TRANSCRIPTOMICS ANALYSIS OF PHLOEM-FEEDING INSECT RESISTANCE IN RICE GERMPLASM

by

MOHAMAD BAHAGIA BIN AB GHAFFAR

A Thesis submitted to
The University of Birmingham
For the degree of
Doctor of Philosophy

School of Biosciences
University of Birmingham
December 2011

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

ABSTRACT

The Brown Plant Hopper (BPH) *Nilaparvata lugens* (Stal) is the most important phloem feeding insect which can cause a serious problem for world rice production especially in Asia. Development of novel control strategies can be facilitated firstly by a comparison of BPH feeding behaviour on varieties exhibiting natural genetic variation, and then elucidation of the underlying mechanisms of resistance using molecular information. First, BPH feeding behaviour on 12 rice varieties with varying resistance was investigated using the Electrical Penetration Graph (EPG) and honeydew clock approaches. Seven feeding behaviours (waveforms) were identified and could be classified into two phases, feeding and non-feeding. Cluster analysis separated 12 varieties into 3 main groups, resistant, moderately resistant and susceptible. Gene expression microarray analysis on all the varieties was then undertaken to identify candidate genes which may contribute to resistance. Insects were not introduced to the rice plant because the research interest was in constitutive rather than inducible genes. The results revealed the difference between resistant and susceptible varieties, and agreed with the EPG and honeydew clock experiment results. Correlation between expression and EPG results, gene ontology (GO) analysis and genetic mapping of known BPH resistance gene markers were conducted to strengthen the candidate genes. Out of 239 genes, a hexose transporter was found in all three analyses and therefore, it was classified as the strongest constitutive candidate gene. The position of this gene is close to *QBph10* marker (RM484) on chromosome 10. This gene is involved in the uptake of glucose or fructose and is found expressed in sink organs, indicating that high expression is associated with the increment in nutrient contents. Consequently, it would also increase BPH feeding ability on that plant. There were also several other strong candidate genes identified.

The amino acid transporter 3 (AAP3) is associated with phagostimulation to BPH feeding. Protein kinase, nucleotide-binding leucine-rich-repeat (NB-LRR) protein, carboxyl methyltransferase (MeJA), fatty acid alpha-oxidase (Alpha-DOX2) and 12-oxo-phytodienoic acid reductases (OPRs) are all known to be involved in defence signal mechanisms and are mostly related to jasmonic acid biosysnthesis. Cytochrome p450s, glutathione S-transferase, peroxidases, beta-glucosidase, metallothionein-like protein (OsMT), zinc finger, alpha/beta hydrolase are all found involved in support roles for the lignification of cell walls and response for repairing or recovering from wounding.

DEDICATION

I would like to specially dedicate this Doctoral dissertation to my late father and mother, AB Ghaffar Bin Sudin and Esah Binti Zainudin. There is no doubt in my mind that without them, who gave me their full support and advice since I was born, I would not be here to complete this process.

I owe my thesis completion to my loving wife (Mazlina) and daughter (Nabila) for always believing in me and extending their full support in every aspect of my life.

Finally, to my God, without your help it would have been impossible for me to complete this study. Thank you.

ACKNOWLEDGMENTS

I would like to gratefully acknowledge my main supervisor, Professor Brian Ford-Lloyd and Dr Jeremy Pritchard who have given me their full support, very good guidance and have also become great friends. I thank them for their patience, advice and assistance in my Ph.D. research programme. I would also like to thank my committee members Professor Jeff Bale and also Professor John Newbury for their generous help in mentoring me. I am also deeply grateful to Dr. Habibbudin (MARDI Officer) for his advice and also for providing me with useful several rice varieties for the experiment. The other MARDI colleagues who also supported and assisted this study are Dr Rahman Milan, Dr Melor, Tengku Malik, En Shukri, Norliza, Ainu Husna, Rabiatul, Suhana, Umi and Datuk Dr Abd Shukur.

I am also grateful to all the past and present members of the S208 Biosciences Lab, for their generous help and support during my Ph.D. programme, especially Fay, Liane and Sue at the Genomic lab. Many Thanks to my fellow graduate students and friends, especially Miss Laura, Miss Tess, Miss Serene, Miss Claire, Miss Jane, Mrs Garima, Mr Rashed, Yunoose, Tony and all the staff in the Bioscience School for their friendship, help and good discussion during the programme. I want to give special thanks to my all family (Noraini, Rosnah, Zaitun, Mohd Shukur, Nia'amad, and Dely Salwah), family in law (Osman, Puteh, Masdar and Marshitah) and Malay Muslim Community in Birmingham (MCIB) for their fully supporting my study.

Finally, I would also like to acknowledge MARDI and the School of Bioscience who financially supported this study.

TABLE OF CONTENTS

LIST OF FIGURES

List of Figures (Continued)

List of Figures (Continued)

LIST OF TABLES

List of Tables

List of Tables

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AAP	Amino Acid Transporter
ANOVA	Analysis of Variance
bph	BPH resistance recessive gene
Bph	BPH resistance dominant gene
BPH	Brown planthopper
cDNA	Complementary DNA
cRNA	Complementary RNA
DNA	Deoxyribonucleic Acid
E1/N4-a	Salivation of sieve element
E2/N4-b	Phloem ingestion
EPG	Electronic Penetration Graph
EST	Expressed Sequence Tag
ET	Ethylene
FAO	Food and Agriculture Organisation
GO	Gene Ontology
HR	Hypersensitive Response
h	Hour
IRRI	International Rice Research Institute
JA	Jasmonic Acid
LOX	Lipoxygenase
LRR	leucine-rich repeat
MARDI	Malaysia Agriculture Research and Development Institute

MIAME	Minimum Information about a Microarray experiment
Min	Minute
mRNA	messenger RNA
N5	Xylem ingestion
NB-LRR	Nucleotide-binding leucine-rich-repeat
NP	None penetration
P	Pathway
PD/N7	Potential drop
PCA	Principal component analysis
PCR	polymerase chain reaction
PI	Proteinase Inhibitors
QTL	Quantitative Trait Loci
RIN	RNA integrity number
RNA	Ribonucleic Acid
SA	Salicylic Acid
s	Second
SD/N6	Derailed stylet mechanics

CHAPTER 1

GENERAL INTRODUCTION AND RESEARCH AIMS

1.1 Introduction

Rice (*Oryza sativa* L.) is an ancient crop and is classified as one of the most economically important cereal foods. It is the staple food for many people around the world especially in Asia where half of the world's population live. As an energy source, rice mainly provides carbohydrates and some proteins, vitamins and fibres for human consumption. In 2007, the Food and Agriculture Organization (FAO) estimated the production areas approximately reached 156 x 10^6 hectares with 660 x 10^6 tons yield (Tester and Langridge, 2010). This crop also plays an important socio-economic role providing the rural population with many job opportunities. According to the FAO of the UN in 2010, China, India, Indonesia, Bangladesh, Vietnam and Thailand were among the top list of world rice production countries (Alexandratos et al., 2006), but only a few from those countries participated in the export market. This can create problems such as those encountered recently. The demand for rice has increased sharply in the international market followed closely by the price. This phenomenon has resulted in global social problems because most people especially in developing countries cannot afford to buy rice. With the world's population increasing each year, immediate action needs to be

undertaken to make sure the production of rice can meet future demand, which is projected to increase to 771.1 M tonnes in 2030 (Nguyen and Ferrero, 2006).

The problem with regard to rice production is associated with several factors including biotic and abiotic. Pests and diseases cause multi-million pound economic losses. Oerke (2005) has claimed that potential rice yield loss due to pests is between 65% to 80% production. The brown planthopper (BPH), *Nilaparvata lugens* (Stål), is one of the major phloem feeding insects which causes serious damage to the plant. They injure the plants through direct sucking of the plant sap, and they also transmit viruses, causing plant wilting or hopper burn when it becomes serious (Catindig et al., 2009).

The use of insecticide to control this problem is already creating other problems especially for the environment. In some cases, without proper planning and with over-spraying of insecticide, this could increase the risk of a potential outbreak of pest because of the in advertent destruction of natural enemies of the pest and creating resistance in the pest (Oerke, 2005). Furthermore, costs of production are also increased. For example, in 2004, the total average for world insecticide sales reached USD10 billion (Oerke, 2005). Introducing varieties with insect resistant characters through a plant breeding approach is an alternative useful technique, however it takes a long time to achieve and the results are also not necessarily consistent. It is believed new molecular approaches can help breeders to solve basic problems in identifying candidate genes which are related to resistance characters. Thus, by manipulating those genes, it is hoped that insects may respond differently as already proven in insect-plant interaction studies on the model plant, *Arabidopsis*. Aphid feeding behaviour is significantly influenced by manipulation of amino acid transporters (AAPs) genes in *Arabidopsis* (Hunt et al., 2006). To date, up

to 22 major genes and several quantitative trait loci (QTL) have already been associated with BPH resistance in cultivars and wild rice species (Santhanalakshmi et al., 2010).

Although many resistance genes have been identified, studies of their interactions, in terms of their plant-insect interactions in rice through molecular approaches are still at early stages especially in relation to phloem feeding insects. Understanding the defence mechanism through genetic information is a big challenge because it involves a very complex process. However, the recent advanced approach involving microarrays is enabling a faster understanding of the processes. Microarrays help analyse the expression of thousands of genes in a single experiment (Thompson and Goggin, 2006). The support of genome wide information and its combination with phenotypic information such as symptom development could provide a precise knowledge for genes‘ involvement in plant defence mechanisms (Reymond, 2001). In addition, the present genomic analysis tools and genomic information also provide opportunities for researcher**s** to home in on specific genes based on their interest**s**.

Therefore, the general target in this project is to exploit this technology by identifying candidate genes which contribute to the development of plant resistance characteristics. Then, these results can be supported with resistance level evaluation experiments to increase the understanding of defence mechanisms. It is belived that this information will help plant breeders to plan their strategies to manipulate specific genes to overcome insect problems in the future. This approach could provide an excellent model for others to utilize and for other crops.

1.2 Aim, scope and contents of this study

This study generally aims to develop suitable strategies using molecular information for controlling the phloem feeding insect brown plant hopper (BPH) in rice. As a model genomic plant, the knowledge discovered from this study could be useful for the management of pests not only in rice but also in others crops especially cereal crops. This study however has covered only constitutive resistance characters because BPH was not introduced to plants at any growing stage. The approach is focussed on identifying candidate genes which are responsible for resistance in the plant. Several specific objectives were:

1. To evaluate the feeding and growth ability of bird cherry-oat aphid (*Rhopalosiphum padi*) as an initial model phloem feeding insect on rice.
2. To evaluate and measure BPH feeding behaviour on 12 rice varieties using the EPG technique and honeydew drop as a resistance level indication. This information will also be used as supporting information for microarray experiments.
3. To identify and characterize constitutive genes that are involved in resistance to BPH over 12 varieties through microarray analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice: Food security and problems in cultivation

Rice (*Oryza sativa*, L.) is the leading food crop for humans, providing dietary energy and protein for half of the world‘s population especially in the Asian region (Shabbir, 2009). Total rice production, however, is expected to face serious challenges and is likely to be more unstable in the near future (Timmer, 2010). A world food crisis happened in 2007-2008, and this caused the price of food in international markets to increase drastically (Timmer, 2010). In addition, it has been predicted that the world population in 2020 will increase 29.7% to 7,593 billion people (Mullin, 1999), which could cause a huge demand for this crop. Therefore, it will be necessary for annual rice production to double to keep up with worldwide demand

The production of rice is mainly in Asian regions in which most countries are classified as poor. It is difficult to transfer new technologies to these areas, and this has become a major constraint on rice production. Most production countries such as Philippines, Indonesia, Vietnam, China, Bangladesh and Malaysia produce barely enough rice for their own populations. These countries also face severe climatic problems such as soil fertility, hurricanes, drought and flood incidence, and unpredictable weather, pests

and diseases. It is evident that rice cultivation areas will face more problems, and inconsistency of rice production is a likely threat in future. Reduced production of rice could also result from the use of less superior rice varieties or poor management.

One of the major hurdles faced by world rice production is pests and diseases problems such as rice blast, bacterial leaf blight, tungro, brown planthopper, yellow rice stem borer and leaf folder (Randhawa et al., 2006). It has been estimated that the yield losses due to rice diseases and pests‘ outbreaks increase up to 25% annually (Khush, 2005). This has caused many farmers to use pesticides heavily to overcome the problem, the usage of which has led to another serious environmental difficulty. This study, however, focusses specifically on those insect pests which are responsible for a major loss of rice production in tropical and subtropical Asia.

2.2 Rice pests and economic importance

Insect pests have the ability to attack almost all parts of a rice plant at any growth stages. There are about 800 insect species which can damage rice whether in the rice field or during the storage period (Dale, 1994), but only a few can be acknowledged as potential major threats. Rice pests also can be divided into several groups according to the way they feed. For example, grain insects such as the stink bugs (*Oebalus pugnax*) remove milk from the developing grain (Patoja et al., 2000), defoliator insects mainly Lepidoptera species such as cutworm (*Spidoptera litura*) feed on the leaves at the larvae stage, and stem borers like *Scirpophaga incertulas* (yellow stem borer) feed within the stem (Pathak and Khan, 1994). However, one of the most important groups is the phloem feeding insects which use phloem sap as their main food source. Aphids and brown planthopper are the two most common insects in this group.

2.2.1. Aphids

Aphids are one of the most threatening insects to agricultural crops around the world. It has been estimated that the annual economic loss due to aphid attacks on food and feed grain has reached $5 billion yearly (Matis et al., 2007). They use anatomically adapted mouthparts, called stylets, for probing and exploring plant tissue as they search for sieve elements with nutritious sap (Dixon, 1985). Their normal feeding behaviour does not only reduce plant production but also has the potential to transmit several viruses that are responsible for major rice disease (Mathew, 1991) such as yellow disease or rice yellows (Yano et al., 1983). They have a short life cycle and the ability to produce their offspring through both asexual and sexual reproduction (Matis et al., 2007). Therefore, an outbreak phenomenon could easily happen in a short time when the conditions suit them.

So far, the biological and economic impacts of aphids attacking rice crops have been rarely studied and have been overlooked for many years. The last review was conducted by Yano et al. in 1983. They listed 37 main aphid species found in rice fields, and most of them did not use rice as their primary host. Yano et al. (1983) also categorized the aphids into two groups based on their feeding sites. The first group known as the rice root aphid lived in and was found to infest the root area. *Tetraneura nigriabdominalis* is one of the important rice root aphid species which has been reported to attack upland rice in Thailand severely especially in the area where a continuous planting system has been applied (VanKeer, 2003). The other important aphid species which is classified in the same group is *Rhopalosiphum rufiabdominalis* (Sasaki) (Doncaster, 1953). It is distributed worldwide and found to attack several species of

Graminae, Cyperaceae and dicots, especially potato and tomato (Patch, 1938; Blackman and Eastop, 1984; Tsai and Liu, 1998; Kindler et al., 2004).

Other aphid species which feed on other parts of the plant such as stems, leaves, flower spikelets or fruits are classified in the second group. Total species numbers based on their variations are also bigger than in the first group (Yano et al., 1983). However, there have been very few reports of these types of aphids causing a major problem to the rice industry. The rusty plum aphid, *Hysteroneura setariae* which belongs to this group is commonly found feeding on leaves and unripened rice grain (Jahn et al., 2005). They live in diverse places all over the world and infect rice at every stage of rice development (Gary et al., 2005). Outbreaks caused by this aphid have been reported in non-irrigated lowland rice, for example in Sierra Loene, Nigeria and India (Dale., 1994; Gary et al., 2005). They are usually associated with the drought season (Akinlosotu, 1977; Gary et al., 2005). *Rhopalosiphum padi* (bird cherry oat aphid) also belongs to the second group. This aphid is a common pest to cereal crops including wheat, barley and oat. Rice plants, however, are only their secondary host, which they infest when their primary hosts are not around. To date, there is no report linking this aphid with any major problem in the rice industry apart from as a vector for the virus disease ‗gialume' (yellow disease or rice yellows) of rice in Italy (Yano et al., 1983).

2.2.2. Brown planthopper

Planthoppers are also categorized as phloem-feeding insects. They constitute 65 species, with three subfamilies, Asiracinae (4 species), Stenocracinae (4 species) and Delphacinae (57 species) (Dupo and Barrion, 2009). Brown planthopper (BPH), *Nilaparvata lugens*

(Stal) belongs to the most economically important subfamily, Delphacinae, family Delphaciedae and Order Hemiptera (Dupo and Barrion, 2009). This small brownish insect is also classified as one of the most important rice pests in Asia, which causes heavy losses of yields due to the destruction of rice crops (Park et al., 2007). The insect is also often found to cause more yield loss than that caused by Lepidoptera pests such as stem borers or leaffolders (Dale, 1994; Gurr et al., 2011). The damage caused by BPH is generally similar to that of the aphids, as it feeds on the phloem sap directly and thereby transmits several types of virus such as rice grassy stunt virus (RGSV), rice ragged stunt virus (RRSV), rice striped virus (RSV), rice black streaked dwarf virus (RBSDV) and south rice black streaked dwarf virus (SRBSDV) (Velusamy and Herichs, 1986; Khush and Brar, 1991; Jena et al., 2005; Gurr et al., 2011).

Similar to aphids, BPH also pierce plant parts and suck the phloem fluid with narrow mouthparts called stylets in order to extract nutrient from the host while injecting toxic into the plants (Sogawa, 1982). In the aphid feeding system, only the stylets go through into the cell as the salivary sheath stops at the sieve element cell wall (Spiller, 1990). Unlike aphids, BPH differs in the maxillary stylet, which is normally accompanied by sheath saliva when it enters the sieve element. Clear evidence of this has been shown in the BPH feeding behaviour study using Electronic Penetration Graph (EPG) by Kimmins (1989) and by Seo et al. (2009). Sieve element feeding waveform patterns in aphids (Tjalingii, 1988) are different compared to the BPH pattern. These feeding mode results show that the symptoms of tissue damage are more severe when compared to aphids. Upon infestation, the rice plants‘ leaf blades start to change colour from yellow to brown before wilting. This phenomenon is known as ―hopper burn” (Sogawa, 1982;

Zheng et al., 2007). Due to that, the development and physiological activities of the plant are disturbed, and this result in dramatic yield reductions.

BPH has been reported to be widely distributed throughout Australia, Bangladesh, Cambodia, China, East Timor, Fiji, India, Indonesia, Japan, Korea, Laos, Malaysia, Myanmar, New Guinea, Pakistan, Palau, Philippines, Taiwan, Thailand, Vietnam and Yap Island (Dupo and Barrion, 2009). Macropterous (BPH with wings) adults have the ability to migrate over long distances (Sogawa, 1982). Catindig et al. (2009) claim that they can fly continuously for at least 30 and up to 48 hs and travel more than 1,000 km over the ocean when the conditions are favourable. They have been found to migrate annually from southern Asia to northern regions in China, Korea and Japan (Tunner et al., 1999).

2.3 Phloem sap as food source for phloem feeding insects

Phloem sap plays significant roles in the transportation system of nutrients in plant nutrition and development, allocating nutrients, water, energy and signals from source to sink organs (Fisher, 2000; Dinant et al., 2010). The nutrients‘ resources which are produced in the leaves or from the active roots (photoassimilates, amino acids and signaling molecules) are delivered to heterotrophic plant tissues by osmosis pressure within the plant (Chen et al., 2001). The phloem sap consists of a wide range of organic and inorganic substances (Ziegler, 1975), with high concentrations of sugar in the form of free amino acids (Douglas, 2006). The composition of phloem sap, however, varies depending on several factors including plant age, temperature and water availability (Douglas, 1993; Geiger and Servaites, 1994; Kehr et al., 1998; Ponder et al., 2000; Corbesier et al., 2001; Karley et al., 2002).

Phloem sap has rich nutrient food sources and is an excellent diet for animals. In addition, it is also generally free of toxins and feeding deterrents which help make it attractive to many animals (Douglas, 2006). Insects under the order of Hemiptera are the main animals which have fully utilized phloem sap as their sole food source. This type of insect is called a phloem feeding insect such as aphids, planthoppers, leaf hoppers, whiteflies, mealybugs and psyllids. All phloem feeding insects, however, need a special feeding mechanism to overcome the barrier created by the phloem itself.

2.4 Plant defence mechanisms

Plants are exposed to a high variation of attackers from herbivorous insects or pathogens. That means plants have to produce special mechanisms in order to protect themselves from those attacks and minimize any damage. The effect on the insects or pathogens can be either direct or indirect. When direct, the insects will be killed by the defence mechanism and their population will decrease. When indirect, the plant will produce a special hormone to attract other parasitoids or predators to protect it from the attacker. The plants also have the ability to activate their defence mechanism whether constantly or just after being attacked by the insect. A plant defence system which always appears or is expressed in any condition or plant stage is referred to as a constitutive character. An inducible mechanism is the only response when the plant is being attacked.

2.4.1 Inducible Plant defences

The term inducible plant defences refers to the activation of certain genes-related defence molecules for plant protection from biotic or abiotic stress factors. This process works

fully when the plant has been affected by both stresses. For example, the signals for the defence mechanism are only activated if herbivores make contact with the plant, and most of the times required some injured. This induced mechanism is involved with a broad range of proteins and other molecules whose synthesis is spatially and temporally controlled (Karban and Baldwin, 1997; Walling, 2000) by certain genes which are programmed, such as in Salicylic Acid (SA) and Jasmonic acid (JA) activities (Reymond, et al., 2004). A classic example was shown by Walling (2000) and Zarate et al. (2007). They found that genes related to SA and JA defence mechanism were differentially expressed in Arabidopsis or tomato plant when attacked by silverleaf whitefly (*Bemisia tabaci*) (Reymond et al., 2004).

The phenomenon on induced resistance started in 1933 (Chester, 1933; Deverall, 1997) and was later separated into direct and indirect defence categories in the early 1970s and 1980s respectively (Dicke and Poecke, 2002). The main key plant signalling compounds involved in this inducible defence response are salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Reymond and Farmer, 1998; Pieterse and Van Loon, 1999; Feys and Parker, 2000; Thomma et al., 2001; Kessler and Baldwin, 2002; Van Osten, 2007). Salicylic acid in plant defence is not only reported to be effective against biotrophic pathogens attack (Van Osten, 2007), but it also works as a long distance messenger molecule within the plant (Neher, 2008). It plays a role in the resistance to pathogens by inducing the production of pathogenesis-related proteins.

Salicylic acid belongs to a diverse group of plant phenolic compounds that usually refer to secondary metabolites which have essential roles in the regulation of plant growth, development and interaction with other organisms (Harborne, 1980; Raskin,

1992). Phenolic compounds also play some role in the biosynthesis of lignin, an important structural component of plant cell walls (Raskin, 1992) and have been associated with the chemical defences of plants against microbes, insects and herbivores (Metraux and Raskin, 1992). Raskin (1992) has suggested that salicylic acid plays a role as a signalling component in plant microbes, required for systemic acquired resistance (SAR) to many viruses, bacteria and fungi, as well as for certain forms of rice-specific disease resistance (Rairdan and Delaney, 2002; Durrant and Dong, 2004). In insect attack, SA was reported to be involved in the activation of proteinase inhibitor, which may provide some protection against insect predation (Raskin, 1992).

JA and ET defences are also important for resistance against necrotrophic pathogens and herbivorous insects (Thomma et al., 2001; Dicke and Van Poecke, 2002; Glazebrook, 2005; Van Osten, 2007). Many studies have supported the important roles of JA in plant defence. JA is involved in the activation of genes encoding protease inhibitors that help protect plants from insect damage (Johnson and Ryan, 1990; Creelman and Mullet, 1997). JA also activates the expression of genes encoding antifungal proteins (Becker and Apel, 1992; Xu et al., 1994; Penninckx et al., 1996; Chaudhry et al., 1994), modulates expression of cell wall proteins (Creelman et al., 1992) and induces genes involved in phytoalexin biosynthesis (Choi et al., 1994; Creelman et al., 1992) and phenolics (Doares et al., 1995). Furthermore, JA is also the source of other volatile aldehydes and alcohols that function in plant defence and wound healing (Creelman and Mullet, 1997).

2.4.2 Constitutive plant defences

Constitutive plant defences are mostly present in the whole life cycle of a plant. Therefore, they do not need either biotic or abiotic stress to be present to activate the process as in inducible types. In insect cases, the constitutive mechanism acts before the attack on the plant occurs and usually works as a first layer defence system. Plant morphological (physical) characters such as cuticles, thorns, trichomes and cell walls are used by the plant as a physical barrier (Peiying et al., 2008). These morphological characters protect or minimize insects‘ ability to contact the plant. For example, Bahlman et al. (2003) claim that the high density of trichomes in resistance wheat variety, Tugela-DN1 plants is able to prevent *Diuraphis noxia* Mordv (Russian aphid wheat) from finding a suitable site for feeding. A stem with a wax surface in resistant rice varieties IR22 and IR62 was found to reduce BPH settling and probing ability (Woodhead and Padgham, 1988). Other studies have suggested that silicon content increases epidermal toughness and strengthens cell walls, which may protect plant tissue from insect pests (Djamin and Pathak, 1967; Nakano et al., 1961; Sasamoto, 1961; Ukwungwu, 1990; Hogendorp, 2008). Wild rice variety, *O. Brachyantha* contains high silica levels which makes plants unattractive to the rice leafhopper (Ramachandran and Khan, 1991).

Some plants can create a chemical barrier by being able to synthesise continuously certain toxic compounds (alkaloids, terpenoids, phenolics), repellents, anti-nutritients and anti-digestive compounds (Liu, 2005), which could be harmful to the insect itself (Mithofer et al., 2005, Peiying et al., 2008). These chemical defence properties are commonly subject to plant secondary metabolites such as proteinase inhibitors (PIs), which are found present constitutively in high concentrations in plant

storage organs and possibly function as protective agents against insects (Greg et al., 2008) or cause them to be less attractive to herbivores (Osbourn, 1996; Tierens et al., 2001). In phloem sap, plants produce two major nutritional problems for the insect, which can be described as the _nitrogen barrier' and the _sugar barrier' (Douglas, 2006). The insect has to overcome the problem before it can use the phloem sap. Nitrogen barriers are related to the low level and quality of nitrogen in the phloem sap.The insect will have little ability to synthesise some of the amino acids which can make up the protein because it is metabolically impoverished (Douglas, 2006).

Unlike inducible genes, these constitutive mechanisms have no ability to change easily in a short period of time although they have been exposed to certain stress such as that from herbivore attack (Karban and Myers, 1989). Constitutive genes only involve a normal function in a plant's life cycle. However, in certain cases, they are needed to support an inducible mechanism which could dramatically increase the effectiveness of plant defence. A classic example is *Bph14*, which is claimed by Du et al. (2009) to be a constitutive resistance gene and is found expressed in leaf sheaths, leaf blades and roots. This gene encodes a coiled-coil, nucleotide-binding and leucine-rich repeat (CC-NB-LRR) protein that activates a salicylic acid signal pathway, and which can cause reduced feeding activity and growth rate in BPH (Du et al., 2009).

This thesis will focus only on constitutive defences, which from the plant's perspective have a more stable consistency compared with inducible characters. The effects are also wider and not specific to certain insects, diseases or other types of stress.

2.5 Electrical penetration graph (EPG) as feeding indicator

Monitoring insect feeding behaviour is an important activity and commonly used to define or indicate plant resistance levels. The plant is considered resistant if the insect is not able to feed normally. This is likely to affect the insect‘s growth and finally reduce insect populations. Monitoring feeding behaviour can be done visually using a microscope, with or without video recording (Kindt, 2004). However the results are of limited value because the information is mainly quantitative especially for phloem feeding insects. Unlike chewing insect, the feeding mechanism for phloem feeding insects takes place inside the plant because they use piercing mouthparts to penetrate the phloem through the plant cell wall and mesophyll to feed. Therefore, visual observation cannot give any information about the events within the tissues of the plant. The ability of insects to reach the phloem sap and commence feeding is different and depends on many factors. In resistant plants, the insect commonly takes several hours to access the phloem region after initial stylet insertion (Prado and Tjallingii, 1994; Tjallingii and Hogen Esch, 1993; Prado and Tjallingii, 1997).

A major breakthrough in this area of research came when a method of electronically monitoring insect feeding behaviour namely electrical penetration graph (EPG) technology was introduced by McLean and Kinsey (1964). This EPG technique was later improved by Tjallingii (1988) and has subsequently gone through many modifications. The basic principle however, is mostly still the same and is composed of a simple electric circuit into which the insect and the plant are incorporated. There is an output wire from the monitor into the soil of a potted plant through which a low-voltage (10^9 Ω) in AC or DC signal is passed. A very thin gold wire about 18 µm is attached to

the dorsum of the insect using silver conductive glue. The insect is then connected to the input of the monitor and placed on the test plant. When theinsect penetrates the plant with its stylets (probing), it will complete the circuit (Tjallingii and Gabrys, 1999) and this generates the electric waveform which can be captured, recorded and visualized by the system. There are many different signals produced which are called waveforms and they are characterized through the amplitude, frequency and other features (Tjallingii, 2006). This entire waveform pattern therefore represents the insect stylet movements which have a relationship with feeding behaviour. At present, these data can be analyzed using software such as STYLET 2.2. The detailed process is explained in the EPG methodology in chapter 4 and chapter 5.

2.5.1 EPG waveform description

The EPG system was first tested and heavily used to study aphids. Then, it has been applied to other piercing insects such as in whiteflies (Janssen et al., 1989; Lei et al., 1998), thrips (Hunter et al., 1993; Harrewijn et al., 1996), mealy bugs (Calatayud et al., 1994), leaf-planthoppers (Backus and Hunter, 1989; Kimmins, 1989; Lett et al., 2001) and also in brown planthopper (Kimmin, Seo et al., 2009; Ghaffar et al 2011). The waveform pattern for each insect can differ because their feeding mechanisms are not the same. Therefore, EPG waveform characterization is the first important step to be carried out before any study begins. For aphids, EPG waveform characterization has already been well established (Tjallingii, 1995) and commonly used as reference for other types of phloem feeding insect including in BPH.

Generally, EPG waveform pattern have been divided into 7 typical types (Ghaffar et al., 2011). The first type namely non-penetration or non-probing phase shows a clear distinction from the other waveforms. A straight line indicates that there is no probing activities happenning. The second waveform represents the start of stylet insertion into the plant until thesieve element is reached. This process commonly takes several hours because the insect stylet has to go through epidermis, mesophyll and other tissues before reaching the sieve element. The movement of this stylet is like a sensor system because the insect has to find and to test a suitable area or type of food for the feeding activity to take place. For aphids, Tjalingii (1995) classified the waveforms as A, B and C. In BPH, Seo et al., (2009) and Ghaffar et al., (2011) classified as N1, N2 and N3. In this study all these inconsistent waveforms have been grouped as „pathway". During this time, three other stylet activities have also appeared regularly such as xylem ingestion (G) as an indicator for insect drinking, derailed stylet mechanics (F) meaning the insect faces difficulty in penetration (Prado and Tjallingii 1994). In BPH, this waveform was named as N5, N6 and N7 (Seo et al., 2009; Ghaffar et al., 2009).

The last two last waveforms, E1 and E2 are the most interesting waveforms which are strongly associated with phloem resistance characteristics. E1 waveform represents sieve element salivation and E2 waveform represents phloem ingestion which is equal to N4-a and N4-b in BPH (Ghaffar et al., 2011). The waveforms for both aphid and BPH however show a clear distinct pattern as shown in figure 23 (chapter 6). The difference could be due to their feeding mechanism. In aphids, only the stylet tip enters the sieve element region but for BPH, the whole stylet bundle goes inside sieve element area (Spiller, 1990, Ghaffar 2011). Sieve element salivation waveform usually appears only

for a short time, however it is an important critical stage for the insect to decide whether to continue to feeding or not. If they proceed to phloem ingestion and remain longer, then the plant will be described as susceptible. A carbohydrate-rich excretion or a sweet waste product, called honeydew which is produced by phloem feeding insects is frequently associated with the phloem ingestion period. The insect must expel this product if they are taking in a large amount of sugar in order to balance osmotic pressure inside their body (Wilkinson et al., 1997). This honeydew information is commonly used as supporting data for EPG study.

2.6 Breeding for insect resistance in rice

2.6.1 Genetic diversity of rice

Rice is a member of the grass family (Gramineae) and belongs to the genus Oryza under tribe Oryzeae. There are only 23 species in the genus *Oryza* (Vaughan et al., 2003), which can be found in the humid tropics and subtropics of Africa, Asia, Central and South America and Australia (Chang, 1976). The most cultivated rice is *Oryza sativa* L, which is grown in 100 countries from the equator to latitude 40^0 south and 50^0 north and to an elevation as high as 3,000 metres above sea level in tropical regions (Juliano, 1993). The other cultivated species is *Oryza glaberrima* Stued, which is found in Africa. It has been claimed, however, that this species will slowly be replaced by the superior variety *O. sativa* in the future (Chang, 1976; Linares, 2002).

Rice cultivated species are mostly diploid (2n=2x=24) with genome AA (Samuel, 2001). However, the wild species under Oryza genus, 20 of which have been reported by

Zhang (2007), contain both diploid (2n=2x=24) and tetraploid (2n=4x=48) forms which are represented by AA, BB, CC, BBCC, CCDD, EE, FF, GG or HHJJ genome (Vaughan et al., 2003). The wild species under *Oryza* genus are considered a useful source for a gene pool provider in rice improvement programmes especially for resistance to biotic or abiotic stresses, and male sterility for hybrid development (QuiChang et al., 1975; Sitch et al., 1989; Kush 1997; Ratnayaka, 1999; Samuels, 2001). For example, *Oryza rufipogon* has a special trait for tolerance to acid sulfate soils and flooding-source, *Oryza meridionalis* for drought tolerance, *Oryza punctata* for multiple pest resistance, *Oryza grandiglumis* for high biomass production and *Oryza officinalis, Oryza eichingeri, Oryza minuta* and *Oryza australiensis* for BPH resistance (Sundaramoorthi et al., 2009; Jena, 2010).

2.6.2 Biodiversity as a source of resistance genes

Presently, the uses of plant breeding for development of plants with insect resistant characteristics are in demand. They are generally seen as an effective way of being environmentally responsible and an economically and socially acceptable method of pest control which plays an integral role in sustainable agricultural systems (Wiseman, 1999). Tolmay (2001) claims that the most important benefit in creating a plant with insect resistant characters is the fact that the pest control occurs independently of the managerial ability, skill and resource level of the producer.

The uses of natural resources through breeding programmes for the development of rice resistant to insects are a better option compared with chemical treatments which could cause other serious problems. Jena (2011) claims that more than twenty-one major BPH resistance genes have already been identified in cultivated rice cultivar (*indica* sp)

and wild species (Table 1). Among them are *Bph1, bph2, Bph3, bph4, Bph6, Bph9, Bph10, bph11, Bph12, Bph13, Bph14, Bph15, Bph18, Bph20* and *Bph21,* which are found in chromosomes 2, 3, 4, 6, 11 and 12 respectively (Hirabayashi et al., 1999; Ishii et al., 1994; Jena et al., 2003; Renganayaki et al., 2002; Sharma et al., 2003; Ren et al., 2004; Yang et al., 2004; Jena et al., 2006). However, not all these genes can be used to overcome the BPH problem in rice (Jairin et al., 2007). One of the reasons is due to the appearance of a BPH biotype which showed a different significant response on different resistant genes (Tanaka and Matsumura, 2000). However, the real issue here is the limited current knowledge about genes that are related to resistance mechanisms (Jairin et al., 2007). The old approaches required substantial time in order to solve the problem but with the introduction of new gene expression analysis and technology, the problem can be resolved in a shorter period of time.

Table 1 shows some varieties including wild types which contain all those BPHresistance genes with wide ranges scaling for resistance level in each variety. However, there are many factors that contribute to these variations. One of them is the use of measurement methods to identify this resistance level such as symptom, total insect or nymph and honeydew production.

Table 1 List of varieties including wild types and their resistance scale, which contain BPH resistance genes tested on BPH biotype 2 or 3; scale 0 indicates highly resistance (HR); 1 resistance (R); 3 moderate resistance (MR); 5 moderate susceptibility (MS); 7 susceptibility (S); 9 high susceptibility (HS).

Varieties	Gene	Resistance rating in three replicates	References
TN1 (check variety)	-	9 9 9	Pham and Bui (1999)
Mudgo /IR64	*Bph1*	5 5 5	Pham and Bui (1999) Cohen et al. (1997)
ASD 7	*bph2*	9 7 7	Pham and Bui (1999)
PTB33	*bph2, Bph3*	- - -	Pham and Bui (1999)
Rathu Heenathi	*Bph3*	3 3 3	Pham and Bui (1999) Khush et al. (1985)
Babawee	*bph4*	7 5 5	Pham and Bui (1999)
ARC 10550	*Bph5*	9 9 9	Pham and Bui (1999) Khush et al. (1985)
Swarnalata	*Bph6*	3 5 3	Pham and Bui (1999), Kabir and Khush (1988)
ARC 15831 (b)	*bph 7*	- - -	Pham and Bui (1999)
T12	*bph7*	5 7 7	Pham and Bui (1999)
Sinsapa	*bph8*	5 5 3	Pham and Bui 1999)
Pokkali	*Bph9*	9 9 9	Pham and Bui (1999)
O. officinalis	*Bph6,bph11, Bph13, Bph14, Bph15*	- - -	Jena et al. (2006) Hirabayashi et al. (1999) Renganyaki et al. (2002) Yang et al. (2004
O. latifolia	*Bph12*	- - -	Yang et al. (2004)
O. australiensis	*Bph10, Bph18*	- - -	Ishii et al. (1994) Jena et al. 2006
O. minuta	*Bph20, Bph 21*	- - -	Rahman et al. (2009)

2.6.3 Molecular breeding

There are very few major differences between conventional and molecular breeding techniques. Their main focus is to identify specific characteristics of a plant and utilize them to develop and improve a cultivar for suitable consumer needs through crossing or gene transfer (transgenic). The common steps usually start with the creation of a gene pool from the germplasm population, then identified superior interest characters of the plant (parent) are assembled, and finally an improved cultivar is developed (progeny) from selected individuals (Moose and Mumm, 2008). The primary goals of plant

breeding have typically targeted resistance character, high yields, good nutritional qualities and other important traits for commercial value.

Conventional breeding selects superior plants through phenotypic characters. On the other hand, molecular breeding is based on genotypes‘ characteristics. Unlike in phenotypic characters, gene information is not influenced by environmental factors such as experiment conditions, place or who did it (Vicente et al., 2005). Therefore, the selection results obtained are more accurate and consistent. In addition, because the plant characteristics are not influenced by environmental factors, the experiment can be done at any plant stage. Consequently, time and research costs are significantly reduced.

The basic common approach in molecular breeding is the use of DNA marker technology, a powerful tool for tagging gene regions which are not available in traditional plant breeding (Prioul et al., 1997). There are several marker techniques commonly used these days. These include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) or microsatellites and single nucleotide polymorphism (SNPs). The markers could have links with specific traits of the plant, and they allow plant breeders to identify their character of interest and make their selection. This exercise is called marker assisted selection (MAS), and it can also help to monitor the transfer of desirable genes from one plant to another such as from parents to their progeny.

In addition, the DNA markers allow genetic information to be described. The advent of molecular markers and statistical techniques has enabled genetic information to be described in detail. For example, a quantitative trait loci (QTL) technique is able to

measure the location of genes on the chromosome which affected plant traits on a quantitative scale (Tanksley, 1993). Several QTL for traits of economic importance in rice have already successfully been mapped with molecular markers such as root traits (Redona and Mackill, 1996; Yadav et al., 1997; Zheng et al., 2000), submergence tolerance (Nandi et al., 1997), yield components (Xiao et al., 1998), blast resistance (Wang et al., 1994) and BPH resistance (Kumari et al., 2010).

2.7 Transcriptomics analysis

A transcriptome is the complete set of transcripts representing all messenger RNA molecules in the cell (Wang et al., 2008). Transcriptomics generally involves a systematic and comprehensive study of all the RNA transcripts of a cell, tissue or organism under defined conditions (Thompson and Goggin, 2006). The evaluation is based on mRNA expression patterns which are highly influenced by the environmental conditions surrounding the genes (Lockhart and Winzeler, 2000), including location, development stage and temperature of the cell. In this study, microarray gene expression analysis, which is now a common method for the measurement of gene experession patterns, was used. This technology was first developed by Mark Schena in 1995 (Chu et al., 2007). Serial analysis of gene expression (SAGE), representation different analysis (RDA) and massively parallel sequence signature (MPSS) are the other methods or technologies that can also be used in transcriptomics analysis.

2.7.1 Principle and technique of microarray

The basic principle behind microarray is the inherent ability of a single stranded nucleic acid (DNA/RNA) to bind to its complementary sequences. The simple process of

microarray involves harvesting RNA from tissues of interest and labelling it as target. Next, this RNA sample is converted into a single stranded type and bound to a prepared single stranded DNA on an array surface (platform) commonly known as probes. The levels of expression are measured at this stage according to the quantity of targets and probes that are successfully binding (hybridizing). Previously, RNA levels were measured using Northern blot or Reversed Transcription Polymerase Chain Reaction (RT-PCR) (Mitchell, 2008). However, this technology allowed only a few genes to be analysed at one time. In contrast, microarray technology enables the expression of thousands of genes simultaneously in one single run to be detected and analysed, which makes it a powerful approach and increasingly important in many genomic studies.

There are different types of probes used on the microarray platform. The most common are cDNA arrays and oligonucleotide arrays (Gibson, 2002; Mitchell, 2008). The first probes on a cDNA microarray are polymerase chain reaction (PCR) products (500 to 5,000 bp; Nambiar et al., 2005) generated from clones of cDNA libraries (Suhaimi, 2009). However, this technique is labour-intensive and could produce a few errors as its construction involves many steps such as the generation of cDNA libraries, culturing thousands of clones, amplifying these clones and spotting them on a suitable surface (Suhaimi, 2009). In contrast, the second type probes of oligonucleotides arrays are synthetic molecules with a shorter target sequence (25 to 60 bp; Nambiar et al., 2005). The construction of this oligonucleotide microarray is based on the availability of sequence information in genome databases. This technique is commonly preferred for commercial production as it provides better capacity, accuracy and reproducibility than

cDNA arrays (Chu et al., 2007; Suhaimi, 2009). Agilent and Affymetrix are the main companies actively involved in producing this oligonucleotide gene chip.

Microarray analysis can be performed by one-colour (intensity-based) or two-colour (ratio-based) platforms depending on the specific applications and needs of the researcher. In the two-colour procedure, the two samples are labelled with different fluorescent dyes such as phycoerythrin, cyanine-3 (Cy3) or cyanine-5 (Cy5), and are hybridized together on a single microarray. This approach only allows comparison on the same array being made and is commonly used for a very narrow specific of interest such as comparing two treatments. Unlike the two-colour technique, only one fluorescent dye (Cy3 or Cy5 dyes) is involved in the one-colour procedure for labelling the hybridization of a single sample to each microarray. This hybridization of a single sample per microarray facilitates comparisons across microarrays and between groups of samples (Patterson et al., 2006). Therefore, experimental design becomes simple and flexible (Patterson et al., 2006) especially when it involves a large number of samples as in this study. The selection approaches between one or two colours are not critical in influencing the end results because it has been shown they are approximately equivalent and provide similar levels of biological insight (Patterson et al., 2006; Paul and Amundson, 2008).

Over the past few years, this technology has been frequently updated in many aspects, from the microarray equipment, the chemicals, measurement (statistical analysis software) to bioinformatics analysis. For example, the numbers of probes printed on the array (oligunucletide) produced by the Agilent Technology company have now increased from 22K to 44k and recently 60K array slides. Therefore, these high density arrays allow more samples to be analysed. In addition, the rapid progress in updating genomic

information such as gene annotation and function is also able to support the efficiency of microarray application.

2.7.2 The application of microarray

DNA is the carrier of genetic information (gene) which can be used to describe or characterize the functions in organisms. RNA acts as a messenger (mRNA), passing the actual information in the DNA chromosome from the nucleus into the cytoplasm of the cell (transcription and translation steps), the place where protein is synthesised (Morange, 2009). This process can be directly associated with gene function and measured through gene expression level. A higher gene expression will indicate that this gene is active (up regulated) and highly significant providing evidence of gene function. In contrast, if the level of expression is low (down regulated), it will indicate that the gene is passive or non-active under the conditions tested. For example, the high expression of genes encoding B-glucosidase in seedlings and young plants has been associated with increased resistance to herbivore and pathogen attack (Forslund et al., 2004; Morant et al., 2008).

The actual biological processes, however, are much more complex. Most biological mechanisms depend on several genes rather than one single gene function. Many genes have contributed their part in activating other genes in certain biological functions such as genes associated with defence signalling pathway. The increase in expression level of jasmonic acid (JA) and salicylic acid (SA) due to aphid attack has also induced a number of other SA- and JA-associated genes such as lipooxygenase (LOX) and genes associated with the production of ROS, particularly hydrogen peroxide (H_2O_2) (Zhu-Salzman et al., 2004; Heidel and Baldwin, 2004). Therefore, to understand

the whole biological mechanism becomes harder without a special tool like microarray technique

The ability of microarrays to analyze thousands of genes in parallel has made it a vital tool in transcriptomics studies. Presently, microarrays are being used to examine a wide range of biological issues for plants, animals and humans (Allemeersch, 2006). The most common application is in analyzing gene expression profiles (Aharoni and Vorst, 2001). This approach uses similarity or dissimilarity in expression patterns to classify gene functions which could be associated with certain mechanisms (Aharoni and Vorst, 2001). In plants, the gene expression patterns can be compared from a variation of samples such as those between different tissues in the same organism (root and leaves), the same tissue on different organisms (root sample from variety A and variety B), or the same tissue but under different conditions (healthy and exposed to biotic or abiotic stresses). For example, Seki et al. (2002) used Arabidopsis cDNA microarrays to profile gene expression under 3 different conditions, drought, cold (low temperature) and high-salinity stress conditions over time. They found 53, 277 and 194 genes from 7000 cDNAs are induced respectively after cold, drought and high-salinity treatments compared to control conditions (Ski et al., 2002). These data suggest that drought factors affect more genes than cold or high salinity conditions (Ski et al., 2002). These expression data could also be used to identify candidate genes which respond to drought stimuli. As a consequence, they also explain the gene function which may contribute to several pathways and gene networks in the drought mechanism. The same concept can also be applied to studying plant response to other conditions such as chemical treatments (fertilizer, hormone or pesticide) and diseases.

2.7.3 Microarray analysis in plant breeding

The evaluation of plant performance for the selection of desirable traits is a basic step in plant breeding. Previously, most of this process was done based only on morphological characters such as high yield, good cosmetics, shape or resistance to stress. However, in any transcriptomics analysis, a plant will also be evaluated according to its gene expression profile and level, thus allowing agronomically important traits to specific genes to be linked. Transcriptomics analysis not only reveals the differential expressions between plant varieties but can also give insights into what is happening at the cellular level such as at different developmental stages, tissues locations or non-stressful conditions.

The genes usually respond to certain conditions or treatment through up- or down-regulation patterns. In disease and insect studies, the related resistance genes usually show high expression patterns in resistant varieties. In contrast, susceptible varieties behave in a different way with low expression levels of the related resistance genes. The evidence of this phenomenon has been shown in microarray analysis performed using healthy and unhealthy plant samples. For example, Baldwin et al. (1999) in their microarray study identified 117 significant genes which responded to pathogen attack. Another similar example was that conducted by Reymond et al. (2000) who found 150 defence-related genes in *Arabidopsis* that showed different regulation patterns when the plant received a mechanical injury from insect attack. Moreover, microarray can also differentiate between sources of attack. Several studies have suggested that phloem feeding insects have less impact on plant gene expression than chewing insects (Fidansef et al., 1999; Heidel and Baldwin, 2004; Kaloshian and Walling, 2005; Thomson and

Goggin, 2006). Another study conducted by De Vos et al. (2005) also identified that different regulation patterns occurred in *Arabidopsis* studies between green peach aphid (*Myzus persicae*), cabbage white butterfly larvae (Pieris rapae) and the bacterial pathogen, *Pseudomonas syringae*.

The most significant advances of microarray are not about identifying related genes, but about determining cellular phenotype and understanding the gene function, which are much more important (Lockhart and Winzeler, 2000). With the assistance of bioinformatic technology, this knowledge can be extended to understanding the actual mechanism involved in plant biological systems. This knowledge will help breeders to plan their strategies to achieve their specific goals. In this study, understanding the defence mechanism in plants against insects, specifically phloem feeding insects was focussed on.

Plant defence mechanism is a very complex process involving many genes, therefore a transcriptomics approach is the best option and could be fundamental in future studies for understanding genetic behaviour.. Although the application in this area is still at an early stage, there have already been successful results. For example, genes which are associated with photosynthesis activity were found to be down regulated in tobacco plants in response to insect attack (Ferry et al., 2004). The same result was also found by Hui et al. (2003). Their study demonstrated that growth-associated transcripts are down regulated but defence-related transcripts show up as regulated. These results provided evidence that genes related with salicylic acid, ethylene, cytokinin and jasmonic acid pathways were regulated simultaneously during herbivore attack (Ferry et al., 2004).

There are many reports that associate salicylic acid, jasmonic acid and ethylene, three important signalling compounds, with plant defence mechanisms (Glazebrook, 2001; Kunkel, 2002; Thomson and Goggin, 2006). It is suggested that these signalling pathways interact within each other (Heidel and Baldwin, 2004). In tomato, for example, SA was able to repress JA-mediated genes such as protein inhibitors (PIs) (Doares et al., 1995) and polyphenol oxidase, affecting resistance against herbivores (Thaler et al., 2002; Heidel and Baldwin, 2004). However, this interaction varies depending on hormonal concentration and the relative timing of induction (Devadas et al., 2002; Thaler et al., 2002). In addition, these interactions can also overlap or have synergistic effects (Schenk et al., 2000; van Wees et al., 2000; Salzman et al., 2005; Thomson and Goggin, 2006).

The application of microarray experiments in rice breeding for insect resistant traits is still at an early stage. So far, only a few studies on brown planthopper attack have been reported, including those of Zhang et al. (2004), Yang et al. (2005) and Yuan et al. (2005). Therefore, there are still many mechanisms that can be explored (Thomson and Goggin, 2006).

