	5	Plasma membrane H+-ATPase [EC:3.6.3.6]	Oxidative phosphorylation	Plasma membrane H+-ATPase, pumps protons out of the cell; major regulator of cytoplasmic pH and plasma membrane potential; part of the P2 subgroup of cation-transporting ATPases (EC:3.6.3.6)
TgtMct230	AmoTS-M269.7b	5	Peptidase (ubiquitin carboxy-terminal hydrolase 2 family)		Hydrolases; Acting on peptide bonds (peptidases); Omega peptidases. Thiol-dependent hydrolysis of ester, thioester, amide, peptide and isopeptide bonds formed

			[EC:3.1.2.15]	by the C-terminal Gly of ubiquitin (a 76-residue protein attached to proteins as an intracellular targeting signal)
TacMag136	AmoTS-M432.6	6	eIF4-gamma/eIF5/eIF2-epsilon domain-containing protein	Translation initiation factor
TgtMct223	AmoTS-M706.4	6	Selenium binding protein (SBP)	Involved in the production of PR proteins

Table 5.3: Table of TDFs from BettaDN cluster tree including putative biochemical pathway and other functional data from KEGG (Kanehisa and Goto 2000; http://www.genome.jp/kegg/).

Clone	Marker	Cluster	Identity	KEGG Putative Pathways	KEGG Putative Function
TcaMag57	AmoTS-M220.7	1	Putative lipoic acid synthetase isoform 1 [EC:2.8.1.8]	Lipoic acid metabolism	In the production of: Oxo-acid dehydrogenase complexes or Glycine cleavage system (H Protein)
TgtMac129	AmoTS-M286.9	1	Acyl-carrier protein, mitochondrial precursor NADH-ubiquitinone oxidoreductase [EC:1.6.5.3; 1.6.99.3]	Oxidative phosphorylation	Acting on NADH or NADPH; With a quinone or similar compound as acceptor. mitochondrial electron transport complex I; NADH coenzyme Q1 reductase
TgtMac116	AmoTS-M318.9	1	WAP2 aspartic proteinase [EC:3.4.23.24]		Hydrolases; Acting on peptide bonds (peptidases); Aspartic endopeptidases
TgtMac121	AmoTS-M383.5	1	Cytochrome P450 like_TBP	C21-steroid hormone metabolism Arachidonic acid biosynthesis	
TgtMct232	AmoTS-M312.1	1	Arf1_5/ArfA-family small GTPase		Cellular Processes and Signaling; GTP-binding proteins. Contains; Atypical Rho GTPases have roles in mitochondrial homeostasis and Apoptosis. ADP-ribosylation factors family signature.
TgtMac122	AmoTS-M378.0	1	Similar to topoisomerase II alpha [EC:5.99.1.3]		The enzyme can introduce negative superhelical turns into double-stranded circular DNA. One unit has nicking-closing activity, and another catalyses super-twisting and hydrolysis of ATP
TgtMac119	AmoTS-M524.6	1	Integral membrane protein NRAMP	Divalent cation-transporter	
TacMag136	AmoTS-M432.6	1	eIF4-gamma/eIF5/eIF2-epsilon domain-containing		Translation initiation factor

			protein		
TcaMac202	AmoTS-M615.1	1	ARM-repeat containing protein [EC 3.6.1.5]		
TacMag141	AmoTS-M351.8	1	2'-hydroxyisoflavone reductase [EC:1.3.1.45]	Isoflavanoid biosynthesis	
TgtMac128	AmoTS-M305.9	1	Isochorismatase hydrolase-like protein [Oryza sativa (japonica cultivar-group)]	Methionine metabolism Selenoamino acid metabolism	
TtgMct195	AmoTS-M468.4b	1	TRR-like domain containing protein		
TgtMct206	AmoTS-M434.3	1	RNA-binding region RNP-1		
TgtMct223	AmoTS-M706.4	1	Selenium binding protein (SBP)		Involved in the production of PR proteins
TtgMct168	AmoTS-M488.3	2	ARIADNE-like protein [EC:6.3.2.19]		Ubiquitin ligase; Forming carbon-nitrogen bonds; Acid-D-amino-acid ligases (peptide synthases)
TtgMct181	AmoTS-M249.0	2	Helix-loop-helix DNA binding domain containing protein		Possible transcription factor
TtgMct193	AmoTS-M577.9	2	Annexin like protein		Calcium ion binding/ calcium dependent phospholipids binding
TcaMag51	AmoTS-M258.0	3	10kD PSII protein	Photosynthesis – antenna protein	
TgtMac132	AmoTS-M499.4	3	Putative VHS1 protein		Cytoplasmic serine/threonine protein kinase; suggested role in G1/S phase progression
TtgMct167	AmoTS-M536.9	3	Defense-related F-box protein		Receptor that recruits phosphorylated substrates to the SCF ubiquitin ligase complex.