CHAPTER 3

FEEDING BEHAVIOUR AND GROWTH PERFORMANCE OF BIRD CHERRY-OAT APHIDS (*Rhopalosiphum padi* L) ON RICE

3.1 Introduction

The bird cherry–oat aphid, *Rhopalosiphum padi* (L.), is commonly known as an economically important phloem-feeding insect on many different cereal crops in Europe (Carter et al., 1980; Loxdale and Brookes, 1988). This aphid can cause damage to hosts through direct feeding and transmitting several virus diseases such as barley yellow dwarf virus (BYDV) (Leather et al., 1989). As in other aphids, it uses anatomically adapted mouthparts, called stylets, for probing and exploring plant tissue, as it searches for sieve elements with nutritious sap (Pollard, 1973; Dixon, 1985).

The bird cherry–oat aphid is mostly categorized as heteroecious and holocyclic, a process involving alternations of parthenogenic and sexual generations (Grönberg, 2006). Its main primary host is bird cherry (*Prunus padus* L.), the wild growing fruit tree native to Europe (Vornan and Gebhardt, 1999). Gramineae, especially grasses, maize, barley, oats and wheat are their secondary hosts (Grönberg, 2006). In winter, the bird cherry-oat aphid lays eggs on bird cherry (Loxdale and Brookes, 1988). Then, it migrates to a secondary host in early summer when most cereals and grasses are at the seedling stage. Therefore, at this time, the bird cherry-oat aphid is provided with an excellent food

source to generate huge populations and form outbreaks. To complete the cycle, the bird cherry-oat aphid will return back to the primary host to lay its eggs in the winter season (Grönberg, 2006). The bird cherry-oat aphid is also able to generate populations through anholocyclic forms which remain entirely on the secondary hosts throughout the year (Simon et al., 1996). This reproductive method with the absence of males can only persist where environmental conditions are favourable (Capinera, 2004).

So far, there have been only very limited reports that aphids including bird cherry-oat aphids cause a major problem in rice. The last review conducted was over 28 years ago by Yano et al. (1983). Bird cherry oat-aphid was found infesting rice in Italy and was responsible for transmitting the virus disease ‗gialume' (yellow disease or rice yellows) (Yano et al., 1983). Their potential threat to rice plants has never been tested.

In this study, the general aims were not only to identify bird cherry-oat aphid performance on several rice varieties but also to move to another level in genomic analysis. Rice is well known as a model plant for cereal crops, while aphids (bird cherry-oat) could play a role as a model for phloem-feeding insects. This combination could be good for creating a model experiment for genomic analysis in future. Morphological and molecular data would enable genomic tools to be manipulated for a better understanding of the mechanism of plant-insect interactions. Therefore, it was anticipated that this approach could provide basic knowledge for subsequent further research into the more important rice-insects' interaction (with the brown planthopper).

3.2 Objectives

Two experiments were designed. The first was to evaluate the response of aphid growth performance to five rice accessions which were chosen randomly from samples provided

by the International Rice Research Institute (IRRI) and the Malaysia Agriculture Research and Development Institute (MARDI). The second objective was to identify the effect of rice age on aphid feeding ability using the EPG (Electrical Penetration Graph) system. The use of EPG technology allows for the monitoring of many aspects of aphid feeding behaviour in detail based on stylet activities (Tjallingii, 1978). Only a Malaysian commercial variety, MR219 was used in this experiment

3.3 Materials and methods

3.3.1 Plant materials

The rice varieties used in this study were provided by the IRRI (International Rice Research Institute) and MARDI (the Malaysia Agriculture Research and Development Institute). In this experiment, only 5 varieties were chosen, namely MR219, IR64, IR123, IR694 and Azucena. All the seeds were germinated in petri dishes on filter paper and then transferred to 5 cm diameter pots containing multipurpose compost (HUMAX). The plants were then maintained in a plant growth room at 24 ± 3 ^{0}C with 60 ± 10% humidity and L16:D8 photoperiod

3.3.2 Insect culture

Bird cherry-oat aphid (*Rhopalosiphum padi*) originated clones were received from Rothamsted Research, UK, and then were maintained in wheat crops (*Triticum aestivum* L) in the insect room, School of Biosciences, University of Birmingham. These continuous culture aphids were then transferred to an early seedling MR219 rice clone and kept in net cages in insect growth facilities with similar conditions as in the growth

room. The rice plant was changed every month in order to maintain the pure colony. Only mature aphids and active aphids were chosen for the EPG experiment

Reproductive rate and aphid growth performance Figure 1 shows a small rounded cage (2.0 cm diameter) which was attached to the lower stem of three-week old rice plants. Bird cherry-oat aphids have been reported to prefer the lower part of the cereal seedling (Gianoli, 1999; Lether and Dixon 1981; Wiktelius, 1987). One week later, a young mature aphid was then transferred into a small rounded tube which had already been attached to the plant using a fine camel brush. Then, monitoring was conducted daily until nymphs were produced. Only one nymph was left in the cage, and the others were discarded, including the original old aphid. Counting was started at this point until the death of the test aphid or a maximum of 24 days. Two parameters were assessed: the total days of nymph survival, and the number of offspring produced. Nymphs that lived less than 2 days were excluded from the analysis. This experiment was conducted for 24 days, and at least 5 samples were collected for each rice variety. A similar process was also undertaken using the second treatment for 8-week old rice.

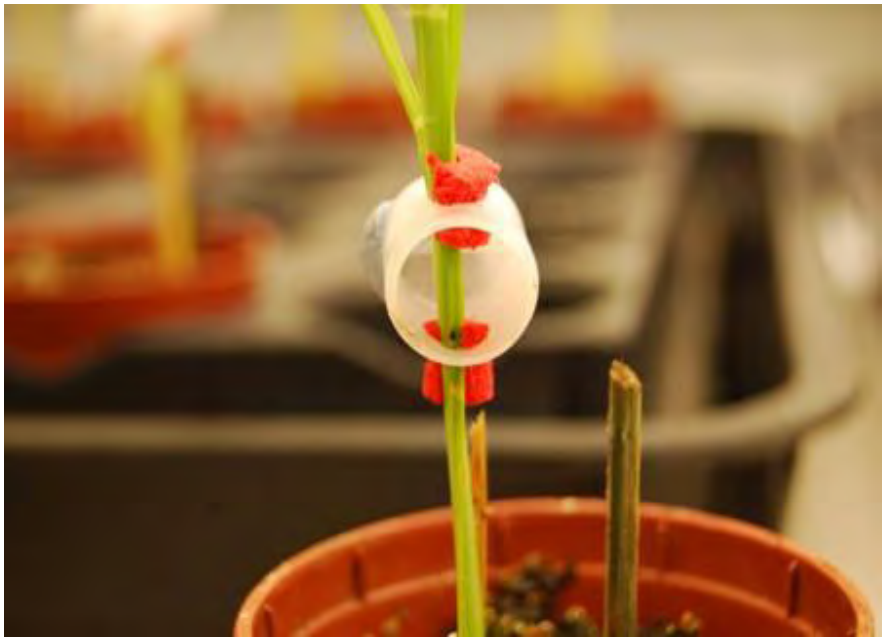


Figure 1 A small rounded clip cage

3.3.3 EPG technique

Aphid feeding behaviour was recorded and classified using a DC electrical penetration graph (EPG) system (Figure 2) as described by Tjallingii (1978, 1988). Adult apterous aphids were selected from the insect culture, according to their size and active behaviour. They were carefully connected to a 3 cm length of 18.5 μm diameter gold wire (EPG system, Wageningen Agricultural University, Wageningen, the Netherlands) with conductive silver glue on their dorsum part and were then left to starve for about an hour. After that, they were wired into a Giga 8- DC EPG amplifier with 10^{9} Ω input resistance and an adjustable plant voltage (Wageningen Agricultural University, Wageningen, the Netherlands) and connected to a rice plant (Figure 3). At the same time, the other electrode, a copper wire about 2 mm in diameter and 10 cm long (serving as the plant electrode) was inserted into the growing medium of the plant and also connected to the

amplifier. The experiment was conducted in the insect house at 24 ± 3 ^{0}C temperature and 60 ± 10% humidity. Illumination was provided continuously for 6 h by a fluorescent mounted lamp above the cage. Recordings of 8 plants and 8 aphids were made on 8 channels simultaneously. All the signals were recorded on a computer hard disk using STYLET 2.2 software (Wageningen Agricultural University, Wageningen, the Netherlands). In this experiment, probing behaviour was recorded for only 6 h and run separately between young (4-week old) and mature (8-week old) rice plants.

Figure 2 GIGA 8-DC Electronic Penetration graph (EPG) circuit system

Figure 3 Aphid connections to a small gold wire on rice leaf

3.3.4 EPG parameters and data analysis

EPG signal analysis and data acquisition were done using the same STYLET 2.2 software. Because this experiment was the first trial to evaluate aphid feeding behaviour on rice using the EPG method, this preliminary study focussed only on 5 important EPG patterns. Those five waveform patterns are non penetration (NP), pathway (C), salivation of sieve element (E1), ingestion of phloem sap (E2) and ingestion of xylem sap (G), classified according to Tjallingii (1990). Any other waveform without any clear pattern such as derailed stylet mechanics (SD) and potential drop (PD) were classified as waveform pathway types. All data were interpreted by the percentage period of time for each EPG waveform type and their frequency as listed below.

1. Mean duration of non penetration waveform pattern (NP)

2. Period of pathway waveform pattern (C).

3. Period of salivation of sieve element waveform pattern (E1).

4. Period of ingestion of phloem sap waveform pattern (E2).

5. Period of derailed stylet mechanics waveform pattern (F)

6. Period of ingestion of xylem sap waveform pattern (G)

7. Total maximum duration of E1 waveform

8. E1 waveform frequency

9. Time to E1 waveform start

10. Total maximum duration of E2 waveform

11. E2 waveform frequency

12. Time to E2 waveform start

3.3.5 Statistical analyses

Summarising statistics including means and standard error were presented using Microsoft Excel. SAS version 9.1 package (SAS Institute, 2008) was used for a more advanced statistical analysis such as Analysis of Variance (ANOVA) and the Mann Whitney Kruskal Wallis test.

3.4 Results

3.4.1 Fecundity and survival rate

There were no significant differences after 24 days for both survival rate (Kruskal Wallis Pr > Chi-Square = 0.479) and total fecundity (Kruskal Wallis Pr > Chi-Square = 0.807)

amongst the five rice genotypes (Table 2). The new born bird cherry-oat aphids could only survive an average 13.4 days. During that time, they were able to produce only a few offspring (average 3.4 nymphs). Figure 4 and figure 5 illustrate clear patterns for both parameters from the day the aphids were born until 24 days later. The percentage survival rate of the aphids drastically declined between the 8- and 10-day experiments by about 60%, especially for varieties IR694, IR123 and Azucena. There were a number of aphid offspring being produced, however, with high variation and an inconsistent pattern for all rice. Aphids started producing their offspring as early as 8 days after they were born in variety MR219, IR694 and Azucena. Most of them produced maximum numbers after 12 to 18 days in all varieties in the study.

Table 2 Means and standard error of total days first instars can survive and total nymphs they produce during 24-day experiment

Accession no	Days of survival	Total nymph produce
MR219	14.0 ±1.9	6.2 ± 3.7
IR694	13.6 ± 4.1	2.4 ± 1.5
IR123	11.7 ± 2.4	0.7 ± 0.4
IR64	15.0 ± 2.2	2.7 ± 1.4
Azucena	12.3 ± 2.9	3.7 ± 2.5
Average	13.4± 1.1	3.4 ± 1.2
Chi-square	3.496	1.611
Pr > Chi-Square	0.479	0.807

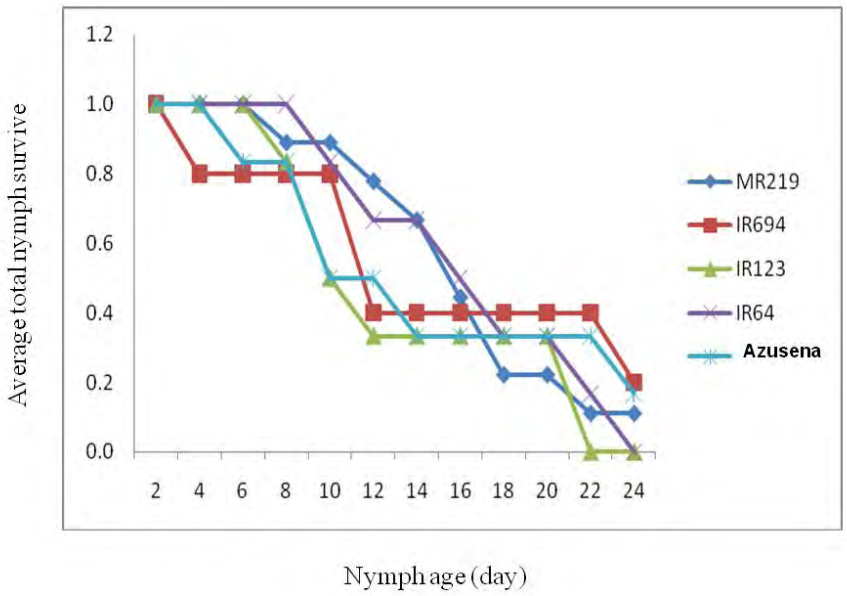


Figure 4 Comparison of total numbers of nymphs (first instar aphid) surviving in days between five rice accessions.

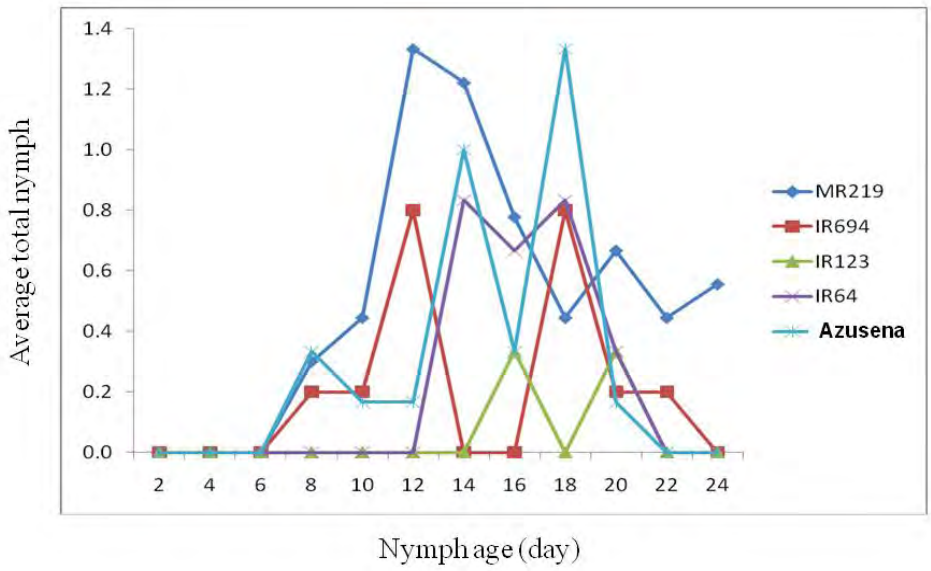


Figure 5 Comparison of total number offspring (nymphs) produced over time by first instar aphids between five rice accessions.

3.4.2 EPG results

All samples with minimum two hours probing activities captured by EPG system were chosen for statistical analysis. Table 3 shows all 12 parameters of aphid feeding behaviour using EPG waveform patterns measured over 6 h. Generally, bird cherry-oat aphids spent most of their time on non penetration type (35.8%) and pathway (39.9%) respectively. Significant differences between young and mature rice plants were only identified related to E2 (ingestion of phloem sap), total percentage period E2 of phloem sap (Pr > Chi-Square: 0.0143), total maximum duration of E2 waveform (Pr > Chi-Square: 0.0160), E2 waveform frequency (Pr > Chi-Square: 0.0470) and time to first E2 waveform (Pr > Chi-Square: 0.0283). These results provided evidence that plant age has a role in influencing aphid behaviour. Bird cherry-oat aphids spent 19.9% of total feeding period for E2 waveform feeding type in young plants, much greater than in mature plants, with only 1.5%. They can stay feeding (E2) longer in young plants with a maximum duration of 1418 s compared to mature plants with only 212 s. Other supporting data such as the total frequency of E2 and the fastest time to E2 were also greater in young plants.

Figure 6 illustrates the EPG waveform of bird cherry-oat aphid feeding in one hour. Generally, non penetration (NP), pathway (C) and xylem EPG waveform patterns appeared only at early aphid feeding stages in young plants (a). In contrast, those three EPG waveforms showed inconsistence patterns in mature plants (b), and these can be seen during all 6 h EPG experiment. In addition, it clearly shows that phloem ingestion (E2) has a shorter time and rarely appears in mature rice plants.

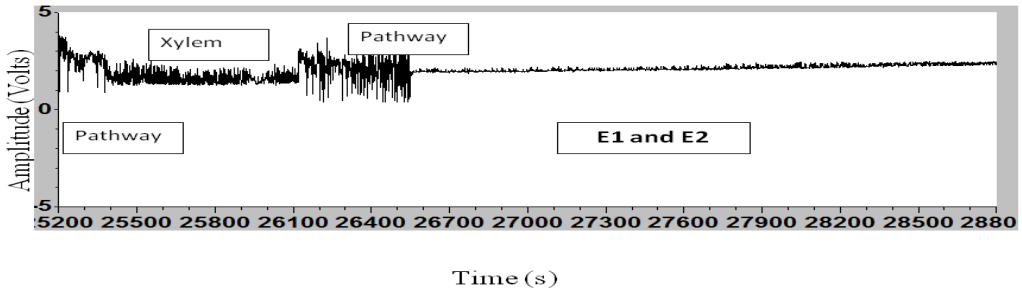
Table 3 Feeding behaviour (mean ± SE of EPG parameters) of bird cherry-oat aphid (*Rhopalisphum padi*) during a 6-h period on 4-week and 8-week old rice plants. (Time in seconds)

Parameters	Means	Young	Mature	Chi-Square	Pr > Chi-Square
Non penetration: NP (%)	35.8 ± 5.7	27.9 ± 6.8	41.8 ± 8.5	0.9899	0.3198
Pathway: P (%)	39.9 ± 4.8	39.5 ± 6.5	40.3 ± 7.0	0.1818	0.6698
Salvation in sieve elements: E1 (%)	4.9 ± 1.5	5.7 ± 2.0	4.3 ± 2.2	1.5568	0.2121
Ingestion of Phloem sap: E2 (%)	9.4* ± 4.2	19.9 ± 8.8	1.5 ± 0.9	5.9949	0.0143
Ingestion of xylem sap: G (%)	9.9 ± 3.6	7.0 ± 4.4	12.1 ± 5.4	0.0052	0.9423
Total maximum duration of E1 waveform (s)	450.3 ±106.2	589.1 ± 175.0	346.1 ± 130.0	1.1393	0.2858
No of E1 waveform frequency	6.8 ± 155	7.2 ± 1.5	6.5 ± 2.0	0.3719	0.5420
Time E1 waveform start (s)	6414.6 ± 1650.2	4167.1 ± 2202.8	8100.3 ± 2327.8	1.8280	0.1764
Total maximum duration of E2 waveform (s)	729.1* ± 266.0	1418.0 ± 529.6	212.4 ± 123.3	5.8036	0.0160
No of E2 waveform frequency	3.6* ± 1.0	6.1 ± 1.8	1.7 ± 0.6	3.9450	0.0470
Time E2 waveform start (s)	11930.8* ± 1831.9	7444.8 ± 2519.3	15295.4 ± 2194	4.8075	0.0283

***P< 0.05 significant different (Kruskal Wallis test)**

A further detail of aphid feeding behaviour throughout the 6 h is shown in figure 7. There was much variation identified during the 6 h feeding period. Once again, only E2 EPG waveform character clearly showed the difference between young and mature plants. Bird cherry-oat aphids can feed better on young plants rather than mature, but their ability decreases towards the end of EPG monitoring time.

a. Young rice plant



b. Mature rice plant

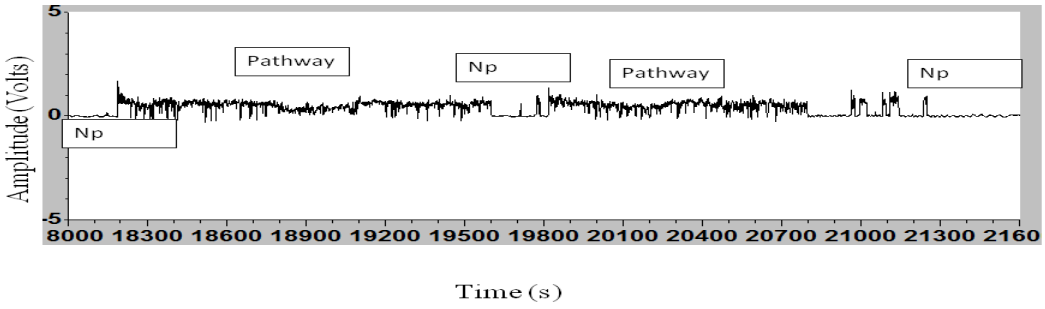


Figure 6 Electrical Penetration Graph (EPG) of aphids feeding on different ages of rice plant in one hour. Comparison of EPG waveform patterns between young (a) and mature (b) rice plants during one hour. Aphids find it easy to penetrate sieve elements in young plants compared to mature plants

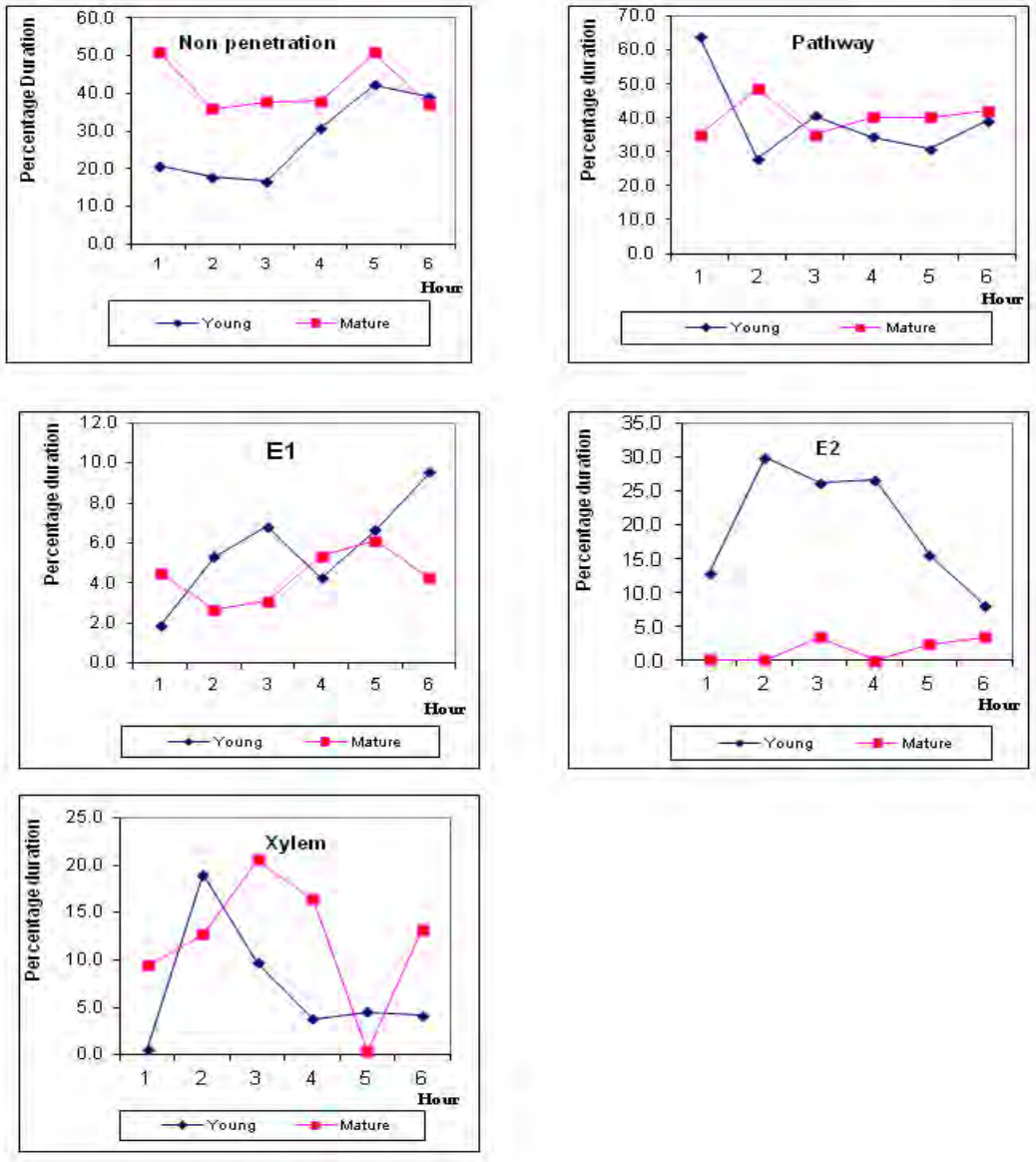


Figure 7 Average total time for five EPG waveform patterns, Non penetration (NP), Pathway (C), Salivation of sieve element (E1), ingestion of phloem sap (E2), derailed stylet mechanics (F) and xylem (G) with a comparison of young and mature rice plants over 6 h

3.5 Discussion

The EPG technique used in this study has provided valuable information on stylet activities via electrical waveform (E2), reflecting the main indicator to describe insect feeding behaviour. In this trial, there was no significant difference in any waveforms except for E2 (phloem ingestion). This waveform type is the most important character that can act as an indicator for host preference of sucking insects. The result suggested that bird cherry-oat aphids feed better on young plants than old plants. The percentage of E2 waveform declined sharply from 19.9% in 4-week old rice plants (young) to 1.5% when the rice plant was more mature (8 weeks old). This result is supported by Traicevski and Ward (2002) who claimed that the frequency and duration of probing during aphid feeding behaviour were affected by the age of the plant. This is similar to the findings made by Karley et al. (2002) in relation to *Myzus persicae* and *Macrosiphum euphorbiae*. They found that both aphids performed better on young rather than mature potato plants.

The percentage of E2 duration found in this experiment from bird cherry-oat aphid on rice was far lower than on other cereal crops. For example, Givovich and Niemeyer (1991) found that aphids spend at E2 waveform about 49 % of their feeding time (349 min from 12 h EPG experiment) on 6 different wheat varieties. Another experiment conducted by Slesak et al. (2001) also found that bird cherry-oat aphids were able to feed for 60% of feeding time (4.8 h from 8 h EPG experiment) on E2 waveform on control a wheat variety. Those values are more than twice those reported here on 4-week old plants.

Data from fecundity and survival rate also support the fact that rice is not suitable for bird cherry-oat aphids even to generate their populations. This was the case in all 6 rice varieties where no significant differences could be identified in all parameters. On average, only 3.4 nymphs were produced (from the parthenogetic process) in 24-day experiments. This value is far lower than that of Leather and Dixon (1981) in their study on several cereal and grass species. They found an average of 24.92 nymphs were produced in oats cv Aster, 23.71 nymphs on cv Trafalgar, 26.38 nymphs on wheat cv Maris Huntsman, 28.54 nymphs on barley cv Maris Otter, 27.83 nymphs on rye grass and 26.3 nymphs on timothy grass. For longevity, bird cherry–oat aphids can only survive for an average of 13 days from the day they were born (first instar) in 6 rice genotypes. This value is very different to that in the study of Taheri and Rastegari (2010). They found that bird cherry–oat aphid could survive an average 21.4 days on 6 wheat varieties. It was even higher in the study of De Celis et al. (1997) with a lifespan of 25.13 days on the Brazilian wheat BR-35 strain. These results clearly suggest that all 6 rice varieties tested in this study are not suitable for bird cherry-oat aphid to survive even as a secondary host plant. This is the main reason why it is only very occasionally found attacking rice.

There is no specific explanation why bird cherry-oat aphids do not like to feed on rice plants. According to Lanning and Eleuterius (1992), rice contains the highest percentage of silica, with 3.2% compared with other cereal crops such as oats (1.4%), barley (0.74%), millet (0.05%), rye (0.01%), sorghum (0.03%) and wheat (0.01%). This high silica content in rice could be one of the factors influencing bird cherry-oat aphid behaviour. This is supported by Yoshida (1975) who found that silica content in the leaf

epidermis acts as a physical barrier to insect penetration. The insect would face even more problems when the rice plant is older because the silica content is also increases (Lewin and Reimann, 1969). The E2 waveform value declined sharply to 1.5% when the rice plant was more mature (8 weeks). Bird cherry-oat aphid stylets also took a longer time to reach the phloem region, which shows that they were not able to ingest phloem sap anymore.

In contrast with silica, nitrogen content was found to act differently. Its level was reported higher during early development stages, with a decline with age (Mattson, 1980). Interestingly, a plant with a low level of N content was found to be associated with a decrease in aphid feeding performance (Hughes and Bazzaz, 2001). This finding was also supported by Leather et al. (1989). They found that the survival, rate of development and fecundity of bird cherry–oat aphids are affected by the crop developmental stage. The characteristics of plant morphology such as hairiness (Ahman et al., 2000), surface layer thickness (Xiang et al., 2008) and waxiness (Tsumuki et al., 1989) could also influence bird cherry-oat aphid behaviour acting as physical barriers. However, there are no reports in relation to rice.

Although the data in this experiment did not provide much information to understand the whole phenomena of bird cherry-oat aphid feeding behaviour, it was enough to conclude that they do not prefer rice as a host plant. This is the reason why they were found to appear only occasionally infesting rice plants. They usually use rice plants only as an alternate or temporary host between the four weather seasons (Yano et al., 1983). The major significance of this study was therefore only to provide experience of the technology of assessing feeding behaviour and interactions between plants and

insects, leading up to the much more valuable assessment of the feeding behaviour of the brown planthopper on rice.

CHAPTER 4

CHARACTERIZATION AND COMPARISON OF FEEDING BEHAVIOUR ON RESISTANCE AND SUSCEPTIBILITY TO BROWN PLANTHOPPER (*N. lugens* Stål) IN RICE GERMPLASM

4.1 Introduction

The brown planthopper (BPH) or *Nilaparvata lugens* (Stål) is one of the most serious rice pests in Asia and has caused heavy losses of yields due to its destruction of rice crops (Jena et al., 2006). The yield loss was caused by direct feeding on plant sap or indirectly through transmission of rice viruses such as ragged stunt virus (RSV) and grassy stunt virus (GSV) into the plant (Velusamy and Herichs, 1986; Khush and Brar, 1991) during this feeding time. Normally, the brown planthopper reduces crop yields between 10 to 30 percent (Win et al., 2009). However, in large infestations, it can completely destroy the crops. The plants become yellow and dry, an effect called "hopper burn", usually an associated outbreak phenomenom. These outbreaks have been reported throughout tropical Asia (Gallagher et al., 1994; Settle et al., 1996), especially when the conditions, including high temperature, relative humidity and rainfall (Kasimoto and Dyck, 1975), along with high levels of nitrogenous fertilizers (Cohen et al., 1997) are favourable for insects to grow. China and Vietnam, two of the largest rice producing countries, suffered

large production losses due to BPH attacks in 2005 and 2008 (Bentur and Viraktamath, 2008; Jena and Kim, 2010).

The applications of chemical insecticides are common practice used to control BPH attack. However, this approach alone could cause other serious problems especially related to the natural ecosystem, human health and increased total operation costs in the long term (Huang et al., 2000; Rola, 1993). The extensive use of chemical pesticides is frequently associated with BPH outbreaks because it can destroy the natural predators of BPH such as *Anagrus nilaparvatae* (Wang et al., 2008) and can create insecticide resistance (Matsumura and Sanada-Morimura, 2010). Furthermore, insecticide control becomes inefficient due to the ability of BHP to migrate from one place to another. The use of natural resources through breeding programmes for the development of rice insect resistance is a better option and more efficient compared with chemical treatment. To date, about 22 major genes and several QTLs associated with BPH resistance have already been identified in rice cultivars and wild species (Yang et al., 2004; Jena et al., 2006; Santhanalakshmi et al., 2010). Among them are *Bph1, bph2, Bph3, bph4, Bph6, Bph9, Bph10, bph11, Bph12, Bph13, Bph14 and Bph15* found on chromosomes 2, 3, 4, 6, and 11 respectively (Hirabayashi et al., 1999; Ishii et al., 1994; Jena et al., 2003; Renganayaki et al., 2002; Sharma et al., 2003; Ren et al., 2004; Yang et al., 2004., Jena et al., 2006). Some of these resistance loci have already been successfully used as parents for breeding programs, and include rice varieties Mudgo *(Bph1),* ASD7 *(bph2),* Rathu Heenathi *(Bph3)* and Babawee *(bph4)* (Athwal et al., 1971; Lakshminarayana and Khush, 1977).

Although many resistance loci have already been discovered, not all can be used to protect the rice plant from BPH attack (Jairin et al., 2007). For example, the *Bph1* resistance gene from the Mudgo variety became susceptible to BPH after 5 years due to the development of the BPH biotype 2 (Jena and Kim, 2010), and similarly with the *bph2* gene in the ASD7 variety. This gene was found to confer susceptibility to the new BPH biotype 3 after 14 years (Jena and Kim, 2010). The process of this resistance-breaking ability continues to happen on other new BPH resistance genes and has been reported in many parts of Asian countries (Seo et al., 2009). It clearly shows that BPH has the ability to overcome the many adaptations plants have evolved as protection. This result also indicates that more research into identified resistance genes is required. The complex resistance mechanism involving sap-feeding pests and their host plants has only recently begun to be understood, and it is clear that the pathway from host location to sustained ingestion of phloem sap can be interrupted at several points, potentially allowing many different types of resistance. A detailed comparison of the similarities and differences in the feeding behaviour of BPH on different rice genotypes varying in resistance will allow underlying mechanisms to be identified, thus providing new targets for control.

The BPH, like other phloem feeding insects, has a special mouthpart consisting of a stylet bundle which serves as a piercing and sucking organ (Sogawa, 1982; Lakshminarayana and Khush, 1977). BPH feeds on the plant by inserting the stylet bundle with an accompanying salivary sheath into the plant (Spiller, 1990), locating the phloem tissue and then regulating the ingestion of the pressurised plant sap (Sogawa, 1982; Seo et al., 2009). This mode of feeding is different from the situation in aphids where the salivary sheath stops at the sieve element cell (Spiller, 1990). This intracellular

penetration type by BPH causes the disruption and degeneration of the organelles of plant cells resulting in cell death (Spiller, 1990). Therefore, it causes more damage to the plant host. Hattori (2001) suggested that the BPH feeding process could be divided into two main phases. The first phase involves the movement of the stylet tip across the plant tissue, while the second phase involves the feeding process (Hattori, 2001) where the stylets enter vascular bundles and ingest the phloem sap. BPH feeding processes are complex but as in aphids, the use of the electrical penetration graph (EPG) technique (Tjallingii, 1978) provides an opportunity for detailed cataloguing of stylet activities during feeding (Tjallingii, 2006).

Several studies have been conducted to investigate BPH feeding behaviour using this technique (Velusamy and Heinrichs, 1986; Seo et al., 2009; Hattori, 2001; Kimmins, 1989; Lösel and Goodman, 1993; Hao, 2008). Most of these studies have correlated EPG waveforms with particular BPH stylet activities, and each study has made its own characterization. The method, which is AC (alternating current)-based, was first used by McLeans and Kingsley (1964), and it was subsequently improved by Tjallingii (1978) using DC (direct current). More recent studies have provided increasing levels of signal detail (e.g. Kimmins, 1989; Seo et al., 2009). The present study exploits the EPG capability by using the DC-EPG technique to compare BPH feeding patterns and so host plant resistance across a range of rice genotypes. In common with other recent studies, the waveforms have been characterised following the descriptions provided by Seo et al. (2009). This research finding has already been successfully published in the recent issue of PLoS journal as shown in appendix J, Ghaffar et al. (2011).

4.2 Research limitations and objectives

This research evaluated 12 rice varieties with different resistance backgrounds and mainly focussed on BPH feeding behaviour, one of the most important characters as an indicator for resistance level. The EPG system was used to monitor BPH feeding behaviour and honeydew clock was used as supporting information. In this experiment, all EPG waveform characterization was based on rice stems, the place that most BPH were found to stay feeding for long periods. The main objective of this study was to characterize and to compare BPH feeding behaviour by utilizing the EPG system as a tool for detailed resistance screening. This information was then used as evidence in the molecular explanation in transcriptomics analysis.

4.3 Materials and methods

4.3.1 The plants

A list of the rice varieties used in the study and their origins is presented in table 4. All the seeds were provided by the IRRI (International Rice Research Institute) and MARDI (the Malaysia Agriculture Research and Development Institute). F1 is a cross between Rathu Heenathi and TN1 was created in 2008 by MARDI. The seeds were germinated in a Petri dish on a filter paper and then transferred to two-inch diameter pots containing soil as a medium. The plants were then maintained in a plant growth room at temperature 24 ± 3 ^{0}C with 60 ± 10% humidity and L16:D8 photoperiod. Only the plants aged between 40-50 days (Hattori and Sogawa, 2002) were used in all the experiments.

Table 4 List of rice varieties and their origin used in this study

No	Variety	Accession numbers	Origin	Resistance level	Reaction to biotype 1	2	3	4	References
1	TN1	11000 (MARDI)	Taiwan	Susceptible	S	S	S	S	Khush and Brar (1991)
2	Azucena	351438 (IRRI)	Philippines	Susceptible	S	S	S	S	Cohen et al. (1997)
3	Nipponbare	318852 (IRRI)	Japan	Susceptible	S	S	S	-	Ikeda and Kaneda (1981)
4	IR694	777182 (IRRI)	Philippines	Unknown	-	-	-	-	-
5	MR232	12047 (MARDI)	Malaysia	Moderate	-	M	-	-	Alias et al. (2001)
6	MR219	11633 (MARDI)	Malaysia	Moderate	-	M	-	-	Alias et al. (2001)
7	IR758	1876352 (IRRI)	Philippines	Unknown	-	-	-	-	-
8	Fujisaka	00444 (MARDI)	Japan	Unknown	-	-	-	-	-
9	IR64	50533 (IRRI)	Philippines	Resistance	S	M	S	S	Cohen et al. (1997)
10	Rathu Heenathi	07637 (MARDI)	Sri Lanka	Resistance	R	R	R	R	Khush and Brar (1991) Alias et al. (2001)
11	Babawee	06246 (MARDI)	Sri Lanka	Resistance	R	R	R	R	Khush and Brar (1991); Alias et al. (2001)
12	F1 (Rathu x TN1)	New (MARDI)	Malaysia	Unknown	-	-	-	-	-

4.3.2 Insect culture

Brown planthoppers (BPH) biotype 2 were originally collected from the MARDI research station at Pulau Pinang, Malaysia. These BPH were then transferred to a mature TN1 rice clone and kept in net cages in an insect room with similar conditions to those described in in chapter three. The rice plant was changed every month in order to maintain the pure colony. Only brycypterous adult females were selected for all the experiments.

4.3.3 Honeydew clock

Honeydew drops were measured following the modification made by Wilkinson and Douglas (1995) and Daniel et al. (2009). This was done by collecting honeydew drops from each individual BPH on treated filter paper with 0.1% bromophenol blue (Sigma-Aldrich Company Ltd., UK) and 0.01 M HCl (Sigma-Aldrich Company Ltd). This yellow treated filter paper turns blue when in contact with honeydew drops. Treated filter paper was attached to the circular plate and a modified clock which could rotate 360^0 over 12 h. The rice plant was arranged horizontally and covered with a modified cage to ensure the correct BPH position (Figure 8). The frequency and volume of honeydew drop production were measured after 12 h. In this experiment, data were collected only if the BPH stayed in the cage more than 3 h after the experiment was started.

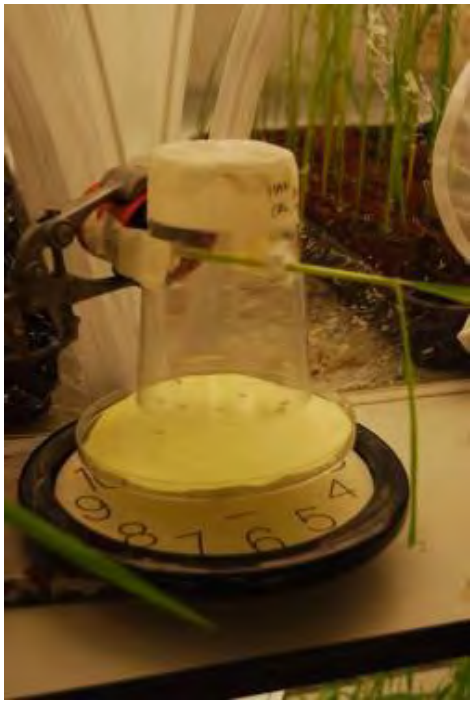


Figure 8 A honeydew clock experiment using bromophenol-blue treated filter paper on a modified clock

4.3.4 EPG technique

BPH feeding behaviour was recorded and classified by using a GIGA-8 DC electrical penetration graph (EPG) amplifier with a 109 Ω input resistance and input bias current less than pA (Wageningen Agricultural University). Only adult brachypterous females (Hattori, 2001; Lösel and Goodman, 1993; Hattori and Sogawa, 2002) were selected from the insect cage according to their size and active behaviour. They were connected with a small gold wire (20 μm diameter) and attached with conductive silver paint at their dorsum part and left to starve for one hour. They were then wired onto the EPG equipment and carefully placed onto the stem of a rice plant (Figure 9). The experiment was conducted in an insect house at 24 ± 3 ^{0}C with 60 ± 10% humidity but continuous photoperiod. Recordings of 4 plants and 4 BPHs were made on 4 channels simultaneously. In this experiment, probing behaviour was recorded for 12 h continuously. Finally, all recorded signals were analysed using Probe 3.4 software version 2007 provided by Wageningen Agricultural University.

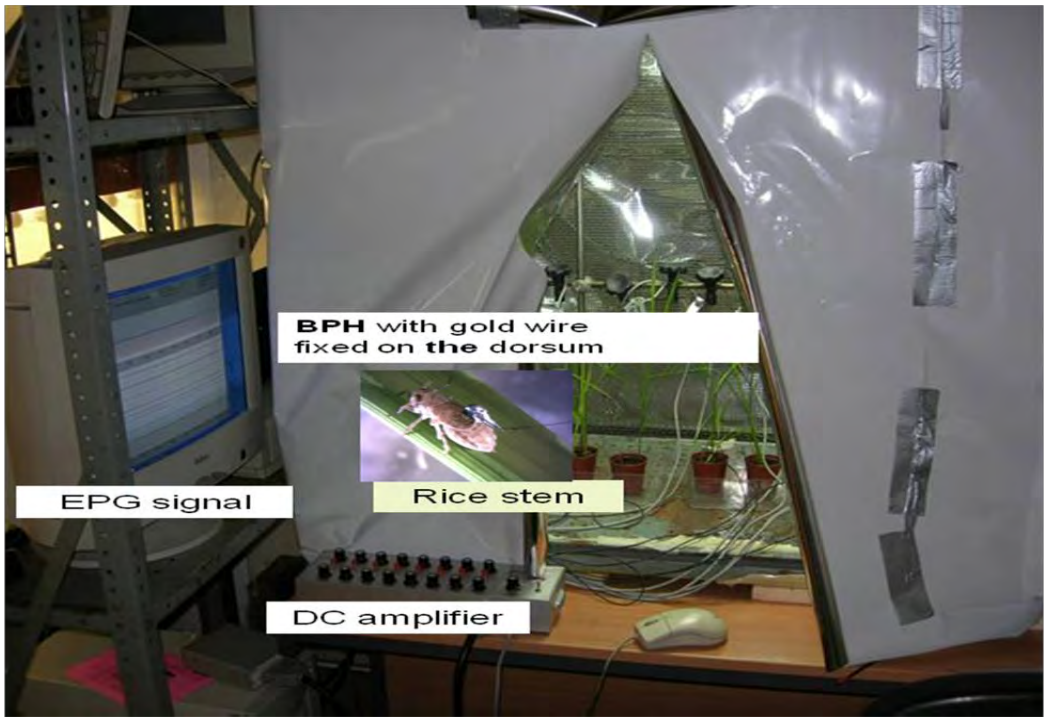


Figure 9 GIGA 8-DC Electrical Penetration graph (EPG) circuit system for BPH on rice stem plants.

4.3.5 Statistical analyses

EPG waveform characterizations, namely NP (non-penetration), pathway, N4-a (sieve element salivation), N4-b (phloem ingestion), N5 (xylem ingestion), N6 (derailed stylet mechanics) and N7 (cell penetration) were identified as decribed by Losel and Goodman (1993), Kimmins (1989) and Seo et al. (2009). Each feeding behaviour component was expressed as a percentage of the total time and its frequency, either for the whole 12 h experimental period or the final 5 h period (8-12 h). All summarising statistics were produced using Excel. SAS version 9.1 (SAS Institute, 2008) was used for more detailed statistical analysis, such as PROC ANOVA for the analysis of variance (ANOVA) and comparison of treatment means (Duncan). However, this analysis was used only for the parameters of honeydew drops and fastest time N4a and fastest time N4b EPG types

within the 12 h experiment. The PROC NPAR1WAY procedure (SAS) for the Kruskal-Wallis test was used for the parameters of percentage duration and frequency of each waveform type. This nonparametric statistical analysis is often used for a suspected non-normal population (Elliott and Hynan, 2011). Mean comparisons of each parameter were conducted using Duncan‘s multiple range test ($P<0.05$). For correlation analysis, PROC CORR (SAS) was conducted on the 12-h experiment to identify the relationships between parameters in this study. Finally, PROC CLUSTER and PROC TREE were used to evaluate the relationships between all 12 varieties. The Euclidean distance coefficient and Ward‘s method (1963) were selected for the cluster analysis.

4.4 Results

4.4.1 Rate of honeydew production

The total number of honeydew drops and their average per hour among rice varieties are shown in table 5. All data show highly significant differences between varieties. Variety IR694 produced the highest total number and average per hour of honeydew drops with 104.3 and 8.9 droplets respectively. However, there was no significant difference identified between the rice varieties, Azucena and TN1. On the other hand, Rathu BPH did not produce a single honeydew drop within the 12 h. Other varieties which produced very low amounts of honeydew drops and statistically can be grouped along with Rathu were IR64, Babawee and F1. Rice variety TN1 was identified as having the fastest time honeydew started to be produced by BPH: only 4 h after introduction onto the plant. Azucena, IR694 and Nipponbare, however were not significantly different. Similarly,

BPH did not produce any honeydew drops on Rathu. BPH took more than 8 h to produce honeydew drops on varieties IR64, Babawee, F1 and MR219.

Table 5 Honeydew production over 12 h by *N. lugens* on 12 rice varieties using the honeydew clock method

	N	Total honeydew droplets in 12 h ± SE	Average honeydew droplets per h ± SE	Fastest time honeydew produce (h)
Azucena	8	79.2 abc (± 15)	7.8 ab (± 1.3)	4.3g (±0.7)
Nipponbare	12	57.4 cd (± 10.6)	5.0 c (± 0.9)	5.7efg (±0.9)
TN1	13	90.7 ab (± 11.1)	7.8 ab (± 0.9)	4.0g (± 0.6)
IR694	11	104.3 a (± 15.6)	8.9 a (± 1.3)	4.5fg (± 0.9)
Fujisaka	10	66.8 bcd (± 20.8)	5.6 bc (± 1.7)	6.8def (± 1.0)
IR758	11	43.1 de (± 15.9)	3.7 cd (± 1.3)	7.9cde (± 1.1)
MR232	10	16.1 ef (± 7.9)	1.3 de (± 0.7)	9.7abc (± 1.0)
MR219	14	40.5 de (± 8.5)	3.5 cd (± 0.7)	7.9cde (± 0.6)
IR64	9	3.7 f (± 2.5)	0.3 e (± 0.2)	11.0ab (± 0.7)
Rathu	9	0.0 f (± 0.0)	0.0 e (± 0.0)	- a
Babawee	16	2.6 f (± 1.3)	0.2 e (±0.1)	8.9bdc (± 0.9)
F1	16	1.5 f (± 0.65)	0.13 e (± 0.05)	10.3abc (± 0.8)
Average		42.2 ** (± 10.8)	3.5 ** (± 1.0)	7.7** (±0.8)

Means± SE within columns followed by the same letters are not significantly different (P > 0.05, Duncan test)
** = Significant at 1% probability level; * = Significant at 5% probability level; ns = Non-significant
'-' = no honeydew observed in 12 h

4.4.2 Characterization of EPG waveform feeding pattern for BPH on rice

Figure 10 shows typical DC-EPG waveform patterns produced by BPH on rice. All characterization was followed according to L**ö**sel and Goodman (1993), Kimmins (1989) and Seo et al. (2009). Non-penetration (NP) waveform was correlated with the absence of feeding activity. The waveform shape is almost a straight line and nearly zeros voltage. In the second type of waveform, namely pathway, BPH started to insert stylets into cell wall tissue and then attempted to penetrate the plant sieve element. At this time, the EPG waveforms produced were quite irregular in form and shape with increased and inconsistent amplitude. Three main EPG patterns, namely N1, N2 and N3 were identified

similar to those identified by Seo et al. (2009) (Figure 10A). N1 waveforms were difficult to identify, appearing only for a few seconds. Generally, N2 waveforms appeared immediately after the NP waveform and consisted of waveform shapes of variable frequency and amplitude. N2 was usually followed by N3, in which the shape was consistent, but with a higher amplitude. Unlike Seo et al. (2009), in the present study the waveforms N1-N3 were combined into one type, the pathway waveform. This helped to reduce the experimental workload in the context of developing a relatively high throughput system for screening germplasm for resistance.

During pathway periods, other waveforms such as N5 (xylem), N6 (derailed stylet mechanics – see below) and N 7 (potential drop) interfered occasionally. The N5 waveform showed a consistent shape (Figure 10C) close to that found by Seo et al. (2009). Interestingly, this shape is also similar to the aphid EPG xylem characterization (Tjallingii, 1978). The other two waveforms, N6 and N7, however, could not be correlated with those seen in other EPG studies. The N6 waveform pattern is similar to N5 but of higher frequency without the consistency of shape (Figure 10D). This N6 waveform was categorized as ‗derailed stylet mechanics' on the grounds that the pattern was similar to that noted by Tjallingii (1988) for aphid feeding. Tjallingii (1978) has also associated derailed stylet mechanics with a mechanical ‗error' impeding the stylets forming a properly functioning bundle. In this study, the N6 waveform generally represented penetration difficulties within the plant tissue (Tjallingii, 1990). The N7 waveform was classified as potential drops; the waveforms suddenly drop from active pathway activities (Figure 10D). N7 waveforms are similar to those noted by Tjallingii (1988), described for aphids believed to correlate with cell penetration. N4-a and N4-b

patterns were clearly (Figure 10C) distinguishable from other waveforms and have been confidently attributed to the sieve element feeding phase (Seo et al., 2009; Kimmins, 1989). N4-a usually appeared in the short gap before N4-b phase started. N4-b waveform meanwhile stayed for a long period, and the strong correlation between honeydew excretion and the N4-b phase (Table 6) provides further evidence of phloem ingestion activity.

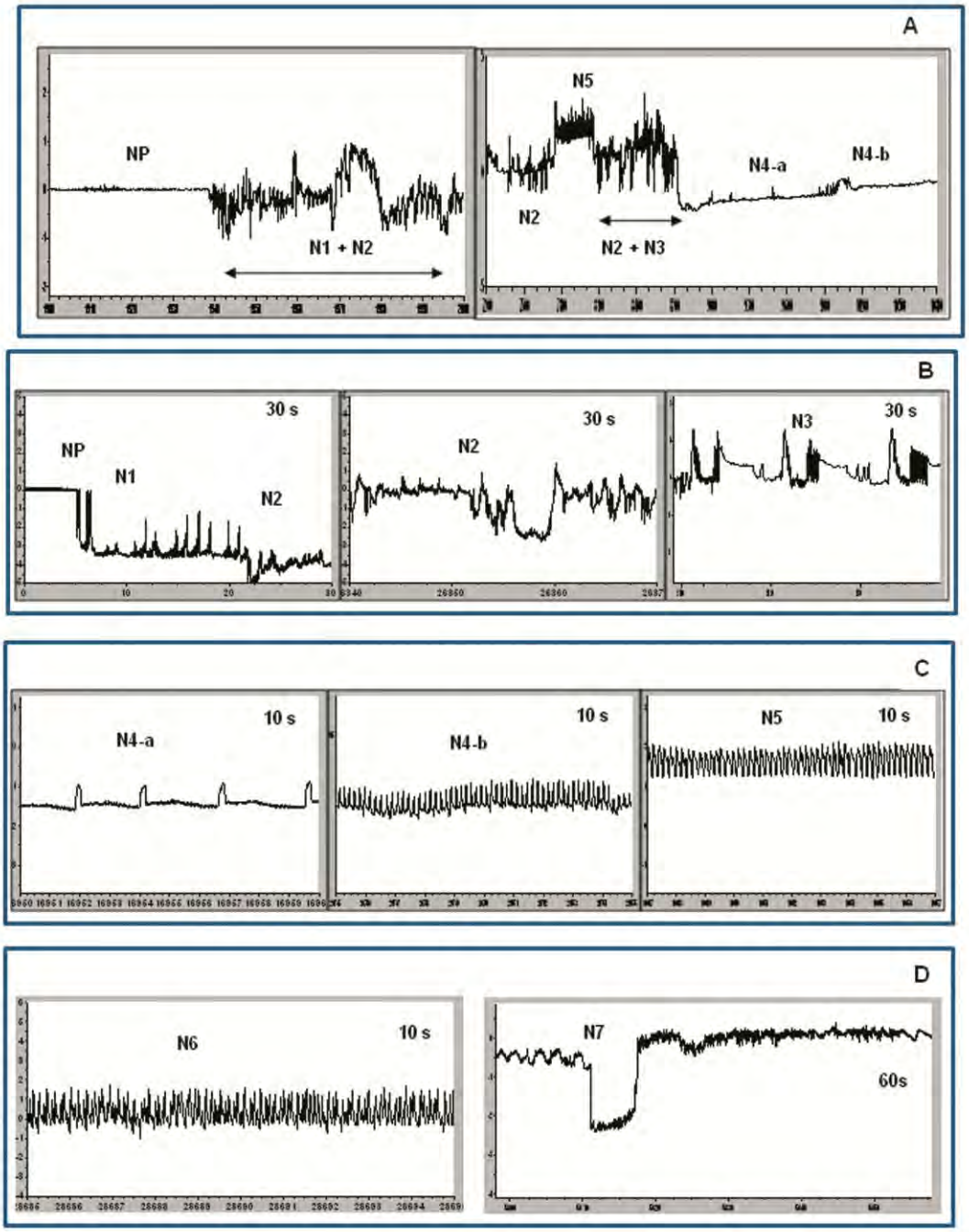


Figure 10 Classification of EPG waveform feeding pattern for BPH in rice. A: Overall typical waveform in two hours. B: Non-penetration (NP), pathway (N1, N2 {irregular mixed} and N3 {transition phase before N4-b start} characterization in 30 s. C: Sieve element salivation (N4-a), phloem (N4-b) and xylem ingestion phase (N5) characterization in 10 s. D: Unclear waveform types; derailed stylet mechanics (N6) and potential drop (N7) characterization in 5 and 60 s

4.4.3 Correlation of *N. lugens* feeding and honeydew production

A complete feeding activity based on percentage EPG waveform duration and honeydew drops in the 12-h experiment is shown in figure 11. Generally, pathway activity (the sum of N1, N2 and N3 phases) decreased over the first 6 h of feeding with a concomitant increase in phloem sap ingestion (N4-b). In contrast, the increase in N4-b activity was paralleled by an increase in honeydew production. In some varieties (notably TN1 and Azucena), there was an initial peak in N4-a activity (salivation), which declined during the later stages of feeding. The other EPG waveforms did not show any clear pattern except for NP. Rice varieties Rathu, Babawee and F1 showed increases in NP percentage duration in the last three hours of the 12 h feeding period.

Linear correlations between EPG waveforms and honeydew drop variables were calculated (Table 6). Strong positive correlations were found between salivation (N4-a), phloem sap ingestion (N4-b) and honeydew drop production. Positive correlations were also found between non-penetration, pathways (N1-N3) and cell penetration (N7) activities. In contrast, pathway behaviour showed a high negative correlation with N4-b waveform ($r = -0.947$, $P=<0.01$), the average rate of honeydew drop production ($r = -0.875$, $P=<0.01$) and the total number of honeydew drops ($r = -0.857$, $P= <0.001$).

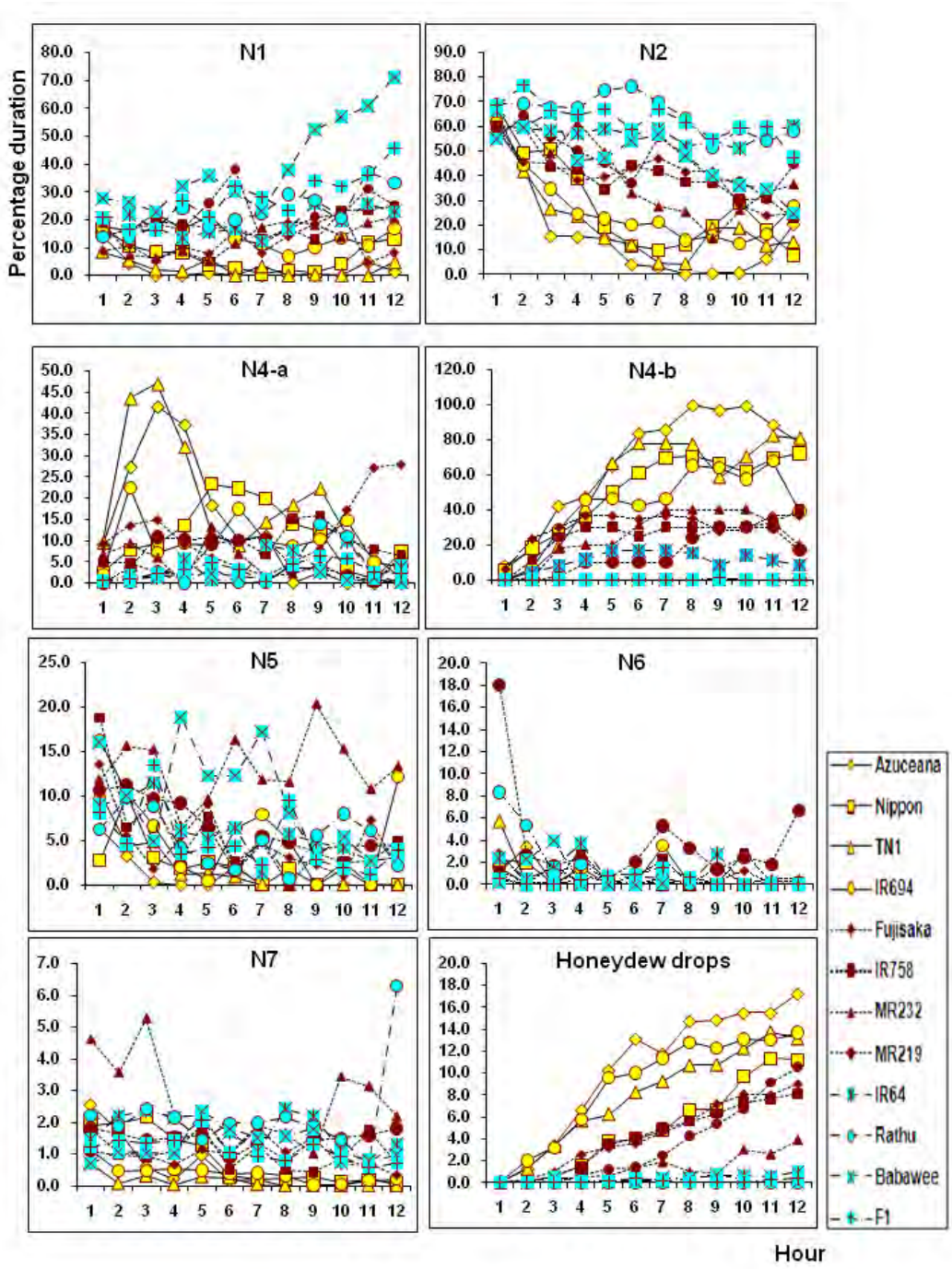


Figure 11 Comparison of EPG waveform and honeydew drop production. The graphs are based on percentage duration for each waveform type, NP (non-penetration), pathway, N4-a (sieve element salivation), N4-b (phloem ingestion), N5 (xylem ingestion), N6 (derailed stylet mechanics) and N7 (potential drop) and honeydew drops for 12 rice varieties. Data were recorded from the first time when BPH made a connection with the plant and then stopped after 12 h

Table 6 Correlation coefficients and significance levels of qualitative EPG and honeydew drop characters among 12 rice accessions

	Pathway	N4-a	N4-b	N5	N6	N7	Average Honeydew drops in 12 h	Total Honeydew drops in 12 h
Non Penetration	0.768** 0.003	−0.700** 0.011	−0.907** <.001	0.607** 0.036	0.007 ns 0.984	0.427 ns 0.166	−0.735** 0.006	−0.719** 0.008
Pathway		−0.693** 0.012	−0.947** <.001	0.450 ns 0.142	0.107 ns 0.740	0.662** 0.019	−0.875** <.001	−0.857** <.001
N4-a			0.663** 0.019	-0.474 ns 0.119	-0.307 ns 0.332	−0.654** 0.021	0.835** <.001	0.833** <.001
N4-b				-0.625** 0.029	-0.037 ns 0.903	−0.629** 0.028	0.837** <.001	0.815** <.001
N5					-0.149 ns 0.645	0.726** 0.008	−0.545* 0.067	−0.519* 0.084
N6						0.131 ns 0.684	-0.096 ns 0.767	-0.09 ns 0.781
N7							−0.774** 0.003	−0.771** 0.003
Average Honeydew drops								0.996** <.001

** = Significant at 1% probability level; * = Significant at 5% probability level; ns = Non-significant

4.4.4 Phloem location

The presence of the salivation waveform (N4-a) indicates the first time the stylets encounter the sieve element. There was no significant difference identified in the time to the first N4-a waveform for BPH across all the rice varieties (Table 7). BPH on Azucena took the shortest time to reach the sieve element of 3.4 h and reached the phloem in a similar time when feeding on Nipponbare, IR694 and TN1. N4-b waveform represents phloem acceptance and successful phloem ingestion. There were significant differences in the time to the first N4-b waveform on the different rice varieties. Based on the frequency of the N4-b waveform, BPH was unable to successfully ingest sieve element sap on Rathu Heenathi and Babawee. The qualitative differences between N4-a and N4-b timings indicate that BPH has a similar ability to locate the sieve element across all varieties, but there is variation in its ability to successfully sustain phloem sap ingestion.

Table 7 Fastest time (h) to N4-a and N4-b waveform patterns within 12-h experiment

Variety	n	N4-a	N4-b
Azucena	7	1.0 ± 0.2	3.4 d (±0.8)
Nipponbare	8	1.2 ± 0.2	3.8 d (± 0.7)
TN1	9	1.8 ± 0.7	4.4 d (± 0.8)
IR694	10	3.4 ± 1.1	5.4 cd (± 1.4)
Fujisaka	11	2.8 ± 1.1	8.1 bc(± 1.6)
IR758	10	3.4 ± 1.1	8.3 bc (± 1.6)
MR232	10	5.2 ± 1.7	8.9 ab (± 1.2)
MR219	10	5.6 ± 1.2	10.1 ab (± 1.0)
IR64	12	5.1 ± 1.3	10.4 ab (± 1.1)
Rathu	8	6.1 ± 1.7	- a
Babawee	10	4.5 ± 1.0	- a
F1	15	4.2 ± 1.0	11.8 a (± 0.2)
Average		3.7 ns ± 0.5	8.2** (±0.9)

Means ± SE within columns followed by the same letters are not significantly different (P > 0.05, Duncan test)
** = Significant at 1% probability level; ns = Non-significant
'-' = no N4-b waveform pattern observed in 12 h

4.4.5 Comparison of duration and frequency of EPG waveforms

The average percentage duration of seven EPG waveforms from BPH on the twelve rice varieties during the final 5 h of the 12-h feeding period was calculated (Table 8). A Kruskal-Wallis nonparametric analysis indicated that all EPG activities varied significantly between the rice varieties except for salivation (N4-a). BPH feeding patterns on Rathu Heenathi and Babawee were markedly different when compared to other varieties. For example, on these two varieties BPH spent around 90% of the time not penetrating (non-penetration - NP) or in pathway. However, no N4-b behaviour was observed. In contrast, BPH feeding on Azucena showed the highest duration (92.5%) of phloem ingestion (N4-b) over this period. Table 9 shows the average frequency of all EPG waveforms in each h over the last 5 h of the experiment. A Kruskal-Wallis nonparametric analysis once again identified that all the EPG characters were highly significantly different amongst the varieties except for N4-a. The table also reveals that

phloem ingestion (N4-b) and derailed stylet mechanics (N6) are highest in TN1, IR694 and Nipponbare.

Table 8 Comparison of different EPG waveform feeding patterns of BPH on different rice varieties for 5 h (8-12h) (percentage duration and standard error)

	N	NP	Pathway	N4-a	N4-b	N5	N6	N7
Azucena	7	1.2e (± 1.0)	5.7c (± 4.1)	0.5 (± 0.5)	92.5a (± 5.1)	0.0c (± 0.0)	0.0c (± 0.0)	0.1ef (± 0.1)
Nipponbare	8	6.4de (± 5.5)	17.2bc (± 8.0)	7.4 (±4.1)	67.9a (± 14.2)	0.9bc (±0.6)	0.0c (± 0.0)	0.2def (± 0.1)
TN1	9	1.1e (± 0.9)	13.2bc (± 8.4)	11.9 (± 5.5)	73.8a (± 8.1)	0.0c (±0.0)	0.0c (± 0.0)	0.0f (±0.0)
IR694	10	11.7cde (± 8.3)	17.2bc (± 6.5)	8.5 (± 5.7)	58.6ab (± 11.7)	3.8ab (± 1.8)	0.0bc (± 0.0)	0.2def (± 0.1)
Fujisaka	11	13.7bcd (± 4.5)	32.0abc (± 8.4)	17.2 (± 7.9)	32.8bcd (± 14.2)	3.1abc (± 1.2)	0.3bc (± 0.2)	0.9abcd (± 0.3)
IR758	10	20.3bcd (±10.5)	31.8abc (± 11.0)	11.2 (±7.4)	32.5bc (± 14.8)	2.8ab (± 1.3)	0.7bc (± 0.6)	0.8bcde (±0.4)
MR232	10	18.3cd (± 9.9)	40.5ab (± 11.5)	2.9 (± 1.9)	34.5bc (± 14.2)	1.5abc (± 0.7)	0.4bc (±0.4)	1.9abcd (± 0.8)
MR219	10	23.2abc (± 7.2)	39.9ab (± 9.0)	1.9 (± 1.2)	26.1cde (± 13.4)	4.4ab (± 1.4)	3.1a (± 1.0)	1.4ab (± 0.4)
IR64	12	22.1abc (± 9.1)	54.9a (± 9.3)	5.3 (± 1.8)	11.4 cde (± 7.6)	4.1ab (± 1.1)	0.6b (± 0.3)	1.6a (± 0.3)
Rathu	8	29.5ab (± 10.6)	57.2a (± 9.3)	6.3 (± 5.3)	0.0e (± 0.0)	4.5a (± 1.3)	0.0c (± 0.0)	2.5ab (±1.5)
Babawee	10	45.2a (± 12.1)	45.5ab (± 10.9)	2.1 (±0.9)	0.0e (±0.0)	5.6ab (± 2.5)	0.1bc (±0.1)	1.5ab (± 0.3)
F1	15	34.2ab (± 8.6)	56.6a (± 7.6)	4.2 (± 1.7)	0.2de (± 0.2)	3.9ab (± 0.8)	0.1bc (± 0.1)	0.9abc (± 0.2)
Average		19.9** (± 3.8)	34.3** (± 5.2)	6.6ns (± 1.4)	35.9** (± 9.0)	2.9** (± 0.5)	0.4** (± 0.3)	1.0** (± 0.2)
Chi-square		42.22	30.56	9.47	57.14	37.76	28.47	38.05
Pr>Chi-square (Kruskal-Wallis P value)		<.0001	0.0013	0.5787	<.0001	<.0001	0.0027	<.0001

Means ± SE within columns followed by the same letters are not significantly different (P > 0.05, Kruskal-Wallis and Duncan test)
** = Significant at 1% probability level; ns = Non-significant

Table 9 Comparison of percentage of time for different EPG waveform feeding patterns of *N. lugens* on different rice varieties for 5 h (8-12 h). (Average percentage frequency and standard error)

	NP	Pathway	N4-a	N4-b	N5	N6	N7
Azucena	5.4 cd (±3.63)	15.3 d (±7.23)	2.597 (±2.59)	72.1 a (±13.36)	0.0 b	0 0c	4.56 d (±2.96)
Babawee	30.3 a (±10.04)	38.7 abc (±4.99)	5.215 (±1.27)	0.0 f	3.13 a (±1.14)	0.12 c (±0.12)	22.49 ab (±4.14)
F1	28.0 a (±7.91)	42.0 ab (±4.53)	4.35 (±1.20)	0.05 ef (±0.05)	3.43 a (±0.75)	0.11 c (±0.11)	21.99 ab (±3.26)
Fujisaka	12.6 abcd (±3.76)	33.8 abc (±6.62)	4.85 (±1.65)	28.22 cde (±13.93)	3.78 a (±1.42)	0.60 bc (±0.52)	16.15 abc (±4.44)
IR64	16.5 abc (±7.66)	44.0 ab (±4.15)	3.71 (±1.54)	2.16 def (±1.51)	5.18 a (±1.94)	1.67 ab (±0.84)	26.83 a (±3.22)
IR694	16.3 abc (±5.13)	32.9 bdc (±5.76)	6.04 (±2.90)	32.20 bc (±12.08)	5.93 a (±2.47)	0.0 c 0	6.67 cd (±3.03)
IR758	19.9 abcd (±10.34)	24.1 bdc (±8.03)	8.28 (±6.55)	33.33 cd (±14.91)	2.21 ab (±1.00)	0.59 bc (±0.52)	11.57 bcd (±4.85)
MR219	14.6 ab (±2.77)	41.3 abc (±3.21)	3.55 (±1.79)	12.19 def (±7.79)	5.31 a (±1.57)	2.83 a (±0.89)	20.22 abc (±3.42)
MR232	16.3 abcd (±7.99)	38.2 abc (±9.15)	2.55 (±1.53)	22.73 cd (±13.02)	2.01 ab (±0.89)	0.42 bc (±0.34)	17.77 abc (±5.09)
Nipponbare	7.4 bcd (±3.43)	24.9 cd (±7.88)	7.41 (±2.84)	51.34 ab (±15.43)	1.75 ab (±0.88)	0.0 c	7.15 cd (±3.35)
Rathu	20.2 a (±4.51)	48.8 a (±3.06)	2.84 (±0.95)	0.0 f	4.56 a (±1.39)	0.0 c	23.62 ab (±4.62)
TN1	2.7 d (±2.10)	26.9 cd (±6.04)	11.77 (±4.24)	58.58 ab (±8.72)	0.0 b	0.0 c	0.0 d
Average	16.78** (±2.05)	35.18** (±1.85)	5.21ns (±0.79)	23.20** (±3.37)	3.26** (±0.41)	0.56** (±0.14)	15.78** (±1.29)
Chi-square	28.01	28.74	9.20	60.42	25.70	37.52	38.83
Pr>Chi-square (Kruskal- Wallis P value)	0.0032	0.0025	0.6034	<.0001	0.0072	<.0001	<.0001

Means ± SE within columns followed by the same letters are not significantly different (P > 0.05, Kruskal-Wallis and Duncan test)

** = Significant at 1% probability level; ns = Non-significant

4.4.6 Cluster analysis

A cluster analysis using Ward‘s method based on Euclidean Distance was performed using 56 activity parameters derived from EPG waveform duration and frequency for the last 5 h of the 12 h feeding period. Fundamentally, this multivariate method involves making pairwise comparisons of all objects (varieties), and then classifying them according to an average linkage method (Ward‘s) and illustrating the object relationships in a dendrogram (Henry et al., 2005). Therefore, the real objective of this analysis is to summarize overall data for classification of resistant and susceptible varieties. Total and average honeydew data for the same last 5 h of feeding were also included in the analysis (Table 10). The resulting dendrogram (Figure 12) divided the 12 rice varieties into three main groups at a 0.15 semi-partial R square value. Group 1 included Azucena, TN1, Nipponbare and IR694. This group showed the greatest distance from the other two groups, namely group 2 - Fujisaka, IR758, MR219 and MR232, while Rathu Heenathi, IR64, Babawee and F1 formed a third group.

In univariate analysis (Table 10), 38 out of 56 feeding activity parameters showed highly significant differences between varieties. These characters mostly related to non-penetration, pathway, N4-b and honeydew drop, and the resistance versus susceptibility could clearly be distinguished for all 12 varieties. Further analysis of the common characteristics of the three groups identified by cluster analysis demonstrated that resistance was associated with high percentage duration of NP, pathway, N5, N6 and N7 EPG waveform characters (Figure 13). In contrast, the susceptible group was associated with the longest duration of N4-b (phloem ingestion). However, N4-a (sieve element salivation) pattern waveform did not statistically differentiate between those groups. The

overview of EPG waveform shapes for the first 6 h shown in figure 14 provided a better picture for an easy comparison between resistant and susceptible rice varieties.

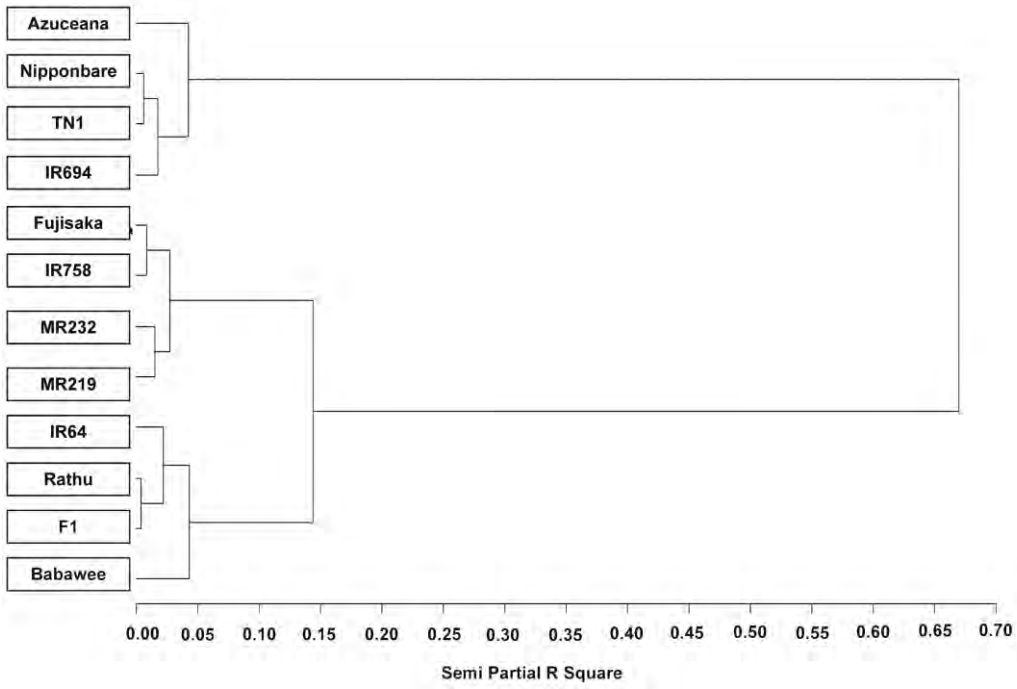


Figure 12 Dendrogram derived using Ward's clustering on 56 characters (SAS, 2008). Twelve rice varieties have been divided into three different groups, namely susceptible group 1(Azucena, TN1, Nipponbare and IR694), moderately resistant group 2 (Fujisaka, IR758, MR232 and MR219) and strongly resistant group 3 (Rathu, IR64, Babawee and F1)

Table 10. List of 56 characters used for cluster analysis and their significance levels from univariate test statistics using CANDISC procedure (SAS software)

No	Characters	Significance level (pr>F)
1	NP (average in 5 h)	0.0016
2	Pathway (average in 5 h)	<.0001
3	N4-a (average in 5 h)	0.5787
4	N4-b (average in 5 h)	<.0001
5	N5 (average in 5 h)	0.0184
6	N6 (average in 5 h)	0.1505
7	N7 (average in 5 h)	0.0045
8	Average honeydew droplets in each h	<.0001
9	Total honeydew droplets (average in 5 h)	<.0001
10	Percentage frequency NP 1 (average in 5 h)	0.0080
11	Percentage frequency Pathway (average in 5 h)	0.0103
12	Percentage frequency N4-a (average in 5 h)	0.3261
13	Percentage frequency N4-b (average in 5 h)	0.0003
14	Percentage frequency N5 (average in 5 h)	0.3225
15	Percentage frequency N6 (average in 5 h)	0.2106
16	Percentage frequency N7 (average in 5 h)	<.0001
17	NP (average in 8^{th} h)	0.0006
18	NP (average in 9^{th} h)	0.0010
19	NP (average in 10^{th} h)	0.0179
20	NP (average in 11^{th} h)	0.0073
21	NP (average in 12^{th} h)	0.0162
22	Pathway (average in 8^{th} h)	<.0001
23	Pathway (average in 9^{th} h)	0.0017
24	Pathway (average in 10^{th} h)	0.0017
25	Pathway (average in 11^{th} h)	0.0002
26	Pathway (average in 12^{th} h)	0.0201
27	N4-a (average in 8^{th} h)	0.4602
28	N4-a (average in 9^{th} h)	0.5107
29	N4-a (average in 10^{th} h)	0.9851
30	N4-a (average in 11^{th} h)	0.3991
31	N4-a (average in 12^{th} h)	0.2513
32	N4-b (average in 8^{th} h)	<.0001
33	N4-b (average in 9^{th} h)	<.0001
34	N4-b (average in 10^{th} h)	<.0001
35	N4-b (average in 11^{th} h)	<.0001
36	N4-b (average in 12^{th} h)	0.0002
37	N5 (average in 8^{th} h)	0.3108
38	N5 (average in 9^{th} h)	0.1659
39	N5 (average in 10^{th} h)	0.2672
40	N5 (average in 11^{th} h)	0.0139
41	N5 (average in 12^{th} h)	0.5633
42	N6 (average in 8^{th} h)	0.4917
43	N6 (average in 9^{th} h)	0.4497
44	N6 (average in 10^{th} h)	0.0187
45	N6 (average in 11^{th} h)	0.1411
46	N6 (average in 12^{th} h)	0.3181
47	N7 (average in 8^{th} h)	0.0053
48	N7 (average in 9^{th} h)	0.0004
49	N7 (average in 10^{th} h)	0.0409
50	N7 (average in 11^{th} h)	0.0106
51	N7 (average in 12^{th} h)	0.2014
52	Honeydew drop (average in 8^{th} h)	0.0003
53	Honeydew drop (average in 9^{th} h)	0.0008
54	Honeydew drop (average in 10^{th} h)	<.0001
55	Honeydew drop (average in 11^{th} h)	<.0001
56	Honeydew drop (average in 12^{th} h)	<.0001

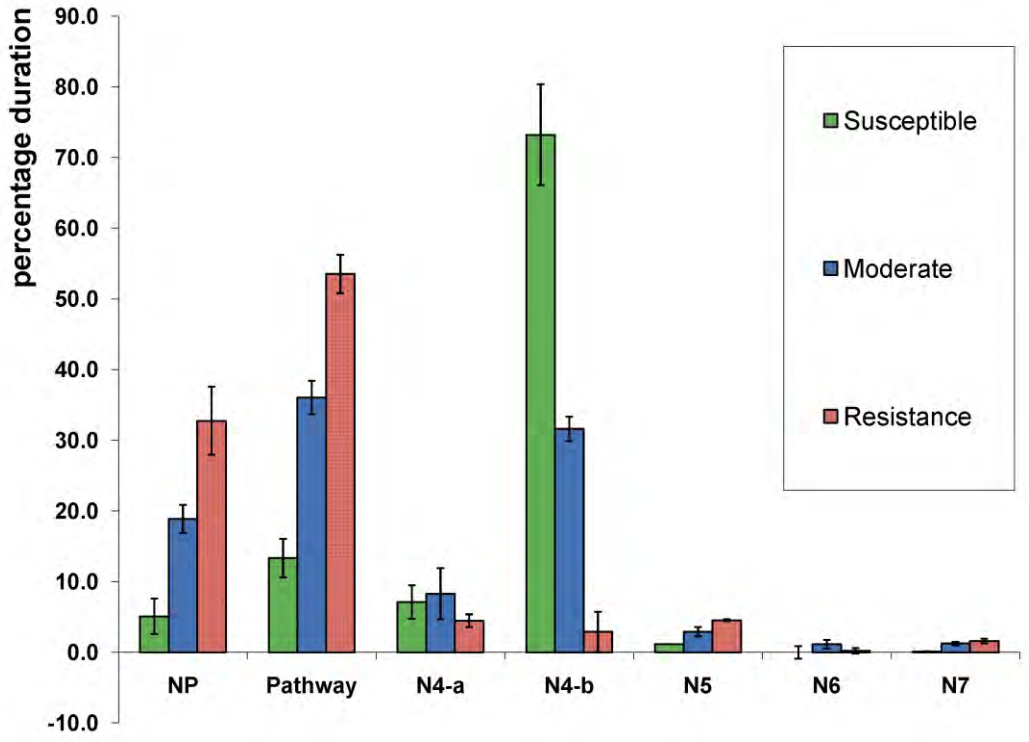


Figure 13 Average percentage duration of 7 types of EPG waveform. The histogram is based on 8 to 12 h (5 h) recording and follows the susceptible, moderate and resistant groups produced by the cluster analysis

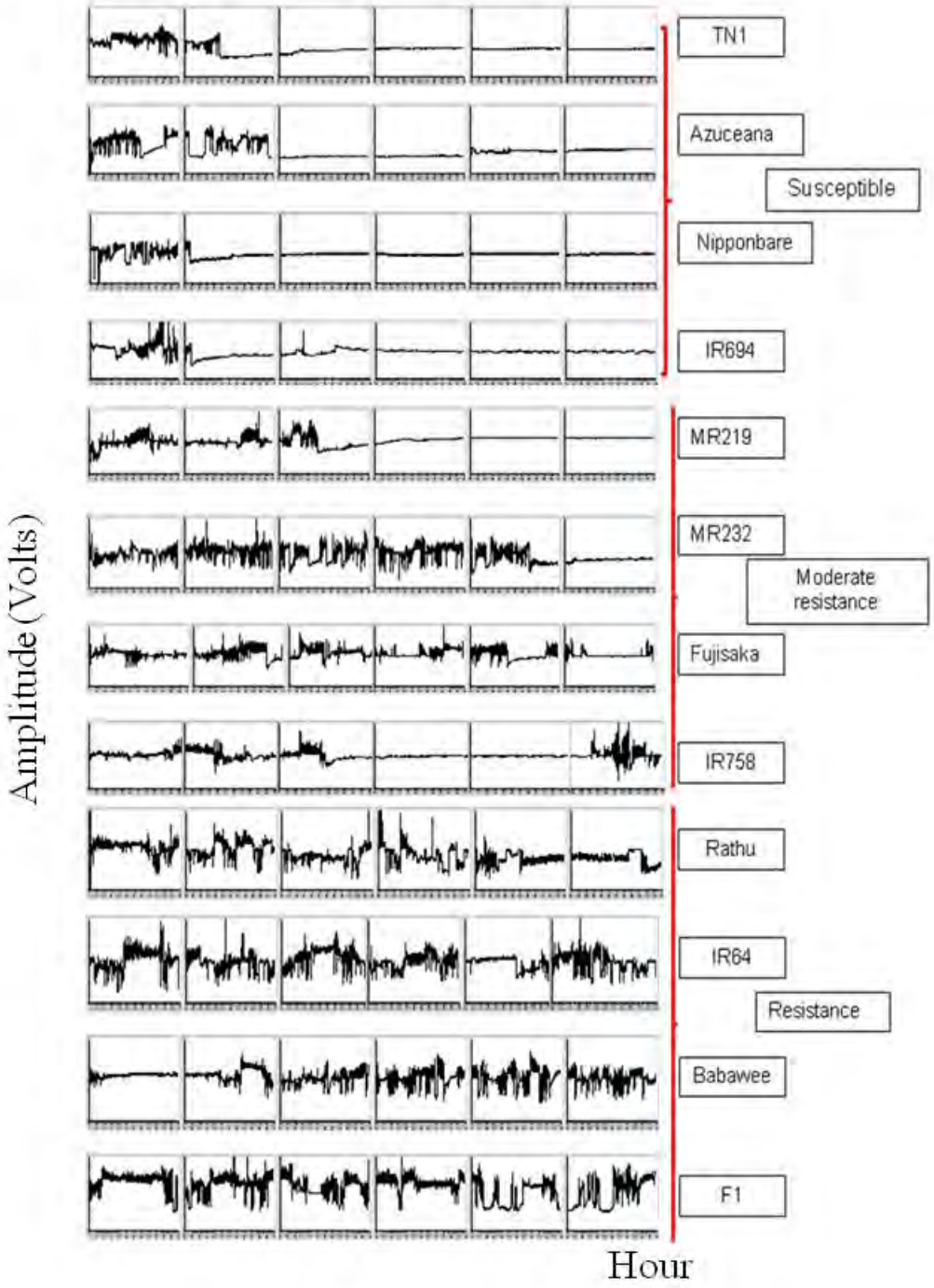


Figure 14 Overview of EPG waveforms found in 12 rice varieties over 6 h. Type 3 and type 4 waveforms are more likely to occur in susceptible rice varieties than moderate and resistant varieties. Susceptible varieties are also less interrupted by other types of EPG waveforms

4.5 Discussion

The electrical penetration graph (EPG) technique is a proven effective tool which allows monitoring of stylet activity and tip position of pierce-sucking insects during plant penetration in their feeding (Sarria et al., 2009). This study has succesfully characterized BPH feeding behaviour using a EPG DC-based electrical penetration graph (EPG), and utilised this to screen 12 rice varieties of differing resistance, facilitating the efficient and detailed classification of rice germplasm for insect resistance.

Seven typical waveforms for BPH were identified, more or less consistent with those previously described for BPH using DC-based EPG (Kimmins, 1989; Lösel and Goodman, 1993; Seo et al., 2009). The most recent histological study of Seo et al. (2009) related to EPG and BPH stylet penetration has provided valuable and detailed information regarding waveform classification. Therefore, their descriptions were chosen as the main guide for EPG characterization. Generally, the sequence of BPH feeding process always starts with non-penetration (NP), and NP is the easiest waveform to describe. A straight line waveform indicates that no feeding activities are happening, or that the stylet has still not inserted itself into the plant. The second waveform, however, was complicated because it produced a variation of frequency, amplitude, voltage level and shape of waveform. Kimmins (1989) classified this waveform into two phases, P2 and P3, while Seo et al. (2009) separated it into three types, N1 (penetration initiation), N2 (salivation and stylet movement) and N3 (extracellular activities). This irregular waveform pattern happens within epidermal and mesophyll cell membranes (Lösel and Goodman, 1993) in the pathway to the phloem, which is one reason used to justify classifying these waveforms as one type, pathway. This gave more confidence in the EPG

classification, focussing only on the objective priority and was experimentally more time-efficient. The N4-a and N4-b were relatively simple to identify because their waveform patterns were consistent with those previously described by Seo et al. (2009). N4-a always occurred just before N4-b appeared. Seo et al. (2009) determined that at this stage the BPH stylet tip was already located in the phloem region but no sap was actually ingested and went on to claim that N4-a is related to intracellular activity in the phloem region on the basis of the different signal amplitude and frequency (Figure 10A) compared to pathway. This phase is close in character to the E1 waveform type (sieve element salivation phase) in aphid studies (Tjallingii, 1985), which was also described on the basis of stylet position, level of voltage, waveform shape and absence of honeydew drops (Seo et al., 2009). By contrast, the duration of N4-b showed a critical difference to the N4-a waveform, being generally sustained over long periods. Associated with N4-b, honeydew drops were produced, providing strong evidence that BPH were ingesting phloem sap at this time.

The other three waveforms, N5, N6 and N7 appeared irregularly from time to time during the pathway period. N5 waveform is similar to P5 as described by Kimmins (1989) and type II waveform as described by Lösel and Goodman (1993). These authors suggest that this waveform is associated with xylem ingestion (cited in Seo et al., 2009). A waveform, N6, not described by other authors has been noted: this waveform pattern appears similar to N5 but with a much higher repetition and frequency and an inconsistent shape. Accordingly, N6 has been classified as ‗derailed stylet mechanics' on the basis of its similarity to the waveform described for aphids (Tjallingii, 1978), and it have been associated it with penetration difficulty. Kimmins (1989) suggests that the

BPH stylet does not puncture cell membranes during the pathway phase leading to the absence of the characteristic cell penetrations of pathway phase in aphid studies. However, in the present study apparent cell penetrations (N7) could be clearly identified (Figure 10D). The discrepancy between the two studies may be attributed to the low input impedence of the EPG amplifier in the previous study (Lösel and Goodman, 1993).

BPH feeding can be divided into two main categories based on the EPG waveforms (Hattori, 2001). The first represents non-ingestion activities, beginning when the BPH first touches the plant, followed by the movement of the stylet tip into the plant through the cell wall, epidermal and mesophyll cell membranes until the stylet reaches the phloem region. EPG waveforms NP, Pathway, N5, N6 and N7 are included in the first category. In the second category, EPG N4-a and N4-b waveforms are included as ingestion activities. Correlation analysis based on the full 12-h feeding period presented in table 6 indicates a strong relation between these two categories. There was a high positive correlation between N4-a, N4-b and honeydew production, but a high negative correlation with NP, pathway, N5, N6 and N7 EPG waveforms. Therefore, a higher proportion of time in the first waveform category is consistent with higher plant resistance to BPH, while more time spent in category two is associated with susceptibility.

In most rice varieties, the total duration of pathway phase decreased after 3 to 4 h and then remained constant over the remaining 8 h. The average times in all 12 rice varieties for BPH to reach N4-b waveform, and then to start to produce honeydew were 8.2 h and 7.7 h respectively.

To focus on varietal differences in category 2 activities, comparisons between the rice varieties were made in the last 5 h of feeding (8-12 h). Using this subset of data, the evaluation of plant resistance in the twelve rice varieties was different to previous reports where data were included from hour zero as in Seo et al. (2009), Hattori (2001) and Kimmins (1989).

BPH clearly responds differently to different rice varieties, spending more than 80% of its time exhibiting the non-ingestion waveform types such as non-penetration or pathway in the varieties (Rathu Heenathi, Babawee and IR64) previously identified as resistant by Brar et al. (2009). A similar result of plant resistance characterization based on EPG was also found by previous researchers using other varieties such as IR56 (Velusamy and Heinrichs, 1986), ASD7 (Khan and Saxena, 1988) and IR 62 (Kimmins, 1989). However, in susceptible varieties such as TN1 (commonly used as a control variety in many BPH experiments), BPH ingested phloem sap for a long period without interruption. Consequently, a longer duration for N4-b waveform could easily be found. Interestingly, N4-a salivation activity for the last 5 h period was not significantly different between the resistant and susceptible rice varieties, indicating that BPH could reach the sieve element region in both resistant and susceptible, but could only ingest the phloem sap in susceptible genotypes. These results support the suggestion of Hattori (2001) that resistance to BPH is determined by phloem-related characters. Phloem-based resistance may have its basis in phloem chemistry (Sogawa, 1982; Chen, 2009), where salicic, oxalic (Chen, 2009: Yoshihara et al., 1979a; Yoshihara et al., 1979b; Yoshihara et al., 1980) and phenolic acids (Chen, 2009; Fisk, 1980), sterols (Shigematsu et al., 1982) and apigenin-C-glycosides (Stevenson et al., 1996; Grayer et al., 1994) have been

implicated in resistance to BPH. The low level of essential amino acids in the phloem could influence BPH feeding (Sogawa, 1982) perhaps representing phago-stimulatory cues. The interaction of plants and herbivorous insects is complex (Thompson and Goggin, 2006) and still not well understood, and further advances in understanding may require molecular approaches (Thompson and Goggin, 2006).

A clear picture of plant resistance based on EPG waveform and honeydew drop data has been presented using cluster analysis. The twelve rice varieties could be classified into three groups. Group 1 was classified as the susceptible group because the average percentage duration of N4-b EPG waveform (category 2) was found to be the highest. In contrast, EPG waveform NP, pathway, N5 and N6 of group 1 showed the lowest values. These results clearly indicate that BPH could easily feed on the phloem sap in this group. As expected, the common control rice variety TN1 was classified in this group 1. The other three varieties in the susceptible group are Azucena, Nipponbare and IR694. Groups 2 and 3 have a much closer relationship, but with group 3 being more resistant than group 2. Consistent with this, the varieties in this group have previously been found to contain the resistance genes *Bph1* in IR64 (Cohen et al., 1997), *bph4* in Babawee, and *Bph3* in Rathu Heenathi (Nemoto et al., 1989; Khush et al., 1985) and the F1 (from the cross between Rathu Heenathi and TN1). BPH spent more time in the non-feeding phase whether in NP, pathway or occasionally in N5 waveform (xylem), possibly to overcome dehydration (Daniels et al., 2009). This result was found to be slightly different to that of Cohen et al. (1997). Although IR64 was classified as resistant, its values for N1+N2+N5 and N6 were the lowest in that group. In addition, the experiment was conducted under full environment control (temperature and relative humidity), which

greatly influenced BPH behaviour (Park, 1983). Furthermore, the classification of Cohen et al. (1997) covered a greater number of parameters including fecundity, nymph survival, feeding rate and an antixenosis test. The parameters were more specific to BPH feeding ability with the limitation of the 12-h period.

The moderately resistant group 2 contained another four varieties, namely MR232, MR219, Fujisaka and IR758. There is very limited information available on their genetic backgrounds, but they are all products of a long history of breeding, with ancestors a likely source of some resistance genes contributing to their moderate resistance, and at least one of the parents of MR219 and MR232 is known to have possessed insect resistance (Habibudin 2009, pers. comm., 21 Nov; Alias et al., 2001).

This study has provided new information on the mechanism of plant resistance to BPH on 12 rice varieties. The results confirmed and extended previous research using the EPG method to quantify BPH feeding behaviour on rice, and allowed the twelve rice varieties to be unequivocally divided into three groups: susceptible, moderately resistant and highly resistant. This study has further demonstrated that BPH has the ability to locate the sieve elements of the different varieties, but there is variation in its ability to begin phloem sap ingestion, thus providing a potential explanation for resistance in these varieties. Future work should focus on the underlying mechanisms at the molecular level. The relatively rapid and inexpensive method of screening germplasm used here can be utilized to identify in genetic resources‘ collections natural sources of genetic variation conferring resistance to BPH in rice, and almost certainly for other pest/crop combinations as well. A firm platform for further genomic and transcriptomics studies to reveal candidate genes for resistance has also now been established.

CHAPTER 5

MICROARRAY ANALYSIS OF RESISTANCE AND SUSCEPTIBILITY TO BROWN PLANTHOPPER (*N. lugens*) IN RICE GERMPLASM

5.1 Introduction

Rice (*Oryza sativa*) is the world's most important staple cereal crop, responsible for providing the main source of food for over half the world's population. The way in which rice is produced, however, has remained static over the past few years causing a shortage of supply to meet the increasing demand led by population growth. One of the most important constraints in achieving higher rice production is losses caused by pests, of which the brown planthopper (BPH) is the most dangerous phloem feeding insect, producing a major problem for the rice industries.

The use of molecular methods to overcome many aspects of agriculture problems has become increasingly important and commonly used in the present day. Plant breeding is one area which has highly benefited from this technology. Molecular techniques can help answer many questions in plant and insect interactions, very complex mechanisms

which are difficult to explain through conventional approaches. For example, although more than 22 genes loci related to BPH resistance in rice have already been identified (Santhanalakshmi. et al., 2010), not all can be used to protect the rice plant from BPH attack (Jairin et al., 2007). It has been reported in several cases that *Bph1* and *bph2* genes have their resistance broken down by BPH biotypes 2 and 3 (Gallun and Khush, 1980; Pathak and Saxena, 1980; Panda and Khush, 1995; Xu et al., 2002). This breakdown process clearly shows that resistance characters are controlled by single genes of major effect rather than polygenic control, which is more durable and viable for insect pest management (Sogawa et al., 2003).

Breeding programmes for quantitative traits with these polygenic insect resistance characters have again still remained poorly understood because they are very difficult to characterize genetically (Xu et al., 2002). These problems can be overcome through molecular marker technologies based on mapping and tagging techniques for quantitative trait locus (QTL) analysis (Sogawa et al., 2003). For example, Alam and Cohen (1998) have identified a total of seven QTLs associated with BPH resistance, located on chromosomes 6 and 12 using double haploid populations of an indica variety, IR64 and a japonica variety, Azucena cross. Phenotypic variance for individual QTLs accounted for between 5.1% and 16.6 %, and most of them were derived from resistant variety IR64. In another study involving a cross between Rathu Heenati and KDML105, the resistant *Bph3* locus was found to be localized on chromosome 6 approximately in a 190 kb interval flanked by the SSR markers RM19291 and RM8072 (Jairin et al., 2007). Other genes from different populations of crosses between Rathu heenati and IR50, which were also associated with BPH resistance characters, were identified on chromosome 3 by

different SSR markers, namely RM186 and RM168 (Gomathi, 2002; Kumari et al., 2010). From the genetic linkage map and QTL analysis in another study involving two F2 populations from crosses between Babawee and TN1, and Babawee and KDML105, the *bph4* locus was found mapped at the same chromosome region as *Bph3* between two flanking markers, RM589 and RM586 (Jairin et al., 2010).

In this study, a different approach using microarrays was chosen allowing for the investigation of thousands genes at one time. This approach is basically based on the level of gene expression, in this case, a gene which influences the resistance level in certain rice varieties from BPH attack. Zhang et al. (2004) in their experiment using Northern blot and cDNA array analysis discovered 14 genes from resistant variety B5 and 44 genes from susceptible MH63. They also found that those genes were mostly grouped under the categories of signalling pathways, oxidative stress/apoptosis, wound-response, drought-inducible and pathogen-related proteins. These classification categories based on gene annotation and function clearly facilitated the understanding of the actual mechanism of plant resistance, especially when phenotype information was also included.

In the experiments, resistance was not induced in the plants by exposing them to BPH. Constitutive resistance genes which have often been overlooked in terms of their importance to breeding programmes were focussed on. Constitutive genes sometimes can be described as housekeeping genes which are needed for the normal function of the cell and probably could dramatically affect the global dynamics of gene expression (Hallinan et al., 2006) although in certain cases they are relatively small in number (Lercher et al., 2002; Hallinan et al., 2006). For instance, a constitutive expression of kinase was found

to trigger a number of cell functions which increased the rate of mitosis and protected the cell from programmed cell death (Marley and Gordon, 2005; Hallinan et al., 2006). Unlike inducible genes, these constitutive genes have no ability to change easily in a short period when they have been exposed to certain stress such as from herbivore attack (Karban and Myers, 1998). Generally, this constitutive mechanism occurs before the attack starts, and they act as a first layer of defence. Sometimes, inducible genes cannot function on its own without the assistance of other constitutive characters. This claim is supported by Agrawal et al. (1999). They found that plant genotypes lacking constitutive expression of cucurbitacins were highly susceptible to mite attack in cucumber crop. In another study by Domingo et al. (2009), the constitutive expression of OsGH3.1 was found to enhance resistance to fungal pathogen in rice when the auxin content was down regulated. Another example is *Bph14*, which is claimed by Du et al. (2009) to be a constitutive resistance gene expressed in leaf sheaths, leaf blades, and roots. This gene encodes a coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) protein, which activates a salicylic acid signal pathway and causes reduced feeding activity and growth rate of the BPH (Du et al., 2009). At the present time, this is the only *Bph* resistance gene which has successfully been cloned and originally came from the wild rice species, *Oryza officinalis*.