TcaMac100	AmoTS-M306.6	3	Plant specific isoprenylated GTP-binding protein		
TgtMct234	AmoTS-M382.7	3	Protein phosphatase 2C [EC:3.1.3.16]	MAPK signaling pathway Calcium signaling pathway	Removes serine- or threonine- bound phosphate group from a range of phosphoproteins. Also acts of phenolic phosphates and phosphamides.
TgtMct210	AmoTS-M304.5	3	TRR-domain containing protein		
TcaMac91	AmoTS-M362.6	4	Elongation factor 1 alpha-subunit (TEF1) [EC:3.6.5.3]		Translation elongation factor
TacMag157	AmoTS-M270.8	4	Peroxisomal ascorbate peroxidase (APX) [EC:1.11.1.11]	Ascorbate and aldarate metabolism Glutathione metabolism	Carbohydrate Metabolism; Ascorbate and aldarate metabolism. Metabolism of Other Amino Acids; Glutathione metabolism.
TcaMac97	AmoTS-M319.3	4	Putative serine/threonine phosphatase [EC:3.1.3.16]	MAPK signaling pathway Calcium signaling pathway	Removes serine- or threonine- bound phosphate group from a range of phosphoproteins. Also acts of phenolic phosphates and phosphamides.
TgtMac125	AmoTS-M262.6	5	Ca2+/H+-exchanging protein (CAX)		H^+/Ca^{2+} exchanger involved in control of cytosolic Ca^{2+} concentration.
TtgMct177	AmoTS-M284.3	5	Methylcrotonoyl-Coenzyme A carboxylase 2 (beta) [EC:6.4.1.4]	Valine, leucine and isoleucine degradation	
TgtMct225	AmoTS-M269.7	5	Plasma membrane H+-ATPase [EC:3.6.3.6]	Oxidative phosphorylation	Plasma membrane H+-ATPase, pumps protons out of the cell; major regulator of cytoplasmic pH and plasma membrane potential; part of the P2 subgroup of cation-transporting ATPases (EC:3.6.3.6)
TgtMct230	AmoTS-M269.7b	5	Peptidase (ubiquitin carboxy-terminal hydrolase 2 family) [EC:3.1.2.15]		Hydrolases; Acting on peptide bonds (peptidases); Omega peptidases. Thiol-dependent hydrolysis of ester, thioester, amide, peptide and isopeptide bonds formed

					by the C-terminal Gly of ubiquitin (a 76-residue protein attached to proteins as an intracellular targeting signal)
TcaMac98	AmoTS-M315.4	6	Calcium-dependent protein kinase 2 [EC:2.7.1.-]	Inositol phosphate metabolism Benzoate degradation via CoA ligation Phosphatidyinositol signaling system	
TtgMct191	AmoTS-M158.3	6	Pdr3 gene PDR-like ABC transporter [EC:3.6.3.-]		Involved in environmental information processing as well as membrane transport.
TcaMac104	AmoTS-M254.4	6	Putative vacuolar ATP synthase subunit H [EC:3.6.3.14]	Oxidative phosphorylation	Hydrogen ion transporting ATP synthase with rotational mechanism
TgtMct207	AmoTS-M387.2	6	Nonphototrophic hypocotyl 1b (Phototropin)		Transcription factor
TgtMct229	AmoTS-M308.8	6	Splicing factor 1 KH-domain containing protein		
TgtMct235	AmoTS-M297.6	6	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [EC:4.1.1.39]	Glyoxylate and dicarboxylate metabolism Carbon fixation	
TgtMct226	AmoTS-M236.2	6	LRR ribonuclease inhibitor subtype containing protein [EC:3.1.-.-]		Binds proteins; protein degradation tagging activity; putative translation initiation inhibitor

Table 5.4: Table of TDFs from Betta *Dn2* cluster tree, including biochemical pathway and other functional data from KEGG (Kanehisa and Goto 2000; http://www.genome.jp/kegg/).