5.2 Overall research objectives

This present study is the first to use the biggest number of rice varieties with different known BPH resistance backgrounds. The aim was to identify candidate genes which are commonly found and constitutively expressed under normal conditions using the Agilent

44K oligonucleotide array. Rice genes can be grouped, annotated and characterized according to expression patterns. Furthermore, the potential of candidate resistance genes in rice is discussed and the applicability of novel genes to crop improvement programmes is proposed.

5.3 Materials and methods

5.3.1 Minimum Information about a Microarray Experiment (MIAME)

Minimum Information about a Microarray Experiment (MIAME) is a standardized procedure for performing microarray experiments. This important step was initiated by the Microarray Gene Expression Database group (MGED; http://www.mged.org), which requires the minimum information needed to be provided along with the results. The aim of the guidelines is to make sure that data presented can be described for better understanding and ease of accessibility. That means the main purpose of these guidelines is to generate and establish a public database for others to use for reference. Among the important contents in MIAME documents are sample and array design description, control elements, experimental design, hybridization procedures and measurements (Brazma et al., 2001). A simple outline structure of these is given in Appendix A.

5.3.2 Plant material and experimental design

The same twelve rice varieties as in chapter 4 were used again in this microarray analysis. However, the plants were divided into three groups, namely susceptible, moderately resistant and resistant. Each group contained four varieties based on the results of cluster analysis in previous experiments. This is the main reason why only two replicates were

used in this experiment design. The seed germination process also followed that in chapter 4. The seeds were germinated on filter paper in Petri dishes and transferred to 5 cm diameter pots with soil after 1 week‘s germination. The plants were then grown under a net structure (60 x 60 x 60 cm) at a temperature of 23-26% and 16L: 8D photoperiod. Each net structure represented a replicate. The plants were maintained until 7 weeks old in the absence of BPH.

5.3.3. Tissue sampling

Tissue samples were collected from plants at 42 days. Only stems 2-3 cm above the main root or soil with size around 0.5 cm thick were used. In order to homogenize the sample, five stems from five rice plants represented one treatment and one replicate. All five stem tissues were mixed immediately in 2 ml microcentrifuge tubes with liquid nitrogen and then kept at -70 ^{0}C.

5.3.4 RNA extraction

RNA extractions in this experiment were conducted using a QIAGEN RNeasy Mini plant kit and followed the manufacturer‘s instructions. 100mg stem tissue samples were placed in liquid nitrogen and immediately ground thoroughly with a mortar and pestle. The tissue powder was decanted into liquid nitrogen cooled RNase free 2ml microcentrifuge tubes, and the liquid nitrogen allowed to evaporate during tissue thawing. 450 µl Buffer RLT were added to the sample tube and immediately vortexed vigorously. The lysate was transferred into a QIA shredder spin column (lilac) placed in a 2 ml collection tube and centrifuged for 2 m at full speed. The supernatant was then transferred carefully to fresh

2ml tubes. 0.5 volume of ethanol (96–100%) was added and mixed by pipetting in order to clear the lysate. The sample (usually 650 µl) was then transferred to an RNeasy spin column (pink) placed in a 2 ml collection tube and centrifuged at 8000 x g (10000 rpm) for 15 s. The flow-through was discarded and 700 µl Buffer RW1 were added to the RNeasy spin column before centrifuging at the same speed and period, 10,000 rpm for 15 s to wash the spin column membrane. The flow-through was discarded again, and the membrane was further washed with 500ul of RPE buffer and was then centrifuged at 10000rpm for 15 s. After discarding the flow-through, the same process was repeated, but this time the centrifuge step was for 2 min. After discarding the flow-through, the RNeasy spin column was placed in a new 1.5 ml collection tube. Finally, 30ul of RNase free water was added to the column and centrifuged for 1 min at full speed. RNA was then immediately stored in a freezer at -70 ^{0}C for further use.

5.3.5 RNA quantification and integrity checking

RNA integrity needed to be quantified before it could be used for microarray analysis. In this experiment, concentrations were determined through two different methods as below.

5.3.5.1 RNA quantification

The total RNA extracted from rice stem samples was quantified by a NanoDrop spectrophotometer (ND-1000 VIS) version 3.2.1. The sample loading area (the receptacle laser cell) needed to be cleaned with RNase free water before measurement. For each RNA sample, first a blank was run in order to reduce error. A single drop of 1 µl was taken from the sample and was loaded onto the receptacle laser cell. Then, it was scanned

by software and the information of the RNA concentration (hg/µl) was provided through the absorbance ratio 260/280 and 260/230. Only a sample with ratios around 2.0 was selected for further analysis. Protein, phenol or alcohol contaminations were related with the 260/280. The 260/230 ratio indicated the presence of genomic DNA.

5.3.5.2 Quality assessment

RNA template quality needed to be assessed before microarray analysis proceeded. It is an important step to make sure that RNA is not degraded during the extraction process which could cause multiple errors in the results. In this experiment, the RNA integrity was detected by the Agilent 2100 bioanalyser using an RNA 6000 Nanochip kit, and all procedures followed the manufacturer‘s guidelines. The compartments of the gel chip (bioanalyser electrodes) were cleaned by filling them with sterile water (300 µl) and vortexing the chip for 10 s. Nine µl of the pre-stained gel (bioanalyzer) were then injected into the G well (black circle) using a specific syringe. The process was continued with another injection in the two G wells of 9 µl each as well as the prepared ladder in its designated well. The marker was then loaded into the same well followed by the RNA sample. Each chip contained 12 sample wells to measure. Finally, the loaded chip was vortexed for 1 min before analysis by the bioanalyzer software for 30 min. The results were read through electrograms based on two distinct peaks representing 18s and 28s ribosomal RNA along with RIN (RNA integrity number). According to Agilent, RIN values above 7.0 are ideal for microarray analysis.

5.3.6 Microarray analysis

5.3.6.1 Design of microarray experiments using one colour method

One colour rice 4x 44K microarray RAP-DB (Agilent Technologies; part number: G2519F and dsign ID: 15241) microarrays were used in this analysis. These represent 43,803 rice genes with one 60-mer oligonucleotide probe representing each sequence. The content sources came from the National Institute of Agrobiological Sciences, RefSeq, GenBank 2007, RAP-DB (Agilent Technologies). The experiment design is a very important step in producing accurate results with minimum error. Six microarray slides were ordered and each of them contained 4 arrays as shown in figure 15 containing all three resistance groups (12 varieties) with 2 replicates. Replicates for each variety were on separate slides and positioned randomly.

Microarray single colour slide design (6 x 4 x 44K)

Plate 1		**Plate 4**	
R1V1	R1V8	R2V3	R2V4
R1V5	R1V12	R2V7	R2V10
Plate 2		**Plate 5**	
R1V3	R1V4	R2V5	R2V6
R1V9	R1V10	R2V11	R2V8
Plate 3		**Plate 6**	
R1V7	R1V2	R2V1	R2V2
R1V11	R1V6	R2V9	R2V12

Resistant Group:	**Susceptible**	**Moderate**	**Resistance**
	V1: TN1	V5: MR219	V9: Rathu Heenathi
	V2: Azusena	V6: MR232	V10: Babawee
	V3: Nipponbare	V7: IR758	V11: IR64
	V4: IR694	V8: Fujisaka	V12: F1
Replicate:	Replicate 1: R1	Replicate 2: R2	

Figure 15 Experiment design for one colour microarray analysis. Layout of 12 rice varieties based on their resistance group with two replicates

5.3.6.2 Sample preparation and hybridization

All standard procedures and guidelines described in this section refer to the Agilent Manual part No. G4140-90040, edition version 5.7, March 2008 on their formal website source (http://www.agilent.com). Figure 16 below shows the flowchart of microarray analysis which can be divided into 4 main steps, namely sample preparation, hybridization, microarray wash and scanning.

There are another four sub-steps in the sample preparation which are the preparation of One-Colour Spike-Mix, labeling reaction, purifying the labeled/amplified RNA and finally quantifying the cRNA (complementary RNA) generated using Agilent's Quick Amp labelling kit-One colour (5190-0442) from the total RNA.

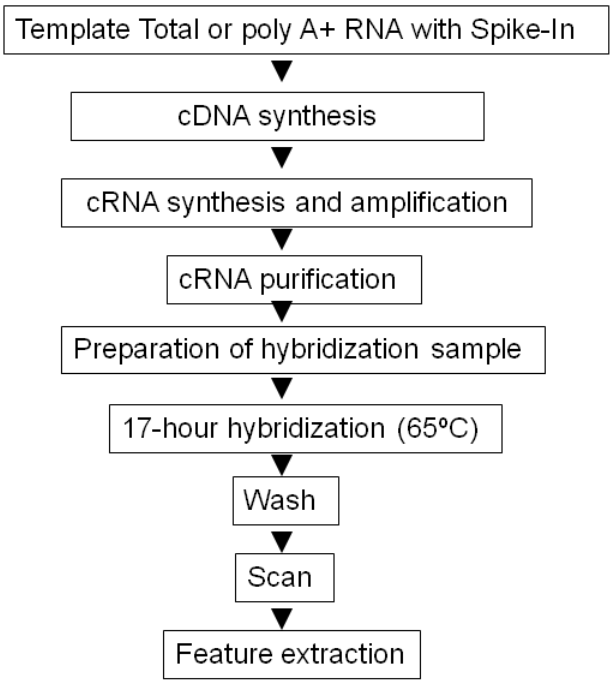


Figure 16 Microarray workflow for sample preparation and array processing

5.3.6.2.1 Preparation labeling reaction

A total of 300 ng RNA were added to the labeling reaction mix containing T7 promoter primer (1.2 ul), spike mix (3.0ul), and RNase free water, making a final volume of 11.5ul. Both primer and template RNA were then denatured in an incubator for 10 min at a temperature of 65^0 C before being placed on ice for another 5 min. The cDNA Master Mix (8.5ul) containing 5x buffer (4ul), DTT (2ul), dNTP's (1ul), mmLV-RT (1ul), RNase out (0.5 ul) was added to the reaction mix prepared for all samples, and they were mixed by flicking and spinning down. These samples were again incubated for 2 h at 40^0 C and were then placed on ice for 5 min to stop the reaction. The transcription master mix (60 ul) containing RNase free water (15.3ul), 4x buffers (20.0ul), DTT (6.0ul), NTP's (8.0ul), preheated 50% PEG (6.4ul), RNase out (0.5ul), inorganic pyrophosphatase (0.6ul), T7 RNA polymerase (0.8ul), and Cy3 dye(2.4ul) was added to each sample. The samples were again mixed by flicking and spinning down before incubating at 40^0 C for another 2 h. The process was continued by labeling the cRNA synthesised samples with Cy3 dye. They were then cleaned following an RNeasy clean up procedure. NanoDrop software was used to quantify the yield of cRNA and Cy3 incorporation in each sample.

5.3.6.2.2 Hybridizations

The blocking reagent was prepared by adding the indicated amount of RNase free water. Then, it was mixed by gently vortexing and spinning down. In a further step, the hybridization sample was prepared using a mixture of cRNA (1.65ug), 10 x blocks (11.0ul) and fragmentation buffer (2.2ul). RNase free water was added to make up the final volume of 55 ul. The samples were incubated at 60^0 C for exactly 30 min to

fragment RNA. Next, 55 ul of 2x GE hybridization buffer were added to stop the fragmentation reaction and were mixed gently by pipetting to avoid introducing bubbles A quick spin was conducted before placing the sample on ice. A clean gasket slide was placed in assembly with the Agilent label facing up and aligned with the rectangular section of the chamber base. 100ul of the sample per array were loaded onto the slide by pipetting gently to avoid bubbles. After that, an array –active side" down was slowly placed onto the SureHyb gasket slide. The –Agilent"-labeled barcode was facing down and the numeric barcode was facing up (Agilent labeled side = Active side). The chamber covers were placed onto the sandwiched slides, and the clamp assembly was slid onto both pieces. The assembled chamber was incubated for 17 h at 65^0 C in an Agilent rotisserie. The wash buffer 2 and staining vessels were prepared and kept at 37^0 C overnight for further use.

5.3.6.2.3 Microarray wash

Two staining troughs with wash buffer 1 at room temperature were prepared for two purposes, the first to disassemble the slide and the other one on the stirrer to wash the slide. All the slides in the assembled chamber were disassembled under wash buffer 1 and were placed in a rack in the stirring wash buffer 1. All the slides were stirred for 1 min in wash buffer 1, after which they were opened. Wash buffer 2 was then poured into the pre-warmed staining trough and stirred. Another slide wash process was performed in wash buffer 2 for just 1 min. The slides were then removed and were washed again for 10 s in 100% acetonitrile, which was followed by another wash for 30 s in a stabilising and drying solution. The slides were slowly removed from the stabilising and drying solution

to allow the reagent to dry off without leaving ―water marks" on them and placed in a slide box.

5.3.6.3 Scanning and feature extraction

The slides were assembled into an appropriate slide holder with the numeric barcode visible and were placed into the scanner carousel. The scan setting was verified for one colour scan and set for automatic file name. In the following step, click Scan **Slot m-n on** the scan control main window (where the letter **m** represents the Start slot where the first slide is located and the letter **n** represents the End slot where the last slide is located) was clicked when the scanner was ready. In this analysis, Gene Pix scanner setting 4000B was supported by Agilent gene expression microarray. Data were acquired by using Agilent feature extraction software version 9.5.3, and the resulting text files were loaded into the Agilent GeneSpring GX software (version 10.0) for further analysis.

.

5.3.7 Statistical analysis

Data from the scanner software were further analyzed using GeneSpring GX10.0 (Agilent). The rice probes were annotated manually through the NCBI website. A quantile method was used for normalization across all arrays. The data were then filtered according to GeneSpring GX 10.0 (Agilent Technologies, Palo Alto, CA, USA) starting with filtering by expression, by flags, by data and finally by error (% CV). One-way ANOVA at $p<0.05$ was used for statistical analysis and then was followed by Volcano plot analysis with Benjamini and Hochberg multiple testing correction. Significant candidate genes were selected based on fold expression differences with a minimum

value of 2.0 and a significance value of at least 0.05. Further evaluation was then conducted only on genes showing significant differences in expression between resistant and susceptible groups, and moderately resistant and susceptible. In order to evaluate the pattern of relationship between gene expression in the plant and BPH behaviour, linear correlation analyses were performed between the microarray expression value and the morphological data from chapter 4. Five characters were chosen, pathway, phloem ingestion, xylem ingestion, total honeydew production and time to first phloem ingestion. Gene expression values used for this correlation analysis are based on the quantile method produced by Genespring software.

5.3.8 Gene Ontology (GO) analysis and classification

All significant candidate genes from the list of comparisons between the groups in the volcano analysis were used for this GO analysis. Those genes were then annotated and defined according to the GO terms directly under the three main categories: molecular function, cellular component and biological process. In this analysis, the oligonucleotide probes from the array were matched with the GO database and categorized into subclasses. At this point, the matched candidate genes were counted in each GO subclass created from comparison with the GO database. This GO analysis was performed using the EasyGO web-based tool <http://bioinformatics.cau.edu.cn/easygo/N

5.3.9 Candidate gene mapping with known QTL markers

The genome browser database GRAMENE (www.gramene.org) provides valuable information through a user-friendly web interface that allowed for comparative assessments using a genetic map (Kim et al., 2009). By selecting markers linked to published BPH resistance QTL, associated information on the candidate genes could be obtained based on the closest distance between loci on the same chromosome. All 239 and 219 significant gene lists were passed through this process and provided an alternative tool to strengthen the candidate gene list.

.

5.4. Results

5.4.1 Quality control/filtering

A total of 21556 probes from 43805 probes passed through the normalization process using the Quantile approach. This result was based only on twenty two samples rather than twenty four samples. The two samples namely MR232 replicate 1 and sample IR694 replicate 2 were discarded from the statistical analysis because of likely RNA contamination or degradedation during preparation. Box plots of RMA-normalized intensity values from each sample and principal component analysis (PCA) are shown in figure 17. Susceptible varieties are clearly separated from resistant and moderately resistant varieties into the PCA component 1(x-axis), PCA component 4 (y-axis) and PCA component 2 (z-axis). Only 415 probes finally passed through the filter after the four-steps filtering process, i.e. by expression, by flags, by data set and by 50% CV values.

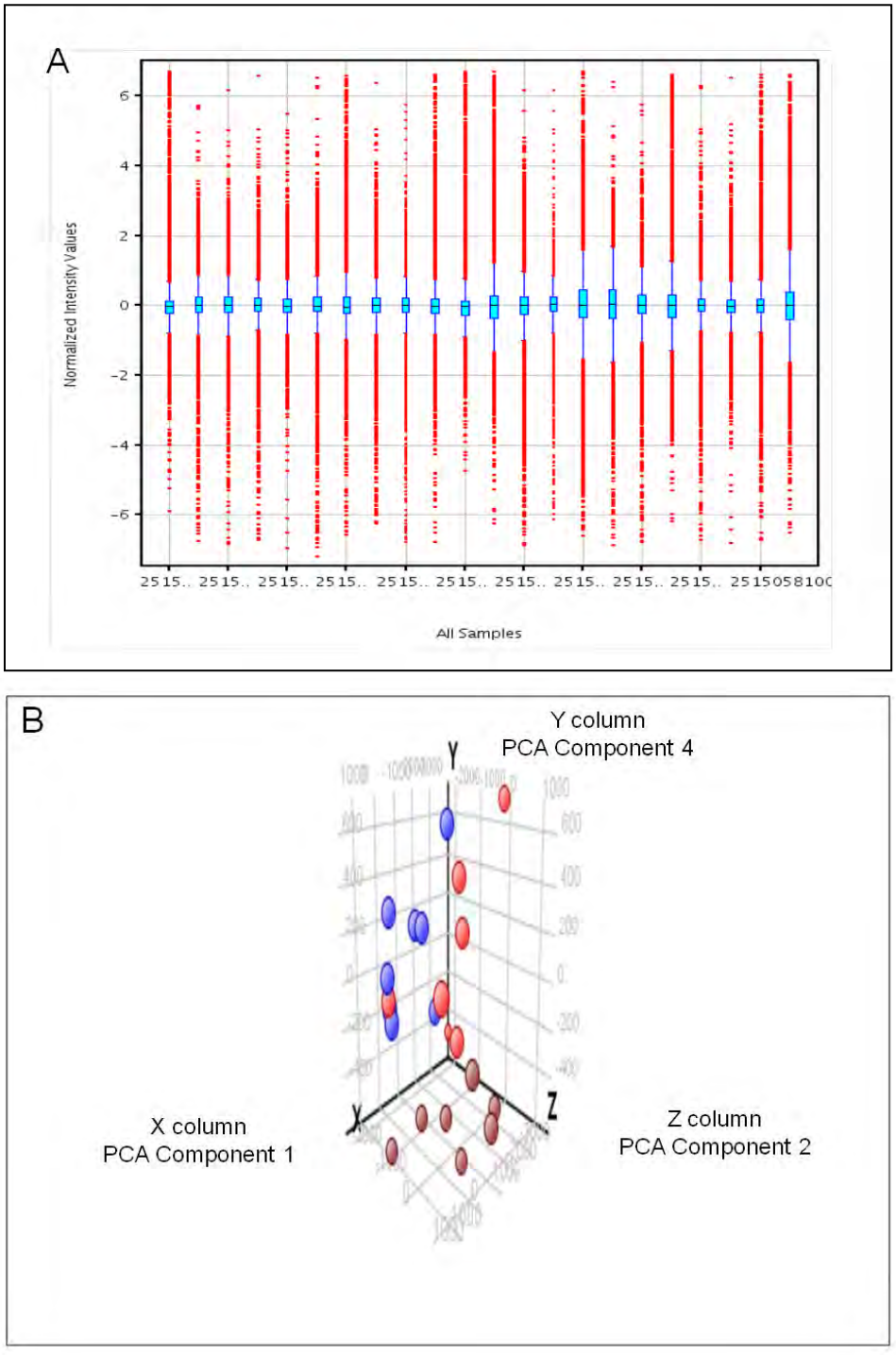


Figure 17 Box plot normalization data and principal component analysis on 22 samples. Principal component analysis (PCA) for susceptible (brown), moderate (red) and resistance (blue)

5.4.2 Determination of significant genes

Statistical analysis using the volcano plot further reduced the number of probes based on a three-combination comparison between the three rice groups at $P<0.05$ and fold difference 2.0 and above. According to Schenk et al. (2000), Voiblet et al. (2001) and Zhang et al. (2004), these values can be treated as significant in terms of transcript concentration. Based on the Venn diagram as shown in figure 18, a total of 239 probes were significantly different in expression between the resistant and susceptible groups, while 219 probes differed in expression between moderately resistant and susceptible. Interestingly, 196 genes were the same in both comparisons, resistant with susceptible and moderately resistant with susceptible. There were no genes that differed significantly between resistant and moderately resistant. This information supported further evidence on how close the relationship between resistant and moderately resistant is.

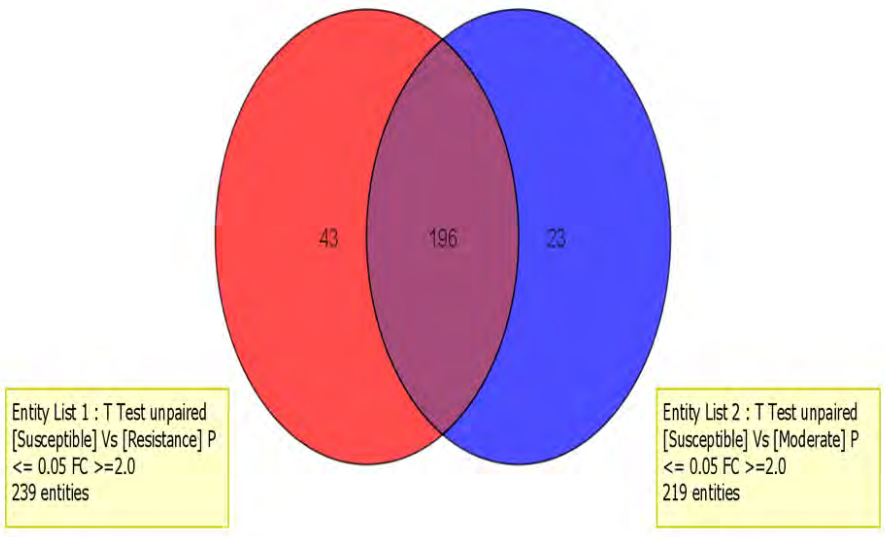


Figure 18 Venn diagram shows the two groups in relation to the number of significant genes. Red represents resistant versus susceptible and blue represents moderately resistant versus susceptible

A detailed classification based on the volcano plot analysis between these three groups is presented in table 11. In this study, no rice varieties were exposed to BPH at any stage. Therefore, gene regulation patterns were not of interest because the focus was on identifying constitutive resistance genes. The results showed that the majority of genes for both resistant versus susceptible or moderately resistant versus susceptible comparisons ranged between 2.0 to 4.0 fold difference value containing 156 and 146 genes respectively. Genes of an unknown function, however, were still large in number, 94 (39%) and 83 (38%) for both comparisons (Appendices B and C). .

Table 11 Number of genes with significant expression differences between groups

Group analysis	Fold difference	Number of significant genes	Total genes
Resistance Vs Susceptible (239 probes)	>2 .00- 4.00	156	239
	>4.00 – 6.00	35	
	>6.00 – 8.00	17	
	>8.00	31	
Moderate resistance Vs Susceptible (219 probes)	>2 .00- 4.00	146	219
	>4.00 – 6.00	30	
	>6.00 – 8.00	13	
	>8.00	29	
Resistance Vs Moderate resistance (0 probe)	>2 .00- 4.00	0	0
	>4.00 – 6.00	0	
	>6.00 – 8.00	0	
	>8.00	0	

Further statistical analysis on different groupings were also conducted to monitor the validity of the results in terms of whether they could have been achieved purely by chance. All rice varieties were randomly assigned to three groups. The results are shown in table 12. In total, only eight genes showed significant expression differences.

Table 12 Number of genes showing significant expression differences between groups following random assignment

Group analysis	Fold difference	Number of significant genes	Total genes
Group A Vs Group B [(TN1 x Rathu) Vs (Azucena x Babawee)]	>2 .00- 4.00	0	0
	>4.00 – 6.00	0	
	>6.00 – 8.00	0	
	>8.00	0	
Group A Vs Group C [(TNxRathu) Vs (Nipponbare x F1)]	>2 .00- 4.00	3	5
	>4.00 – 6.00	0	
	>6.00 – 8.00	1	
	>8.00	1	
Group B Vs Group C [(Azucena x Babawee) Vs (Nipponbare x F1)]	>2 .00- 4.00	1	3
	>4.00 – 6.00	0	
	>6.00 – 8.00	0	
	>8.00	3	

5.4.3 Strong candidate genes associated with BPH resistance

All the significant genes differentially expressed between resistant and susceptible varieties or moderately resistant and susceptible varieties (239 and 219 respectively) are important. However, the total number is still large and difficult to explain in detail. Therefore, further study focussed on only 239 candidate genes from the resistant and

susceptible groups. The results from three methods were chosen to strengthen the candidacy of the genes, which are described below. Having decided this, it still needs to be reiterated that more than 80 % of the resistant and moderately resistant were identical according to their expression pattern compared to the susceptible.

5.4.3.1 Correlation of gene expression with morphological BPH feeding behaviour

The full results of the correlation analysis between gene expression values of the 12 rice genotypes and the five selected morphological BPH feeding behaviour parameters from chapter 4 are given in appendix E. The results show that the resistant varieties produced a positive correlation on EPG waveform for pathway (N1+N2+N3), xylem ingestion (N5) and the time to first BPH stylet reaching the phloem sap. Negative correlations were identified for phloem ingestion, EPG waveform (N4b) and total honeydew production. Overall, the total number of genes associated with resistance, however, was less than for susceptibility. Only 89 genes from 239 genes were found to have a negative correlation with phloem ingestion. A gene from *Arabidopsis thaliana* At3g17850 mRNA for putative protein kinase (Os03g0711800) showed a very high correlation with almost all morphological BPH behaviour characters. This gene had the highest negative correlation with phloem ingestion (r=-0.8480) and time to first phloem ingestion (r=-0.8687). Figure 19 clearly shows the exact resistance pattern of this gene. The high expression level of gene coding for protein kinase had significantly associated with the reduced of BPH phloem ingestion duration (Figure 19A). Consequently, the time to BPH stylet reaching the phloem sap region also increased (Figure 19B). However, from 15 genes annotated as protein kinases, only 4 negatively responded with phloem ingestion.

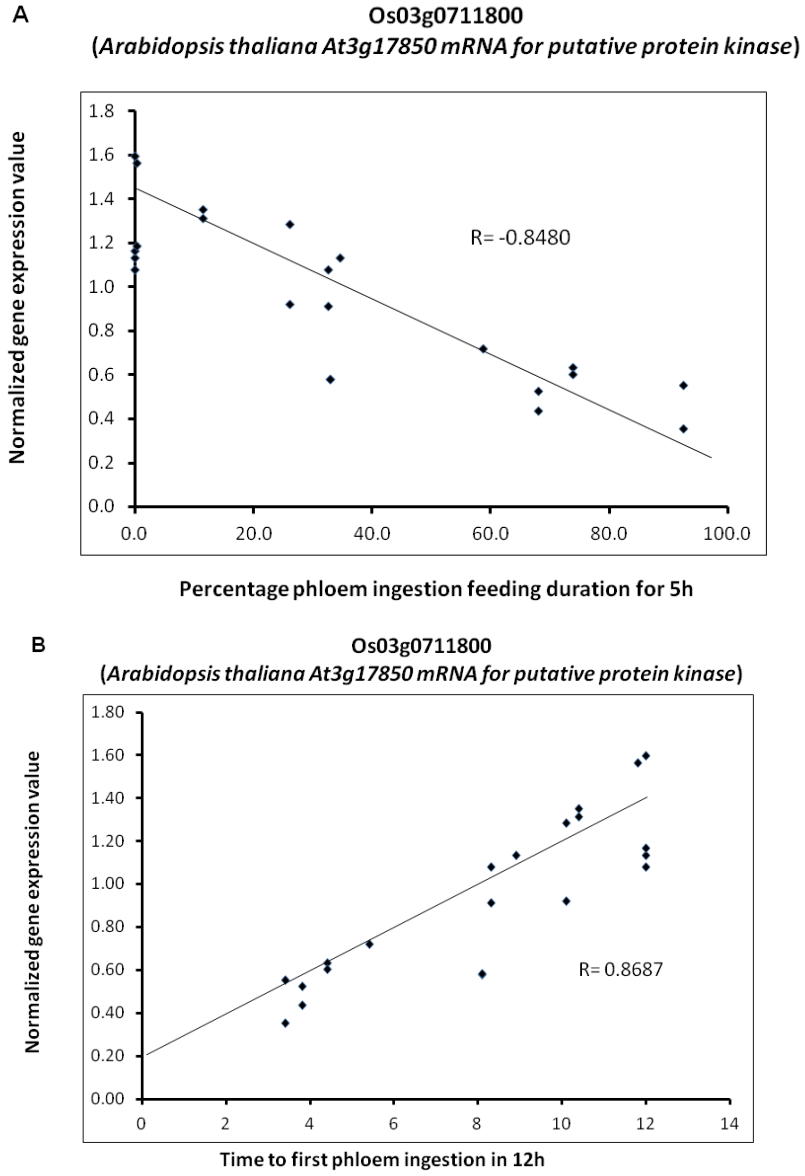


Figure 19 Relationship between phloem feeding over a 5h period (A) and time to first phloem ingestion (B) with normalized expression of gene Os03g0711800 annotated as *Arabidopsis thaliana* At3g17850 mRNA for putative protein kinase. Expression is significantly decreased for phloem ingestion and increased for time to first phloem ingestion in resistant varieties. Each point is a single expression value for an individual variety from microarray data for the preset study plotted against the percentage average time of phloem ingestion from the same variety (data from Ghaffar et al., 2011). Each of 12 varieties is represented twice. Line shows the linear correlation with R values of -0.8480 and 0.8667

For susceptible varieties, a positive correlation is shown between phloem ingestion and total honeydew production, with gene expression level, while a negative correlation was found with pathway and time to first phloem ingestion. Gene Os08g0434100 from *Oryza sativa* (japonica cultivar-group) mRNA for ribonuclease is a good example of a candidate gene associated with the susceptible character. This gene produced the highest positive correlation value for phloem ingestion (r= 0.87) and total honeydew drop (0.8737). Figure 20 clearly demonstrates that a high level of gene expression extends the percentage duration of phloem ingestion (Figure 20A) and therefore, the total honeydew production is also increased (Figure 20B).

It is also important to highlight the fold difference value in the microarray experiment because it represents the quality of the sample. In this analysis, *Arabidopsis thaliana* clone 1204 mRNA (Os02g0243300) showed the highest fold difference value (25.26). There were several genes with known annotation that produced a good combination of high correlation and high fold difference value associated with resistance genotype characteristics. Among them were *Arabidopsis thaliana* putative glutamate receptor protein GLR3.4b (GLR3.4) mRNA, GLR3.4-2 allele (Os07g0522600), the *Daucus carota* transposable element TdcA1-ORF2 mRNA (Os08g0208300) and *Oryza sativa* nucleotide-binding leucine-rich-repeat protein 1 mRNA (Os12g0199100).

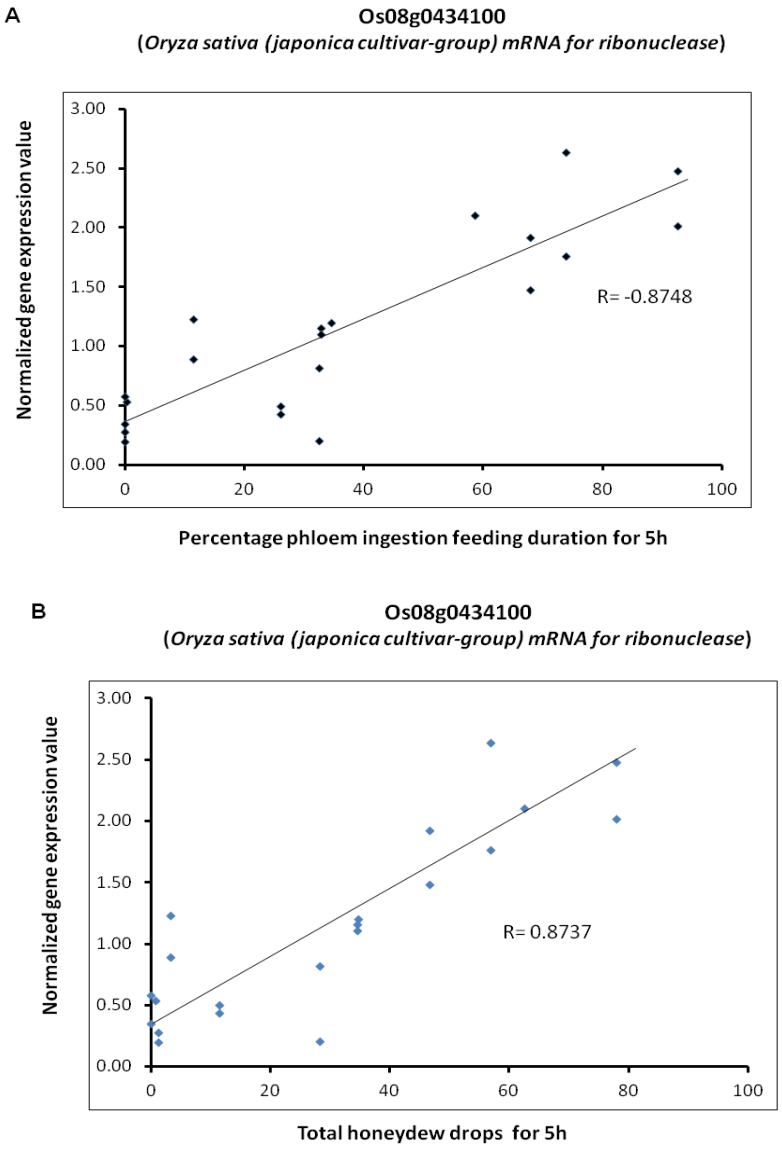


Figure 20 Relationship between phloem feeding (A) and total honeydew drops (B) over a 5h period with normalized expression of gene Oso8g0434100 annotated as *Oryza sativa* (japonica cultivar-group) mRNA for ribonuclease. Expression is significantly increased in susceptible varieties. Each point is a single expression value for an individual variety from microarray data for the preset study plotted against the percentage average time of phloem ingestion and total honeydew drop from the same variety (data from Ghaffar et al., 2011). Each of 12 varieties is represented twice. Line shows the linear correlation with R values of 0.8748 and 0.8737 respectively

5.4.3.2 Gene ontology (GO) enrichment analysis

Functional annotation using the Gene Ontology controlled vocabulary is standard practice for many microarray experiment results. It is used to identify possible molecular biological processes that govern the response of differentially expressed genes such as BPH resistance. In this study, a gene Ontology (GO) enrichment analysis process was performed using the EasyGO web-based tool <http://bioinformatics.cau.edu.cn/easygo/N (Zhou and Su, 2007) with an FDR adjusted p-value of ≤0.05 as the cut-off. Results can be categorized into three main classes, namely cellular biological process, cellular component and molecular function (Ashburner et al., 2000). The distributions of the identified gene sets over the different GO functional categories are shown in table 13. The evaluation is based on a comparison of the percentage of differentially expressed genes belonging to each functional category with the degree of representation of the respective functional category in the genome (De Vos et al., 2005).

The distribution of GO analysis showed that the biological process featured the highest number of GO term annotations and classifications with 30 categories (Appendix F). Thirteen categories represent GO term annotations in cellular component (Appendix G) with only three categories featuring GO term annotations in molecular function (Appendix H). A relatively large number of genes (63) of the GO term category _response to stimulus‘ (GO: 0050896) were found. Interestingly, the _response to biotic stimulus‘ (GO: 0009607) category rated very highly with 28 genes in total (Figure 21). Other GO terms also in this group were _response to external stimulus‘ (GO: 0009605, 14 genes), _response to endogenous stimulus‘ (GO: 0009719, 35 genes) and _response to stress _ (GO:0006950, 23 genes).

For the second GO term sub-class under the cellular component category, cell part (GO: 0005618) and cell were equal (GO: 0044464) in number with 79 genes each. A large proportion was also found in ‗intracellular' (GO: 0005622, 57 genes), ‗intracellular part' (GO: 0044424, 57 genes) and ‗cytoplasm' (GO: 0005737, 53 genes). For molecular function, ‗catalytic activity' (GO: 0003824) comprised the highest number with 80 genes, while ‗transferase activity' (GO: 0016740) had 41 genes and ‗transporter activity' (GO: 0005215) another 17.

Table 13 List of GO terms under biological process, molecular function and cellular component categories for the 239 significant genes (results from comparison between resistant and susceptible) using EasyGO web tool analysis

GO term	Ontology Type	GO description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0050896	Biological Process	Response to stimulus	63	6072	1.20E-07	3.00E-05
GO:0009607	Biological Process	Response to biotic stimulus	28	2011	1.20E-06	0.00015
GO:0065007	Biological Process	Biological regulation	35	3245	1.80E-05	0.0014
GO:0009605	Biological Process	Response to external stimulus	14	797	4.00E-05	0.0024
GO:0009991	Biological Process	Response to extracellular stimulus	6	146	7.40E-05	0.0036
GO:0032502	Biological Process	Developmental process	22	1840	0.00012	0.0049
GO:0019748	Biological Process	Secondary metabolic process	15	1021	0.00016	0.0054
GO:0007165	Biological Process	Signal transduction	29	3051	0.00057	0.0076
GO:0007275	Biological Process	Multicellular organismal development	18	1460	0.00032	0.0076
GO:0009719	Biological Process	Response to endogenous stimulus	35	3895	0.00049	0.0076
GO:0050789	Biological Process	Regulation of biological process	30	3067	0.0003	0.0076
GO:0032501	Biological Process	Multicellular organismal process	18	1464	0.00033	0.0076
GO:0006810	Biological Process	Transport	17	1386	0.00048	0.0076
GO:0050794	Biological Process	Regulation of cellular process	29	3051	0.00057	0.0076

Continue table

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0023046	Biological Process	Signaling process	29	3051	0.00057	0.0076
GO:0051234	Biological Process	Establishment of localization	17	1386	0.00048	0.0076
GO:0051179	Biological Process	Localization	17	1386	0.00048	0.0076
GO:0023060	Biological Process	Signal transmission	29	3051	0.00057	0.0076
GO:0023052	Biological Process	Signaling	29	3090	0.00069	0.0087
GO:0009791	Biological Process	post-embryonic development	11	781	0.0015	0.018
GO:0040007	Biological Process	Growth	9	584	0.0021	0.024
GO:0006950	Biological Process	Response to stress	23	2464	0.0023	0.025
GO:0000003	Biological Process	Reproduction	12	961	0.0025	0.025
GO:0006629	Biological Process	Lipid metabolic process	12	957	0.0024	0.025
GO:0030154	Biological Process	Cell differentiation	10	739	0.0031	0.028
GO:0048869	Biological Process	Cellular developmental process	10	739	0.0031	0.028
GO:0065008	Biological Process	Regulation of biological quality	5	206	0.0031	0.028
GO:0006519	Biological Process	Cellular amino acid and derivative metabolic process	13	1149	0.0039	0.032
GO:0044281	Biological Process	Small molecule metabolic process	13	1149	0.0039	0.032
GO:0009987	Biological Process	Cellular process	62	9072	0.0054	0.044

Continue table

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0003824	Molecular Function	Catalytic activity	80	10047	4.60E-05	0.0027
GO:0005215	Molecular Function	Transporter activity	18	1437	0.00027	0.0078
GO:0016740	Molecular function	Transferase activity	41	4799	0.0005	0.0097
GO:0030312	Cellular component	External encapsulating structure	27	2595	0.00023	0.0071
GO:0005618	Cellular component	Cell wall	27	2595	0.00023	0.0071
GO:0044464	Cellular component	Cell part	79	10811	0.0005	0.0083
GO:0005623	Cellular component	Cell	79	10833	0.00053	0.0083
GO:0005737	Cellular component	Cytoplasm	53	7085	0.0017	0.019
GO:0005739	Cellular component	Mitochondrion	35	4228	0.0018	0.019
GO:0016020	Cellular component	Membrane	37	4657	0.0027	0.023
GO:0044444	Cellular component	Cytoplasmic part	43	5646	0.0029	0.023
GO:0044424	Cellular component	Intracellular part	57	8195	0.0051	0.036
GO:0043231	Cellular component	Intracellular membrane-bounded organelle	51	7469	0.0097	0.047
GO:0005622	Cellular component	Intracellular	57	8397	0.0078	0.047
GO:0043227	Cellular component	Membrane-bounded organelle	51	7469	0.0097	0.047
GO:0005576	Cellular component	Extracellular region	5	272	0.0096	0.047

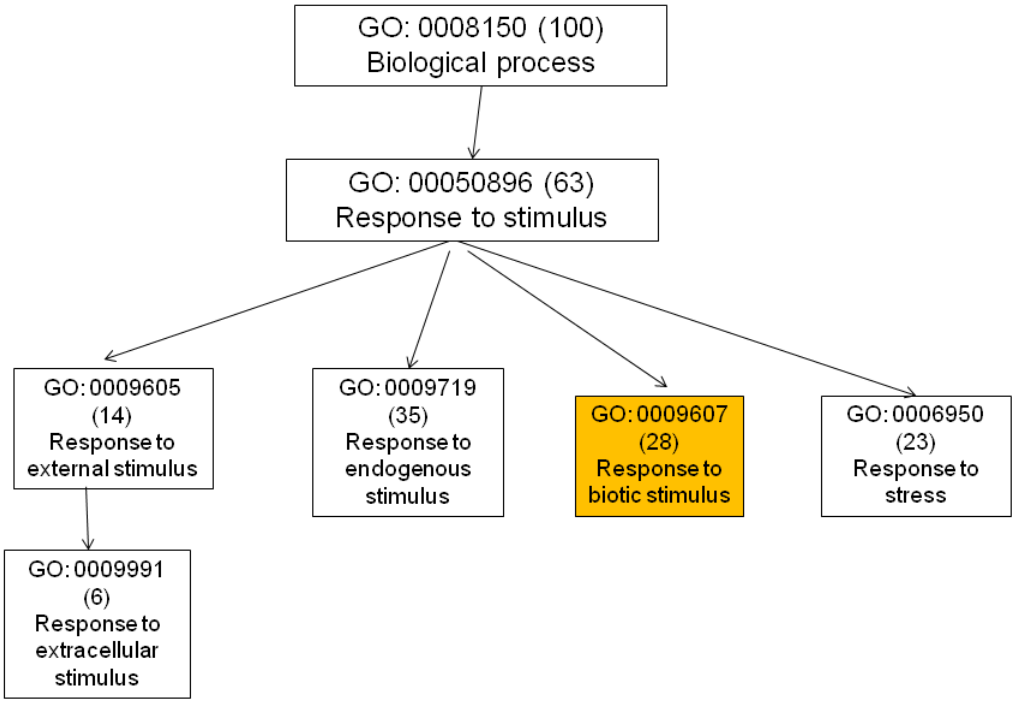


Figure 21 GO term sub-class under response to stimulus category. Response to biotic stimulus (GO: 0009607) has highest significant level (FDR)

5.4.3.2 .1 Genes for response to biotic stimulus genes

For further evaluation, this study focussed on only GO term sub-class response to biotic stimulus. The aim was to detail and narrow down the number of genes until significant specific genes of interest were found. The list of twenty-eight genes belonging to this GO term and their EasyGO descriptions are shown in table 14. In this evaluation, fold difference value and the five correlated feeding characters, namely pathway, phloem ingestion, xylem ingestion, total honeydew production and time BPH starts phloem ingestion from the previous analysis were also included to support these findings and strengthen the candidacy of the gene list. The combination of all that information allowed

for the identification of 28 genes under GO term response to biotic stimulus and their subsequent allocation into 5 groups.

The first group contains five genes classified according to their resistance characters. A positive correlation was found with pathway, xylem ingestion and time BPH starts ingestion of the phloem. In contrast, negative correlations were found on characters phloem ingestion and honeydew production. Gene Os07g0522600 annotated as a glutamate receptor 3.4 precursor produced the highest correlation for pathway (r=0.69), phloem ingestion, total honeydew drop (r=0.69), time BPH starts ingesting the phloem (r=0.72) and phloem ingestion (r=-0.68). Gene Os08g0539700 *Oryza sativa* PibH8 mRNA and gene Os12g0199100 *Oryza sativa* nucleotide-binding leucine-rich-repeat protein also showed a high correlation for the same character glutame receptor gene. Although gene Os12g0118400 (*Arabidopsis thaliana* At3g10840 mRNA) for putative alpha/beta hydrolase showed a moderate correlation value, the highest fold difference value of 17.40 indicated that it was a highly interesting gene.

The second group is associated with transporter activities. This group comprises 7 genes and most of them showed high positive correlation values with BPH phloem ingestion and total honeydew drops. The most outstanding gene was a zinc transporter 1 precursor, putative (Os04g0613000). This gene not only demonstrated a high fold difference value of 10.07 but also a correlation value above r=0.7. The other two genes of interest were Os02g0102200 (*Arabidopsis thaliana* putative amino acid carrier At1g77380) and Os08g0127100 (*Arabidopsis thaliana* At5g40780/K1B16_3 mRNA). Fold difference and correlation values were higher compared to others within the group.

Tranferase activities characterise the third group. Four out of six genes were found to be associated with peroxidase enzymes with almost all of them possessing high correlation values especially for gene Os08g0113000 (*Arabidopsis thaliana* class III peroxidase ATP32 mRNA). The correlation value with pathway, phloem ingestion, total honeydew drop and time BPH starts feeding on the phloem were all above 0.8. Gene Os03g0389700 (*Morinda citrifolia* mRNA for 3-deoxy-D-arabino-heptulosonate) in this group also showed a significantly high correlation with phloem ingestion (r=0.77) and time BPH starts ingesting the phloem (r=0.80).

The fourth group also contains 6 genes which show similar high correlation values with the same characters as in group 3. All genes are referred to as showing receptor protein kinase activity. Gene Os02g0228300 (*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA) and gene Os12g0182300 (*Arabidopsis thaliana* putative receptor protein kinase) are both highly interesting. Correlation values for phloem ingestion and time BPH starts feeding on the phloem were among the highest of 28 genes belonging to GO term response to biotic stimulus.

The final group 5 comprises only 4 genes which show other molecular functions such as hydrolase, catalytic or unknown molecular function activities. All genes in this group had fold difference values above 4.0 and showed correlation values around r=0.60.

Table 14 Genes in the GO _response to biotic stimulus' category with fold difference values and their linear coefficient correlation with key BPH feeding pathway components

UniGene symbol	Fold Change	Pathway	Phloem ingestion	Xylem ingestion	Average honeydew drops (1h)	Starting time BPH ingest the phloem	Description (main substrate)
Group 1 (Negative correlation with phloem ingestion)							
Os12g0118400	17.40	0.56	-0.57	0.57	0.61	0.61	*Arabidopsis thaliana* At3g10840 mRNA for putative alpha/beta hydrolase, complete cds, clone:
Os06g0323100	5.35	0.43	-0.37	0.35	-0.40	0.36	*Arabidopsis thaliana* clone 37493 mRNA, complete sequence.
Os07g0522600	4.10	0.69	-0.68	0.56	-0.69	0.72	*Arabidopsis thaliana* putative glutamate receptor protein GLR3.4b (GLR3.4) mRNA, GLR3.4-2 allele.
Os12g0199100	6.87	0.62	-0.62	0.65	-0.63	0.64	*Oryza sativa* nucleotide-binding leucine-rich-repeat protein 1 mRNA
Os08g0539700	2.33	0.60	-0.66	0.64	- 0.64	0.69	*Oryza sativa* PibH8 mRNA
Group 2 (transporter activities)							
Os04g0613000	10.07	-0.75	0.80	-0.73	0.71	-0.81	*Arabidopsis thaliana* putative zinc transporter (ZIP1) mRNA
Os02g0102200	4.75	-0.70	0.77	-0.73	0.67	-0.78	*Arabidopsis thaliana* putative amino acid carrier (At1g77380) mRNA.
Os10g0539900	2.90	-0.66	0.71	-0.54	0.68	-0.73	*Hordeum vulgare* mRNA for hexose transporter (stp1 gene).
Os04g0454200	2.64	-0.54	0.65	-0.71	0.56	-0.67	*Oryza sativa* OsMST1 mRNA for monosaccharide transporter 1

Table continue

UniGene symbol	Fold Change	Pathway	Phloem ingestion	Xylem ingestion	Average honeydew drops (1h)	Starting time BPH ingest the phloem	Description (main substrate)
Os01g0872600	2.01	-0.35	0.45	-0.44	0.40	-0.49	*Arabidopsis thaliana* At1g72120/F28P5_2 mRNA, (Peptide transporter PTR2-B, putative)
Os02g0580900	2.08	-0.55	0.63	-0.66	0.63	-0.65	*Arabidopsis thaliana* At3g21670/MIL23_23 mRNA,.
Os08g0127100	4.94	-0.77	0.83	-0.82	0.77	-0.85	*Arabidopsis thaliana* At5g40780/K1B16_3 mRNA, (LHT)
Group 3 (Transferase activities)							
Os12g0112000	7.02	-0.59	0.66	-0.64	0.64	-0.66	*Nicotiana tabacum* mRNA for peroxidase,
Os08g0113000	4.58	-0.80	0.83	-0.69	0.83	-0.84	*Arabidopsis thaliana* class III peroxidase ATP32 mRNA,
Os07g0677200	3.12	-0.69	0.71	-0.59	0.77	-0.71	*Oryza sativa* peroxidase (POX22.3) mRNA,.
Os09g0471100	2.44	-0.64	0.70	-0.69	0.68	-0.74	*Gossypium hirsutum* gaiacol peroxidase (pod5) mRNA
Os03g0389700	3.95	-0.69	0.77	-0.68	0.74	-0.*80*	*Morinda citrifolia* mRNA for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, DS1
Os02g0719600	4.22	-0.50	0.63	-0.63	0.57	-0.63	*Atropa belladonna* AbSAMT1 mRNA for S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase

Table continue

UniGene symbol	Fold Change	Pathway	Phloem ingestion	Xylem ingestion	Average honeydew drops (1h)	Starting time BPH ingest the phloem	Description (main substrate)
Group 4 (Kinase activities)							
Os02g0228300	3.57	-0.75	0.84	-0.79	0.78	-0.85	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA.
Os06g0557700	2.68	-0.75	0.79	-0.75	0.71	-0.85	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA.
Os12g0182300	2.14	-0.76	0.82	0.78	0.77	-0.86	*Arabidopsis thaliana* putative receptor protein kinase
Os01g0878300	4.49	-0.75	0.80	-0.64	0.78	-0.82	*Arabidopsis thaliana* putative receptor protein kinase (At1g28440)
Os06g0557100	2.28	-0.60	0.67	-0.73	0.60	-0.73	*A.thaliana* receptor-like protein kinase mRNA
Os03g0440900	2.17	-0.57	0.68	-0.69	0.59	-0.71	*Arabidopsis thaliana* Unknown protein (At5g21090) mRNA,
Group 5 (Other activities)							
Os04g0513400	5.88	-0.67	0.72	-0.65	0.67	-0.72	*Pinus contorta* beta-glucosidase mRNA.
Os12g0448900	4.94	-0.65	0.73	-0.71	0.68	-0.76	*Oryza sativa* fatty acid alpha-oxidase mRNA.
Os01g0369900	4.20	-0.61	0.63	-0.55	0.67	-0.66	*Oryza sativa* RRJ4 mRNA for 12- oxophytodienoic acid reductase,
Os04g0474800	4.20	-0.58	0.59	-0.37	0.63	-0.64	*Prunus serotina* amygdalin hydrolase isoform AH I precursor (AH1) mRNA,.