Clone	Marker	Cluster	Identity	KEGG Putative Pathways	KEGG Putative Function
TcaMag74	AmoTS-M304.6	1	Cytochrome P450 like_TBP	C21-steroid hormone metabolism Arachidonic acid biosynthesis	
TcaMac93	AmoTS-M352.0	1	FATB acyl-ACP thioesterase [EC:3.1.2.14 3.1.2.-]	Fatty acid biosynthesis	oleoyl-[acyl-carrier-protein] hydrolase; acyl-[acyl-carrier-protein] hydrolase
TcaMac95	AmoTS-M331.2	1	Acyl-coenzyme A oxidase 2, peroxisomal precursor [EC:1.3.3.6]	Fatty acid metabolism alpha-Linolenic acid metabolism Biosynthesis of unsaturated fatty acids	A flavoprotein (FAD). Acts on CoA derivatives of fatty acids with chain lengths from 8 to 18.
TcaMac97	AmoTS-M319.3	1	Putative serine/threonine phosphatase [EC:3.1.3.16]	MAPK signaling pathway Calcium signaling pathway	Removes serine- or threonine- bound phosphate group from a range of phosphoproteins. Also acts of phenolic phosphates and phosphamides.
TacMag162	AmoTS-M204.0	1	PapD-like; [EC:3.6.3.14]	Oxidative phosphorylation Photosynthesis	F0 sector of membrane-bound ATP synthase, subunit A; A multisubunit non-phosphorylated ATPase that is involved in the transport of ions. Large enzymes of mitochondria, chloroplasts and bacteria with a membrane sector (Fo, Vo, Ao) and a cytoplasmic-compartment sector (F1, V1, A1). The F-type enzymes of the inner mitochondrial and thylakoid membranes act as ATP synthases. All of the enzymes included here operate in a rotational mode, where the extramembrane sector (containing 3 alpha- and 3 beta-subunits) is connected via the delta-subunit to the membrane sector by several smaller subunits. Within this complex, the gamma- and epsilon-subunits, as well as the 9-12 c

					subunits rotate by consecutive 120_degree_ angles and perform parts of ATP synthesis. This movement is driven by the H+ electrochemical potential gradient. The V-type (in vacuoles and clathrin-coated vesicles) and A-type (archebacterial) enzymes have a similar tructure but, under physiological conditions, they pump H+ rather than synthesize ATP.
TtgMct190	AmoTS-M154.6	1	Na+/H+ antiporter (NHX1)		NHX1 (NA+/H+ EXCHANGER); sodium ion transmembrane transporter/sodium:hydrogen antiporter
TgtMac111	AmoTS-M551.6	2	Chloroplast lumen common protein family		
TtgMct181	AmoTS-M249.0	2	Helix-loop-helix DNA binding domain containing protein		Possible transcription factor
TgtMac112	AmoTS-M504.4	2	Repressor protein Dr1		TATA-binding protein-associated phosphoprotein
TtgMct164	AmoTS-M620.6	2	SEU3A protein		
TacMag161	AmoTS-M201.4	2	Topoisomerase II alpha [EC:5.99.1.3]		The enzyme can introduce negative superhelical turns into double-stranded circular DNA. One unit has nicking-closing activity, and another catalyses super-twisting and hydrolysis of ATP
TgtMct209	AmoTS-M324.8	2	Protein H2A		Histone protein
TcaMag53	AmoTS-M246.3	3	Palmitoyl-ACP thioesterase [EC:3.1.2.22]	Fatty acid elongation in mitochondria	
TacMag147	AmoTS-M381.5	3	NB-ARC domain containing protein		
TacMag160	AmoTS-M246.6	3	NADH dehydrogenase subunit1 [EC:1.6.5.3]	Oxidative phosphorylation Ubiquinone biosynthesis	Oxidoreductases; Acting on NADH or NADPH; With a quinone or similar compound as acceptor