.

5.4.4 Mapping candidate genes with known BPH markers to rice QTL

The list of 82 BPH markers for QTL used in this evaluation is shown in appendix I. The genome web browser under http://www.gramene.org/ was used for the screening process. Out of the 239 candidates, only 7 genes were found located within a BPH resistance marker locus region: RM1103 (Park et al., 2008), RM261, RM185, RM17/12 (Kumari et al., 2010), RM19291, RG1 (Jairin et al., 2007) and RM484 (Sun et al., 2005). The list of BPH resistance genes involved were *Bph1* (chromosome 12), *Bph3* (chromosome 6), *Bph10(t)* (chromosome 12), *Bph12(t)* (chromosome 4), *Bph15* (chromosome 8) and *QBph10* (chromosome 12), respectively. Interestingly, four genes in the list of table 15 refer to the known BPH resistant varieties, Rathu Heenathi and IR64.

Gene Os12g0631200 (*Arabidopsis thaliana* clone 108517 mRNA, zinc finger, putative, expressed) was found to be the closest to a BPH marker at only 37*k*bp distance. Figure 22 shows how the mapping process and locus distance measurement were conducted. This gene also produced a high expression correlation with BPH phloem ingestion (r=0.79). The other two genes, Os12g0571100 (*Oryza sativa* metallothionein-like protein mRNA) and Os10g0539900 (*Hordeum vulgare* mRNA for hexose transporter) were also found close to BPH resistance markers at 57kbp and 85kbp respectively. These genes also had a high positive correlation with BPH phloem ingestion (r=0.76 and 0.71 respectively). Interestingly, this hexose transporter (STP1) gene was strengthened in terms of its candidacy because it was the only gene which appeared twice: once in this analysis and also in the GO term response to biotic stress. This gene is associated with *Qbph10* located on chromosome 10, and there is strong evidence that it plays a special role in attracting BPH to feed on the plant.

With the exception of gene Os06g0120200 (unknown expressed protein), the other three genes were found to have negative correlations with the BPH phloem ingestion character. Gene Os04g0196300 (unknown expressed protein) and Os04g0379300 (*Arabidopsis thaliana* clone U18492 unknown protein (At2g31740) mRNA) are both associated with wild rice species, *Oryza latifolia*. Those genes are linked to *Bph12(t)* on chromosome 4 and are 100kbp distant from their reference marker. The last candidate gene, Os08g0440100 (1, 6-bisphosphate aldolase precursor) is associated with Rathu Heenathi and showed a high fold difference value (5.67). This gene location, however, was the furthest from the reference BPH marker at 242 kbp. In addition, this gene had the lowest correlation with the phloem ingestion character (r=-0.45).

Table 15 Closest map positioned to BPH genes using reference markers (taken from Gramene website)

Gene	Donor	Marker	Candidate gene map position (TIGR and Unigen symbols)	Location of BPH references marker And distance (bp)	Gene description	Fold difference	Phloem ingestion (Correlation)
Bph1	IR64	RM1103 Park DS et al. (2008)	LOC_Os12g38300 Os12g0571100 23,481,607 -23,482,258	23,539,431-23,539,654 (57kbp)	*Oryza sativa* metallothionein-like protein mRNA, complete cds	4.99	0.76
Bph12(t)	B14 *[Oryza latifolia]*	RM261 Kumari et al. (2010)	LOC_Os04g11980 Os04g0196300 6,563,327-6,564,094	6,574,386-6,574,528 (103kbp)	Unknown expressed protein	15.84	-0.55
Bph12(t)	B14 [*Oryza latifolia]*	RM185 Kumari et al. (2010)	LOC_Os04g31000 Os04g0379300 18,348,456-18,358,985	18,579,750-18,579,967 (221kbp)	*Arabidopsis thaliana* clone U18492 unknown protein (At2g31740) mRNA, complete)	2.92	-0.65
Bph3	Rathu heenathi	RM19291 Jairin et al. (2007)	LOC_Os06g02960 Os06g0120200 1,089,059-1,091,626	1,215,874-1,216,040 (124kbp)	Unknown expressed protein	9.28	0.6
Bph15	PTB33/ Rathu heenathi	RG1 Jairin et al. (2007)	LOC_Os08g34150 Os08g0440100 21,404,694-21,406,066	21,645,653-21,646,067 (240kbp)	*Avena sativa* fructose 1,6-bisphosphate aldolase precursor, mRNA, complete cds; nuclear gene for chloroplast product.	5.67	-0.45
Bph10(t)	*Oryza rufipogon*	RM17/RM12 Kumari et al. (2010)	LOC_Os12g43560 Os12g0631200 26,991,676-26,996,384	26,954,647-26,954,957 (37kbp)	*Arabidopsis thaliana* clone 108517 mRNA, complete sequence. (zinc finger, putative, expressed)	2.54	0.79
QBph10	Rathu heenathi	RM484 Sun et al. (2005)	LOC_Os10g39440 Os10g0539900 20,976,750-20,981,273	21,066,719-21,067,037 (85kbp)	*Hordeum vulgare* mRNA for hexose transporter (stp1 gene).	2.90	0.71

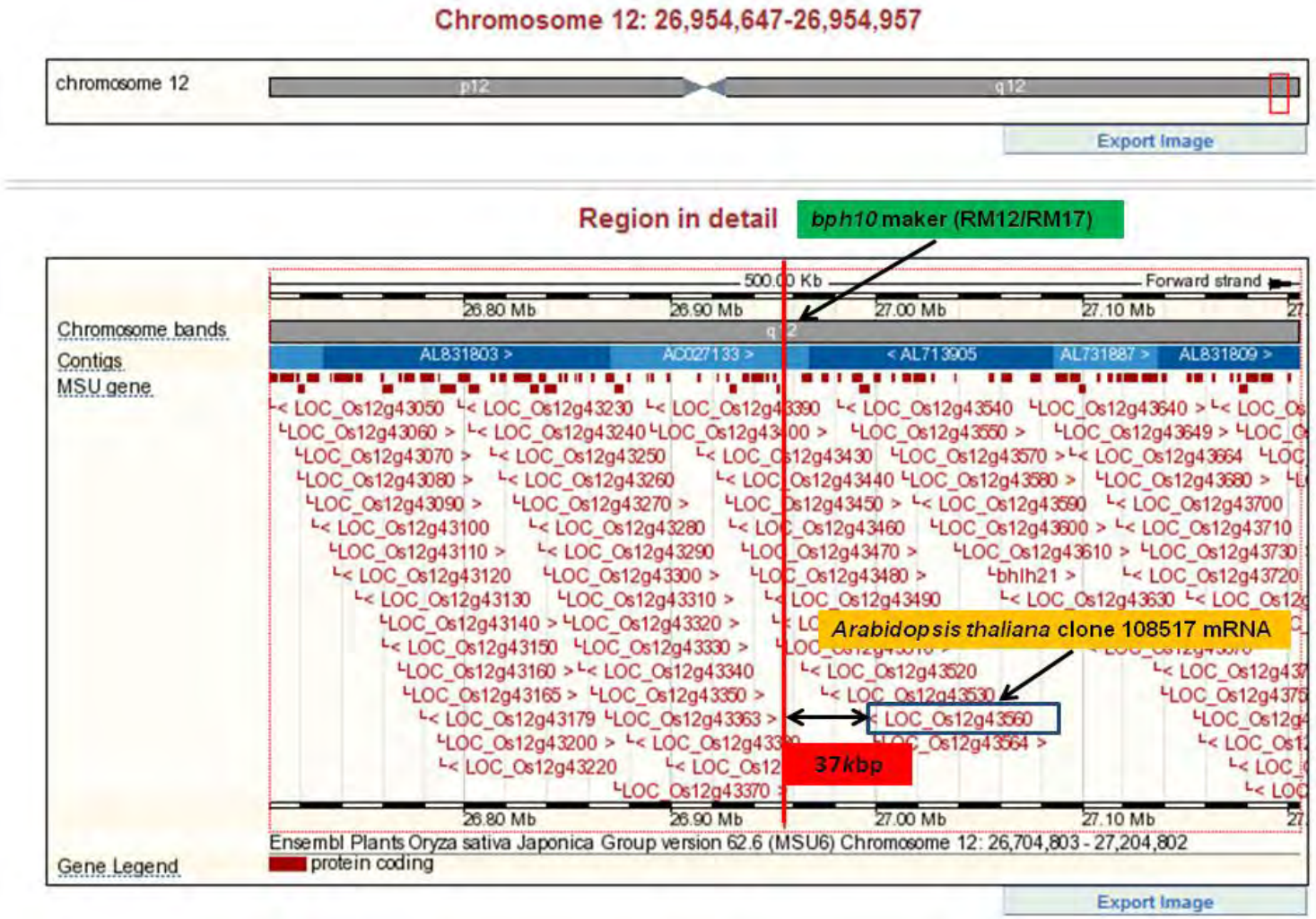


Figure 22 Example of the position and distance of a candidate gene in relation to known mapped QTL markers for BPH resistance

5.5 Discussion

One unique feature of this experiment was that the plant material was not subjected to BPH infection as is common in other gene expression studies. The genes that were aimed at being identified were expressed constitutively and not induced post-infection. These types of gene can be more durable and sustained over a long period because they have no ability to change easily in a short period when herbivores circumvent them (Karban and Myers, 1998). Selections for constitutive characters are also easy to achieve because they are not too complex or complicated. Inducible characters need very sensitive and specific conditions to activate their expression levels, such as plant damage levels or total insect numbers (Underwood et al., 2000). Sometime, inducible resistance characters refer only to a specific pathogen and herbivorous insect (Åhman, 2009). Therefore, it is difficult to achieve the target with the breeding selection of this character. Unlike in constitutive character, the selection can be made at an early stage even without insect infection as has been shown in this study.

In this transcriptomics study, more than 80 percent of genes (196 genes) were found expressed similarly between resistant and moderately resistant varieties in comparison to susceptible. This same pattern was also found in a previous morphological study where the BPH feeding behaviour was characterized using electrical penetration graph (EPG) and honeydew drops experiments. This could be further evidence to support the claim that moderate and resistant varieties could have a close genetic relationship due to a long history of breeding (Ghaffar et al., 2011). There is very limited supporting information available on the genetic backgrounds of the rice varieties except for moderately resistant varieties MR219 and MR232. It has been reported that at least one

of their parents is known to have possessed insect resistance (Alias et al., 2001). Moreover, this result also provided strong evidence that the relationship between molecular and morphological data is significant. It will create opportunities to investigate further in detail the interaction between BPH and plants.

5.5.1 Novel approach to strengthening the candidacy of BPH resistance genes

Although microarray experiments enable the analysis of the expression patterns of thousands of genes at one time, further activities are still needed to interpret the results. The 239 candidate genes for resistance still represent a big number and are difficult to explain and use in a conventional way. However, the various genomic analyses available through the web and particularly the support of the morphological data have increased the efficiency in the interpretation of the microarray data and added a further unique feature to this study.

Three different strategies were utilized to summarize and identify the strongest candidate genes related to BPH resistance. The first approach used a simple linear correlation analysis; data were compared between gene expression in the present study and the BPH feeding behaviour characters in chapter 4 (Ghaffar et al., 2011). Parameters related to BPH phloem ingestion duration and time to first BPH ingestion of the phloem were considered to be the most important in determining resistance level phenotypically. Therefore, the genes with a high positive correlation to phloem feeding duration were classified as being associated to susceptibility. In contrast, a high positive correlation for time to first phloem ingestion was categorized as a reflection of plant resistance.

Interestingly, the results show that the numbers of genes expressed in susceptible varieties (150 genes) are almost twice those for resistance (89). This could indicate that susceptible varieties contain more active genes than resistant.

Gene ontology analysis is a common practice used for summarizing microarray results. GO provides an extra advantage by standardizing or controlling the vocabulary (Meng et al., 2009) which can help to describe and understand better which genes contribute to the defence function. The results have confirmed this statement because it explains the distribution of the constitutive resistance candidate genes. The majority were found classified under GO class ‗response to stimulus' (GO: 0050896) with 63 genes, and GO sub-class ‗response to biotic stimulus' (GO: 0009607) (28 genes) (Table 13.and figure 22). This is important evidence which shows that these microarray data are reliable and successfully differentiate between susceptibility and resistance genes to BPH in this rice germplasm.

Mapping candidate genes identified from microarray experiments with known QTL BPH gene by way of mapped markers is highly significant in this study. This has allowed identification of a possible linkage of the 239 candidate genes with the known QTL BPH gene markers on the rice chromosomes and to estimate the candidates' distance from these known QTL BPH gene. To date, at least 22 major *Bph* resistance genes (QTL) have been indentified in cultivars and wild rice species (Santhanalakshmi et al., 2010), which has given further opportunity to compare known QTL BPH gene markers with the candidate genes. As a result, 7 out of 239 candidate genes have been discovered to fall close to the locus of BPH gene markers.

In this analysis, Gene Os12g0631200 (*Arabidopsis thaliana* clone 108517 mRNA) has the shortest distance to the *Bph10* marker at only 37 kbp. The other two genes, Os12g0571100 (*Oryza sativa* metallothionein-like protein mRNA) and Os10g0539900 (*Hordeum vulgare* mRNA for hexose transporter (stp1 gene)) are also located close to their reference BPH gene markers at 57kbp and 85kbp respectively. Also of interest is the source of the resistance. Four genes belong to Rathu Heenathi (*Bph3, Qbph10, Bph15*) and IR64 (*Bph1*), two of the varieties used in this analysis. This indicates that resistance genes from both are likely to represent or contribute significantly to the differentiation between resistance and susceptibility. In addition, one of the varieties in the resistant group, F1 is a cross between TN1 and Rathu Heenathi itself. It clearly shows that Rathu Heenathi is a good parent due to the heritable resistance character in its progeny. This variety has already been used extensively in rice breeding programs in Asia since 1980 (Khush et al., 1985; Jairin et al., 2007).

5.5.2 Strong candidate genes for BPH resistance

It should be borne in mind that the initial analysis indicated that all 239 genes in this experiment were potential candidates for resistance. They have significant differential expressions which can be used to differentiate between resistant and susceptible rice to BPH attack. The three approaches taken and which have been discussed before allow for more focussed hypotheses of gene function to be made. As a result, the gene encoding hexose transporter (Os10g0539900) could be classified as the strongest gene associated with constitutive resistance character to BPH attack. This gene has appeared twice, through gene ontology analysis under the GO subclass ‗response to biotic stimulus' and

mapping candidate genes with known QTL BPH gene marker, *QBph10*, which relates to the Rathu Heenathi resistant variety. However, this gene also has a strong high positive correlation with the BPH feeding behaviour for phloem ingestion and total honeydew production. Therefore, it confers susceptibility rather than resistance when the expression level is higher.

Plant hexose transporters are plasma membrane carriers and function as proton (HC)/hexose symporters (Bush, 1993; Gear et al., 2000) and belong to a large superfamily of transmembrane facilitators (MFS, major facilitator superfamily) (Marger and Saier, 1993; Büttner and Sauer, 2000). In the *Arabidopsis* genome, hexose transporters are classified as a member of the sugar transporter proteins (STPs) family, which includes over 50 transporters (Schofield et al., 2009). This hexose transporter refers to a gene associated with *Hordeum vulgare* mRNA (STP1), which is commonly found expressed in sink organs such as roots and green fruits (Gear et al., 2000; Weschke et al., 2003). Hexose transporters have been reported to be involved in the uptake of glucose or fructose (Sauer et al., 1994; Kühn et al., 2003; Baxter et al., 2005; Fridman et al., 2004, Frankel et al., 2007). They play an important role supplying sugars to cells for plant growth and development. This process provides strong evidence that a hexose transporter gene with high expression could help make plants more susceptible to BPH attack. The plant becomes more attractive because it has more nutrients available to the herbivore, and an increased expression of hexose transporter genes has been observed to be induced following aphid feeding (Frankel et al., 2007).

There are several studies suggesting that *Arabidopsis* STP genes also have some role in plant defence and plant cell death based on their expression profile (Nørholm et

al., 2006). STP4 was found to be induced when the plant had been infected by a pathogen (Fotopoulos et al., 2003; Truernit et al., 1996; Nørholm et al., 2006), STP3 was induced by wounding (Büttner et al., 2000; Nørholm et al., 2006), and STP1 mRNA levels increased upon treatment with defence-related products such as salicylic acid and methyl jasmonate (Schenk et al., 2000; Moran and Thompson, 2001; Nørholm et al., 2006).

There are seven other genes grouped under transporter activity in the same GO sub-class ‗response to biotic stimulus'. As for hexose transporters, most of the genes have a similar main function in nutrient or ion distribution. All of them are found to respond positively with BPH phloem ingestion. Like a hexose transporter, a monosaccharide transporter 1 (Os04g0454200) has similar functions in sink tissues such as delivering and distributing nutrients to any part of the plant (Sauer and Stadler, 1993). It is also claimed that this gene plays a significant role in programmed cell death (Nørholm et al., 2006) in response to pathogen infection or after wounding (Truernit et al., 1996).

Gene Os02g0102200, annotated as an amino acid permease (AAP3) is also expressed in sink tissues, mainly root tissue, suggesting a potential role in the uptake and distribution of amino acids into the cells surrounding the phloem (Okumoto et al., 2004). Amino acids are important components of the diet of sap feeding insects (Ortiz-Lopez et al., 2000) and can also act as phagostimulants (Srivastava and Auclair, 1975) A number of these AAPs have been implicated in the regulation of sieve element amino acid levels (AAP6 (Hunt et al., 2010)) and aphid performance (AAP1 (Kemp, 2011, unpublished data). If gene Os02g0102200 is involved in loading amino acids into the phloem, then

higher activities could facilitate phloem location by BPH by way of following intercellular amino acid gradients. High positive correlation values for phloem ingestion and time to first phloem ingestion support this hypothesis. It is little known that this gene is involved directly with plant defence. However, Ramputh and Brown (1996) reported that it is associated with GABA (γ-aminobutyric acid), the gene that causes phytophagous larvae of the oblique-banded leaf rollers (OBLR) (*Chohstoneura rosaceana*), retarded development and reduced survival rates.

There are another two interesting candidate genes found in this gene mapping approach. Gene Os12g0631200 coding for zinc finger is the closest gene with reference *Bph10* marker at only 37kbp locus distance. Zinc finger represents the sequence motifs which are classified according to the arrangement of the zinc-binding amino acids and plays a critical role in interactions with other molecules (Takatsuji, 1998). Therefore, it is important for many biological processes in plants. This could be the reason why the expression level is high in susceptible varieties in this study. Eulgem et al. (1999) claim the gene is involved in controlling induction of cell death and hypersensitive response (HR) and is mostly associated with disease reactions

The distance between gene encoding Metallothionein-like protein (OsMT) and *Bph1* marker is also close at 57kbp locus distance and has a similarly high positive correlation with phloem ingestion as zinc finger. This OsMTs gene contains only eleven genes in the rice genome sequence (Zhou et al., 2006). A recent study however showed that Metallothionein is involved in many biological processes including plant defence, for example in maintaining homeostasis of essential metals, metal detoxification (Cobbett and Goldsbrough, 2002; Hall, 2002; Delhaize et al., 2004), scavenging reactive oxidant

species (ROS) (Akashi et al., 2004; Wong et al., 2004) and protecting against intracellular oxidative damage (Zhou et al., 2006). There was very limited information associating this gene with insects unless for pathogen and abiotic stress.

It is important to highlight a gene associated with peroxidase. It has been reported that the rice peroxidase family is composed of138 genes (Passardi et al., 2004) and plays many roles in plant development including defence systems (Chittoor et al 1997). This gene has been implicated in the defence against herbivores through the JA pathway. Elevated peroxidase has been hypothesized to increase the level of cross linking in the cell wall making it more difficult to digest (Ralph, 2004). Peroxidases are implicated in the detoxification of reactive oxygen species (ROS) that can occur in response to herbivore attack (Hoang, 2010). In the present study, 4 significant genes were annotated as peroxidase (Os12g011200, Os08g01130000, Os07g0677200, Os09g0471100) and grouped as _transferase activities'. However, in all cases BPH feeding, as measured by a faster time to phloem location and a greater amount of phloem ingestion, was associated with higher peroxidase expression levels. Increased levels of peroxidase were induced following BPH feeding on rice (Rani and Jyothsna, 2010). Higher constitutive levels of peroxidase may reflect lower levels of ROS that can form a defence against herbivores.

Gene Os02g0719600 (*Atropa belladonna* AbSAMT1 mRNA for S-adenosyl-L-methionine (salicylic acid carboxyl methyltransferase) is also in the same group of _transferase activities' and has similar expression patterns as the peroxidase gene. Higher expression is associated with greater phloem ingestion duration suggesting its constitutive expression facilitates BPH feeding. Acid carboxyl methyltransferase is a key enzyme for jasmonate-regulated plant responses, which involve airborne signals that mediate

interplant communication for defence responses (Farmer and Ryan, 1990; Seo et al., 2001). It is also important for the synthesis of methyl salicylate (MeSA) which plays a role in plant defence and response to abiotic challenge (Kwon et al., 2009). However, in contrast to the current data it has been reported to have a higher expression following pathogen attach (Xu et al., 2006; Kwon et al., 2009). Interestingly, Ren et al., (2004) reported this gene was directly associated with *bph2* gene in rice plants.

Wei et al. (2009) reported that Gene Os12g0448900 coding for *Oryza sativa* fatty acid alpha-oxidase (Alpha-DOX2) is also involved in JA biosysnthesis which is important for defence signal pathway, and that it is a dioxygenase that synthesizes 13-hydroperoxylinolenic acid from linolenic acid in JA biosynthesis. They found that alpha-DOX2 expression significantly increased in both susceptible and resistant rice plants attacked by BPH. In this study, high expression was found in susceptible rather than resistance varities. This result clearly indicates that high expression alpha-DOX2 is associated with susceptible varieties even without BPH attack. Koeduka et al. (2005) claimed that this gene is also involved in the defence sytem against pathogen infection and heavy metal stress.

Another group of genes which highly influence BPH feeding behaviour are genes with protein kinase annotation. In this analysis, putative protein kinases represent the majority. They are one of the largest gene families in plants. In *Arabidopsis,* protein kinase represents 4% of all genes (Chevalier and Walker, 2005). There are around 1,400 protein kinases in the rice genome, and they have a diverse range of functions, including defence signaling (Ding et al., 2009) Of the 239 significantly differentially expressed genes, 15 were annotated as protein kinases. This indicates that this type of gene may

play a significant role in the plant defence system against BPH. Most of them are highly correlated with BPH feeding behaviour. Genes Os02g0228300, Os12g0182300, Os01g0878300 and Os06g0557700 have correlation values over 0.75. Interestingly, not all these genes show the same expression patterns because 4 of them are negatively correlated. Gene Os03g0711800 (*Arabidopsis thaliana* At3g17850 mRNA for putative protein kinase) produces the highest negative correlation with phloem ingestion data. Protein kinases can be classified according to their primary sequence and type of protein phosphorylation activity. Protein kinases are also enzymes with specific properties which have an important role in cellular communication (signalling) and gene duplication (Chevalier and Walker, 2005). Protein kinases have been shown to regulate aphid resistance signaling pathways in tomatoes, and increased expression of a protein kinase has been correlated with resistance to BPH in rice (Wei et al., 2009)

A number of genes whose annotation suggest they are related to defence showed high expression in more susceptible varieties. These included cytochrome p450s (Os06g0639800) and glutathione S-transferase (Os09g0467200), which are often induced following herbivore attack and therefore associated with plant defence. However, higher constitutive levels may generate lower levels and/or a faster turnover of secondary metabolites that could interfere with BPH feeding. As in any protein kinase case, these are large gene families with diverse functions as evidenced by the observation that one glutathione S-transferase (Os09g0467200) showed higher expression in susceptible varieties while another gene (Os10g0527800) showed reduced expression. B-glucosidase (Os4g0513400) also had higher expression in susceptible varieties. This enzyme can be involved in the production of toxic aglucose that forms a defence against chewing

herbivores (Poulton and Li., 1994) through a bitter taste and by releasing toxic hydrogen cyanide when tissue has been disrupted (Zagrobelny et al., 2004). However, the more specific phloem feeding mechanism of BPH may allow it to avoid this.

The phloem has its own defence mechanisms, including sieve plate blockage by callose deposition. A high level of expression of glucosyltransferase may facilitate BPH feeding by generating a high rate of callose turnover, which may have been selected during domestication as efficient phloem translocation was chosen over herbivore resistance.

The rice blast resistance gene, Os08g0539700 (*Oryza sativa* PibH8) is also found among the 239 genes and falls into the GO subclass _response to biotic stimulus'. This gene is a member of the nucleotide binding site (NBS) and leucine-rich repeat (LRR) class of plant disease resistance (R) genes and belongs to a small gene family (Wang et al., 2001). This gene was found to produce high constitutive expression in the resistant rice varieties and associated with shorter BPH phloem ingestion and a longer time for BPH reach phloem. In pathogenesis, this gene was reported as up-regulated by the environment when conditions favour the infection (Wang et al., 2001). Resistance factors like jasmonic acid, salicylic acid, ethylene and probenazole are also claimed to influence the expression level of this gene (Wang et al., 2001).

As PibH8 gene is a member of the nucleotide binding site (NBS) and leucine-rich repeat (LRR), it could therefore share several features with gene Os12g0199100 (*Oryza sativa* nucleotide-binding leucine-rich-repeat (NB-LRR) protein 1). The NB-LRR class resistance genes (R) involved act as receptors in signal transduction pathways that are triggered as a resistance response against pathogens (Hammond-Kosack and Jones, 1997;

Thirumalaiandi et al., 2008). The NBS region is important for ATP binding for the overall functionality of the R-gene product (Walker et al., 1982; Saraste et al., 1990; Thirumalaiandi et al., 2008). Ori et al. (1997) have reported the involvement of this classified R gene in resistance processes characterized by a hypersensitive response (HR). This class of gene is already proven as being involved in resistance against bacteria, fungi, viruses, nematodes and aphids (Timmerman et al., 2000; Thirumalaiandi et al., 2008). In rice, there is evidence that this gene provides a high level of resistance against the green rice leafhopper (GRH), *Nephotettix cincticeps* (Fujita et al., 2009).

The gene with the best combination of the correlation values which refer to BPH resistance mechanism in GO subclass ‗response to biotic stimulus' is Gene Os07g0522600 coding for a putative glutamate receptor protein GLR3.4. It produced the highest correlation for pathway (r=0.69), phloem ingestion (r=-0.68), total honeydew drop (r=0.69) and the time BPH starts ingesting the phloem (r=0.72). Davenport (2002) suggests that GLRs function as constitutively active non-selective cation channels, a suggestion supported by when they were observed in plasma membranes (Demidchik et al., 2007). There are several roles played by the gene which include regulation of hypocotyl elongation (Lam et al., 1998; Dubos et al., 2003), sensing of mineral nutrient status (Kim et al., 2001), regulating carbon/nitrogen balance (Kang and Turano, 2003), resisting aluminium toxicity (Sivaguru et al., 2003) and cold (Meyerhoff et al., 2005), root meristem function (Li et al., 2006; Walch-Liu et al., 2006), as well as jasmonate-mediated defence mechanisms (Kang et al., 2006, Stephens et al., 2008). The explanation of the defence mechanism, however, is very limited.

The final gene which needs to be highlighted in this study is gene Os12g0118400 (putative alpha/beta hydrolase). Although this gene also has a negative response to BPH feeding behaviour, it has the highest fold difference values (17.40) in the GO sub-class ‗response to biotic stimulus'. This gene is common to several hydrolytic enzymes of widely differing phylogenetic origin and catalytic function (Ollis et al., 1992) and has been reported to be involved in cell wall development, detoxification processes (Urs et al., 1998) and programmed cell death (Mishra et al., 2010).

In summary, this study has proved the usefulness of transcriptomics approaches for identifying candidate BPH resistance genes even where there is no BPH interference in the plant. This provides an advantage for an early selection process in a plant breeding program. Moreover, it is also able to explain the molecular mechanism of a plant defence system. The results clearly clarify the important role of constitutive genes for early protection against BPH attack. Although this discussion has managed to cover only a few of the significantly differentially expressed genes from the list of 239, the results presented here are able to facilitate the prioritization of strong candidate genes for further detailed investigation such as in functional genomic study. Another important step is to test the hypothesis proposed in this study. It is strongly suggested that the three genes coding for hexose transporter (Os10g0539900), metallothionein-like protein (Os12g0571100) and zinc finger (Os12g0631200) are used for cloning testing similar to what has been done on *Bph14* by Du et al. (2009). These three selected genes have been classified as the strongest potential candidate genes in this study based on their close association with known BPH gene markers and BPH feeding ability.

CHAPTER 6

SUMMARY AND LIMITATIONS

6.0 Summary

Rice is being attacked by many herbivores including phloem feeding insects, which have already caused rice production major problems. Brown planthopper (BPH) is one of the most significant phloem feeding insects which can destroy rice plants in a short time. Considering the fact that rice is a staple food for many millions of people, controlling BPH is extremely important to overcome these problems. The use of host plant resistance is common practice in any commercial agriculture crop for environmental safety and saving costs over a long period. However, to identify suitable resistance genes is a major challenge in this approach. In addition, the mechanism of plant defence is also a complex process to understand. Recent advances in genomics and bioinformatics studies, however, have helped to solve those issues.

In this study, three main experiments were conducted to achieve the objectives. The first two experiments are associated with aphid and BPH feeding behaviour. A transcriptomics approach was used in the third experiment to identify candidate resistance genes through gene expression level. A combination of morphological and molecular data has produced significantly useful information to support the findings.

6.1 Bird cherry-oat aphid (*Rhopalisphum padi*) feeding and growth performance on rice

EPG is one common method used in much research for the detailed monitoring of feeding behaviour studies focussing on phloem feeding insect types. So far, this is the first study to

investigate the feeding behaviour of aphids using a DC-EPG-based approach on rice. Interest has previouly focussed more on other cereal crops such as wheat. In this study, bird cherry-oat aphid (*Rhopalisphum padi*) was chosen because it is the closest local aphid which has previously been associated with rice. The results from this preliminary study, however, have revealed that bird cherry-oat aphid do not prefer rice as a primary host. This EPG study confirmed that the total duration aphids are able to ingest phloem sap (E2) even on a young plant is only 19 % of the total duration of 6 h. It is far lower than on several varieties of wheat with more than 49 % of E2 duration (Givovich and Niemer., 1991; Slesak et al., 2001). This duration reduces sharply to only 1.5 % on mature plants. Therefore, this process affects their survival rate. It was found that they can survive only 13 days on average, which is still lower compared with the 21.4 days (Taheri and Restegari, 2010) and 25.13 days (De Celis et al., 1997) on wheat plants. During that time, only 3.4 nymphs were produced. The performance on other rice varieties did not have any effect because it was not significant when tested. This phenomenon caused difficulty generating aphid culture on rice for further study.

6.2 Variation of resistance level rice varieties based on BPH feeding performance

BPH is a small brownish insect classified as one of the most serious pests of rice. Asian countries are the places most affected, where outbreaks are frequently found and the heaviest losses of yield have been reported (Park et al., 2007). Consequently, this insect was significantly more interesting than aphids. Another EPG test with the support of a honeydew clock experiment was conducted to study its feeding behaviour across several rice varieties from the collection. The results show that the DC-EPG-based characterizations found are more or less consistent with those previously described (Kimmins, 1989; Lösel and

Goodman, 1993; Seo et al., 2009), especially for character N4a (sieve element ingestion) and N4b (phloem ingestion) waveforms. Interestingly, two other new waveforms which have never been highlighted before in rice studies are also included in these characterizations. The first waveform, N6 was classified as ‗derailed stylet mechanic' and was associated with stylet penetration difficulty, while the second waveform, N7 was correlated with cell penetration activities close to a potential drop in aphids.

Like aphids, BPH is in the same phloem feeding group that secretes soluble saliva (watery) and salivary sheath (gel) during sap sucking (Cooper et al., 2010; Konishi et al., 2009). Watery saliva contains numerous digestion and oxidation enzymes that seem to play roles in establishing and maintaining feeding sites by assisting stylet penetration, suppressing plant defences and/or inducing changes in plant physiology that benefit their feeding and nutrition (Miles, 1999; Will et al., 2007). The difference between aphids and BPH is reported as being the way they use the salivary sheath to enter the sieve element region. Spiller (1990) claims that in aphids, only the stylets go through into the cell and the salivary sheath stops at the sieve element cell wall. In BPH, the maxillary stylets are accompanied by sheath saliva which enters the sieve element during sap sucking. This sheath saliva can easily be seen in phloem sap even after the BPH ceases feeding (Sogawa, 1982). This mechanism could result in blockage of the vascular bundle and interference with the translocation of nutrients especially if it involves greater amounts of sheath saliva inside the phloem sap (Wang et al., 2004). Therefore, this mode of feeding can cause more destruction to the plant tissue than with aphids. It could also explain why the waveforms of BPH and aphids are different as shown in figure 23.

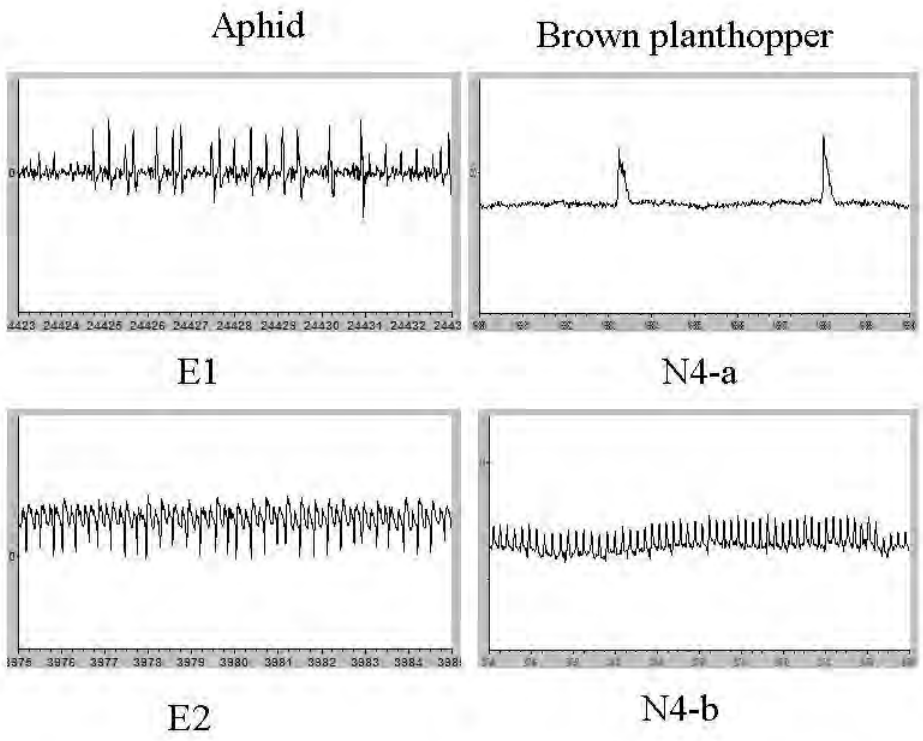


Figure 23 Comparison of waveform patterns between aphid and BPH. E1; Sieve element salvation for aphid, N4-a; Seive element for BPH, E2; Phloem ingestion for aphid, N4-b; Phloem ingestion for BPH.

So far, this is also the first experiment which has successfully screened resistance to BPH using the EPG method on the largest accession number with 12 rice varieties. The results confirm that the EPG system is applicable to mass screen study. Based on these EPG parameters, the twelve varieties have been separated into three groups using the Ward cluster analysis. The highly resistant group has a very low percentage of N4b duration of less than 3 percent. This group includes Rathu heenathi, Babawee, IR64 and F1 variety. They are consistent with previous studies which also confirmed that this group contains certain resistant genes. *Bph1* gene was found in IR64 (Cohen et al., 1997), *bph4* in Babawee, and *Bph3* in Rathu Heenathi (Nemoto et al., 1989; Khush et al., 1985) and the F1 (from the cross between Rathu Heenathi and TN1). In contrast, the susceptible group has the highest N4b duration with over 73 percent overall, and it is represented by TN1, Azucena, Nipponbare

and IR694. The other group containing variety MR219, MR232, Fujisaka and IR75 was classified as moderate and was close to resistance character. A long history of breeding could have contributed to this character, where the resistance genes have an ancestor donor such as in MR219 and MR232 (Alias et al., 2001).

This study has demonstrated that BPH has the ability to locate its stylet in sieve elements even in resistance varieties. The difference begins at phloem sap ingestion because it retreats to proceed, thus providing a clue that the chemical content in phloem sap may play some role in the defence mechanism. Silicic, oxalic (Chen, 2009; Yoshihara et al., 1979a; Yoshihara et al., 1979b; Yoshihara et al., 1980) and phenolic acids (Chen., 2009; Fisk., 1980), sterols (Shigematsu et al., 1982) and apigenin-C-glycosides (Stevenson et al., 1996; Grayer et al., 1994) are among the chemicals inside the phloem, which have been reported to contribute to the resistance character to BPH. In addition, the total amount of essential amino acids could also influence BPH feeding performance (Sogawa, 1982), perhaps representing phago-stimulatory cues. The real process in detail, however, is very complex and needs further explanation.

6.3 Gene expression study

Microarray is one of the most common methods these days which could help answer many questions in relation to biological systems. The ability to detect and analyse the expression of thousands of genes simultaneously in one single analysis has made microarray a unique approach. In addition, with the assistance of genomic information, its application has become more widespread and benefitted many areas of study. As a plant breeder, the main objective is not only to identify resistance candidate genes to BPH in rice plants, but at the same time to also try to understand the real mechanism behind the scenes.

The uniqueness of this experiment was that the samples were not subjected to BPH infection as commonly practised previously. This study focussed only on constitutive genes which are more durable, with a wide coverage and stable in the long term. Constitutive character did not change easily (Karban and Baldwin, 1997) and needed specific conditions or induction to activate as in inducible genes (Ahman, 2009). The microarray analysis results showed that 239 genes from 41,000 genes (Rice Agilent gene chips) have a significantly different expression level between the resistance and susceptible groups. Another 219 genes were found to have a significantly different expression level between the moderate resistance and susceptible groups. There was no significance found when the resistance and moderate resistance groups were compared. Interestingly, these results are almost the same as those produced by the EPG experiment which provided full evidence of the reliability of the morphological and molecular data. These data also support the previous claim that moderate and resistance varieties could have a close genetic relationship due to the long history of the breeding process as in MR219 and MR232 (Alias et al., 2001).

Despite the great potential of this microarray experiment, it is important to keep in mind that further analysis is needed to support the efficiency which would provide useful for the establishment of the hypotheses. In addition, 239 genes are still a large number and need to be summarized followed the interest of this study. Therefore, the following three different approaches were conducted in this study.

Simple linear correlation analysis was used to connect information between morphological and molecular data. This first approach allowed for the identification of the relationship between gene expressions and BPH feeding behaviour. The results finally separated 239 candidate genes into two big groups. The genes with a high positive correlation expression with N4-b (phloem ingestion) were classified as susceptible varieties. In contrast, high negative correlations with N4-b were grouped as resistance. Ontology (GO) analysis

through the web-based tool EasyGO was used in the second approach. This method helped to describe and understand better which genes contribute to the defence function. Interestingly, the _response to biotic stimulus‘ (GO: 0009607) category, which is related to the main objective of this study was found to have the highest significant level (FDR value=0.00015) with 28 genes. Generally, gene coding for protein kinase, nutrient transporter and peroxidase were clearly the main products and appeared several times in the annotation description list. All these genes are highly positive correlated with N4-b duration.

The final approach was more specific because it referred directly to the known bph genes based on their reference markers. All 239 candidate genes were screened using GRAMENE web to identify their locus position within the known reference BPH gene markers. As a result, only 7 genes were found matched within the known bph markers regions identified by Sanju et al. (2010) (RM261, RM185, RM17/RM12), Jairin et al. (2007) (RM19291, RG1), Sun et al. (2005) (RM484) and Park et al. (2008) (RM1103). Out of the seven, three genes belong to Rathu Heenathi (*Bph3*, Qbph10, *Bph15*), which indicates that genes from Rathu Heenathi are likely to contribute significantly to the differentiation between the resistance and susceptible groups. It is clear evidence that Rathu Heenathi is a good parent due to the heritable resistance character in its progeny, F1. This variety has been used extensively in rice breeding programmes in Asia since 1980 (Khush et al., 1985; Jairin et al., 2007). Gene Os10g0539900 encoding hexose transporter is the only gene which has been found in all 3 approaches. This could indicate that this gene is the strongest constitutive candidate gene associated with BPH resistance character.

6.4 Different constitutive gene expression as a strategy to prevent BPH attack

The successful defence against herbivores depends greatly on the ability of plants to protect themselves at an early stage (Maffei et al., 2007) and usually refers to constitutive characters as they appear in the absence of the attacker (Bodenhausen, 2007). Their strategy is to create a border around the target area, the place where the herbivore starts to attack. The success of this mechanism is highly correlated with the insect feeding mode. BPH is classified as a phloem feeding insect which is highly specialized in its mode of feeding and presents a unique stress on plant fitness (Douglas, 2006). It is able to stay feeding for a very long period when the feeding site is well established (Thompson, 2006). In this discussion, several candidate constitutive defence genes were divided into several categories based on their functions and roles.

6.4.1 Genes involved in attraction factors

Results from the correlation analysis show that many of the candidate genes have a positive relationship with susceptible characters. This clearly reveals that those genes with high expression may create a special constitutive character in susceptible varieties which may attract the attention of BPH feeding activities. One of the main factors is plants with higher nutritional contents because BPH like other herbivores requires this nutrient for its growth. Nutrient transporters are among those important genes which have been reported to have an influence on nutrient contents and their concentration in plants (Kreuzwieser and Gessler, 2010). There were seven candidate genes under GO sub-class ‗response to biotic stimulus' which were found annotated with a transporter function. Among them was gene encoding hexose transporter (STP1), which is commonly found expressed in sink organs such as roots

and green fruits (Gear et al., 2000; Weschke et al., 2003). This gene is involved in the uptake of glucose or fructose (Sauer and Stadler., 1993; Kühn et al., 2003; Baxter et al., 2005; Fridman et al., 2004; Frankel et al., 2007). The other two genes, monosaccharide transporter 1 and amino acid permease (AAP3) also have a similar function in sink tissues in delivering or distributing nutrients and acid amino to many parts of the plant (Okumoto et al., 2004; Sauer and Stasler, 1993). This process provides strong evidence that the transporter genes such as hexose, monosaccharide transporter 1 and amino acid permease (AAP3) with higher expression could contain a higher nutrient and sugar content. Several reports have also claimed that acid amino is able to act as phagostimulants (Leckstein and Llewellyn, 1974) and play roles in host selection by eliciting insect feeding and oviposition (Bernays and Chapman, 1994; Chiozza et al., 2010). These processes clearly facilitate BPH feeding on susceptible rice varieties that have high nutrient transporter expression genes. A high positive correlation value for phloem ingestion and time to first phloem ingestion supports this hypothesis.

6.4.2 Non attraction factors (toxification and detoxification)

The reverse effect on BPH feeding behaviour appeared when it retreated to locate its stylet from the phloem region especially in resistance varieties as shown in the EPG experiment. This could have been caused by toxic compounds which have been suggested play a significant role in plant defence against herbivores especially related to their ingestion process (Majorczyk, 2009). In this study based on the present gene annotation, there were no genes that were found to act directly on this constitutive defence mechanism. Generally, the genes found to be associated with this toxic character responded positively to the BPH feeding performance such as cytochrome p450s and glutathione S-transferase. Therefore, the results suggest that those genes with high expression act as detoxification rather than

toxification, which can help BPH feed better. This hypothesis could be true because the insect has been reported able to manipulate glutathione S-transferase and neutralize the toxic effects caused by eletrophilic substrates (Grant and Matsumura, 1989; Mittapalli et al., 2007)

Gene cytochrome p450s and glutathione S-transferase are also often claimed to influence the secondary metabolite process which could generate a defence mechanism. Therefore, a higher constitutive level may generate a lower level and/or a faster turnover of secondary metabolite that could interfere with BPH feeding. These genes also have large gene families with diverse functions as evidenced by the observation that one of the genes showed the opposite response to BPH feeding behaviour.

There are several other genes reported involved in this toxification process. Among them are gene encoding for B-glucosidae (Poulton and Li, 1994), amygdalin hydrolase isoform (Zagrobelny et al., 2004) and peroxidases (Felton et al., 1989, 1992; Dowd, 1994; Dowd and Norton, 1995). It has been claimed that many of these genes produce toxicity compounds that cause the plant to taste bitter and less nutritious to the insect (Felton et al., 1989, 1992; Dowd, 1994; Dowd and Norton, 1995; Zagrobelny et al., 2004). The levels of gene expression, however, show a reverse pattern. The higher expression level was associated with the plant being more susceptible to BPH attack. The insect may once again exploit specific chemicals such as cytochrome p450s and glutathione S-transferase to neutralize toxicity and allow it to feed, but further studies are needed to test this hypothesis.

6.4.3 Genes involved in defence signals

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are major components for hormones involved in defence signalling networks and often refer to inducible defence characters (Glazebrook, 2001; Kessler and Baldwin, 2002; Pieterse and Dicke, 2007; Reymond and Farmer, 1998; Thomma et al., 2001; Van Oosten et al., 2008). These hormones

are able to activate several defence mechanisms such as in producing plant secondary metabolites, for example plant volatilise, or in repairing wounded areas as a response to pathogen or herbivorous attacks. There are several genes in the candidate list found to support or associated with this defence process. Gene coding for acid carboxyl methyltransferase (MeJA), fatty acid alpha-oxidase (Alpha-DOX2) and 12-oxo-phytodienoic acid reductases (OPRs) are major compounds in the JA biosysnthesis process (Tao et al., 2003).

Acid carboxyl methyltransferase has been claimed to be able to induce an increase in the number of direct chemical defences of plants (such as nicotine and proteinase inhibitors) resulting in a significant improvement in plant resistance to herbivore damage (Tao et al., 2003). Consequently, acid carboxyl methyltransferase activity is also found to increase rapidly following an herbivorous attack, and these signal molecules activate the plant defence-related genes to produce various chemical defences (Tao et al., 2003; Xu et al., 2006; Kwon et al., 2009). Alpha-DOX2 has also been reported to behave in the same way in increasing the expression after BPH attack in both susceptible and resistant rice plants (Wei et al., 2009). Interestingly, both genes were found associated with the *bph2* gene at chromosome 12 (Ren et al., 2004).

Another group of genes which has been reported involved in defence signals is that with protein kinase annotation (Ding et al., 2009). These genes are claimed to play a central role in signalling during pathogen recognition (Romeis, 2001) and subsequently activate downstream proteins through phosphorylation which eventually lead to a defence response, most often in the form of a hypersensitive response (HR) (Eck, 2007). Protein kinases are one of the largest gene families in plants. In rice, protein kinases represent around 1,400 proteins in the rice genome and have a diverse range of functions including some in defence signals (Ding et al., 2009). Putative protein kinases also represent the majority of genes annotated in

the candidate list, and several of them have high correlation values above r=0.8 with phloem ingestion parameter. This indicates that this type of gene may play a significant role in plant defence systems against insects such as BPH. Wei et al. (2009) have confirmed that expression of protein kinases increases especially in the susceptible lines after BPH treatment, which is similar to what this study found in certain kinases genes even without the appearance of BPH itself. Protein kinases have been shown to regulate aphid resistance signalling pathways in tomatoes and an increased expression of a protein kinase correlated with resistance to BPH in rice (Wei et al., 2009)

Protein kinases have also been reported to interact directly with a pathogen‘s avirulence resistance genes (R genes), and encoding for nucleotide-binding leucine-rich-repeat (NB-LRR) protein (Eck, 2007). There are approximately over 400 NB-LRR encoding genes in the *Oryza sativa* genome (Meyer et., 2003; Monosi et al., 2004; McHale et al., 2006), mostly associated with phatogen infection (McHale et al., 2006). These genes play the role of receptors in signal transduction pathways that are a triggered resistance response in a series of plant defence responses, such as activation of an oxidative burst, calcium and ion fluxes, mitogen-associated protein kinase cascade, induction of pathogenesis-related genes and the hypersensitive response (Belkhadir et al., 2004; Hammond and Parker, 2003; Nimchuk et al., 2003; Pedley and Martin, 2005; McHale et al., 2006; Thirumalaiandi et al., 2008). Several studies have already proved that NB-LRR genes are involved in initiating a signal transduction for plant defence against bacteria, fungi, viruses, nematodes and aphids (Timmerman et al., 2000; Thirumalaiandi et al., 2008). This study found that high expression of NB-LRR in resistant varieties has been associated with a reduction in the total time that BPH ingest the phloem. Therefore, the results reveal that this gene may have a direct effect on BPH feeding ability.