TgtMac126	AmoTS-M289.7	3	Ubiquinol-cytochrome C reductase complex [EC:1.10.2.2]	Oxidative phosphorylation	Acting on diphenols and related substances as donors; With a cytochrome as acceptor
TacMag163	AmoTS-M196.4	3	DEAD BOX RNA helicase [EC:3.6.1.-]	Starch and sucrose metabolism Folate biosyntesis	
TtgMct187	AmoTS-M165.0	3	Putative Kinesin		Microtubule motor protein
TcaMag73	AmoTS-M207.3	3	Os04g0566500 PAZ_argonaute_like domain		This domain is named PAZ after the proteins Piwi Argonaut and Zwille. This domain is found in two families of proteins that are involved in post-transcriptional gene silencing. These are the Piwi family and the Dicer family, that includes the Carpel factory protein. The function of the domains is unknown but has been suggested to mediate complex formation between proteins of the Piwi and Dicer families by hetero-dimerisation. PAZ can bind the characteristic two-base 3' overhangs of siRNAs, indicating that although PAZ may not be a primary nucleic acid binding site in Dicer or RISC, it may contribute to the specific and productive incorporation of siRNAs and miRNAs into the RNAi pathway
TcaMac92	AmoTS-M335.6	3	Putative beta-glucosidase [EC:3.2.1.21]	Cyanoamino acid metabolism Starch and sucrose metabolism Phenypropanol biosynthesis	
TtgMct177	AmoTS-M284.3	3	Methylcrotonoyl-Coenzyme A carboxylase 2 (beta) [EC:6.4.1.4]	Valine, leucine and isoleucine degradation	
TcaMac99	AmoTS-M309.2	3	Splicing factor, CC1-like family protein		
TgtMct211	AmoTS-M291.0	3	Shaggy-like kinase [EC:2.7.1.-]	ErbB signaling pathway Cell cycle	Glycogen synthase kinase 3 beta

				Wnt signaling pathway Hedgehog signaling pathway Circadian rhythm	
TacMag153	AmoTS-M307.3	3	Putative dioxygenase PcbC		Putative iron/ascorbate oxidoreductase family protein. Oxidoreductases; Acting on paired donors, with O2 as oxidant and incorporation or reduction of oxygen. And/or chlorophyll a/b binding light-harvesting protein PcbC
TacMag146	AmoTS-M289.1	3	Potential cadmium /Zn transporting ATPase HMA1 [EC:3.6.3.3] [EC:3.6.3.5]		A P-type ATPase that undergoes covalent phosphorylation during the transport cycle. This enzyme also exports Cd2+ and Pb2+.
TacMag159	AmoTS-M258.7	3	FKBP12 interacting protein		Contains taxilin domain: Taxilin contains an extraordinarily long coiled-coil domain in its C-terminal half and is ubiquitously expressed. It is a novel binding partner of several syntaxin family members and is possibly involved in Ca2+-dependent exocytosis in neuroendocrine cells [1]. Gamma-taxilin, described as leucine zipper protein Factor Inhibiting ATF4-mediated Transcription (FIAT), localizes to the nucleus in osteoblasts and dimerises with ATF4 to form inactive dimers, thus inhibiting ATF4-mediated transcription
TcaMac85	AmoTS-M458.8	4	Putative Zinc transporter zupT		Metal cation transporter; Involved in environmental information processing and membrane transport.
TtgMct173	AmoTS-M324.2	4	Putative zinc finger DHHC domain containing protein [EC:2.3.1.-]	Sphingolipid metabolism	Thought to be involved in protein-protein interactions and palmitoyltransferase activity.
TcaMac88	AmoTS-M416.6	4	Putative protein kinase Xa21		Resistance gene linked to surface recognition and resistance to pathogen
TtgMct186	AmoTS-	4	Malate dehydrogenase	Citrate cycle	

	M166.9		[NADP], chloroplast precursor [EC:1.1.1.82]	Pyruvate metabolism Glyoxylate and dicarboxylate metabolism Carbon fixation Reductive carboxylate cycle (CO_2 fixation)	
TtgMct197	AmoTS-M399.3	4	Carbonic anhydrase [EC:4.2.1.1]	Energy Metabolism; Nitrogen metabolism: reduction and fixation	
TcaMac104	AmoTS-M254.4	4	Putative vacuolar ATP synthase subunit H [EC:3.6.3.14]	Oxidative phosphorylation	Hydrogen ion transporting ATP synthase with rotational mechanism
TgtMac130	AmoTS-M285.4	4	Putative RING-H2 finger protein RHB1a		Protein binding or zinc ion binding
TtgMct199	AmoTS-M390.9	4	WD40 domain containing TOR family protein		WD40 repeat protein similar to S. cerevisiae LST8 (YNL006W) component of both TOR1 and TOR2 protein/phosphatidylinositol kinase complexes
TgtMct207	AmoTS-M387.2	4	Nonphototrophic hypocotyl 1b (Phototropin)		Transcription factor. phototropin photoreceptor, protein-serine/threonine kinase
TgtMct229	AmoTS-M308.8	4	Splicing factor 1 KH-domain containing protein		
TgtMct226	AmoTS-M236.2	4	LRR ribonuclease inhibitor subtype containing protein [EC:3.1.-.-]		Binds proteins; protein degradation tagging activity; putative translation initiation inhibitor
TacMag152	AmoTS-M316.1	4	Putative immunophilin [EC:5.2.1.8]	Calcium signaling pathway	FKBP-type peptidyl-prolyl cis-trans isomerase; involved in genetic information processing; folding, sorting and degradation; protein folding and associated processing
TtgMct183	AmoTS-M219.7	4	Tropinone reductase [EC:1.1.1.206]	Alkaloid biosynthesis II	Biosynthesis of Secondary Metabolites
TtgMct196	AmoTS-M427.4	4	Putative Zn-dependent protease with chaperone		Genetic information processing; folding, sorting and degradation.

			function [EC:3.4.24.-]		
TcaMag54	AmoTS-M245.3	5	Plasma membrane P-type proton pump ATPase (Ha1) [EC:3.6.1.-]		Involved in Na^{+}/K^{+}, H^{+}/K^{+}, Ca^{++} and Mg^{++} transport
TgtMac123	AmoTS-M343.9	5	2-isopropylmalate synthase B [EC:2.3.3.13]	Valine, leucine and isoleucine metabolism	
TgtMct210	AmoTS-M304.5	5	TRR-domain containing protein		
TgtMct234	AmoTS-M382.7	5	Protein phosphatase 2C [EC:3.1.3.16]	MAPK signaling pathway Calcium signaling pathway	Removes serine- or threonine- bound phosphate group from a range of phosphoproteins. Also acts of phenolic phosphates and phosphamides.
TacMag139	AmoTS-M368.5	6	E3 ubiquitin protein ligase UPL1 [EC:6.3.2.19]		
TacMag157	AmoTS-M270.8	6	Peroxisomal ascorbate peroxidase (APX) [EC:1.11.1.11]	Ascorbate and aldarate metabolism Glutathione metabolism	Carbohydrate Metabolism; Ascorbate and aldarate metabolism. Metabolism of Other Amino Acids; Glutathione metabolism.
TtgMct165	AmoTS-M595.1	6	HEAT repeat family protein		Receptor tail, linking cargo to vesicle
TtgMct191	AmoTS-M158.3	6	PDR-like ABC transporter [EC:3.6.3.-]		Involved in environmental information processing as well as membrane transport.
TacMag133	AmoTS-M502.2	6	HATPase_c [EC:2.7.13.3]		HATPase_c; Histidine kinase-like ATPases; This family includes several ATP-binding proteins for example: histidine kinase, DNA gyrase B, topoisomerases, heat shock protein HSP90, phytochrome-like ATPases and DNA mismatch repair proteins
TgtMct232	AmoTS-M312.1	6	Arf1_5/ArfA-family small GTPase		Cellular Processes and Signaling; GTP-binding proteins. Contains; Atypical Rho GTPases have roles in