The classic example gene associated with NB-LRR is *Bph14*, the only successful gene being cloned at the present time (Du et al., 2009). *Bph14,* however, encodes a more specific gene annotation with a coiled-coil, nucleotide-binding and leucine-rich repeat (CC-NB-LRR) protein. Although the 44k Agilent genechip contains CC-NB-LRR gene annotation, it did not appear in the candidate list because it is a unique gene, specific to *Bph14,* which the gene derives from the wild rice variety, *O. Officinalis* (Li et al., 2011). NB-LRR gene in this study is probably a general constitutive resistance gene from the three resistance varieties, IR64 (*Bph1*), Rathu heenathi (*Bph3*) and Babawe (*bph4*).

6.4.4 Genes involved in cell walls

The reinforcement of cell walls is important in plant defence mechanism because they are the first place where the infection from pathogens or herbivores begins. Plants use complex compounds such as cellulose and lignin to produce physical structures such as thick cell walls (Boudet, 1998), wax, thorns, or sticky resin (Bodenhausen, 2007) to prevent and create a physical barrier against infection. Gene encoding for peroxidases is found involved in several important roles in the process of cell wall lignification such as cell wall enhancement and the deposition of cell wall appositions, both of which can involve the polymerization of lignin or suberin (Hammerschmidt and Kuc, 1982; Espelie et al., 1986) and the cross-linking of wall glycoproteins or polysaccharides (Bradley et al., 1992; Fry, 1986; Iiyama et al., 1994), while gene encoding for the beta-glucosidase enzyme plays a support role in the lignification process (Whetton and Sederoff, 1995).

Generally, plants also have other defence mechanisms to support the efficiency of lignifications of cell walls based on the wounding response for repairing or recovering lesions (Herrmann, 1995). There are several genes in the candidate list which were found involved in this mechanism. For example, gene encoding for fatty acid alpha-oxidase mRNA acts in the

following: repairing stress-induced damage in membranes and regulating the fluidity of membranes and permeability to toxic ions (Holmberg and Bulow, 1998; Way et al., 2005); gene encoding metallothionein-like protein (OsMT) protecting against intracellular oxidative damage (Zhou et al., 2006); gene encoding zinc finger involving controlling the induction of cell death (Eulgem et al., 1999), and putative alpha/beta hydrolase involved in programme cell death (Mishra et al., 2010). All these, however, had a high expression in susceptible varieties except for alpha/beta hydrolase. To date, there has been very limited information associating alpha/beta with defence mechanisms.

6.6 Limitations and future work

The results presented in this experiment are basically based on the present rice genome annotation data base. More than 38% from all on the candidate gene list are still without a known description function, meaning that nobody has assigned a function to those genes in the genome database yet. However, as knowledge of this genomic study is being rapidly updated based on present trends especially into rice as a model plant for cereal crops, so it will help to develop and discover many opportunities in the future. For the time being, all these unknown functions of genes remain potential areas to be explored later.

In this study, all 12 varieties have been chosen because of known resistant/susceptible characteristics, but some of them without any such prior knowledge. After the EPG experiment was conducted, these 12 varieties have been separated into three categories. Coincidentally, the two varieties in the susceptible group, namely Azucaena and Nipponbare are in Japonica subspecies. In contrast, all 4 varieties in the resistant group are in Indica subspecies. Therefore, the effect of different gene blocks existing between those subspecies could also be associated with the 239 candidate genes from the microarray analysis. Since the more important factor in this study‘s objectives is BPH resistance, the influence from

subspecies group of Japonica can be dismissed. In addition, this is the reason why the three approaches, correlation, gene ontology and QTL mapping information were introduced in order to help validate the resistance candidate genes.

The current plan in the near future is to validate these microarray results using an RT-PCR experiment on the selected candidate genes. This validation process could not be done during this study due to the difficulty faced in identifying a suitable housekeeping gene as a control treatment which could represent all the 12 rice varieties in the experiment. Four housekeeping genes suggested by Jain et al. (2006), namely Actin 11 (AK100267), Eukaryotic elongation factor 1-alpha (AK061464), Ubiquitin 5 (AK061988) and 18S ribosomal RNA (AK059783) were tested; however, none of them were succesful. There are many factors which could have influenced the results especially those involved in technical areas such as RNA quality, RNA volume or concentration, primer selection, the modification of the PCR machine, including time, temperature and the number of cycles. These problems become harder when they involve a large variety of plants as in this study and could take a long period of time to solve.

In this thesis, a large number of genes that are significantly involved in differentiating between resistances and are susceptible to brown plant hopper based on expression value has been discoverd. This information could both benefit and provide many opportunities for the exploration of gene functions in future research. The transcriptomics results, however, can only make it easier to create these hypotheses. Therefore, the hypotheses proposed in this study should be tested to confirm the contribution of those candidate genes in constitutive defence mechanisms against BPH. It is strongly suggested that the three genes‘coding for hexose transporter (Os10g0539900*),* metallothionein-like protein (Os12g0571100) and zinc finger (Os12g0631200), which were found through the fine mapping method with known BPH markers, should be the first to enter the cloning process for a functional genomic test.

These three selected genes have been classified as the strongest candidate genes based on their close association with known BPH gene markers and BPH feeding ability. To date, only *Bph14* has successfully been cloned (Du et al., 2009), which has been the inspiration to add another collection of BPH resistance genes for cloning for breeding purposes in the future.

Another area of research which could receive special consideration in future work is the study of the variation of nutritional value of the phloem sap in the twelve rice varieties. This information could be important to support the results of gene expression studies and could suggest that high nutrient content in the phloem sap could play a major role as a resistance indicator or attractor. The end result could give a better understanding of the relationship between gene expression values, BPH feeding behavior and phloem sap quality. The information of this chemical analysis could thefore support both present and future experiments. Phloem sap collection can be achieved through the stylectomy technique, the method that is commonly used in this area of study.

LIST OF REFERENCES

Agrawal, A.A., Gorski, P.M. and Tallamy D.W. (1999) Polymorphism in plant defence against herbivory: constitutive and induced resistance in *Cucumis sativus*. Journal of Chemical Ecology 25:2285–2304

Aharoni, A. and Vorst, O. (2001) DNA microarrays for functional plant genomics. Plant Molecular Biology 48: 99–118

Ahman, I., Tuvesson, S. and Johansson, M. (2000) Does Indole alkaloid Gramine confer resistance in barley to aphid *Rhopalosiphum padi*? Journal of Chemical Ecology, vol. 26, no. 1

Ahman, I. (2009) Breeding for inducible resistance against insects – applied plant breeding aspects. IOBC-WPRS bulletin 44:121-130

Akashi, K., Nishimura, N., Ishida, Y. and Yokota, A. (2004) Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon. Biochem. Biophys. Res. Com. 323:72-78

Akinlosotu, T.A. (1977) Outbreak of the rusty plum aphid, *Hysteroneura setariae* Th. (Homoptera, Aphididae), on rice (*Oryza sativa* L.) in Ibadan, Nigeria. Ghana. J. Agric.Sci. 10: 149-150

Alam, S.N. and Cohen, M.B. (1998) Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. Theor Appl Genet. 97:1370–1379

Alexandratos, N., Bruinsma, J. and Boedeker, G. (2006) World Agriculture: Towards 2030/2050. Interim Report. Prospects for Food, Nutrition, Agriculture and Major Commodity Group*s*. Global Perspective Studies Unit, Food and Agriculture Organization of the United Nations

Alias, I. Mohamad, H., Othman, O., Saad, A. and Habibuddin, H. (2001) Pembentukan dan prestasi varieti padi baru MR219. Malaysia Agriculture Research and Development Institute pp.283-293

Allemeersch, J. (2006) Statistical analysis of microarray data: applications in platform comparison, compendium data, and array CGH. Ph.D.Thesis. Electrical engineering, Katholieke Universitiet, Leuven, Belgium

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., et al. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 25: 25–29

Athwal, D.S., Pathak, M.D., Bacalangco, E. and Pura, C. (1971) Genetics of resistance to brown planthoppers and green leaf hoppers in Oryza sativa L. Crop Sci. 11: 747-750

Backus, E.A., Hunter, W.B., (1989) Comparison of feeding behavior of the potato leafhopper *Empoasca fabae* (Homoptera: Cicadellidae) on alfalfa and broad bean leaves. Environmental Entomology 18, 473-503

Bahlman, L., Govender, P. and Botha A.M (2003) Leaf epicuticular wax ultrastucture and trichomes presences on Russian wheat aphid (*Diuraphis noxia*) resistant and susceptible leaves, African Entomology 11:59-64

Baldwin, D., Crane, V. and Rice, D. (1999) A comparison of gel-based, nylon filter and microarray techniques to detect differential RNA expression in plants. Curr. Opin. Plant Biol. 2:96–103

Baxter, C.J., Carrari, F., Bauke, A., Overy, S., Hill, S.A., Quick, P.W., Fernie, A.R. and Sweetlove, L.J. (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. Plant Cell Physiol. 46:425–437

Becker, W. and Apel, K. (1992) Isolation and characterization of a cDNA clone encoding a novel jasmonate-induced protein of barley (*Hordeum vulgare* L.). Plant Mol. Biol. 19:1065–67

Belkhadir, Y., Subramaniam, R. and Dangl, J.L. (2004) Plant disease resistance protein signaling: NBS-LRR proteins and their partners. CurrOpin Plant Biol. 7:391-399

Bentur, J.S. and Viraktamath, B.C. (2008) Rice planthoppers strike back. Current Science vol 95, no. 4:441-443

Bernays, E.A. and Chapman R.F. (1994) Host-plant selection by phytophagous insects, Chapman & Hall, New York

Blackman, R. L. and Eastop, V.F. (1984) Aphids on the world‘s crops: An identification guide. John Wiley and Sons, Chichester, UK

Bodenhausen, N. (2007) *Arabidopsis thaliana* response to insect feeding: new components controlling defence gene expression and plant resistance. Ph.D Thesis. Université de Lausanne, Switzerland

Boudet, A.M. (2000) Lignins and lignification: Selected issues. Plant Physiol. Biochem. 38 (1/2): 81−96

Bradley, D.J., Kjellbom, P. and Lamb, C.J. (1992) Elicitor and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defence response. Cell 70: 21-30

Brar, D.S., Virk, P.S., Jena, K.K. and Khush, G.S. (2009) Breeding for resistance to planthoppers in rice. Planthopper: new threats to the sustainability of intensive rice production systems in Asia. IRRI: 401–27

Brazma, A., Hingamp, P., Quackenbush, J., Sherlock, G., Spellman, P., et al. (2001). Minimum information about a microarray experiment (MIAME)—toward standards for microarray data. Nature Genetics 29:365 – 371

Bush, D.R. (1993) Proton-coupled sugar and amino acid transporters in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 513–542

Böttner, M. and Sauer, N. (2000) Review: Monosaccharide transporters in plants: structure, function and physiology. Biochimica et Biophysica Acta 1465: 263-274

Böttner, M., Truernit, E., Baier, K., Scholz-Starke, J., Sontheim, M., Lauterbach, C., Huss, V.A.R. and Sauer, N. (2000) AtSTP3, a green leaf-specific, low affinity monosaccharide-H+ symporter of *Arabidopsis thaliana*. Plant, Cell Environ. 23:175–184

Calatayud, P.A., Rahbe, Y., Tjallingii, W.F., Tertuliano, M., Ru, B.l., Le Ru, B., (1994) Electrically recorded feeding behaviour of cassava mealybug on host and non-host plants. Entomologia Experimentalis et Applicata 72, 219-232

Capinera, J. L. (2004) Encyclopedia of Entomology. New York: Kluwer Academic

Carter, N., McLean, I.F.G., Watt, A.D. and Dixon, A.F.G. (1980) Cereal aphids: a case study and review. In: Advances in Applied Biology, (ed). TH Coaker, pp. 271–348. New York: Academic

Catindig, J.L.A., Arida, G.S., Baehaki, S.E., Bentur, J.S., Cuong, L.Q., Norowi, M., Rattanakarn, W., Sriratanasak, W., Xia, J. and Lu, Z. (2009) Situation of planthoppers in Asia. In Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia, pp. 191–220. Eds K.L. Heong and B. Hardy. Los Banos, Philippines: International Rice Research Institute

Chang, T.T. (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. Euphytica 25(1): 425-441

Chaudhry, B., Mueller-Uri, F., Cameron- Mills, V., Gough, S., Simpson, D., et al. (1994) The barley 60 kDa jasmonate-induced protein (JIP60) is a novel ribosome-inactivating Protein. Plant J. 6:815–24

Chen, H. Y. (2009) Variation in planthopper-rice interactions: possible interactions among three species? In Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia, pp. 315-326. Eds K.L. Heong and B. Hardy. Los Banos, Philippines: International Rice Research Institute

Chen, S.X., Petersen, B.L., Olsen, C.E., Schulz, A. and Halkier, B.A. (2001) Long-distance phloem transport of glucosinolates in Arabidopsis. Plant Physiol. vol.127:194–201

Chester, K. (1933) The problem of acquired physiological immunity in plants. Quarterly Rev. Biol. 8:275-324

Chevalier, D. and Walker, J.C. (2005) Functional genomics of protein kinases in plants. Briefings in Functional Genomic and Proteomics vol.3 no 4:362–371

Chiozza, M., O'Neal, M. and MacIntosh, G. (2010) Constitutive and induced differential accumulation of amino acid in leaves of susceptible and resistant soybean plants in response to the soybean aphid (Hemiptera: Aphididae). Environmental Entomology 39, 3:856-864

Choi, D., Bostock, R.M., Avdiushko, S. and Hildebrand, D.F. (1994) Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes antimicrobial isoprenoids in *Solanum tuberosum* L'. Proc. Natl. Acad. Sci. USA. 91:2329–33

Chu, T.T., Fink, M.Y., Mong, J.A., John, G., Auger, A.P., Ge, Y. and Sealfon S.C. (2007) Effective use of microarrays in neuroendocrine research. J Neuroendocrinol vol.19 no.3:145-161

Cobbett, C. and Goldsbrough, P. (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu. Rev. Plant Biol. 53:159-182

Cohen, M.B., Alam, S.N., Medina, E.B. and Bernal, C.C. (1997) Brown planthopper, *Nilaparvata lugens*, resistance in rice cultivar IR64: mechanism and role in successful N-*lugen*s management in Central Luzon, Philippines'. Entomologia Experimentalis Et Applicata 85: 221-229

Corbesier, L., Havelange, A., Lejeune, P., Bernier, G. and Perilleux, C. (2001) N content of phloem and xylem exudates during the transition to flowering in *Sinapis alba* and *Arabidopsis thaliana*. Plant, Cell and Environment 24: 367–375

Cooper, W., Dillwith, J.W. and Puterka G.J (2010) Physiological Ecology: Salivary Proteins of Russian Wheat Aphid (Hemiptera: Aphididae). Environ. Entomol. 39(1): 223-231

Creelman, R.A., Tierney, M. L. and Mullet, J.E. (1992) Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc. Natl. Acad. Sci. USA. vol.89:4938–41

Creelman, R.A. and Mullet, J. E. (1997) Biosynthesis and action of jasmonate in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:355–81

Dale, D. (1994) Insect pests of the rice plant—their biology and ecology. Ref. 52:364–85

Daniels, M., Bale, J.S., Newbury, H.J., Lind, R.J. and Pritchard, J. (2009) A sublethal dose of thiamethoxam causes a reduction in xylem feeding by the bird cherry-oat aphid (*Rhopalosiphum* padi), which is associated with dehydration and reduced performance. Journal of Insect Physiology 55:758-765

Davenport, R. (2002) Glutamate receptors in plants, Ann. Bot. 90:549-557

De Celis, V.R., Gassen, D., Valente, V.L. and De Oliveira, A.K. (1997) Longevity, fecundity and embryogenesis in Brazilian aphids. Pesquisa agropecuaria brasileira 32(2):137-146

Delhaize, E., Ryan, P. R., Hebb, D. M., Yamamoto, Y., Sasaki, T. and Matsumoto, H. (2004) Engineering high-level aluminium tolerance in barley with the ALMT1 gene. Proc. Natl. Acad. Sci. 101:15249-15254

Demidchik, V. and Maathuis, F.J.M. (2007) Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development, New Phytol. 175:387-404

Devadas, S.K., Enyedi, A. and Raina, R. (2002) The *Arabidopsis hrl1* mutation reveals novel overlapping roles for salicylic acid, jasmonic acid and ethylene signalling in cell death and defence against pathogens. The Plant Journal 30:467–80

Deverall, B.J. (1997) Disease control through natural plant defences. International Turfgrass Society Research Journal vol. 8

De Vos, M., Van Oosten, V.R., Van Poecke, R.M.P., Van Pelt, J.A., Pozo M.J., et al. (2005) Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Mol. Plant-Microbe Interact. 18:923–37

Dicke, M. and Van Poecke, R.M.P. (2002) Signaling in plant-insect interactions: Signal transduction in direct and indirect plant defence In: Plant Signal Transduction: Frontiers in Molecular Biology pp.289-316

Dinant, S., Bonnemain, J., Girousse, C. And Kehr, J. (2010) Phloem sap intricacy and interplay with aphid feeding. Comptes Rendus Biologies Volume: 333, Issue: 6-7, pp.504-515

Ding, X., Richter, T., Chen, M., Fujii, M., Seo, Y.S. et al. (2009) A Rice Kinase-Protein Interaction Map. Plant Physiology vol. 149:1478–1492

Dixon, A.F.G (1985) Aphid Ecology. Blackie, Glasgow

Djamin, A. and Pathak, M.D (1967) Role of silica in resistance to asiatic rice borer, Chilo suppressalis Walker in rice varieties. J. Econ. Entomol. 60:347-351

Doares, S.H., Narvaez-Vasquez, J., Conconi, A. and Ryan, C.A. (1995) Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol. 108:1741–46

Domingo, C., Andres, F., Tharreau, D., Iglesias, D.J. and Talon, M. (2009) Constitutive expression of OsGH3.1 reduces auxin content and enhances defence response and resistance to a fungal pathogen in rice. Mol Plant Microbe Interact. 22(2):201–10

Doncaster, J. P. (1956) The rice root aphid. Bull. Entomol. Res. 47: 741–747

Douglas, A.E. (1993) The nutritional quality of phloem sap utilized by natural aphid populations. Ecological Entomology 18:31–38

Douglas A.E. (2006) Phloem-sap feeding by animals: problems and solutions. Journal of Experimental Botany vol.57, no. 4:747–754

Dowd, P.F. (1994) Enhanced maize (*Zea mays* L.) pericarp browning: Associations with insect resistance and involvement of oxidizing enzymes. J. Chem. Ecol. 20:2497-2523

Dowd, P.F. and Norton, R.A. (1995) Browning-associated mechanisms of resistance to insects in corn callus tissue. J. Chem. Ecol. 21:583-600

Dubos, C., Huggins, D., Grant, G.H., Knight, M.R. and Campbell, M.M. (2003) A role for glycine in the gating of plant NMDA-like receptors, Plant J. 35, pp.800-810

Du, B., Zhang, W., Liu, B., Hu, J., Wei, Z., Shi, Z., He, R., Zhu, L., Chen, R., Han, B. and He, G. (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. PNAS vol. 106 no. 52: 22163–22168

Dupo, A.L.B. and Barrion, A.T. (2009) Taxonomy and general biology of delphacid planthoppers in rice agroecosystems. In Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia, pp. 3–155. Eds K.L. Heong and B. Hardy, Los Banos, Philippines: International Rice Research Institute

Durrant, W.E. and Dong, X. (2004) Systemic acquired resistance. Annual Review of Phytopathology 42:185–209

Eck, L.V. (2007) Aphid-induced transcriptional regulation in near-isogenic wheat. Faculty of Natural and Agricultural Science, Department Genetic University Pretoria, Pretoria

Elliott, A.C. and Hynan, L.S.A. (2011) A SAS® macro implementation of a multiple comparison post hoc test for a Kruskal–Wallis analysis. Computer methods and programs in biomedicine 102: 75–80

Espelie, K.E., Franceschi, V.R. and Kolattukudy, P.E. (1986) Immunocyto-chemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound-healing potato tuber tissue. Plant Physio. l81:487-492

Eulgem, T., Rushton, P.J., Schmelzer, E., Hahlbrock, K. and Somssich, I.E. (1999) Early nuclear events in plant defence signalling:rapid gene activation by WRKY transcription factors. The EMBO Journal vol.18 no.17:4689–4699

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

Felton, G.W., Donato, K., Delvfcchio, R.J. and Duffy, S.S. (1989) Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J.Chem. Ecol. 15:2667-2694

Felton, G.W., Donato, K.K., Broadway, R.M. and Dliffey, S.S. (1992) Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. J. Insect Physiol. 38:277-285

Ferry, N., Edwards, M.G., Gatehouse, J.A. and Gatehouse, A.M.R. (2004) Plant–insect interactions: molecular approaches to insect resistance. Current Opinion in Biotechnology 15:155–161

Feys, B.J. and Parker, J.E. (2000) Interplay of signalling pathways in plant disease resistance. Trends Genet.16:449-455

Fisher, D.B. (2000) Long-distance transport. In: Buchanan B, Gruissem W, Jones R, eds. Biochemistry and molecular biology of plants. Rockville, USA: American Society of Plant Physiologists pp.730–784

Fisk, J. (1980) Effects of hydrogen. Entomol. Exp. Appl. 27:211-222

Forslund, K., Morant, M., Jørgensen, B., Olsen, C.E., Asamizu, E., Sato, S., Tabata, S. and Bak, S. (2004) Biosynthesis of the nitrile glucosides rhodiocyanoside A and D and the cyanogenic glucosides lotaustralin and linamarin in *Lotus japonicus*. Plant Physiol. 135:71–84

Fotopoulos, V., Gilbert, M.J., Pittman, J.K., Marvier, A.C., Buchanan, A.J., Sauer, N., Hall, J.L. and Williams, L.E. (2003) The monosaccharide transporter gene, AtSTP4, and the cell-wall invertase, *Atbetafruct1*, are induced in Arabidopsis during infection with the fungal biotroph *Erysiphe cichoracearum*. Plant Physiol. 132:821–829

Frankel, N., Nunes-Nesi, A., Balbo, I., Mazuch, J., Centeno, D., Iusem, N.D., Fernie, A.R. and Carrari, F. (2007) ci21A/Asr1 expression influences glucose accumulation in potato tubers. Plant Mol Biol. 63:719–730

Fridman, E., Carrari. F., Liu, Y.S., Fernie. A.R. and Zamir, D. (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science 305:1786–1789

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

Fujita, D., Myint, K.K.M., Matsumura, M. and Yasui, H. (2009) The genetics of host-plant resistance to rice planthopper and leafhopper. In: Heong KL, Hardy B, editors. Planthoppers: new threats to the sustainability of intensive rice production systems in Asia. Los Baños (Philippines): International Rice Research Institute, pp.389-400

Gallagher, K. D., Kenmore, P. E. and Sogawa. K. (1994) Judicial use of insecticides deter planthopper outbreaks and extend the life of resistant varieties in Southeast Asian rice. In: R. F. Denno and J.T. Perfect (eds). Planthoppers: Their Ecology and Management.Chapman and Hall, New York pp.599–614

Gallun, R.L. and Khush, G.S. (1980) Genetic factors affecting expression and stability of resistance. In: Maxwell FG, Jennings PR (eds) Breeding plants resistant to insects., John Wiley and Sons, New York pp.63–85

Gary, C. J., Azan, L.P. and Jocelyn, B.P. (2005) Effect of Nitrogen Fertilizer on the Intrinsic Rate of Increase of *Hysteroneura setariae* (Thomas) (Homoptera: Aphididae) on Rice (*Oryza sativa* L.). Environ. Entomol. 34(4): 938-943

Gear, M.L., McPhillips, M.L., Patrick, J.W. and McCurdy, D.W. (2000) Hexose transporters of tomato: molecular cloning. Expression analysis and functional characterization. Plant Mol Biol. 44:687–697

Geiger, D.R., and Servaites, J.C. (1994) Diurnal regulation of photosynthetic carbon metabolism in C-3 plants. Annual Reviews of Plant Physiology 45:235–256

Ghaffar, M.B. A.B., Pritchard, J. and Ford-Lloyd, B. (2011) Brown planthopper (*N. lugens* Stal) feeding behaviour on rice germplasm as an indicator of resistance. PLoS One Vol: 6 iss:7 pg:e22137

Gianoli, E. (1999) Within-plant distribution of *Rhopalosiphum padi* on wheat seedlings is affected by induced responses. Entomologia Experimentalis et Applicata 93(2):227-230

Gibson G. (2002) Microarrays in ecology and evolution: a preview. Molecular Ecology 11(1): 17-24

Givovich, A. and Niemeyer, H.M. (1991) Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*. Entomologia Experimentalis et Applicata 59:79-85

Glazebrook, J. (2001) Genes controlling expression of defence responses in Arabidopsis – 2001 status. Current Opinion in Plant Biology 4:301–308

Gomathi, S.K. (2002) Identification of genetic locus associated with resistance to brown planthopper *(Nilaparvata lugens Sta°l*) in rice (*Oryza sativa L.*). PhD thesis, Tamil Nadu Agricultural University, Coimbatore

Grant, D.F. and Matsumura, F. (1989) Glutathione S-transferase 1and 2 in susceptible and resistant insecticide resistant Aedes aegypti. Pesticide Biochemistry and Physiology 33:132-143

Grayer, R. J., Kimmins, F.M., Stevenson, P.C., Stevenson, J.B. and Wijayagunesekera, H.N.P. (1994) Phenolics in rice phloem sap as sucking deterrents to the brown planthopper (*Nilaparvata lugens*). Acta Hortic. 381:391-394

Grönberg, N. (2006) Induction of pathogenesis-related genes, PR-17a and N-methyltransferase, in barley infested by the aphid Rhopalosiphum padi, Master's thesis, Södertörns högskola, University College, Stockholm, Sweden

Gurr, G.M., Liu, J., Read, D.M.Y., Catindig, J.L.A., Cheng, J.A., Lan, L.P. and Heong, K.L. (2011) Parasitoids of Asian rice planthopper (Hemiptera: Delphacidae) pests and prospects for enhancing biological control by ecological engineering. Ann Appl Biol. 158:149–176

Hall, J. L. (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53:1-11

Hallinan, J., Bradley, D. and Wiles, J. (2006) Effects of constitutive gene expression on the dynamics of random Boolean networks., in: IEEE Congress on Evolutionary Computation pp. 2355–2361

Hammerschmidt, R. and Kuc, J. (1982) Lignification as a mechanism for induced systemic resistance in cucumber. Physiol. Pl. Path. 20:61–71

Hammond-Kosack, K.E. and Jones, J.D.G. (1997) Plant disease resistance genes. Annual Review of Plant Physiology and Plant Molecular Biology. vol. 48, 575-607

Hammond-Kosack, K.E. and Parker, J.E. (2003) Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. Curr Opin Biotechnol. 14:177-193

Harbome, J.B. (1980) Plant phenolics. In Secondary Plant Products, ed. E. A. Bell, B. V. Charlwood , Berlin: Springer-Verlag pp.329-402

Harrewijn, P., Tjallingii, W.F., Mollema, C., (1996) Electrical recording of plant penetration by western flower thrips. Entomologia Experimentalis et Applicata 79, 345-353

Hao, P., Liu, C., Wang, Y., Chen, R., Tang, M., et al. (2008) Herbivore induced callose deposition on the sieve plates of rice: an important mechanism for host resistance. Plant Physiol. 146: 1810-1820

Hattori, M. (2001) Probing behaviour of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae) on a non-host barnyard grass, and resistant and susceptible varieties of rice. Appl Entomol Zool 36: 83-89

Hattori, M. and Sogawa, K. (2002) Oviposition behaviour of the rice brown planthopper, *Nilaparvata lugens* (Stal), and its electronic monitoring. Journal of Insect Behaviour 15: 283-293

Heidel, A.J. and Baldwin, I.T. (2004) Microarray analysis of salicylic acid and jasmonic acid-signalling in responses of Nicotiana attenuate to attack by insects from multiple feeding guilds. Plant, Cell and Environment 27:1362–1373

Henry, D.B., Tolan, P.H. and Gorman-smith, D. (2005) Cluster Analysis in Family Psychology Research. Journal of Family Psychology 19(1):121-132

Herrmann K. M. (1995) The Shikimate Pathway: Early Steps in the Biosynthesis of Aromatic Compounds. The Plant Cell vol.7:907-919

Hirabayashi, H., Kaji, R., Angeles, E.R., Ogawa, T., Brar, D.S. and Khush, G.S. (1999) RFLP analysis of a new gene for resistance to brown planthopper derived from *O. officinalis* on rice chromosome 4 (in Japanese). Breed Sci Suppl. 49:48

Hoang, A. (2010) The Role of Peroxidase in the Defence Response of Buffalograss to Chinch Bugs. Thesis dissertation, University of Nebraska, Lincoln.

Hogendrorp, B.K. (2008) Effects of silicon-based fertilizer applications on the development and reproduction of insect pests associated with greenhouse-grown crops. PhD Thesis. University of Illinois

Holmberg, N, and Bulow, L. (1988) Improving stress tolerance in plants by gene transfer, Trends Plant Sci. 3:61–66

Howe, G.A. and Schaller, A. (2008) Direct Defences in plants and their induction by wounding and insect herbivores. In: Induced plant resistance to herbivory: Springer, Science. pp 7- 30

Huang, J., Qiao, F. and Zhang, L. (2000) Farm Pesticide, Rice Production and Human Health. Health (San Francisco)

Hughes, L. and Bazzaz, F.A. (2001) Effects of elevated CO2 on five plant-aphid interactions. Entomologia Experimentalis et Applicata 99(1):87-96

Hui, D.Q., Iqbal, J., Lehmann, K., Gase, K., Saluz, H.P. and Baldwin, I.T. (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. Plant Physiol. 131:1877-1893

Hunt, E. J., Pritchard, J., Bennett, M.J., Zhu, X., Barrett, D.A., Allen, T., Bale, J.S. and Newbury, H.J. (2006) The *Arabidopsis thaliana* / *Myzus persicae* model system demonstrates that a single gene can influence the interaction between a plant and a sap-feeding insect. Mol. Ecol. 15:4203–4213

Hunt, E., Gattolin, S., Newbury, H.J., Bale, J.S., Tseng, H.M., Barrett, D.A. and Pritchard, J. (2010) A mutation in amino acid permease AAP6 reduces the amino acid content of the Arabidopsis sieve elements but leaves aphid herbivores unaffected. J. Exp. Bot. 61:55–64

Hunter, W.B., Ullman, D.E., Moore, A., (1993) Electronic monitoring: characterizing the feeding behavior of western flower thrips (Thysanoptera: Thripidae). In: Elsbury, M.M., Backus, E.A., Ullman, D.E. (Eds.), History, Development, and Application of AC Electronic Insect Feeding Monitors. Thomas Say Publications in Entomology. Entomological Society of America., Maryland, pp. 73-85

Ikeda, R. and Kaneda C. (1981) Genetic analysis of resistance to brown planthopper, *Nilaparvata lugens* (Stal), in rice. Jpn. J. Breed. 31:279-285

Ishii, T., Brar, D.S., Multani, D.S. and Khush, G.S. (1994) Molecular tagging on genes for brown planthopper resistance and earliness introgressed *Oryza australiensis* into cultivated rice, *Oryza sativa*. Genome 37:217-221

Jahn, G.C., Almazan, L.P. and Pacia, J.B. (2005) Effect of nitrogen fertilizer on the intrinsic rate of increase of *Hysteroneum setariae* (Thomas) (Homoptera: Aphididae) on rice (*Oryza sativa* L.). Environ Entomol. 34:938–943

Jairin, J., Teangdeerith, S., Leelagud, P., Phengrat, K., Vanavichit, A. and Toojinda, T. (2007) Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. Sci. Asia 33: 347–352

Jairin, J., Sansen, K., Wongboon, W. and Kothcharerk, J. (2010) Detection of a brown planthopper resistance gene *bph4* at the same chromosomal position of *Bph3* using two different genetic backgrounds of rice. Breeding Science 60:71–75

Janssen, J.A.M., Tjallingii, W.F., van Lenteren, J.C., (1989) Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. Entomologia Experimentalis et Applicata 52, 69-81

Jena, K.K., Pasalu, I.C., Rao, Y.K., Varalaxmi, Y., Krishnaiah, K., et al. (2003) Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). Euphytica 129: 81-88

Jena, K.K., Jeung, J.U., Lee, J.H., Cho, H.C. and Brar, D.S. (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18*(t), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet. 112:288–297

Jena, K.K. (2010) The species of the genus Oryza and transfer of useful genes from wild species into cultivated rice, *O. sativa.* Breeding Science 60:518-523

Jena, K.K. and Kim, S.M. (2010) Current Status of Brown Planthopper (BPH). Resistance and Genetics. Rice 3(2-3), 161-171

Johnson, R. and Ryan, C.A. (1990) Wound-inducible potato inhibitor II genes: enhancement of expression by sucrose. Plant Mol. Biol. 14:527–36

Juliano, B.O. (1993) Rice in Human Nutrition. FAO/IRRI. FAO Food and Nutrition Series No.26., FAO, Rome

Kabir, M.A. and Khush, G.S. (1988) Genetic analysis of resistance to brown planthopper in rice, *Oryza sativa* L. Plant Breed. 100:54-58

Kaloshian, I. and Walling, L.L. (2005) Hemipterans as plant pathogens. Annual Review of Phytopathology 43:491–421

Kang, J. and Turano, F.J. (2003) The putative glutamate receptor 1.1 (AtGLR1.1) functions as a regulator of carbon and nitrogen metabolism in *Arabidopsis thaliana.* Proc Natl Acad Sci USA. 100: 6872–6877

Kang, S., Kim, H.B., Lee, H., Choi, J.Y., Heu, S., Oh, C.J., Kwon, S.I. and An, C.S. (2006) Over-expression in Arabidopsis of a plasma membrane-targeting glutamate receptor from small radish increases glutamate-mediated Ca21 influx and delays fungal infection. Mol Cells 21:418–427

Karban, R. and Myers, J.H. (1989) Induced plant response to herbivory. Annu. Rev. Ecol. Syst. 20:331-48

Karban, R. and Baldwin, I.T. (1997) Induced Responses to Herbivory. University of Chicago Press, Chicago, IL

Karley, A.J., Douglas, A.E. and Parker, W.E. (2002) Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. Journal of Experimental Biology. 205: 3009–3018

Kasimoto, R. and Dyck, V.A. (1976) Climate and rice insect. In: Proceedings of the Symposium on Climate and Rice. IRRI 565 pages

Kehr, J., Hustiak, F., Walz, C., Willmitzer, L. and Fisahn, J. (1998) Transgenic plants changed in carbon allocation pattern display a shift in diurnal growth pattern. The Plant Journal 16:497–503

Kemp, H., Newbury, J., Bale, J. and Pritchard, J. (2008) Investigating plant-aphid interactions in Arabidopsis; using functional genomics to investigate the role of the plant amino acid transporter AAP1. American Society of plant biology. http://abstracts.aspb.org/pb2008/public/P14/P14040.html (Oct 21, 2011)

Kessler, A., and Baldwin, I.T. (2002) Plant responses to insect herbivory: The emerging molecular analysis. Annu. Rev.Plant Biol. 53: 299-328

Khan, Z.R. and Saxena, R.C. (1988) Probing behaviour of three biotypes of *Nilaparvata lugens* (Homoptera: Delphacidae) on different resistant and susceptible rice varieties. Journal of Economic Entomology 81: 1338-1345

Khush, G.S., Rezaul-Karim, A.N.M.R. and Angeles, E.R. (1985) Genetics of resistance of rice cultivar ARC10550 to Bangladesh brown planthopper biotype. J Genet. vol.64: 121-125.

Khush, G.S. and Brar, D.S. (1991) Genetics of resistance to insects in crop plants. Advances in Agronomy 45:223-274

Khush, G.S. (2005) What it will take to feed 5.0 billion rice consumers by 2030. Plant Mol. Biol. 59:1–6

Kim, C.K., Kim, J.H., Shin, Y.H., Park, S.H., Yun, D.W., Ahn, B.O., Kim, D.H., Park, B.S. and Hahn, J.H. (2009) A genome browser database for rice (*Oryza sativa*) and Chinese cabbage (*Brassica rapa*). Afr. J. Biotechnol. vol. 8 (20): 5253 –5259

Kim, S.A., Kwak, J.M., Jae, S.K., Wang, M.H. and Nam, H.G. (2001) Over-expression of the AtGluR2 gene encoding an Arabidopsis homolog of mammalian glutamate receptors impairs calcium utilization and sensitivity to ionic stress in transgenic plants. Plant Cell Physiol. 42:74–84

Kimmins, F.M. (1989) Electrical penetration graphs from *Nilaparvata lugens* on resistant and susceptible rice varieties. Entomol. Exp. Appl. 50:69–79

Kindler, D., Hesler, L., Elliott, N., Shufran, K. and Springer, T. (2004) Cereal and Grass Hosts of the Rice Root Aphid, *Rhopalosiphum rufiabdominalis* (Sasaki), and a Description of an Efficient Greenhouse Rearing Technique. J. Agric. Urban Entomol. 21(1): 51–59

Kindt F (2004) Behavioural study on the feeding of Western flower thrips related to Tomato spotted wilt virus transmission and host plant susceptibility. Ph.D. Thesis .van Wageningen Universiteit

Koeduka, T., Matsui, K., Hasegawa, M., Akakabe, Y. and Kajiwara, T. (2005) Rice fatty acid alpha-dioxygenase is induced by pathogen attack and heavy metal stress: activation through jasmonate signaling. J. Plant Physiol. 2005, 162:912–920

Kreuzwieser, J. and Gessler, A. (2010) Global climate change and tree nutrition: influence of water availability. Tree Physiol 30 (9): 1221-1234

Kühn, C., Hajirezaei, M.R., Fernie, A.R., Roessner-Tunali, U., Czechowski, T., Hirner, B. and Frommer, W.B. (2003) The sucrose transporter StSUT1 localizes to sieve elements in potato tuber phloem and influences tuber physiology and development. Plant Physiol. 131:102–113

Kumari, S., Sheba, J.M., Marappan, M., Ponnuswamy, S., Seetharaman, S., Pothi, N., Subbarayalu, M., Muthurajan, R. and Natesan, S. (2010) Screening of IR50 3 Rathu Heenati F7 RILs and Identification of SSR Markers Linked to Brown Planthopper (*Nilaparvata lugens* Sta°l) Resistance in Rice (*Oryza sativa* L.) Mol Biotechnol. 46:63–71

Kunkel, B.N. (2002) Cross talk between signaling pathways in pathogen defence. Current Opinion in Plant Biology 5, 325–331

Kush, G.S. (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35:25–34

Kwon, S., Hamada, K., Matsuyama, A., Yasuda, M., Nakashita, H. and Yamakawa, T. (2009) Biotic and abiotic stresses induce AbSAMT1, encoding S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase, in Atropa belladonna. Plant Biotechnology 26: 207–215

Lakshminarayana, A. and Khush, G.S. (1977) New genes for resistance to the brown planthoppers. Crop Science 17: 96-100

Lam, H.M., Chiu, J., Hsieh, M.H., Meisel, L., Oliveira, I.C., Shin, M. and Coruzzi, G. (1998) Glutamate-receptor genes in plants, Nature 396:125-126

Lanning, F. C. and Eleuterius, L. N. (1992) Silica and Ash in Seeds of Cultivated Grains and Native Plants. Annals of Botany 151-160

Leather, S. R. and Dixon, A.F.G. (1981) The effect of cereal growth stage and feeding site on the reproductive activity of the bird-cherry aphid, *Rhopalosiphum padi*. Annals of Applied Biology, 97(2), 135-141

Leather, S.R., Walters, K.F.A. and Dixon, A.F.G. (1989) Factors determining the pest status of the bird cherry-oat aphid , *Rhopalosiphum padi* (L .) (Hemiptera: Aphididae), in Europe : a study and review. Growth (Lakeland):345-360

Leckstein, P.M. and Llewellyn, M. (1974) Role of amino acids in diet intake and selection and utilization of dipeptides by *Aphis fabae*. J. Insect Physiol. 20: 877- 885

Lei, H., Tjallingii, W.F., Van Lenteren, J.C., (1998) Probing and feeding characteristics of the greenhouse whitefly in association with host-plant acceptance and whitefly strains. Entomologia Experimentalis et Applicata 88, 73-80

Lercher, M.J., Urrutia, A.O. and Hurst, L.D. (2002) Clustering of housekeeping genes provides a unified model of gene order in the human genome. Nature Genetics 31(2):180–183

Lett, J.M., Granier, M., Grondin, M., Turpin, P., Molinaro, F., Chiroleu, F., Peterschmitt, M., Reynaud, B., (2001) Electrical penetration graphs from Cicadulina mbila on maize, the fine structure of its stylet pathways and consequences for virus transmission efficiency Entomologia Experimentalis et Applicata 101, 93-109

Lewin, J. and Reimann, B.E.F. (1969) Silicon and Plant Growth. Annual Review of Plant Physiology 20(1), 289-304

Li, J., Zhu, S., Song, X., Shen, Y., Chen, H., Yu, J., Yi, K., Liu, Y., Karplus, V.J., Wu, P., et al. (2006) A rice glutamate receptor-like gene is critical for the division and survival of individual cells in the root apical meristem. Plant Cell 18: 340–349

Li, J., Chen, Q., Wang, L., Liu, J., Shang, K. and Hua, H. (2011) Biological effects of rice harbouring *Bph14* and *Bph15* on brown planthopper, *Nilaparvata lugens*. Pest Management Science Vol.67, Issue 5:528–534

Linares, O.F. (2002) African rice (*Oryza glaberrima*): history and future potential. Proc. Natl Acad. Sci. USA 99, 16360–16365

Liu, Y. (2005) Investigating insect molecular responses to two plant defence proteins and characterizing a novel insecticidal protein from Arabidopsis. Ph.D Thesis. Department of Entomology, Texas A&M University

Iiyama, K., Lam, T.B.T. and Stone, B.A. (1994) Covalent cross-links in the cell wall. Plant Physiol. 104: 315–320

Lockhart, D.J. and Winzeler, E.A. (2000) Genomics, gene expression and DNA arrays. Nature 405:827–836

Lösel, P.M. and Goodman, L.J. (1993) Effects on the feeding behaviour of *Nilaparvata lugens* (Stål) of sub lethal concentrations of the foliarly applied nitromethylene heterocycle 2-nitromethylene-1, 3-thiazinan-3-yl-carbarnal dehyde. Physiol Entomol. 18:67-74

Loxdale, H.D. and Brookes, C.P. (1988) Electrophoretic study of enzymes from cereal aphid populations . *V. Spatial* and temporal genetic similarity of holocyclic populations of the bird-cherry oat aphid, *Rhopalosiphum padi* (L) (Hemiptera :Aphididae), in Britain. East. 241-249

Maffei, M.E., Mithofer, A. and Boland, W. (2007) Before gene expression: early events in plant–insect interaction. Trends. in Plant Science vol.12 no.7

Majorczyk, A.M. (2009) Chemical Defence Mechanisms of *Arabidopsis thaliana* Against Insect Herbivory: The Role of Glucosinolate Hydrolysis Products. Thesis: Master of Science (MS), Bowling Green State University, Biological Sciences

Marger, M.D. and Saier, M.H. Jr. (1993) A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends in Biochemical Sciences vol. e 18, Issue 1, January, pp. 13-20

Marley, S.B. and Gordon, M.Y. (2005) Chronic myeloid leukemia: Stem cell derived but progenitor cell driven. Clinical Science 109(1): 13 – 25

Matis, J.H., Kiffe, T.R., Matis, T.I. and Stevenson, D.E. (2007) Stochastic modeling of aphid population growth with nonlinear, power-law dynamics. Mathematical Biosciences 208:469-494

Matsumura, M. and Sanada-morimura, S. (2010) Recent Status of Insecticide Resistance in Asian Rice Planthoppers. East Asia 44:225-230

Matthews, R.E.F. (1991) Plant Virology. 3rd Edn., Academic Press, San Diego, pp: 835

Mattson, W.J. (1980) Herbivory in Relation to Plant Nitrogen Content. Annual Review of Ecology and Systematics 11(1):119-161

McHale, L., Tan, X., Koehl, P. and Michelmore, W.R. (2006) Review: Plant NBS-LRR proteins: adaptable guards. Genome Biology 2006:7:212

McLean, D.L. and Kinsey, M.G. (1964) A technique for electronically recording aphid feeding and salivation. Nature 202, 1358–1359

Meng, S., Brown, D.E., Ebbole, D.J., Torto-Alalibo, T., Oh, Y.Y., Deng, J., Mitchell, T.K. and Dean, R.A. (2009) Gene Ontology annotation of the rice blast fungus, *Magnaporthe oryzae.* BMC Microbiol. 9(Suppl 1):S8

Métraux J.P. and Raskin, I. (1993) Role of phenolics in plant disease resistance. In I Chet, ed, Biotechnology in Plant Disease Control. Wiley-Liss, New York pp.191-209

Meyerhoff, O., Mu¨ller, K., Roelfsema, M.R.G., Latz, A., Lacombe, B., Hedrich, R., Dietrich, P. and Becker, D. (2005) AtGLR3.4, a glutamate receptor channel like gene is sensitive to touch and cold. Planta 222:418–427

Meyers, B.C., Kozik, A., Griego, A., Kuang, H. and Michelmore, R.W. (2003) Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell 15:809-834

Miles, P. W. (1999) Aphid saliva. Biol. Rev. 74: 41-85

Mishra, A., Pandey, D., Goel, A. and Kumar, A. (2010) Molecular Cloning and In silico Analysis of Functional Homologues of Hypersensitive Response Gene(s) Induced During Pathogenesis of Alternaria Blight in Two Genotypes of Brassica. J Proteomics Bioinform, vol. 3(8):244-248

Mitchell, M.C. (2008) Conserving Brassica Crop Wild Relative Diversity: Exploiting data from Ecogeography and the Transcriptome. Ph.D. Thesis. School of Biosciences, The University of Birmingham

Mithofer, A., Wanner, G. and Boland, W. (2005) Plant resistance towards insect herbivores: a dynamic interaction. Plant Physiol. 137: 1160–1168

Mittapalli, O., Neal, J.J. and Shukle, R. H. (2007) Tissue and life stage specificity of glutathione S-transferase expression in the Hessian fly, Mayetiola destructor: Implications for resistance to host allelochemicals. Journal of Insect Science vol.7, no. 20:1536-2442

Monosi, B., Wisser, R.J., Pennill, L. and Hulbert, S.H. (2004) Full-genome analysis of resistance gene homologues in rice. Theor Appl Genet. 109:1434-1447

Moose, S.P. and Mumm, R.H. (2008) Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. Plant Physiology 147:969-977

Moran, P.J. and Thompson, G.A. (2001) Molecular responses to aphid feeding in Arabidopsis in relation to plant defence pathways. Plant Physiol. 125:1074–1085.

Morange, M. (2009) The Central Dogma of Molecular Biology A Retrospective after Fifty Years. Resonance: 236-247

Morant, A.V., Jørgensen, K., Jørgensen, C., Paquette, S.M., Sánchez-Pérez, R., Møller, B.L. and Bak, S. (2008) β-Glucosidases as detonators of plant chemical defence. Phytochemistry 69:1795–1813

Mullins, I.M. (1999) Evolutionary Relationships in Oryza Inferred from the Gene that Encodes the 10 kDa Prolamin (Seed Storage Protein) Polypeptide. Master's of Science In Biology. Faculty of the Virginia Polytechnic Institute and State University

Nakano, K., Abe, G., Taketa, N. and Hirano, C. (1961) Silicon as an insect-resistance component of host plant, found in relation between the rice stem-borer and the rice plant. Jap. J. Appl. Entomol. Zool. 5: 17-27

Nambiar, S. and Hengge, U.R. (2005) Gene expression patterns in melanoma reveal two independent predictors. Workshop: Microarray technologies in clinical oncology; potential and perspectives. June 30, Roma

Nandi, S., Subudhi, P.K., Senadhira, D., Manigbas, N.L., Sen-Mandi, S. and Huang, N. (1997) Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol. Gen. Genet. 255:1–8.

Nemoto, H., Ikeda, R. and Kaneda, C. (1989) New Gene for rice resistance to brown planthopper, *Nilaparvata lugens* stal, in rice. Japanese Journal of Breeding 39: 23-28

Neher, O.T. (2008) Disease control and plant defence pathways induced by *Bacillus mojavensis* isolate 203-7 and *Bacillus mycoides* isolate BMJ. Montana State University, Bozeman, Montana

Nguyen, N. and Ferrero, A. (2006) Meeting the challenges of global rice production. Paddy and Water Environment 4(1):1-9

Nimchuk, Z., Eulgem, T., Holt, B.F. and Dangl, J.L. (2003) Recognition and response in the plant immune system. Annu Rev Genet. 37:579-609

Nørholm, M.H.H., Hussam, H., Nour-Eldin, Brodersen, P., Mundy, J. and Halkier, B.A. (2006) Expression of the Arabidopsis high-affinity hexose transporter STP13 correlates with programmed cell death. FEBS Letters 580 (2006) 2381–2387

Oerke, E.C. (2005) Crop losses to pests. The Journal of Agricultural Science 144(01), 31

Okumoto, S., Schmidt, R., Tegeder, M., Fischer, W. N., Rentsch, D., Frommer, W.B. and Koch, W. (2004) High Affinity Amino Acid Transporters Specifically Expressed in Xylem Parenchyma and Developing Seeds of Arabidopsis. The Journal of Biological System Chemistry vol. 277, no. 47, Issue of November 22, pp. 45338–45346

Ollis, D.L., Shea, E., Cygler, M., Dijkstra, B., Frolow, F., et al. (1992) The hydrolase fold. Protein Engineering vol.5 no.3 pp.197-211

Ori, N., Eshed, Y., Paran, I., Presting, G., Aviv, D., et al. (1997) The I2C family from the wilt disease resistance locus I2 belongs to the nucleotide binding leucine-rich repeat superfamily of plant resistance genes. Plant Cell 9:521–32

Ortiz-Lopez, A., Chang, H.C. and Bush, D.R. (2000) Review Amino acid transporters in plants. Biochimica et Biophysica Acta 1465: 275-280

Osbourn, A. (1996). Saponins and plant defence - a soap story. Trends Plant Sci. 1:4-9

Panda, N. and Khush, G.S. (1995) Host plant resistance to insects. CAB International

Pantoja, A., Garcia, C.A. and Duque, M.C. (2000) Population dynamics and effects of *Oebalus ornatus* (Hemiptera: Pentatomidae) on rice yield and quality in South western Colombia. J. Econ. Entomol. 93: 276-279

Park, C.G. (1983) Effect of Temperatures and Relative Humidities on the Development of Brown Planthopper, *Nilaparvata lugen* (Stål). Korean J Plant Protection (4):262-270

Park, D.S., Lee, S.K., Lee, J.H., Song, M.Y., Song, S.Y., Kwak, D.Y., Yeo, U.S., Jeon, N.S., Park, S.K., Yi, G. et al. (2007) The identification of candidate rice genes that confer resistance to the brown planthopper (*Nilaparvata lugens*) through representational difference analysis. Theoretical and Applied Genetics 115:537–547

Park, D.S., Song, M.Y., Park, S.K., Lee, S.K., Lee, J.H., Song, S.Y., Eun, M.Y., Hahn, T.R., Sohn, J.K., Yi, G., Nam, M.H. and Jeon, J.S. (2008) Molecular tagging of the *Bph1* locus for resistance to brown planthopper (*Nilaparvata lugens* Sta˚ l) through representational difference analysis. Mol. Genet. Genom. 208:163–172

Patch, E.M. (1938) Food-plant catalogue of the aphids of the world. Maine Agric. Exp. Stn. Bull. 393: 36–431

Pathak, M.D. and Saxena, R.C. (1980) In: Smith H (ed) Breeding plants resistant to insects. Pergamon, Oxford. pp.61–81

Pathak, M.D. and Khan, Z.R. (1994) Insect Pests of Rice, International Rice Research Institute, Manila, The Philippines

Patterson, T.A., Lobenhofer, E.K., Fulmer-Smentek, S.B., Collins, P.J., Chu, T.M., et al., (2006) Performance comparison of one-colour and two-colour platforms within the Microarray Quality Control (MAQC) project. Nature Biotechnology vol.24 no 9:1140-1150

Paul, S. and Amundson, S.A. (2008) Development of gene expression signatures for practical radiation biodosimetry. Int J Radiat Onc Biol Phys. 71:1236 –1244

Pedley, K.F. and Martin, G.B. (2005) Role of mitogen-activated protein kinases in plant immunity. Curr Opin Plant Biol. 8:541-547

Penninckx, I.A.M.A., Eggermont, K., Terras, F.R.G., Thomma, B.P.H.J., De Samblanx, GW, et al. (1996) Pathogen-induced systemic activation of a plant defence gene in Arabidopsis follows a salicylic acid–independent pathway involving components of the ethyleneand jasmonic acid responses. PlantCell In press

Pham, T.M. and Bui, B.B. (1999) Evaluation of rice varieties for resistance to brown plant hopper in the Mekong delta. Omon Rice Journal 7:5–11

Pieterse, C.M.J. and Van Loon, L.C. (1999) Salicylic acid-independent plant defence pathways. Trends Plant Sci. 4: 52-58

Pieterse, C.M.J. and Dicke, M. (2007) Plant interactions with microbes and insects: From molecular mechanisms to ecology. Trends Plant Sci. 12:564-569

Pollard, D.G. (1973) Plant penetration by feeding aphids (Hemiptera, Aphidoidea) a review. Bull. Entomol. Res. 62:631–714

Ponder, K.L., Pritchard, J., Harrington, R. and Bale, J.S. (2000) Difficulties in location and acceptance of phloem sap combined with reduced concentration of phloem amino acids explain lowered performance of the aphid *Rhopalosiphum padi* on nitrogen deficient barley (*Hordeum vulgare*) seedlings. Entomologia Experimentalis et Applicata 97:203–210

Poulton, J.E. and Li, C.P. (1994) Tissue level compartmentation of (R)-amygdalin and amygdalin hydrolase prevents large scale cyanogenesis in undamaged prunus seeds. Plant Physiol. 104: 29–35

Prioul, J.L., Quarrie, S., Causse, M. and Vienne, D. (1997) Dissecting complex physiological functions through the use of molecular quantitative genetics. J. Exp. Bot. (1997) 48 (6): 1151-1163

Prado, E., and Tjallingii, W.F. (1994) Aphid activities during sieve element punctures. Entomologia Experimentalis et Applicata 72:157-165

Prado, E., and Tjallingii, W.F, (1997) Effects of previous plant infestation on sieve element acceptance by two aphids. Entomologia Experimentalis et Applicata 82:189-200

Prado, E., and Tjallingii, W.F. (1999) Effects of experimental stress factors on probing behaviour by aphids. Entomologia Experimentalis et Applicata 90(3):289-300.

QuiChang, T.T., Qu, S.H., Pathak, M.D., Ling, K.C. and Kauffman, H.E. (1975) The search for disease and insect resistance in rice germplasm. In: Frankel OH, Hawkes JG (eds) Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, pp 183–200

Rahman, M.L., Jiang, W., Chu, S.H., Qiao, Y., Ham, T.H., Woo, M.O., Lee, J., Khanam, M.S., Chin, J.H., Jeung, J.U., et al. (2009) High resolution mapping of two brown planthopper resistance genes, *Bph20(t*) and *Bph21(t),* originating from *Oryza minuta*. Theor.Appl. Genet. 119: 1237–1246

Rairdan, G.J. and Delaney, T.P. (2002) Role of salicylic acid and Nim1/npr1 in race-specific resistance in Arabidopsis. Genetics 161:803–811

Ralph, J., Bunzel, M., Marita, J.M., Hatfield, R.D., Lu, F., et al. (2004) Peroxidase-dependent cross-linking reactions of p-hydroxycinnamates in plant cell walls. Phytochemistry Reviews 3:79–96

Ramachandran, R. and Khan, Z.R. (1991) Mechanisms of resistance in wild rice *Oryza brachyantha* to rice leaffolder *Cnaphalocrocis medinalis* (Guene´ e) (Lepidoptera: Pyralidae). J. Chem. Ecol. 17: 41- 65

Ramputh, A.L. and Bown, A.W. (1996) Rapid and y-aniinobutyric acid synthesis and the inhibition of the growth and development of oblique-banded leaf-roller larvae. Plant Physiol 111:1 349- 1352

Randhawa, G.J., Bhalla, S., Chalam, V.C., Tyagi, V., Verma, D.D. and Hota, M. (2006) Document on Biology of Rice (*Oryza sativa* L.) in India. New Delhi: National Bureau of Plant Genetic Resources

Rani, P.U. and Jyothsna, Y. (2010) Biochemical and enzymatic changes in rice plants as a mechanism of defence. Acta Physiol Plant 32:695–701

Raskin, I. (1992) Roles of Salicylic Acid in Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:439-463

Ratnayaka, I. (1999) Agrobacterium-mediated gene transformation of rice: Comparison of callus and shoot apex derived plants. A Dissertation Submitted to Texas A&M University, Texas

Redona, E.D. and Mackill, D.J. (1996) Mapping quantitative trait loci for seedling vigor in rice using RFLPs. Theoretical and Applied Genetics 92, 395–402

Renganayaki, K., Fritz, A.K., Sadasivam, S., Pammi, S., Harrington, S.E., et al. (2002) Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, O. sativa. Crop Science 42:2112-2117

Ren, X., Wang, X. , Yuan, H., Weng, Q. , Zhu, L. and He, G. (2004) Mapping quantitative trait loci and expressed sequence tags related to brown planthopper resistance in rice. Plant Breeding 123:342-348

Reymond, P. and Farmer, E.E. (1998) Jasmonate and salicylate as global signals for defence gene expression. Curr. Opin. Plant Biol. 1:404-411

Reymond, P., Weber, H., Damond, M. and Farmer, E.E. (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis, Plant Cell 12:707–720

Reymond, P. (2001) DNA microarrays and plant defence. Plant Physiology and Biochemistry 39(3-4):313-321

Reymond, P., Bodenhausen, N., Van Poecke, R.M., Krishnamurthy, V., Dicke, M. and Farmer, E.E. (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16:3132–3147

Rola, A.C. and Pingali, L.P. (1993) Pesticides, Rice Productivity, and Farmers' Health: An Economic Assessment. Los Baños, Philippines, and Washington D.C: International Rice Research Institute and World Resource Institute

Romeis, T. (2001) Protein kinases in the plant defence response. Current Opinion in Plant Biology. 4:407–414

Salzman, R.A., Brady, J.A., Finlayson, S.A., et al. (2005) Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. Plant Physiology 138:352–368

Samuels, M.N. (2001) Optimization of APEX-Mediate DNA transformation in rice. Ph.D Thesis. Louisiana State University and Agricultural and Mechanical College, US

Santhanalakshmi, S., Saikumar, S., Shubhada, Roa., Saiharini, A., Khera, P., Shashidar, H.E and Kadirvel, P. (2010) Mapping genetic locus linked to brown planthopper resistance in rice *Oryza sativa* L. International Journal of Plant Breeding and Genetic 4 (1): 13-22

Saraste, M., Sibbad, P.R. and Wittinghoer, A. (1990) The P-loop-a common motif in ATP- and GTP-binding proteins. Trends in Biochemical Sciences. vol. 15, no.11:430-434

Sarria, E., Cid, M., Garzo, E. and Fereres, A. (2009) Excel Workbook for automatic parameter calculation of EPG data. Computers and Electronics in Agriculture 67(1):35-42

Sasamoto, K. (1961) Resistance of the rice plant applied with silicate and nitrogenous fertilizers to the rice stem borer, *Chilo suppressalis* Walker. Proc. of the Faculty of Liberal Arts Education 3, Yamanashi University, Japan 3: pp. 73

Sauer, N. and Stadler, R. (1993) A sink specific H1/monosaccharide cotransporter from *Nicotiana tabacum*: cloning and heterologous expression in baker‘s yeast. Plant J 4:601–610

Sauer, N., Baier, K., Gahrtz, M., Stadler, R., Stolz, J. and Truernit, E. (1994) Sugar transport across the plasma membranes of higher plants. Plant Mol. Biol. 26:1671–1679.

SAS Institute, (2008) SAS version 9.1 package

Schena, M., Shalon, D., Davis, R..W. and Brown P.O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995; 270: 467–470

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. and Manners, J.M. (2000) Co-ordinated plant defence responses in Arabidopsis revealed by microarray analysis. Proceedings of the National Academy of Sciences, USA :11655–11660

Schofield, R.A., Bi, Y.M., Kant, S. and Rothstein, S.J. (2009) Over-expression of STP13, a hexose transporter, improves plant growth and nitrogen use in *Arabidopsis thaliana* seedlings. Plant Cell Environ. 32: 271–285

Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., et al. (2002) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J. 31:279–92

Seo, H.S., Song, J.T., Cheong, J.J., Lee, Y.H., Lee, Y.W., Hwang, I., Lee, J.S. and Choi, Y.D. (2001) Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. Proc. Natl. Acad. Sci. USA 98: 4788–4793

Seo, B.Y., Kwon, Y.H., Jung, J.K. and Kim, G.H. (2009) Electrical penetration graphic waveforms in relation to the actual positions of the stylet tips of *Nilaparvata lugens* in rice tissue. Journal of Asia-Pacific Entomology 12: 89-95

Settle, W. H., Ariawan, H., Astuti, E.T., Cahyana, W., Hakim, A.L., Hindayana, D., et al. (1996) Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. Ecology 77:1975–1988

Sharma, N., Ketipearachchi, Y., Murata, K., Torii, A., Takumi, S., et al. (2003) RFLP/AFLP mapping of a brown planthopper (*Nilaparvata lugens* Stal) resistance gene *Bph1* in rice. Euphytica 129: 109-117

Shigematsu, Y., Murofushi, N., Ito, K., Kaneda, C., Kawabe, S. and Takahashi, N. (1982) Sterols and asparagine in the rice plant, endogenous factors related to resistance against the brown planthopper (*Nilaparvata lugens*). Agric. Biol. Chem. 46:2877-2879

Simon, J.C., Carrel, E., Hebert, P.D.N., Dedryver, C.A., Bonhomme, J., and Gallic, J.F. (1996) Genetic diversity and mode of reproduction in French populations of the aphid Rhopalosiphum padi L. Heredity 76: 305-313

Sitch, L.A., Dalmacio, R.D. and Romero, G.O. (1989) Crossability of wild Oryza species and their potential use for improvement of cultivated rice. Rice Genet Newslett 6:58–60

Sivaguru, M., Pike, S., Gassmann, W. and Baskin, T.I. (2003) Aluminum rapidly depolymerizes cortical microtubules and depolarizes plasma membrane: evidence that these responses are mediated by a glutamate receptor. Plant Cell Physiol. 44: 667–675

Slesak, E., Slesak, M. and Gabrys, B. (2001) Effect of methyl jasmonate on hydromix acid content, protease activity, and bird cherry-oat aphid, *Rhopalosiphum padi* (L.) probing. Journal of Chemical Ecology vol. 27, no. 12

Sogawa, K. (1982) The rice brown planthopper: feeding physiology and host plant interactions. Annu. Rev. Entomol. 27:49–73

Sogawa, K., Juan, Z. and Zhong-hai Q. (2003) Applications of DNA-marker to analyze rice planthopper resistance genes. Chinese J Rice Sci. 17:37-46

Spiller, N.J. (1990) An ultrastructural study of the stylet pathway of the brown planthopper, *Nilaparvata lugens*. Entomol Exp Appl. 54:191-193

Srivastava, P.N. and Auclair, J.L. (1975) Rôle of single amino acids in phagostimulation, growth, and survival of *Acyrthosiphon pisum.* Journal of Insect Physiology vol. 21, Issue 11:1865-1871

Stephens, N.R., Qi, Z. and Spalding, E.P. (2008) Glutamate Receptor Subtypes Evidenced by Differences in Desensitization and Dependence on the GLR3.3 and GLR3.4 Genes. Plant Physiology vol.146:529–538

Stevenson, P.C., Kimmins, F.M., Grayer, R.J. and Raveendranath S. (1996) Schaftosides from rice phloem as feeding inhibitors and resistance factors to brown planthoppers, *Nilaparvata lugens*. Entomol. Exp. Appl. 80:246-249

Suhaimi, A.H.M. (2009) Relationships between hypothalamic gene expression and the resumption of ovulation in postpartum beef cows. Ph.D. Thesis. The University of Queensland

Sun, L., Su, C., Wang, C., Zhai, H. and Wan, J. (2005) Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. Breed Sci. 55:391–396

Sundaramoorthi, J., Babu, C. and Ram, S.G. (2009) Molecular diversity in the primary and secondary gene pools of genus Oryza. Plant Syst. Evol. 279:115-123

TaheRi, S., RazmJou, J. and RaSTegaRi, N. (2010) Fecundity and Development Rate of the Bird Cherry-oat Aphid , *Rhopalosiphum padi* (L) (Hom : Aphididae) on Six Wheat Cultivars.' Plant Protect. Sci. 46(2):72-78

Takatsuji, H. (1998) Review: Zinc-finger transcription factors in plants. CMLS, Cell. Mol. Life Sci. 54:582–596

Tanaka, K. and Matsumura, M. (2000) Development of virulence to resistant rice varieties in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), immigrating into Japan. Appl. Entomol. Zool. 35:529-533

Tao, X., Qiang, Z., Qiang, X., Wenqing, Z., Guren, Z. and Dexiang, G. (2002) Effects of herbivore-induced rice volatiles on the host selection behaviour of brown planthopper, *Nilaparvata lugens*. Chinese Science Bulletin vol. 47 no. 16

Technologies, A. One-Colour Gene Expression Microarray Solution Dual-mode delivers the best of both worlds. Retrieved from www.agilent.com/chem/onecolour (Oct 19,2010)

Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. Science (New York). 327(5967):818-22

Thaler, J.S., Fidantsef, A.L. and Bostock, R.M. (2002) Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defence related proteins, herbivore, and pathogen performance in tomato.Journal of Chemical Ecology 28, 1131–1159

Thirumalaiandi, R., Selvaraj, M.G., Rajasekaran, R. and Subbarayalu, M. (2008) Cloning and characterization of resistance gene analogs from underexploited plant species. Electr. J. Biotechnol. 11: 1-11

Thomma, B.P.H.J., Penninckx, I.A.M.A., Cammue, B.P.A. and Broekaert, W.F. (2001) The complexity of disease signalling in Arabidopsis. Curr. Opin. Immunol. 13: 63-68

Thompson, G.A. and Goggin F.L. (2006) Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. Journal of Experimental Botany, vol. 57, no. 4:755–766

Timmer, C.P. (2010) Rice and structural transformation. In : Sushil Pandey, Derek Byerlee, David Dawe, Achim Dobermann, Samarendu Mohanty, Scott Rozelle, and Bill Hardy, editors. (2010) Rice in the global economy: strategic research and policy issues for food security. Los Baños (Philippines): International Rice Research Institute. pp 37-59

Timmerman, V., Frew, G.M. and Weeden, N.F. (2000) Characterization and linkage mapping of R-gene analogous DNA sequences in pea (*Pisum sativum* L.). Theoretical and Applied Genetics vol. 101, no.1-2:241-247

Tierens, K.F.M., Thomma, B.P.H.J., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., Mauch-Mani, B., Cammue, B.P.A. and Broekaert, W.F. (2001). Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of Arabidopsis to microbial pathogens. Plant Physiol. 125:1688-1699

Tjallingii, W.F. (1978) Electronic recording of penetration behaviour by aphids. Entomologia Experimentalis et Applicata 24:721-730

Tjallingii, W.F. (1985) Electrical nature of recorded signals during stylet penetration by aphids. Entomologia Experimentalis Et Applicata 38: 177-186

Tjallingii, W.F. (1988) Electrical recording of stylet penetration activities. In *Aphids:*Their Biology, Natural Enemies and Control, vol. B., ed. AK Minks, P Harrewijn, Amsterdam: Elsevier pp.95–108

Tjallingii, W. F. (1990) Stylet penetration parameters from aphids in relation to host-plant resistance. Symp. Biol. Hung. 39:411-419

Tjallingii, W.F. (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. Journal of Experimental Botany 57: 739-745

Tjallingii, W. F. and Gabrys, B. (1999) Anomalous stylet punctures of phloem sieve elements by aphids. Entomol. Exp. Appl. 91, 97-103

Tjallingii WF, Hogen Esch T. (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiological Entomology 18:317-328

Tolmay, V.L. (2001) Resistance to biotic and abiotic stress in the Triticeae. Hereditas 135: 239-242

Traicevski, V. and Ward, S. (2002) Probing behaviour of *Aphis craccivora* Koch on host plants of different nutritional quality. Ecological Entomology. 213-219

Tanksley, S.D. (1993) Mapping polygenes. Annu Rev Genet 27:205-233

Truernit, E., Schmid, J., Epple, P., Illig, J. and Sauer, N. (1996) The sink-specific and stress-regulated Arabidopsis STP4 gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge. The Plant Cell 8:2169–2182

Tsai, J. H. and Liu, Y.H. (1998) Effect of temperature on development, survivorship, and reproduction of rice root aphid (Homoptera: Aphididae*).* Environ. Entomol 27: 662–666

Tsumuki, H., Kanehisa, K. and Kawada, K. (1989) Leaf surface wax as a possible resistance factor of barley to cereals aphids. Appl. Entomol. Zool. 24:295–301

Turner, R.W., Song, Y.H. and Uhm, K.B. (1999) Numerical simulations of rice planthopper (*Nilaparvata lugens* (Stål) and *Sogatella furcifera* Horvarth (Homoptera: Delphacidae) migration as a component in an internet surveillance system. Bull. Entomol. Res. 89(6):557-569

Ukwungwu, M. N. 1990. Host plant resistance in rice to the African striped borer, *Chilo zacconius* Bles. (Lepidoptera: Pyralidae). Insect Sci. Appl. 11: 639-647

Underwood, N., Morris, W., Gross, K. and Lockwood III, J.R. (2000) Induced resistance to Mexican bean beetles in soybean: variation among genotypes and lack of correlation with constitutive resistance. Oecologia 122:83–89

Urs, W., Beatrice, M., Meinhard, H., Anita, J., Sepp, D.K., Helmut, S. and Robert, D. (1998) The defence-related rice gene *Pir7b* encodes an alpha/beta hydrolase fold protein exhibiting esterase activity towards naphthol AS-esters. Eur. J. Biochem. 254, 32237

Van Keer, K. (2003) On-farm agronomic diagnosis of traditional upland rice swidden cropping systems in Northern Thailand. Ph. D. Thesis, Katholieke Universiteit Leuven

Van Oosten, V. (2007) Induced Pathogen and Insect Resistance in Arabidopsis: Transcriptomics and Specificity of Defence. PhD Thesis, Wageningen University, the Netherlands

Van Oosten, .R.V., Bodenhausen, N., Reymond, P., Van Pelt, J.A., Loon, LCV, Dicke, M. and Pieterse, C.M.J. (2008) Differential effectiveness of microbially induced resistance against herbivorous insects in Arabidopsis. Mol. Plant Microbe Interact 21:919–930

Van Wees, S.C.M., de Swart, E.A.M., van Pelt, J.A., van Loon L.C. and Pieterse, C.M.J., (2000) Enhancement of induced disease resistance by simultaneous activation of salicylate and jasmonate-dependent defence pathways in *Arabidopsis thaliana.* Proceedings of the National Academy of Sciences, USA. 97:8711–8716

Vaughan, D.A. and Morishima, H. (2003) Biosystematics of genus Oryza. Chapter 1.2. In: CW Smith, C.W. and Dilday, R.H. eds. Rice. Origin, History, Technology, and Production. John Wiley and Sons Inc., Hoboken, New Jersey pp.27-65

Velusamy, R. and Heinrichs, E.A. (1986) Electronic monitoring of feeding behaviour of *Nilaparvata lugens* (Homoptera: Delphacidae) on resistant and susceptible rice cultivars. Environ. Entomol. 15:678–682

Vicente, M.C., de Guzman, F.A., Engels, J. and Rao, V.R. (2005) Genetic characterization and its use in decision making for the conservation of crop germplasm. In: The role of biotechnology, Proceedings. Turin, pp.121-128

Voiblet, C., Duplessis, S., Encelot, N., and Martin, F. (2001) Identification of symbiosis-regulated genes in *Eucalyptus globulus*-Pisolithus tinctorius ectomycorrhiza by differential hybridization of arrayed cDNAs. Plant J. 25:181–191

Vornan, B. and Gebhardt, K. (1999) Application of cpDNA and RAPD-Markers in characterization of clone collections of wild cherries and performance of micropropagated plus trees. In: Proceed. Int. Congress –Applications of biotechnology to forest genetics", Vitoria-Gasteiz, Spain, Eds. Espinel S, Ritter E.; Biofor. pp.61-71

Walch-Liu, P., Liu, L.H., Remans, T., Tester, M. and Forde, B.G. (2006) Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in *Arabidopsis thaliana*. Plant Cell Physiol. 47:1045–1057

Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) Distantly related genes in the alpha and beta subunits of ATP synthetase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide-binding fold. EMBO Journal vol.1, no.8:945-951

Walling, L.L. (2000) The myriad plant responses to herbivores. J Plant Growth Regul. 19:195–216

Wang, G.L., Mackill, D.J., Bonman, M., McCouch, S.R., Champoux, M.C. and Nelson, R.J. (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136: 1421–1434

Wang, Z.X., Yamanouchi, U., Katayose, Y., Sasaki, T. and Yano, M. (2001) Expression of the *Pib* rice-blast-resistance gene family is up-regulated by environmental conditions favouring infection and by chemical signals that trigger secondary plant defences. Plant Molecular Biology 47: 653–661

Wang, X., Ren, X., Zhu, l. And He, G. (2004) OsBi1, a rice gene, encodes a novel protein with a CBS-like domain and its expression is induced in responses to herbivore feeding

Wang, H.Y., Yang, Y., Su, J.Y., Shen, J.L., Gao, C.F., et al. (2008) Assessment of the impact of insecticides on *Anagrus nilaparvatae* (Pang et Wang) (Hymenoptera : Mymanidae), an egg parasitoid of the rice planthopper, *Nilaparvata lugens* (Hemiptera : Delphacidae). Crop Protection 27: 514-522

Ward, J.H. (1963) Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association. 58:236–244

Way, H., Chapman, S., McIntyre, L., Casu, R., Xue, G.P., Manners, J. and Shorter, R. (2005) Identification of differentially expressed genes in wheat undergoing gradual water deficit stress using a subtractive hybridisation approach. Plant Science 168:661–670

Wei, Z., Hu, W., Lin, Q., Cheng, X., Tong, M., Zhu, L., Chen, R. and He, G. (2009) Understanding rice plant resistance to the Brown Planthopper (*Nilaparvata lugens*): A proteomic approach. Proteomics 9: 2798–2808

Weschke, W., Panitz, R., Gubatz, S., Wang, Q., Radchuk, R., Weber, H. and Wobus, U. (2003) The role of invertases and hexose transporters in controlling sugar ratios in maternal and filial tissues of barley caryopses during early development. Plant J. 33: 395–411

Whetton, R. and Sederoff, R. (1995) Lignin biosynthesis. Plant Cell 7:1001–1013

Wiktelius, S. (1987) Distribution of *Rhopalosiphum padi* (Homoptera: Aphididae) on spring barley plants. Annals of Applied Biology 110:1-7

Wilkinson, T.L. and Douglas, A.E. (1995) Aphid feeding, as influenced by disruption of the symbiotic bacteria: an analysis of the pea aphid (*Acyrthosiphon pisum*). Journal of Insect Physiology 41: 635-640

Wilkinson, T.L., Ashford, D.A., Pritchard, J. and Douglas A.E. (1997) Honeydew sugars and osmoregulation in the pea aphid Acyrthosiphon pisum. Journal of Experimental Biology 200, 2137–2143

Will, T., Tjallingii W.F., Thonnessen, A. and van Bel, A.J.E. (2007) Molecular sabotage of plant defence by aphid saliva. Proc. Natl. Acad. Sci. U.S.A. 104: 10536-10541

Win, S.S., Muhamad, R., Ahmad, Z.A.M. and Adam, N.A. (2009) Life Table and Population Parameter of *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae) on Rice. Journal of Biological Sciences 9(8):904-908

Wiseman, B.R. (1999) Successes in plant resistance to insects, In J. A. Wisemand and B. R. Webster (eds.), Proceedings, Thomas Say Publications in Entomology. Entomological Society of America, Lanham, MD pp.3-15

Wong, H. L., Sakamoto, T., Kawasaki, T., Umemura, K. And Shimamoto, K. (2004) Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant Physiol. 135:1447-1456

Woodhead, S., and Padgham, D.E. (1988) The effect of plant surface characteristics on resistance of rice to the brown planthopper, *Nilaparvata lugens*. Entomol. Exp. Appl. 47:15-22.

Xiang-shun, H., Hui-yan, Z., Zu-qing, H., Dong-hong, L. and Yu-hong, Z. (2008) EPG Comparison of *Sitobion avenae* (Fab.) Feeding Behaviour on Three Wheat Varieties. Agricultural Sciences in China 7:180-186.

Xiao, J., Li, J., Grandillo, S., Ahn, S.N., Yuan, L., Tanksley, S.D., and McCouch, S.R. (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150: 899–90

Xu, R., Song, F. and Zhen, Z. (2006) OsBISAMT1, a gene encoding S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, is differentially expressed in rice defence responses. Molecular Biology Reports 33:223–231

Xu, X.F., Mei, H.W., Luo, L.J., Cheng, X.N. and Li, Z.K. (2002) RFLP facilitated investigation of the quantitative resistance of rice to brown planthopper (*Nilaparvata lugens*). Theor Appl Genet. 104:248–253

Xu, Y., Chang, P.F.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., et al. (1994) Plant defence genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell 6:1077–85

Yadav, R., Courtois, B., Huang, N. and Mclaren, G. (1997) Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor Appl Genet 94:619–632

Yang, H.Y., You, A.Q., Yang, Z.F., Zhang, F., He, R.F., et al. (2004) High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 110: 182-191

Yang, Z., Zhang, F., He, Q., and He, G. (2005) Molecular dynamics of detoxification and toxin-tolerance genes in brown planthopper (*Nilaparvata lugens* Sta°l., Homoptera: Delphacidae) feeding on resistant rice plants. Archives of Insect Biochemistry and Physiology 59:59–66

Yano, K., Miyake, T. and Eastop, V.F. (1983) The biology and economic importance of rice aphids (Hemiptera: Aphididae): A review. Bull. ent. Res. 73:539-566

Yoshida, S. (1975) The physiology of silicon in rice. Food fertilizer tech. Centre technical bull. no.25

Yoshihara, T., Sogawa, K., Pathak, M.D., Juliano, B. and Sakamura, S. (1979a) Soluble silicic acid as a sucking inhibitory substance on rice against the brown planthopper (Delphacidae, Homoptera). Entomol. Exp. Appl. 26:314-322

Yoshihara, T., Sogawa, K., Pathak, M.D. and Villareal, R. (1979b) Comparison of oxalic acid concentration in rice varieties susceptible to the brown planthopper. Int. Rice Res. Newsl. 4:10-11

Yoshihara, T., Sogawa, K., Pathak, M.D., Juliano, B. and Sakamura, S. (1980) Oxalic acid as a sucking inhibitor of the brown planthopper in rice (Delphacidae, Homoptera).‘ Entomol. Exp. Appl. 27:149-155

Yuan, H., Chen, X., Zhu, L. and He, G. (2005) Identification of genes responsive to brown planthopper *Nilaparvata lugens* Sta° 1 (Homoptera: Delphacidae) feeding in rice. Planta 221: 105–112

Zagrobelny, M., Bak, S., Rasmussen, A.V., Jørgensen, B., Naumann, C.M. and Møller, B.L. (2004) Cyanogenic glucosides and plant–insect interactions. Phytochemistry 65:293–306

Zarate, S.I., Kempema, L.A. and Walling, L.L. (2007) Silverleaf whitefly induces salicylic acid defences and suppresses effectual jasmonic acid defences. Plant Physiol. 143:866-875

Zhang, F., Zhu, L. and He, G. (2004) Differential gene expression in response to brown planthopper feeding in rice. Journal of Plant Physiology 161:53–62

Zhang, Q. (2007) Strategies for developing Green Super Rice. PNAS. vol.104 no.42:16402–16409

Zheng, H.G., Babu, R.C., Pathan, M.S., Ali, M.L., Huang, N., Courtois, B. and Nguyen, H.T. (2000) Quantitative trait loci for root penetration ability and root thickness in rice: comparison of genetic backgrounds. Genome 43:53–61

Zheng, Y.L., Xu, L., Wu, J.C., Liu, J.L. and DuanMu, H.L. (2007) Time of occurrence of hopperburn symptom on rice following root and leaf cutting and fertilizer application with brown planthopper, *Nilaparvata lugens* (Stål) infestation.Crop Protection 26: 66–72

Zhou, G., Xu, F., Li, J.,Yang, L. and Liu, J.Y. (2006) Molecular Analyses of the Metallothionein Gene Family in Rice (*Oryza sativa* L.). Journal of Biochemistry and Molecular Biology vol. 39, no. 5:595-606

Zhou, X. and Su, Z. (2007) EasyGO: Gene Ontology-based annotation and functional enrichment analysis tool for agronomical species, BMC Genomics 8: 246

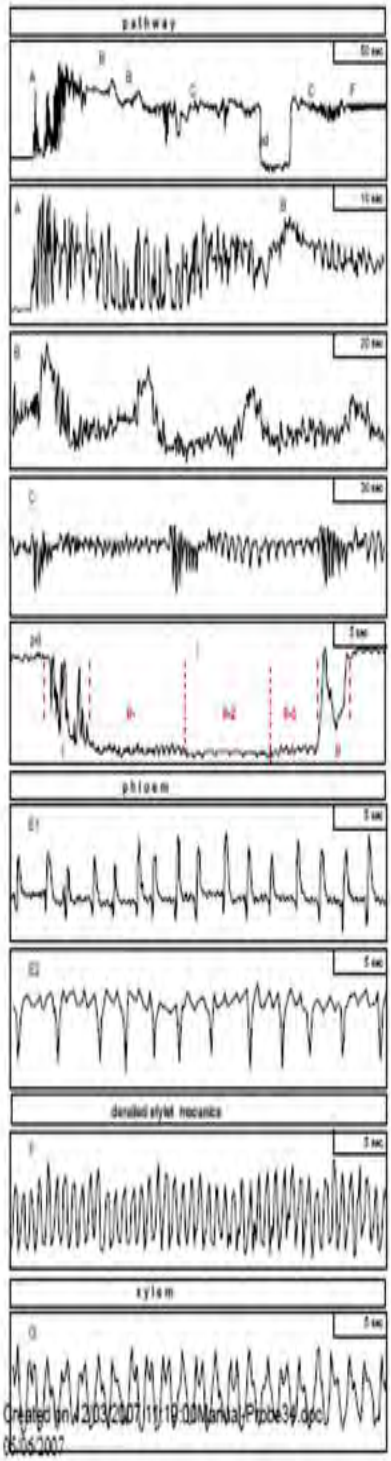
Ziegler, H. (1975) Nature of transported substances. In: Zimmerman MH, Milburn JA, eds. Encyclopedia of plant physiology, New series, vol. 1. Berlin: Springer-Verlag, 59–100

List of Appendices

Appendix A-MIAME/Plant data

MIAME/PLANT frame work	Experiment information
Array Design Description	
Manufacturer	Agilent
Chip type	4x 44K rice one colour genechip
Plant Experiment Design	
Pooling	
Number of plants in each pool	5
When pooled	7 weeks old
Genotype pooled	Individual
Planted on the same day	Yes
Experimental design	
Number of blocks	2
Randomised between blocks	Yes
Plant sample used, extract preparation and labelling	
Biosource properties	
Germplasm Accession	12
Starting material	Seed
Development stage	7 weeks old (vegetative stage)
Organism part	Plant stem
Biomaterial manipulations	
Growth substrate	Multipurpose compost (HUMAX)
Growth environment	Control growth room
Environmental conditions	
Light duration	16L: 8D
Light intensity	250-280 µmmol.m-^{2}s-^{1}
Light source	Fluorescent lamps
Humidity	60 ± 10%
Watering conditions	Manually
Temperature	24 ± 3 ^{0}C
Spacing/density of the plant	5 cm
Pots	5 cm diameter
Growth/Control agents	None
Harvesting conditions	Growth room temperature
Treatment type	None
Isolation techniques	Stem removed by scaple and flash frozen in liquid nitrogen
Extraction method	Qiagen plant mini kit (see chapter 5)
Labelling	As Agilent manufactures instruction (see chapter 5)

Appendix B: Aphid EPG Waveforms and correlations



EPG wave form[1]	characteristics: relative[2] amplitude	rep. rate	volt. level	el. origin	correlations: plant tissue	aphid activity	remarks
A	100	5-10	e	R	epidermis	cuticle penetration	first wave-form, elec trical stylet contacts
B	75	0.2-0.3	e	R	epidermis / mesophyll	sheath salivation	Waveforms overlap. Therefore, ABC mostly lumped as 'stylet pathway' activity (C) in EPG analysis.
C	30	mixed	e	R	all tissues	many activities during pathway	
pd				emf	all living cells	stylet puncture	3 phases, II with 3 sub-phases:
II-1						salivation ?	non-pers. virus inoculation
II-2						salivation ?	non-pers. virus inoculation
II-3						ingestion	non-pers. virus acquisition
E1e		2-7	e	emf	mesophyll (?)	unknown	same activity as E1?
E1		2-7		emf	sieve elements	salivation	persistent virus inoculation
E2							
w		4-9		emf	sieve elements	(watery?)	salivation persistent virus
p	5	0.5-4		R	sieve elements	(passive)	ingestion acquisition
F	5	11-19	e	R/emf	all tissues	derailed stylet mechanics	'penetration difficulties'
G							
w		4-9	e	emf	xylem	active ingestion	'drinking'
p	0-60	4-9	e	R	,,	unknown	only occasionally shown

[1] p: peaks, w: waves. [2] useless to provide for emf signals.

Created on 4/3/2007 11:19:00 Manual_Probe34.doc 05/06/2007

Manual book in STYLET 2.2 software

Appendix C. List of 239 significant genes between susceptible and resistance

Probe ID	Fold change	Description
A_71_P100387	2.50	*Arabidopsis thaliana* unknown protein (At1g16310) mRNA, complete cds.|PLN
A_71_P100388	2.54	*Arabidopsis thaliana* unknown protein (At1g16310) mRNA, complete cds.|PLN
A_71_P100413	2.72	*Oryza sativa* metallothionein-like type 2 (OsMT-2) mRNA, complete cds.|PLN
A_71_P100622	2.04	*Arabidopsis thaliana* putative DNA-binding protein (At1g68810) mRNA, complete cds.|PLN
A_71_P100654	2.05	*Oryza sativa* 1-deoxy-D-xylulose 5-phosphate reductoisomerase precursor, mRNA, complete cds; nuclear gene for plastid product.|PLN
A_71_P100971	2.58	Unknown expressed protein
A_71_P100986	4.74	*Arabidopsis thaliana* clone 10986 mRNA, complete sequence.|PLN
A_71_P100990	2.13	*Homo sapiens*, clone MGC:35476 IMAGE:5195029, mRNA, complete cds.|PRI
A_71_P101402	4.20	*Oryza sativa* RRJ4 mRNA for 12-oxophytodienoic acid reductase, complete cds.|PLN
A_71_P101412	2.07	*Arabidopsis thaliana* unknown protein (At1g28380) mRNA, complete cds.|PLN
A_71_P101498	4.27	*L.esculentum* mRNA for RNA-directed RNA polymerase.|PLN
A_71_P101565	3.08	*Arabidopsis thaliana* unknown protein (At3g18270) mRNA, complete cds.|PLN
A_71_P101582	2.05	*Arabidopsis thaliana* At3g51780/ORF3 mRNA, complete cds.|PLN
A_71_P101654	4.49	*Arabidopsis thaliana* putative receptor protein kinase (At1g28440) mRNA, complete cds.|PLN
A_71_P101830	2.63	*Arabidopsis thaliana* clone 23166 mRNA, complete sequence.|PLN
A_71_P101986	2.02	*Zea mays* nuclear matrix protein 1 (NMP1) mRNA, complete cds.|PLN
A_71_P101999	3.29	*Arabidopsis thaliana* At5g10830 mRNA, complete cds.|PLN
A_71_P102127	3.48	*Oryza sativa* subtilase mRNA, complete cds.|PLN
A_71_P102137	2.31	Unknown expressed protein
A_71_P102230	2.18	*Arabidopsis thaliana* unknown protein (At1g68140) mRNA, complete cds.|PLN
A_71_P102333	3.82	*Oryza sativa* clone C26554 UMP synthase (UMPS1) mRNA, complete cds.|PLN
A_71_P102363	2.38	*Pisum sativum* mRNA for raffinose synthase (rfs gene).|PLN
A_71_P102369	2.43	*Arabidopsis thaliana* putative kinase (At5g14270) mRNA, complete cds.|PLN
A_71_P102373	3.12	*Arabidopsis thaliana* clone RAFL15-16-B02 (R20452) unknown protein (At4g32930) mRNA, complete cds.|PLN

A_71_P102388	8.77	Unknown expressed protein
A_71_P102670	2.60	*Caenorhabditis elegans* R151.8A protein (R151.8A) mRNA, complete cds.|INV
A_71_P103010	3.62	Unknown expressed protein
A_71_P103011	6.14	Unknown expressed protein
A_71_P103012	17.67	Unknown expressed protein
A_71_P103133	2.93	Unknown expressed protein
A_71_P103375	2.10	*Arabidopsis thaliana* clone 37307 mRNA, complete sequence.|PLN
A_71_P103383	2.21	*Arabidopsis thaliana* At1g28600/F1K23_6 mRNA, complete cds.|PLN
A_71_P103421	2.27	*Arabidopsis thaliana* fatty acid multifunctional protein (AtMFP2) (F17A9.1) mRNA, complete cds.|PLN
A_71_P103437	2.24	*Oryza sativa* chloroplast carbonic anhydrase mRNA, complete cds.|PLN
A_71_P103449	3.78	Unknown expressed protein
A_71_P103494	3.60	*Oryza sativa* mRNA for asparaginyl endopeptidase, complete cds.|PLN
A_71_P103761	7.97	Unknown expressed protein
A_71_P103779	2.14	*Arabidopsis thaliana* At3g25290/MJL12_25 mRNA, complete cds.|PLN
A_71_P103913	2.01	*Arabidopsis thaliana* At1g72120/F28P5_2 mRNA, complete cds.|PLN
A_71_P104017	2.15	*Panicum miliaceum* mRNA for plastidic aspartate aminotransferase , complete cds.|PLN
A_71_P104157	7.65	*Zea mays* plasma membrane integral protein ZmPIP1-5 mRNA, complete cds.|PLN
A_71_P104210	4.22	*Atropa belladonna* AbSAMT1 mRNA for S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, complete cds.|PLN
A_71_P104266	2.31	*Oryza sativa* mRNA for Pib, complete cds.|PLN
A_71_P104413	9.39	*Quercus ilex* mRNA for putative chloroplast terpene synthase (16 gene).|PLN
A_71_P104669	6.49	Unknown expressed protein
A_71_P105137	3.84	Unknown expressed protein
A_71_P105148	2.69	*Arabidopsis thaliana* clone U10014 unknown protein (At5g27730) mRNA, complete cds.|PLN
A_71_P105257	2.10	*Arabidopsis thaliana* clone 15975 mRNA, complete sequence.|PLN
A_71_P105431	2.08	*Hordeum vulgare* clone HV_CEb0009E08f CONSTANS-like protein CO7 (CO7) mRNA, partial cds.|PLN
A_71_P105469	2.27	*Arabidopsis thaliana* unknown protein (At1g79910) mRNA, complete cds.|PLN
A_71_P105737	3.65	*Arabidopsis thaliana* clone U18226 unknown protein (At4g00330) mRNA, complete cds.|PLN
A_71_P105812	2.18	Unknown expressed protein

A_71_P105912	25.26	*Arabidopsis thaliana* clone 1204 mRNA, complete sequence.|PLN
A_71_P106481	3.57	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA, complete cds.|PLN
A_71_P106638	2.08	*Arabidopsis thaliana* putative protein phosphatase-2C (At1g79630) mRNA, complete cds.|PLN
A_71_P106784	2.40	*Rattus norvegicus* 270 kDa ankyrin G isoform mRNA, partial cds.|ROD
A_71_P106798	2.53	*Arabidopsis thaliana* At1g69640/F24J1.22 mRNA, complete cds.|PLN
A_71_P106872	2.62	Unknown expressed protein
A_71_P106926	2.46	*Arabidopsis thaliana* clone 9503 mRNA, complete sequence.|PLN
A_71_P107075	2.80	Unknown expressed protein
A_71_P107161	4.75	*Arabidopsis thaliana* putative amino acid carrier (At1g77380) mRNA, complete cds.|PLN
A_71_P107269	2.08	*Arabidopsis thaliana* At3g21670/MIL23_23 mRNA, complete cds.|PLN
A_71_P107414	2.38	*Arabidopsis thaliana* At3g17850 mRNA for putative protein kinase, complete cds, clone: RAFL16-77-O03.|PLN
A_71_P107518	2.19	*Arabidopsis thaliana* putative protein kinase (At1g67890) mRNA, complete cds.|PLN
A_71_P107893	3.95	*Morinda citrifolia* mRNA for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, DS1.|PLN
A_71_P108313	2.17	*Arabidopsis thaliana* Unknown protein (At5g21090) mRNA, complete cds.|PLN
A_71_P108358	2.29	*Arabidopsis thaliana* flavanone 3-hydroxylase-like protein (At5g24530) mRNA, complete cds.|PLN
A_71_P108538	2.11	Unknown expressed protein
A_71_P108638	3.38	*Arabidopsis thaliana* mRNA for sulfate transporter, complete cds.|PLN
A_71_P109098	3.00	*Arabidopsis thaliana* putative WRKY-type DNA binding protein (At2g46400) mRNA, complete cds.|PLN
A_71_P109258	2.14	Unknown expressed protein
A_71_P109290	4.87	Unknown expressed protein
A_71_P109333	2.89	Unknown expressed protein
A_71_P109502	3.77	Unknown expressed protein
A_71_P109692	17.89	Unknown expressed protein
A_71_P110206	17.45	Unknown expressed protein
A_71_P110348	2.64	*Arabidopsis thaliana* aminophospholipid flippase (ALA1) mRNA, complete cds.|PLN
A_71_P110868	2.77	*Callistephus chinensis* flavone synthase II (CYP93B5) mRNA, complete cds.|PLN
A_71_P110869	2.73	*G.max* mRNA for putative cytochrome P450, clone CP5.|PLN
A_71_P110946	2.76	*Arabidopsis thaliana* clone 104017 mRNA, complete

		sequence.|PLN
A_71_P110950	2.85	*Arabidopsis thaliana* clone C105054 unknown protein (At1g22930) mRNA, complete cds.|PLN
A_71_P111055	2.67	*Arabidopsis thaliana* clone 146543 mRNA, complete sequence.|PLN
A_71_P111145	4.20	*Prunus serotina* amygdalin hydrolase isoform AH I precursor (AH1) mRNA, complete cds.|PLN
A_71_P111730	5.36	*Arabidopsis thaliana* unknown protein (At5g42500) mRNA, complete cds.|PLN
A_71_P111756	5.04	Unknown expressed protein
A_71_P111907	5.88	Pinus contorta beta-glucosidase mRNA, complete cds.|PLN
A_71_P111914	6.03	Unknown expressed protein
A_71_P111916	2.77	Unknown expressed protein
A_71_P112075	6.77	Unknown expressed protein
A_71_P112265	15.84	Unknown expressed protein
A_71_P112410	2.48	*Oryza sativa* putative aminotransferase mRNA, partial cds.|PLN
A_71_P112485	3.09	*Oryza sativa* gibberellin C-20 oxidase mRNA, complete cds.|PLN
A_71_P112717	5.52	*Medicago truncatula* anthocyanidin reductase (BAN) mRNA, complete cds.|PLN
A_71_P112774	2.15	*Allium cepa* mRNA for invertase.|PLN
A_71_P112787	2.14	*Arabidopsis thaliana* putative S-receptor kinase (At4g32300) mRNA, complete cds.|PLN
A_71_P112828	2.94	*Arabidopsis thaliana* clone U10011 putative carbonyl reductase (At1g01800) mRNA, complete cds.|PLN
A_71_P112844	4.13	Corn mRNA for cysteine proteinase, clone CCP, complete cds.|PLN
A_71_P112916	3.85	*Arabidopsis thaliana* UDP-glucose glucosyltransferase (At1g22360) mRNA, complete cds.|PLN
A_71_P112930	3.73	*Homo sapiens* mRNA for KIAA1038 protein, partial cds.|PRI
A_71_P112946	2.64	*Oryza sativa* OsMST1 mRNA for monosaccharide transporter 1, complete cds.|PLN
A_71_P113124	10.07	*Arabidopsis thaliana* putative zinc transporter (ZIP1) mRNA, complete cds.|PLN
A_71_P113180	2.03	*Mus musculus*, Similar to hypothetical protein FLJ10743, clone MGC:38260 IMAGE:5324875, mRNA, complete cds.|ROD
A_71_P113184	2.92	*Arabidopsis thaliana* clone U18492 unknown protein (At2g31740) mRNA, complete cds.|PLN
A_71_P113349	2.53	*Arabidopsis thaliana* putative protein (At5g35160) mRNA, complete cds.|PLN
A_71_P113373	2.04	*Arabidopsis thaliana* clone 29744 mRNA, complete sequence.|PLN
A_71_P113466	2.13	*Musa acuminata* putative 0-deacetylbaccatin III-10-O-acetyl transferase-like protein mRNA,

		partial cds.|PLN
A_71_P113476	2.63	*Oryza sativa* (japonica cultivar-group) mRNA for aspartic proteinase, complete cds.|PLN
A_71_P113773	4.70	Unknown expressed protein
A_71_P113814	3.07	Unknown expressed protein
A_71_P113838	3.47	Unknown expressed protein
A_71_P113941	2.19	*Triticum aestivum* Na+/H+ antiporter (NHX1) mRNA, complete cds.|PLN
A_71_P114079	2.59	*Oryza sativa* (japonica cultivar-group) mRNA for qSH-1, complete cds.|PLN
A_71_P114236	2.59	*Arabidopsis thaliana* clone RAFL15-05-K10 (R20381) putative lipase (At1g09390) mRNA, complete cds.|PLN
A_71_P114315	2.35	*Arabidopsis thaliana* clone 8156 mRNA, complete sequence.|PLN
A_71_P114413	2.46	*Arabidopsis thaliana* clone 25342 mRNA, complete sequence.|PLN
A_71_P114501	2.37	*Arabidopsis thaliana* calreticulin, putative (At1g08450) mRNA, complete cds.|PLN
A_71_P114621	2.67	*Arabidopsis thaliana* putative receptor protein kinase (At5g48380) mRNA, complete cds.|PLN
A_71_P114864	2.22	Unknown expressed protein
A_71_P114907	3.83	*Arabidopsis thaliana* unknown protein (At3g18660) mRNA, complete cds.|PLN
A_71_P115683	3.42	Panax ginseng ACBP mRNA for Acyl-CoA-binding protein, complete cds.|PLN
A_71_P115765	3.84	Unknown expressed protein
A_71_P115769	9.28	Unknown expressed protein
A_71_P115993	2.77	Unknown expressed protein
A_71_P116025	3.81	*Solanum tuberosum* mRNA for cytochrome P450 (CYP71D4 gene).|PLN
A_71_P116071	7.82	*Arabidopsis thaliana* unknown protein (At3g14800) mRNA, complete cds.|PLN
A_71_P116147	4.15	Maize mRNA for putative protein kinase.|PLN
A_71_P116417	4.78	Unknown expressed protein
A_71_P116535	2.23	Unknown expressed protein
A_71_P116723	2.05	*Arabidopsis thaliana* unknown protein (At4g00880) mRNA, complete cds.|PLN
A_71_P116733	2.94	*Arabidopsis thaliana* At1g21000/F9H16_1 mRNA, complete cds.|PLN
A_71_P116856	2.28	*A.thaliana* receptor-like protein kinase mRNA, complete cds.|PLN
A_71_P116858	2.68	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA, complete cds.|PLN
A_71_P116926	5.35	*Arabidopsis thaliana* clone 37493 mRNA, complete sequence.|PLN