					mitochondrial homeostasis and Apoptosis. ADP-ribosylation factors family signature.
TgtMac117	AmoTS-M299.1	7	UNC50		Golgi membrane protein- partner of the ARF
TtgMct189	AmoTS-M156.9	7	Putative hydroxymethylglutaryl coenzyme A synthase [EC:2.3.3.10]	Synthesis and degradation of ketone bodies Valine, leucine and isoleucine degradation Butanoate metabolism	
TtgMct188	AmoTS-M159.9	7	TASK5 mRNA		Potassium channel family
TacMag141	AmoTS-M351.8	8	2'-hydroxyisoflavone reductase [EC:1.3.1.45]	Isoflavanoid biosynthesis	
TtgMct166	AmoTS-M558.1	8	RanBPM-related		GTP activating protein
TcaMag51	AmoTS-M258.0	8	10kD PSII protein	Photosynthesis – antenna protein	
TcaMac202	AmoTS-M615.1	8	ARM-repeat containing protein		
TgtMct201	AmoTS-M626.9	8	NRPC1 (nuclear RNA polymerase C 1)		DNA directed RNA polymerase
TgtMct223	AmoTS-M706.4	8	Selenium binding protein (SBP)		Involved in the production of PR proteins

Table 5.5: Table of TDFs from wounded cluster tree, including biochemical pathway and other functional data from KEGG (Kanehisa and Goto 2000; http://www.genome.jp/kegg/).

Clone	Marker	Cluster	Identity	KEGG Putative Pathways	KEGG Putative Function
TgtMac124	AmoTS-M314.2	1	Single-stranded nucleic acid binding protein (whGRP-1)		Wheat glycine rich RNA binding protein.
TcaMac87	AmoTS-M420.4	1	Chaperone protein dnaJ-like	Stabilizes polypeptides to intermediate conformation	
TtgMct174	AmoTS-M306.4	1	Myosin heavy chain		
TacMag156	AmoTS-M273.5	1	NADH dehydrogenase (Ubiquinone oxidoreductase)	Oxidative phosphorylation Ubiquinone biosynthesis	Oxidoreductases; Acting on NADH or NADPH; With a quinone or similar compound as acceptor
TgtMct225	AmoTS-M269.7	2	Plasma membrane H+-ATPase [EC:3.6.3.6]	Oxidative phosphorylation	Plasma membrane H+-ATPase, pumps protons out of the cell; major regulator of cytoplasmic pH and plasma membrane potential; part of the P2 subgroup of cation-transporting ATPases (EC:3.6.3.6)
TgtMct230	AmoTS-M269.7b	2	Peptidase (ubiquitin carboxy-terminal hydrolase 2 family) [EC:3.1.2.15]		Hydrolases; Acting on peptide bonds (peptidases); Omega peptidases. Thiol-dependent hydrolysis of ester, thioester, amide, peptide and isopeptide bonds formed by the C-terminal Gly of ubiquitin (a 76-residue protein attached to proteins as an intracellular targeting signal)
TgtMac123	AmoTS-M343.9	2	2-isopropylmalate synthase B [EC:2.3.3.13]	Valine, leucine and isoleucine metabolism	
TgtMac127	AmoTS-M300.6	2	UNC-50 family protein		Golgi membrane protein- partner of the ARF

TtgMct189	AmoTS-M156.9	2	Putative hydroxymethylglutaryl coenzyme A synthase [EC:2.3.3.10]	Synthesis and degradation of ketone bodies Valine, leucine and isoleucine degradation Butanoate metabolism	
TtgMct196	AmoTS-M427.4	4	Putative Zn-dependent protease with chaperone function [EC:3.4.24.-]		Genetic information processing; folding, sorting and degradation.
TtgMct186	AmoTS-M166.9	4	Malate dehydrogenase [NADP], chloroplast precursor [EC:1.1.1.82]	Citrate cycle Pyruvate metabolism Glyoxylate and dicarboxylate metabolism Carbon fixation Reductive carboxylate cycle (CO_2 fixation)	
TgtMct235	AmoTS-M297.6	4	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [EC:4.1.1.39]	Glyoxylate and dicarboxylate metabolism Carbon fixation	
TtgMct177	AmoTS-M284.3	4	Methylcrotonoyl-Coenzyme A carboxylase 2 (beta) [EC:6.4.1.4]	Valine, leucine and isoleucine degradation	
TgtMct211	AmoTS-M291.0	4	Shaggy-like kinase [EC:2.7.1.-]	ErbB signaling pathway Cell cycle Wnt signaling pathway Hedgehog signaling pathway Circadian rhythm	Glycogen synthase kinase 3 beta