A_71_P116998	6.38	Unknown expressed protein
A_71_P117119	2.37	*Arabidopsis thaliana* Unknown protein (At1g16670) mRNA, complete cds.|PLN
A_71_P117233	2.99	*H.vulgare* mRNA for xyloglucan endotransglycosylase-like protein (XEA).|PLN
A_71_P117254	3.98	*Cicer arietinum* mRNA for copper containing amine oxidase (DAO).|PLN
A_71_P117272	3.09	*Zea mays* NADPH HC toxin reductase (hm1) mRNA, hm1-W22 allele, complete cds.|PLN
A_71_P117415	7.38	Unknown expressed protein
A_71_P117418	2.23	*Arabidopsis thaliana* unknown protein (At2g39570/F12L6.23) mRNA, complete cds.|PLN
A_71_P117839	3.12	*Oryza sativa* peroxidase (POX22.3) mRNA, complete cds.|PLN
A_71_P117865	11.66	*Arabidopsis thaliana* At3g26770/MDJ14_21 mRNA, complete cds.|PLN
A_71_P117871	4.10	*Arabidopsis thaliana* putative glutamate receptor protein GLR3.4b (GLR3.4) mRNA, GLR3.4-2 allele, complete cds; alternatively spliced.|PLN
A_71_P118087	13.38	Unknown expressed protein
A_71_P119575	2.61	*Arabidopsis thaliana* At2g39210/T16B24.15 mRNA, complete cds.|PLN
A_71_P119587	2.09	Unknown expressed protein
A_71_P119764	4.94	*Arabidopsis thaliana* At5g40780/K1B16_3 mRNA, complete cds.|PLN
A_71_P119943	3.19	*Oryza sativa* (japonica cultivar-group) mRNA for monodehydroascorbate reductase, partial cds. |PLN
A_71_P120004	4.33	Unknown expressed protein
A_71_P120037	8.78	*Daucus carota* transposable element TdcA1-ORF2 mRNA, partial cds.|PLN
A_71_P120062	2.92	*Arabidopsis thaliana* putative receptor serine/threonine kinase (At1g29750) mRNA, complete cds.|PLN
A_71_P120188	6.59	*Arabidopsis thaliana* unknown protein (At1g80110) mRNA, complete cds.|PLN
A_71_P120304	4.58	*Arabidopsis thaliana* class III peroxidase ATP32 mRNA, complete cds.|PLN
A_71_P120316	5.97	*Arabidopsis thaliana* unknown protein (At4g02210) mRNA, complete cds.|PLN
A_71_P120393	2.33	*Oryza sativa* PibH8 mRNA, complete cds.|PLN
A_71_P120550	4.20	*Oryza sativa* (japonica cultivar-group) mRNA for ribonuclease, complete cds, clone:C30227.|PLN
A_71_P120580	3.74	Unknown expressed protein
A_71_P120661	5.67	*Avena sativa* fructose 1,6-bisphosphate aldolase precursor, mRNA, complete cds; nuclear gene for chloroplast product.|PLN

A_71_P120688	2.14	Unknown expressed protein
A_71_P120753	2.59	Unknown expressed protein
A_71_P120761	2.84	*Arabidopsis thaliana* unknown protein (At2g36430) mRNA, complete cds.|PLN
A_71_P120848	6.49	*Arabidopsis thaliana* unknown protein (At3g04140) mRNA, complete cds.|PLN
A_71_P120920	3.45	Unknown expressed protein
A_71_P121143	2.49	Unknown expressed protein
A_71_P121309	5.33	Unknown expressed protein
A_71_P121750	2.54	*Arabidopsis thaliana* putative potassium transporter (At2g35060) mRNA, complete cds.|PLN
A_71_P121767	2.38	*Arabidopsis thaliana* unknown protein (At5g39780) mRNA, complete cds.|PLN
A_71_P121872	2.10	*Arabidopsis thaliana* clone C105073 unknown protein (At4g11570) mRNA, complete cds.|PLN
A_71_P121933	2.44	*Gossypium hirsutum* gaiacol peroxidase (pod5) mRNA, complete cds.|PLN
A_71_P122101	2.91	*Solanum tuberosum* mRNA for CDSP34 protein.|PLN
A_71_P122103	8.10	Unknown expressed protein
A_71_P122112	8.83	*Euphorbia esula* putative flavonol synthase-like protein mRNA, complete cds.|PLN
A_71_P122176	2.81	Vicia faba mRNA for putative potassium transporter (hak1 gene).|PLN
A_71_P122206	4.88	*Oryza sativa* subsp. japonica putative glutathione S-transferase OsGSTU17 mRNA, complete cds.|PLN
A_71_P122249	4.15	*Arabidopsis thaliana* clone 108568 mRNA, complete sequence.|PLN
A_71_P122268	2.24	*Olea europaea* RUB1 conjugating enzyme (ORCE) mRNA, complete cds.|PLN
A_71_P122520	2.75	Unknown expressed protein
A_71_P122651	20.02	Unknown expressed protein
A_71_P122780	6.94	Unknown expressed protein
A_71_P122813	2.04	*Citrus jambhiri* blight-associated protein p12 precursor mRNA, complete cds.|PLN
A_71_P122835	5.50	*Nicotiana tabacum* UDP-glucose:salicylic acid glucosyltransferase (SA-GTase) mRNA, complete cds.|PLN
A_71_P123076	3.79	*Arabidopsis thaliana* unknown protein (At3g22550) mRNA, complete cds.|PLN
A_71_P123152	2.81	*Arabidopsis thaliana* AT5g28840/F7P1_20 mRNA, complete cds.|PLN
A_71_P123200	4.42	Unknown expressed protein
A_71_P123310	3.61	*Arabidopsis thaliana* cytochrome p450, putative (At1g64900) mRNA, complete cds.|PLN
A_71_P123372	2.90	*Hordeum vulgare* mRNA for hexose transporter (stp1 gene).|PLN

A_71_P123474	3.03	*Nicotiana tabacum* centrin (CEN1) mRNA, complete cds.|PLN
A_71_P123501	8.54	*Arabidopsis thaliana* partial mRNA for hypothetical protein, clone 105C20.|PLN
A_71_P123516	3.68	Unknown expressed protein
A_71_P123575	2.53	*Arabidopsis thaliana* unknown protein (At4g14740) mRNA, complete cds.|PLN
A_71_P123744	9.90	Rice mRNA for aspartic protease, complete cds.|PLN
A_71_P123762	3.52	*Solanum chacoense* cytochrome P450 mRNA, complete cds.|PLN
A_71_P123885	3.21	*Oryza sativa* subsp. japonica putative glutathione S-transferase OsGSTU12 mRNA, complete cds.|PLN
A_71_P124174	7.71	Unknown expressed protein
A_71_P124178	2.16	*Arabidopsis thaliana* clone 30996 mRNA, complete sequence.|PLN
A_71_P124211	6.18	Unknown expressed protein
A_71_P124262	3.74	Unknown expressed protein
A_71_P124595	3.49	*Sorghum bicolor* mRNA for protein serine/threonine kinase RLK1.|PLN
A_71_P124627	2.94	Maize Adh2-N mRNA for alcohol dehydrogenase 2.|PLN
A_71_P124724	4.60	*Nicotiana tabacum* mRNA for hsr203J, complete cds.|PLN
A_71_P124792	3.01	*Arabidopsis thaliana* putative carboxypeptidase (At5g09640) mRNA, complete cds.|PLN
A_71_P124915	3.28	Unknown expressed protein
A_71_P125002	5.08	Unknown expressed protein
A_71_P125033	2.78	Unknown expressed protein
A_71_P125105	12.15	Unknown expressed protein
A_71_P125138	2.54	Unknown expressed protein
A_71_P125168	2.42	*Oryza sativa* receptor serine/threonine kinase mRNA, partial cds.|PLN
A_71_P125211	6.49	*Avena strigosa* mRNA for beta-amyrin synthase (bAS1 gene).|PLN
A_71_P125246	2.12	*Oryza sativa* metallothionein-like protein mRNA, complete cds.|PLN
A_71_P125335	14.30	*Oryza sativa* OSKgamma mRNA for shaggy-related protein kinase gamma, complete cds.|PLN
A_71_P125516	4.24	*Arabidopsis thaliana* clone RAFL14-93-K05 (R20243) unknown protein (At5g23570) mRNA, complete cds.|PLN
A_71_P125519	6.87	*Oryza sativa* nucleotide-binding leucine-rich-repeat protein 1 mRNA, complete cds.|PLN
A_71_P125539	2.54	*Arabidopsis thaliana* clone 108517 mRNA, complete sequence.|PLN
A_71_P125580	4.94	*Oryza sativa* fatty acid alpha-oxidase mRNA, complete cds.|PLN
A_71_P125681	2.77	*C.lacryma*-jobi mRNA for alpha-coixin 17kDa.|PLN
A_71_P125859	3.46	*Solanum tuberosum* StCBP mRNA for citrate binding protein,

		complete cds.|PLN
A_71_P126158	4.99	*Oryza sativa* metallothionein-like protein mRNA, complete cds.|PLN
A_71_P126254	2.93	Unknown expressed protein
A_71_P126304	8.35	*Arabidopsis thaliana* At1g76730 mRNA for unknown protein, complete cds, clone: RAFL21-46-B03.|PLN
A_71_P126426	2.49	*Arabidopsis thaliana* unknown protein (At4g36860) mRNA, complete cds.|PLN
A_71_P126487	4.47	Unknown expressed protein
A_71_P126491	9.15	*Oryza sativa* unknown mRNA.|PLN
A_71_P126492	3.72	Unknown expressed protein
A_71_P126605	2.14	*Arabidopsis thaliana* putative receptor protein kinase, ERECTA (At2g26330) mRNA, complete cds.|PLN
A_71_P126609	2.52	*Arabidopsis thaliana* putative protein (At5g04550) mRNA, complete cds.|PLN
A_71_P126612	3.14	*Oryza sativa* putative phytosulfokine peptide precursor (PSK3) mRNA, complete cds.|PLN
A_71_P126614	17.94	Unknown expressed protein
A_71_P126761	11.55	Unknown expressed protein
A_71_P126795	2.50	Unknown expressed protein
A_71_P126799	10.82	Unknown expressed protein
A_71_P126800	14.93	*Arabidopsis thaliana* At2g30530/T6B20.12 mRNA, complete cds.|PLN
A_71_P126802	17.40	*Arabidopsis thaliana* At3g10840 mRNA for putative alpha/beta hydrolase, complete cds, clone: RAFL17-30-F07.|PLN
A_71_P126819	15.01	*Arabidopsis thaliana* putative phospholipid cytidylyltransferase (At2g38670) mRNA, complete cds.|PLN
A_71_P126852	7.02	*Nicotiana tabacum* mRNA for peroxidase, complete cds, clone:tpoxC1.|PLN
A_71_P126881	2.45	*Arabidopsis thaliana* unknown protein (At1g80110) mRNA, complete cds.|PLN
A_71_P128402	9.45	Unknown expressed protein
A_71_P128422	12.80	Unknown expressed protein
A_71_P128629	20.83	*Phaseolus vulgaris* NBS-LRR resistance-like protein J78 (J78) mRNA, complete cds.|PLN
A_71_P128640	8.27	Unknown expressed protein

Appendix D List of 219 significant genes between susceptible and moderate resistance

ID	Fold Change	Description
A_71_P100387	2.10	*Arabidopsis thaliana* unknown protein (At1g16310) mRNA, complete cds.|PLN
A_71_P100388	2.15	*Arabidopsis thaliana* unknown protein (At1g16310) mRNA, complete cds.|PLN
A_71_P100413	3.57	*Oryza sativa* metallothionein-like type 2 (OsMT-2) mRNA, complete cds.|PLN
A_71_P100971	2.74	Unknown expressed protein
A_71_P100986	4.11	*Arabidopsis thaliana* clone 10986 mRNA, complete sequence.|PLN
A_71_P101101	2.12	*Arabidopsis thaliana* AT5g54860/MBG8_12 mRNA, complete cds.|PLN
A_71_P101402	2.73	*Oryza sativa* RRJ4 mRNA for 12-oxophytodienoic acid reductase, complete cds.|PLN
A_71_P101412	2.23	*Arabidopsis thaliana* unknown protein (At1g28380) mRNA, complete cds.|PLN
A_71_P101498	4.10	*L.esculentum* mRNA for RNA-directed RNA polymerase.|PLN
A_71_P101565	3.14	*Arabidopsis thaliana* unknown protein (At3g18270) mRNA, complete cds.|PLN
A_71_P101615	5.98	Unknown expressed protein
A_71_P101654	3.49	*Arabidopsis thaliana* putative receptor protein kinase (At1g28440) mRNA, complete cds.|PLN
A_71_P101830	2.22	*Arabidopsis thaliana* clone 23166 mRNA, complete sequence.|PLN
A_71_P101999	3.34	*Arabidopsis thaliana* At5g10830 mRNA, complete cds.|PLN
A_71_P102127	3.13	*Oryza sativa* subtilase mRNA, complete cds.|PLN
A_71_P102333	3.60	*Oryza sativa* clone C26554 UMP synthase (UMPS1) mRNA, complete cds.|PLN
A_71_P102363	2.80	*Pisum sativum* mRNA for raffinose synthase (rfs gene).|PLN
A_71_P102369	2.64	*Arabidopsis thaliana* putative kinase (At5g14270) mRNA, complete cds.|PLN
A_71_P102373	2.61	*Arabidopsis thaliana* clone RAFL15-16-B02 (R20452) unknown protein (At4g32930) mRNA, complete cds.|PLN
A_71_P102388	8.41	Unknown expressed protein
A_71_P102670	2.42	Caenorhabditis elegans R151.8A protein (R151.8A) mRNA, complete cds.|INV
A_71_P102963	3.93	Unknown expressed protein
A_71_P103010	3.81	Unknown expressed protein
A_71_P103011	11.78	Unknown expressed protein
A_71_P103012	67.07	Unknown expressed protein
A_71_P103133	3.54	Unknown expressed protein
A_71_P103162	2.30	*Oryza sativa* mRNA for RicMT, complete cds.|PLN
A_71_P103383	2.70	*Arabidopsis thaliana* At1g28600/F1K23_6 mRNA, complete

		cds.|PLN
A_71_P103437	2.12	*Oryza sativa* chloroplast carbonic anhydrase mRNA, complete cds.|PLN
A_71_P103449	3.03	Unknown expressed protein
A_71_P103494	3.27	*Oryza sativa* mRNA for asparaginyl endopeptidase, complete cds.|PLN
A_71_P103761	23.89	Unknown expressed protein
A_71_P104017	2.29	*Panicum miliaceum* mRNA for plastidic aspartate aminotransferase , complete cds.|PLN
A_71_P104157	6.15	*Zea mays* plasma membrane integral protein ZmPIP1-5 mRNA, complete cds.|PLN
A_71_P104210	3.53	*Atropa belladonna* AbSAMT1 mRNA for S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, complete cds.|PLN
A_71_P104413	5.25	*Quercus ilex* mRNA for putative chloroplast terpene synthase (16 gene).|PLN
A_71_P104669	5.11	Unknown expressed protein
A_71_P105137	2.70	Unknown expressed protein
A_71_P105148	2.68	*Arabidopsis thaliana* clone U10014 unknown protein (At5g27730) mRNA, complete cds.|PLN
A_71_P105257	2.26	*Arabidopsis thaliana* clone 15975 mRNA, complete sequence.|PLN
A_71_P105469	2.02	*Arabidopsis thaliana* unknown protein (At1g79910) mRNA, complete cds.|PLN
A_71_P105737	3.79	*Arabidopsis thaliana* clone U18226 unknown protein (At4g00330) mRNA, complete cds.|PLN
A_71_P105905	2.28	*Arabidopsis thaliana* AT5g56750/MIK19_22 mRNA, complete cds.|PLN
A_71_P105912	23.49	*Arabidopsis thaliana* clone 1204 mRNA, complete sequence.|PLN
A_71_P106481	3.15	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA, complete cds.|PLN
A_71_P106784	2.63	*Rattus norvegicus* 270 kDa ankyrin G isoform mRNA, partial cds.|ROD
A_71_P106798	2.34	*Arabidopsis thaliana* At1g69640/F24J1.22 mRNA, complete cds.|PLN
A_71_P106872	2.25	Unknown expressed protein
A_71_P106926	2.28	*Arabidopsis thaliana* clone 9503 mRNA, complete sequence.|PLN
A_71_P107075	3.24	Unknown expressed protein
A_71_P107161	5.09	*Arabidopsis thaliana* putative amino acid carrier (At1g77380) mRNA, complete cds.|PLN
A_71_P107518	2.82	*Arabidopsis thaliana* putative protein kinase (At1g67890) mRNA, complete cds.|PLN
A_71_P107893	3.77	*Morinda citrifolia* mRNA for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, DS1.|PLN
A_71_P107988	16.30	Unknown expressed protein

A_71_P108313	2.29	*Arabidopsis thaliana* Unknown protein (At5g21090) mRNA, complete cds.|PLN
A_71_P108315	3.35	*Oryza sativa* subsp. indica polyprotein mRNA, complete cds.|PLN
A_71_P108535	2.10	*Solanum tuberosum* BEL1-related homeotic protein 30 (Bel30) mRNA, complete cds.|PLN
A_71_P108638	3.16	*Arabidopsis thaliana* mRNA for sulfate transporter, complete cds.|PLN
A_71_P109098	2.14	*Arabidopsis thaliana* putative WRKY-type DNA binding protein (At2g46400) mRNA, complete cds.|PLN
A_71_P109315	2.35	*Arabidopsis thaliana* unknown protein (At5g65840) mRNA, complete cds.|PLN
A_71_P109333	2.98	Unknown expressed protein
A_71_P109692	14.28	Unknown expressed protein
A_71_P110206	14.22	Unknown expressed protein
A_71_P110348	2.53	*Arabidopsis thaliana* aminophospholipid flippase (ALA1) mRNA, complete cds.|PLN
A_71_P110449	2.07	*Arabidopsis thaliana* clone RAFL15-01-M05 (R20306) putative axi 1 protein (At2g03280) mRNA, complete cds.|PLN
A_71_P110636	2.09	*Phaseolus lunatus* ClpB (clpB) mRNA, complete cds; nuclear gene for chloroplast product.|PLN
A_71_P110868	2.50	*Callistephus chinensis* flavone synthase II (CYP93B5) mRNA, complete cds.|PLN
A_71_P110869	2.23	*G.max* mRNA for putative cytochrome P450, clone CP5.|PLN
A_71_P110946	2.57	*Arabidopsis thaliana* clone 104017 mRNA, complete sequence.|PLN
A_71_P110950	2.28	*Arabidopsis thaliana* clone C105054 unknown protein (At1g22930) mRNA, complete cds.|PLN
A_71_P111055	3.07	*Arabidopsis thaliana* clone 146543 mRNA, complete sequence.|PLN
A_71_P111145	3.67	*Prunus serotina* amygdalin hydrolase isoform AH I precursor (AH1) mRNA, complete cds.|PLN
A_71_P111148	2.16	*Prunus serotina* prunasin hydrolase isoform PHA precursor, mRNA, complete cds.|PLN
A_71_P111730	4.03	*Arabidopsis thaliana* unknown protein (At5g42500) mRNA, complete cds.|PLN
A_71_P111907	5.70	Pinus contorta beta-glucosidase mRNA, complete cds.|PLN
A_71_P111914	7.08	Unknown expressed protein
A_71_P111916	7.47	Unknown expressed protein
A_71_P112075	11.39	Unknown expressed protein
A_71_P112265	13.88	Unknown expressed protein
A_71_P112485	3.63	*Oryza sativa* gibberellin C-20 oxidase mRNA, complete cds.|PLN
A_71_P112717	5.34	*Medicago truncatula* anthocyanidin reductase (BAN) mRNA, complete cds.|PLN
A_71_P112828	2.95	*Arabidopsis thaliana* clone U10011 putative carbonyl reductase (At1g01800) mRNA, complete cds.|PLN

A_71_P112844	3.28	Corn mRNA for cysteine proteinase, clone CCP, complete cds.|PLN
A_71_P112916	3.82	*Arabidopsis thaliana* UDP-glucose glucosyltransferase (At1g22360) mRNA, complete cds.|PLN
A_71_P112930	3.47	*Homo sapiens* mRNA for KIAA1038 protein, partial cds.|PRI
A_71_P112946	2.21	*Oryza sativa* OsMST1 mRNA for monosaccharide transporter 1, complete cds.|PLN
A_71_P113124	7.18	*Arabidopsis thaliana* putative zinc transporter (ZIP1) mRNA, complete cds.|PLN
A_71_P113184	3.11	*Arabidopsis thaliana* clone U18492 unknown protein (At2g31740) mRNA, complete cds.|PLN
A_71_P113349	2.53	*Arabidopsis thaliana* putative protein (At5g35160) mRNA, complete cds.|PLN
A_71_P113373	2.36	*Arabidopsis thaliana* clone 29744 mRNA, complete sequence.|PLN
A_71_P113476	2.86	*Oryza sativa* (japonica cultivar-group) mRNA for aspartic proteinase, complete cds.|PLN
A_71_P113675	33.44	Unknown expressed protein
A_71_P113773	4.84	Unknown expressed protein
A_71_P113814	2.93	Unknown expressed protein
A_71_P113838	2.68	Unknown expressed protein
A_71_P113941	2.40	*Triticum aestivum* Na+/H+ antiporter (NHX1) mRNA, complete cds.|PLN
A_71_P114079	2.57	*Oryza sativa* (japonica cultivar-group) mRNA for qSH-1, complete cds.|PLN
A_71_P114236	2.51	*Arabidopsis thaliana* clone RAFL15-05-K10 (R20381) putative lipase (At1g09390) mRNA, complete cds.|PLN
A_71_P114315	2.63	*Arabidopsis thaliana* clone 8156 mRNA, complete sequence.|PLN
A_71_P114413	2.61	*Arabidopsis thaliana* clone 25342 mRNA, complete sequence.|PLN
A_71_P114415	2.11	*Oryza sativa* OsETTIN1 mRNA for Arabidopsis ETTIN-like protein 1, complete cds.|PLN
A_71_P114501	2.54	*Arabidopsis thaliana* calreticulin, putative (At1g08450) mRNA, complete cds.|PLN
A_71_P114621	2.70	*Arabidopsis thaliana* putative receptor protein kinase (At5g48380) mRNA, complete cds.|PLN
A_71_P114907	3.61	*Arabidopsis thaliana* unknown protein (At3g18660) mRNA, complete cds.|PLN
A_71_P115683	3.36	Panax ginseng ACBP mRNA for Acyl-CoA-binding protein, complete cds.|PLN
A_71_P115765	3.62	Unknown expressed protein
A_71_P115993	4.33	Unknown expressed protein
A_71_P116025	2.30	*Solanum tuberosum* mRNA for cytochrome P450 (CYP71D4 gene).|PLN
A_71_P116071	4.92	*Arabidopsis thaliana* unknown protein (At3g14800) mRNA, complete cds.|PLN

A_71_P116147	3.52	Maize mRNA for putative protein kinase.|PLN
A_71_P116417	2.67	Unknown expressed protein
A_71_P116723	2.21	*Arabidopsis thaliana* unknown protein (At4g00880) mRNA, complete cds.|PLN
A_71_P116733	3.00	*Arabidopsis thaliana* At1g21000/F9H16_1 mRNA, complete cds.|PLN
A_71_P116856	2.27	*A.thaliana* receptor-like protein kinase mRNA, complete cds.|PLN
A_71_P116858	2.28	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA, complete cds.|PLN
A_71_P116926	2.65	*Arabidopsis thaliana* clone 37493 mRNA, complete sequence.|PLN
A_71_P116998	5.16	Unknown expressed protein
A_71_P117119	2.49	*Arabidopsis thaliana* Unknown protein (At1g16670) mRNA, complete cds.|PLN
A_71_P117233	3.40	*H.vulgare* mRNA for xyloglucan endotransglycosylase-like protein (XEA).|PLN
A_71_P117254	3.19	*Cicer arietinum* mRNA for copper containing amine oxidase (DAO).|PLN
A_71_P117272	3.75	*Zea mays* NADPH HC toxin reductase (hm1) mRNA, hm1-W22 allele, complete cds.|PLN
A_71_P117415	7.77	Unknown expressed protein
A_71_P117746	2.12	*Arabidopsis thaliana* unknown protein (At3g22970) mRNA, complete cds.|PLN
A_71_P117839	3.38	*Oryza sativa* peroxidase (POX22.3) mRNA, complete cds.|PLN
A_71_P117865	5.82	*Arabidopsis thaliana* At3g26770/MDJ14_21 mRNA, complete cds.|PLN
A_71_P118087	8.01	Unknown expressed protein
A_71_P118372	2.16	Unknown expressed protein
A_71_P119384	4.56	Unknown expressed protein
A_71_P119552	2.11	*Oryza sativa* partial mRNA for putative potasium transporter (HAK16 gene).|PLN
A_71_P119575	2.77	*Arabidopsis thaliana* At2g39210/T16B24.15 mRNA, complete cds.|PLN
A_71_P119764	3.96	*Arabidopsis thaliana* At5g40780/K1B16_3 mRNA, complete cds.|PLN
A_71_P119943	3.36	*Oryza sativa* (japonica cultivar-group) mRNA for monodehydroascorbate reductase, partial cds.|PLN
A_71_P120004	3.75	Unknown expressed protein
A_71_P120037	5.64	*Daucus carota* transposable element TdcA1-ORF2 mRNA, partial cds.|PLN
A_71_P120062	2.95	*Arabidopsis thaliana* putative receptor serine/threonine kinase (At1g29750) mRNA, complete cds.|PLN
A_71_P120188	5.51	*Arabidopsis thaliana* unknown protein (At1g80110) mRNA, complete cds.|PLN
A_71_P120304	3.32	*Arabidopsis thaliana* class III peroxidase ATP32 mRNA, complete cds.|PLN

A_71_P120316	12.27	*Arabidopsis thaliana* unknown protein (At4g02210) mRNA, complete cds.|PLN
A_71_P120393	2.27	*Oryza sativa* PibH8 mRNA, complete cds.|PLN
A_71_P120550	2.99	*Oryza sativa* (japonica cultivar-group) mRNA for ribonuclease, complete cds, clone:C30227.|PLN
A_71_P120580	3.83	Unknown expressed protein
A_71_P120661	7.69	*Avena sativa* fructose 1,6-bisphosphate aldolase precursor, mRNA, complete cds; nuclear gene for chloroplast product.|PLN
A_71_P120688	2.43	Unknown expressed protein
A_71_P120761	2.50	*Arabidopsis thaliana* unknown protein (At2g36430) mRNA, complete cds.|PLN
A_71_P120848	4.88	*Arabidopsis thaliana* unknown protein (At3g04140) mRNA, complete cds.|PLN
A_71_P120920	4.16	Unknown expressed protein
A_71_P121309	4.19	Unknown expressed protein
A_71_P121750	2.43	*Arabidopsis thaliana* putative potassium transporter (At2g35060) mRNA, complete cds.|PLN
A_71_P121767	2.06	*Arabidopsis thaliana* unknown protein (At5g39780) mRNA, complete cds.|PLN
A_71_P121840	2.13	*Arabidopsis thaliana* serine/threonine protein kinase-like protein (T30N20_200) mRNA, complete cds.|PLN
A_71_P121872	2.27	*Arabidopsis thaliana* clone C105073 unknown protein (At4g11570) mRNA, complete cds.|PLN
A_71_P121933	2.15	*Gossypium hirsutum* gaiacol peroxidase (pod5) mRNA, complete cds.|PLN
A_71_P122034	2.11	*Arabidopsis thaliana* At3g41950 mRNA sequence.|PLN
A_71_P122101	3.40	*Solanum tuberosum* mRNA for CDSP34 protein.|PLN
A_71_P122103	6.20	Unknown expressed protein
A_71_P122112	7.50	*Euphorbia esula* putative flavonol synthase-like protein mRNA, complete cds.|PLN
A_71_P122206	5.01	*Oryza sativa* subsp. japonica putative glutathione S-transferase OsGSTU17 mRNA, complete cds.|PLN
A_71_P122249	3.90	*Arabidopsis thaliana* clone 108568 mRNA, complete sequence.|PLN
A_71_P122268	2.47	*Olea europaea* RUB1 conjugating enzyme (ORCE) mRNA, complete cds.|PLN
A_71_P122300	3.97	Unknown expressed protein
A_71_P122520	3.58	Unknown expressed protein
A_71_P122651	15.11	Unknown expressed protein
A_71_P122780	7.84	Unknown expressed protein
A_71_P122835	5.66	*Nicotiana tabacum* UDP-glucose:salicylic acid glucosyltransferase (SA-GTase) mRNA, complete cds.|PLN
A_71_P123076	3.35	*Arabidopsis thaliana* unknown protein (At3g22550) mRNA, complete cds.|PLN
A_71_P123152	2.17	*Arabidopsis thaliana* AT5g28840/F7P1_20 mRNA, complete cds.|PLN

A_71_P123200	2.40	Unknown expressed protein
A_71_P123310	3.54	*Arabidopsis thaliana* cytochrome p450, putative (At1g64900) mRNA, complete cds.|PLN
A_71_P123372	3.16	*Hordeum vulgare* mRNA for hexose transporter (stp1 gene).|PLN
A_71_P123474	3.84	*Nicotiana tabacum* centrin (CEN1) mRNA, complete cds.|PLN
A_71_P123501	8.75	*Arabidopsis thaliana* partial mRNA for hypothetical protein, clone 105C20.|PLN
A_71_P123516	2.97	Unknown expressed protein
A_71_P123575	2.42	*Arabidopsis thaliana* unknown protein (At4g14740) mRNA, complete cds.|PLN
A_71_P123744	17.58	Rice mRNA for aspartic protease, complete cds.|PLN
A_71_P123762	2.54	*Solanum chacoense* cytochrome P450 mRNA, complete cds.|PLN
A_71_P123885	3.98	*Oryza sativa* subsp. japonica putative glutathione S-transferase OsGSTU12 mRNA, complete cds.|PLN
A_71_P124174	7.49	Unknown expressed protein
A_71_P124178	2.75	*Arabidopsis thaliana* clone 30996 mRNA, complete sequence.|PLN
A_71_P124211	4.10	Unknown expressed protein
A_71_P124262	2.69	Unknown expressed protein
A_71_P124428	13.88	Unknown expressed protein
A_71_P124595	4.24	*Sorghum bicolor* mRNA for protein serine/threonine kinase RLK1.|PLN
A_71_P124724	3.42	*Nicotiana tabacum* mRNA for hsr203J, complete cds.|PLN
A_71_P124792	2.84	*Arabidopsis thaliana* putative carboxypeptidase (At5g09640) mRNA, complete cds.|PLN
A_71_P124858	2.22	Populus trichocarpa mRNA for laccase, lac1 gene, partial.|PLN
A_71_P124915	3.54	Unknown expressed protein
A_71_P125002	2.63	Unknown expressed protein
A_71_P125105	56.86	Unknown expressed protein
A_71_P125168	2.16	*Oryza sativa* receptor serine/threonine kinase mRNA, partial cds.|PLN
A_71_P125211	13.73	*Avena strigosa* mRNA for beta-amyrin synthase (bAS1 gene).|PLN
A_71_P125246	3.14	*Oryza sativa* metallothionein-like protein mRNA, complete cds.|PLN
A_71_P125335	33.80	*Oryza sativa* OSKgamma mRNA for shaggy-related protein kinase gamma, complete cds.|PLN
A_71_P125516	4.72	*Arabidopsis thaliana* clone RAFL14-93-K05 (R20243) unknown protein (At5g23570) mRNA, complete cds.|PLN
A_71_P125519	4.33	*Oryza sativa* nucleotide-binding leucine-rich-repeat protein 1 mRNA, complete cds.|PLN
A_71_P125539	2.20	*Arabidopsis thaliana* clone 108517 mRNA, complete sequence.|PLN
A_71_P125580	5.45	*Oryza sativa* fatty acid alpha-oxidase mRNA, complete cds.|PLN

A_71_P125859	2.39	*Solanum tuberosum* StCBP mRNA for citrate binding protein, complete cds.|PLN
A_71_P126158	4.35	*Oryza sativa* metallothionein-like protein mRNA, complete cds.|PLN
A_71_P126254	2.48	Unknown expressed protein
A_71_P126304	7.82	*Arabidopsis thaliana* At1g76730 mRNA for unknown protein, complete cds, clone: RAFL21-46-B03.|PLN
A_71_P126426	2.56	*Arabidopsis thaliana* unknown protein (At4g36860) mRNA, complete cds.|PLN
A_71_P126487	4.07	Unknown expressed protein
A_71_P126491	13.72	*Oryza sativa* unknown mRNA.|PLN
A_71_P126492	7.19	Unknown expressed protein
A_71_P126605	2.27	*Arabidopsis thaliana* putative receptor protein kinase, ERECTA (At2g26330) mRNA, complete cds.|PLN
A_71_P126609	2.21	*Arabidopsis thaliana* putative protein (At5g04550) mRNA, complete cds.|PLN
A_71_P126612	2.37	*Oryza sativa* putative phytosulfokine peptide precursor (PSK3) mRNA, complete cds.|PLN
A_71_P126614	14.52	Unknown expressed protein
A_71_P126761	10.91	Unknown expressed protein
A_71_P126799	9.92	Unknown expressed protein
A_71_P126800	12.39	*Arabidopsis thaliana* At2g30530/T6B20.12 mRNA, complete cds.|PLN
A_71_P126802	18.00	*Arabidopsis thaliana* At3g10840 mRNA for putative alpha/beta hydrolase, complete cds, clone: RAFL17-30-F07.|PLN
A_71_P126819	15.18	*Arabidopsis thaliana* putative phospholipid cytidylyltransferase (At2g38670) mRNA, complete cds.|PLN
A_71_P126852	4.17	*Nicotiana tabacum* mRNA for peroxidase, complete cds, clone:tpoxC1.|PLN
A_71_P126881	3.65	*Arabidopsis thaliana* unknown protein (At1g80110) mRNA, complete cds.|PLN
A_71_P128402	8.57	Unknown expressed protein
A_71_P128422	10.81	Unknown expressed protein
A_71_P128629	16.49	*Phaseolus vulgaris* NBS-LRR resistance-like protein J78 (J78) mRNA, complete cds.|PLN
A_71_P128640	7.60	Unknown expressed protein

Appendix E Correlation value between microarray expression value with several BPH morphological experiment data (Electrical Penetration graph (EPG) and honeydew drop

Agilent ID	UniGene symbol	Fold difference	Pathway N (1+2+3)	Phloem ingestion (N4b)	Xylem ingestion (N5)	Total honeydew drop	Time to first N4b	Description
AK099702	Os01g0106900	2.05	-0.59	0.66	-0.49	0.64	-0.65	*Oryza sativa* 1-deoxy-D-xylulose 5-phosphate reductoisomerase precursor, mRNA, complete cds; nuclear gene for plastid product.|PLN
AK061853	Os01g0130000	2.50	-0.46	0.48	-0.22	0.46	-0.45	*Arabidopsis thaliana* unknown protein (At1g16310) mRNA, complete cds.|PLN
AK060150	Os01g0130100	2.54	-0.77	0.81	-0.62	0.78	-0.81	*Arabidopsis thaliana* unknown protein (At1g16310) mRNA, complete cds.|PLN
AK058378	Os01g0138900	3.08	-0.68	0.75	-0.73	0.70	-0.76	*Arabidopsis thaliana* unknown protein (At3g18270) mRNA, complete cds.|PLN
AK069318	Os01g0149200	2.72	-0.57	0.63	-0.55	0.64	-0.66	*Oryza sativa* metallothionein-like type 2 (OsMT-2) mRNA, complete cds.|PLN
AK061224	Os01g0170000	2.38	-0.53	0.63	-0.62	0.61	-0.66	*Pisum sativum* mRNA for raffinose synthase (rfs gene).|PLN
AK064209	Os01g0198000	4.27	0.43	-0.51	0.53	-0.49	0.56	*L.esculentum* mRNA for RNA-directed RNA polymerase.|PLN
AK062484	Os01g0214500	4.74	-0.74	0.77	-0.66	0.77	-0.81	*Arabidopsis thaliana* clone 10986 mRNA, complete sequence.|PLN
AK104740	Os01g0214800	2.13	-0.55	0.63	-0.72	0.66	-0.68	*Homo sapiens*, clone MGC:35476 IMAGE:5195029, mRNA, complete cds.|PRI
AK068593	Os01g0218100	2.04	-0.76	0.76	-0.62	0.74	-0.77	*Arabidopsis thaliana* putative DNA-binding protein (At1g68810) mRNA, complete cds.|PLN
AK100444	Os01g0348600	2.27	-0.52	0.59	-0.53	0.56	-0.62	*Arabidopsis thaliana* fatty acid multifunctional protein (AtMFP2) (F17A9.1) mRNA, complete cds.|PLN
AK067903	Os01g0366100	3.78	0.58	-0.60	0.57	-0.60	0.64	Unknown expressed protein
AK100034	Os01g0369900	4.20	-0.61	0.63	-0.55	0.66	-0.66	*Oryza sativa* RRJ4 mRNA for 12-oxophytodienoic acid reductase, complete cds.|PLN
AK064570	Os01g0510500	2.58	0.40	-0.52	0.57	-0.51	0.57	Unknown expressed protein
AK064005	Os01g0538000	7.97	0.52	-0.60	0.60	-0.58	0.61	Unknown expressed protein

AK106137	Os01g0539400	3.62	0.68	-0.70	0.66	-0.70	0.70	Unknown expressed protein
AK070057	Os01g0540800	6.14	0.57	-0.63	0.65	-0.59	0.63	Unknown expressed protein
AK059464	Os01g0541000	17.67	0.47	-0.52	0.55	-0.52	0.54	Unknown expressed protein
AK067597	Os01g0559600	3.60	-0.69	0.78	-0.70	0.71	-0.79	*Oryza sativa* mRNA for asparaginyl endopeptidase, complete cds.|PLN
AK061216	Os01g0565600	3.12	0.59	-0.59	0.59	-0.59	0.62	*Arabidopsis thaliana* clone RAFL15-16-B02 (R20452) unknown protein (At4g32930) mRNA, complete cds.|PLN
AK066561	Os01g0612600	2.18	-0.45	0.50	-0.35	0.39	-0.47	*Arabidopsis thaliana* unknown protein (At1g68140) mRNA, complete cds.|PLN
AK067056	Os01g0639600	2.93	-0.58	0.60	-0.43	0.65	-0.64	Unknown expressed protein
AK060890	Os01g0639900	2.24	-0.63	0.76	-0.77	0.70	-0.78	*Oryza sativa* chloroplast carbonic anhydrase mRNA, complete cds.|PLN
AK111374	Os01g0648700	2.43	-0.61	0.66	-0.64	0.68	-0.73	*Arabidopsis thaliana* putative kinase (At5g14270) mRNA, complete cds.|PLN
AK105142	Os01g0649200	2.10	-0.69	0.79	-0.87	0.75	-0.82	*Arabidopsis thaliana* clone 37307 mRNA, complete sequence.|PLN
AK102591	Os01g0650200	2.21	-0.63	0.69	-0.57	0.71	-0.71	*Arabidopsis thaliana* At1g28600/F1K23_6 mRNA, complete cds.|PLN
AK102535	Os01g0679200	3.82	0.48	-0.55	0.65	-0.54	0.59	*Oryza sativa* clone C26554 UMP synthase (UMPS1) mRNA, complete cds.|PLN
AK069379	Os01g0680900	2.14	-0.70	0.68	-0.66	0.72	-0.71	*Arabidopsis thaliana* At3g25290/MJL12_25 mRNA, complete cds.|PLN
AK061091	Os01g0708500	2.63	-0.74	0.77	-0.72	0.77	-0.81	*Arabidopsis thaliana* clone 23166 mRNA, complete sequence.|PLN
AK101454	Os01g0716500	3.29	-0.75	0.78	-0.69	0.78	-0.83	*Arabidopsis thaliana* At5g10830 mRNA, complete cds.|PLN
AK063360	Os01g0726000	8.77	0.40	-0.47	0.48	-0.47	0.51	Unknown expressed protein
AK105476	Os01g0757900	2.60	0.38	-0.46	0.46	-0.41	0.48	*Caenorhabditis elegans* R151.8A protein (R151.8A) mRNA, complete cds.|INV
AK100351	Os01g0795400	3.48	-0.66	0.72	-0.78	0.66	-0.75	*Oryza sativa* subtilase mRNA, complete cds.|PLN
AK061108	Os01g0798200	2.31	0.78	-0.81	0.79	-0.81	0.84	Unknown expressed protein
AK070208	Os01g0831200	2.05	-0.61	0.72	-0.72	0.66	-0.71	*Arabidopsis thaliana* At3g51780/ORF3 mRNA, complete cds.|PLN
AK070435	Os01g0869500	2.02	-0.60	0.65	-0.60	0.70	-0.71	*Zea mays* nuclear matrix protein 1 (NMP1) mRNA, complete

								cds.|PLN
AK060248	Os01g0872600	2.01	-0.35	0.45	-0.44	0.41	-0.49	*Arabidopsis thaliana* At1g72120/F28P5_2 mRNA, complete cds.|PLN
AK069324	Os01g0878300	4.49	-0.75	0.80	-0.64	0.79	-0.82	*Arabidopsis thaliana* putative receptor protein kinase (At1g28440) mRNA, complete cds.|PLN
AK064908	Os01g0958700	2.07	-0.52	0.60	-0.52	0.56	-0.63	*Arabidopsis thaliana* unknown protein (At1g28380) mRNA, complete cds.|PLN
AK060685	Os02g0102200	4.75	-0.70	0.77	-0.73	0.69	-0.78	*Arabidopsis thaliana* putative amino acid carrier (At1g77380) mRNA, complete cds.|PLN
AK110925	Os02g0121700	9.39	-0.69	0.70	-0.56	0.68	-0.77	*Quercus ilex* mRNA for putative chloroplast terpene synthase (16 gene).|PLN
AK065610	Os02g0129700	2.18	-0.74	0.79	-0.77	0.70	-0.85	Unknown expressed protein
AK102507	Os02g0159200	2.27	-0.57	0.60	-0.40	0.64	-0.62	*Arabidopsis thaliana* unknown protein (At1g79910) mRNA, complete cds.|PLN
AK068418	Os02g0173200	2.62	-0.68	0.75	-0.71	0.73	-0.78	Unknown expressed protein
AK109630	Os02g0178100	2.08	-0.61	0.60	-0.42	0.59	-0.61	*Hordeum vulgare* clone HV_CEb0009E08f CONSTANS-like protein CO7 (CO7) mRNA, partial cds.|PLN
AK066602	Os02g0216300	2.80	-0.58	0.65	-0.62	0.64	-0.67	Unknown expressed protein
AK072080	Os02g0224100	2.08	-0.52	0.60	-0.62	0.55	-0.66	*Arabidopsis thaliana* putative protein phosphatase-2C (At1g79630) mRNA, complete cds.|PLN
AK111620	Os02g0228300	3.57	-0.75	0.84	-0.79	0.80	-0.85	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA, complete cds.|PLN
AK105563	Os02g0243300	25.26	0.58	-0.59	0.59	-0.63	0.62	*Arabidopsis thaliana* clone 1204 mRNA, complete sequence.|PLN
AK107164	Os02g0297200	6.49	0.43	-0.49	0.59	-0.52	0.56	Unknown expressed protein
AK101296	Os02g0457500	2.40	-0.69	0.74	-0.65	0.68	-0.79	*Rattus norvegicus* 270 kDa ankyrin G isoform mRNA, partial cds.|ROD
AK071231	Os02g0526000	2.69	-0.73	0.78	-0.71	0.79	-0.79	*Arabidopsis thaliana* clone U10014 unknown protein (At5g27730) mRNA, complete cds.|PLN
AK072036	Os02g0580900	2.08	-0.55	0.63	-0.66	0.63	-0.65	*Arabidopsis thaliana* At3g21670/MIL23_23 mRNA, complete cds.|PLN
AK059625	Os02g0582400	2.46	0.55	-0.62	0.71	-0.66	0.69	*Arabidopsis thaliana* clone 9503 mRNA, complete sequence.|PLN

AK062638	Os02g0642300	2.10	-0.59	0.65	-0.51	0.65	-0.68	*Arabidopsis thaliana* clone 15975 mRNA, complete sequence.|PLN
AK070415	Os02g0719600	4.22	-0.50	0.63	-0.63	0.58	-0.63	*Atropa belladonna* AbSAMT1 mRNA for S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, complete cds.|PLN
AK103058	Os02g0745700	2.53	-0.63	0.68	-0.49	0.70	-0.72	*Arabidopsis thaliana* At1g69640/F24J1.22 mRNA, complete cds.|PLN
AK105059	Os02g0797500	2.15	-0.56	0.61	-0.49	0.61	-0.64	*Panicum miliaceum* mRNA for plastidic aspartate aminotransferase , complete cds.|PLN
AK073656	Os02g0818500	2.31	0.53	-0.59	0.58	-0.56	0.61	*Oryza sativa* mRNA for Pib, complete cds.|PLN
AK112048	Os02g0821400	3.65	-0.73	0.78	-0.68	0.76	-0.83	*Arabidopsis thaliana* clone U18226 unknown protein (At4g00330) mRNA, complete cds.|PLN
AK102174	Os02g0823100	7.65	-0.80	0.81	-0.66	0.84	-0.83	*Zea mays* plasma membrane integral protein ZmPIP1-5 mRNA, complete cds.|PLN
AK108744	Os02g0828300	3.84	0.41	-0.51	0.62	-0.52	0.55	Unknown expressed protein
AK068438	Os03g0122300	2.29	-0.59	0.61	-0.61	0.62	-0.65	*Arabidopsis thaliana* flavanone 3-hydroxylase-like protein (At5g24530) mRNA, complete cds.|PLN
AK066932	Os03g0161200	3.38	-0.72	0.77	-0.64	0.77	-0.79	*Arabidopsis thaliana* mRNA for sulfate transporter, complete cds.|PLN
AK073082	Os03g0164100	2.11	0.52	-0.53	0.49	-0.53	0.58	Unknown expressed protein
AK067971	Os03g0181100	3.77	0.45	-0.46	0.33	-0.40	0.43	Unknown expressed protein
AK066877	Os03g0189100	2.89	-0.67	0.74	-0.77	0.68	-0.78	Unknown expressed protein
AK101653	Os03g0321700	3.00	-0.80	0.85	-0.82	0.83	-0.89	*Arabidopsis thaliana* putative WRKY-type DNA binding protein (At2g46400) mRNA, complete cds.|PLN
AK059185	Os03g0326100	2.64	-0.68	0.73	-0.65	0.68	-0.79	*Arabidopsis thaliana* aminophospholipid flippase (ALA1) mRNA, complete cds.|PLN
AK059247	Os03g0389700	3.95	-0.69	0.77	-0.68	0.75	-0.80	*Morinda citrifolia* mRNA for 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, DS1.|PLN
AK099659	Os03g0401100	2.19	-0.46	0.54	-0.43	0.51	-0.59	*Arabidopsis thaliana* putative protein kinase (At1g67890) mRNA, complete cds.|PLN
AK063543	Os03g0422300	17.45	0.53	-0.55	0.58	-0.58	0.61	Unknown expressed protein
AK061730	Os03g0440900	2.17	-0.57	0.68	-0.69	0.61	-0.71	*Arabidopsis thaliana* Unknown protein (At5g21090) mRNA, complete cds.|PLN

AK109832	Os03g0596200	4.87	0.66	-0.67	0.60	-0.68	0.70	Unknown expressed protein
AK070048	Os03g0695400	6.49	0.50	-0.56	0.58	-0.57	0.61	*Arabidopsis thaliana* unknown protein (At3g04140) mRNA, complete cds.|PLN
AK106515	Os03g0711800	2.38	0.90	-0.85	0.72	-0.85	0.87	*Arabidopsis thaliana* At3g17850 mRNA for putative protein kinase, complete cds, clone: RAFL16-77-O03.|PLN
AK063237	Os03g0785100	17.89	0.54	-0.56	0.58	-0.59	0.63	Unknown expressed protein
AK062754	Os03g0854800	2.14	0.54	-0.59	0.66	-0.58	0.56	Unknown expressed protein
AK100972	Os04g0101400	2.73	-0.68	0.76	-0.68	0.72	-0.76	*G.max* mRNA for putative cytochrome P450, clone CP5.|PLN
AK103323	Os04g0170500	8.27	0.53	-0.54	0.56	-0.57	0.58	Unknown expressed protein
AK069845	Os04g0173300	2.49	0.67	-0.65	0.58	-0.67	0.67	Unknown expressed protein
AK073111	Os04g0196300	15.84	0.53	-0.55	0.57	-0.57	0.61	Unknown expressed protein
AK072639	Os04g0302300	2.14	0.79	-0.77	0.66	-0.79	0.77	*Arabidopsis thaliana* putative S-receptor kinase (At4g32300) mRNA, complete cds.|PLN
AK071948	Os04g0311400	4.13	-0.67	0.77	-0.85	0.69	-0.79	Corn mRNA for cysteine proteinase, clone CCP, complete cds.|PLN
AK105783	Os04g0320700	3.85	-0.67	0.74	-0.64	0.69	-0.80	*Arabidopsis thaliana* UDP-glucose glucosyltransferase (At1g22360) mRNA, complete cds.|PLN
AK109611	Os04g0377600	2.03	-0.77	0.82	-0.69	0.80	-0.83	*Mus musculus*, Similar to hypothetical protein FLJ10743, clone MGC:38260 IMAGE:5324875, mRNA, complete cds.|ROD
AK065694	Os04g0379300	2.92	0.57	-0.65	0.58	-0.63	0.68	*Arabidopsis thaliana* clone U18492 unknown protein (At2g31740) mRNA, complete cds.|PLN
AK108662	Os04g0415000	2.76	-0.71	0.75	-0.60	0.72	-0.78	*Arabidopsis thaliana* clone 104017 mRNA, complete sequence.|PLN
AK063893	Os04g0431300	2.67	-0.67	0.72	-0.70	0.63	-0.77	*Arabidopsis thaliana* clone 146543 mRNA, complete sequence.|PLN
AK062183	Os04g0439100	2.85	-0.72	0.80	-0.68	0.78	-0.81	*Arabidopsis thaliana* clone C105054 unknown protein (At1g22930) mRNA, complete cds.|PLN
AK072059	Os04g0454200	2.64	-0.54	0.65	-0.71	0.58	-0.67	*Oryza sativa* OsMST1 mRNA for monosaccharide transporter 1, complete cds.|PLN
AK100820	Os04g0474800	4.20	-0.58	0.59	-0.37	0.63	-0.64	*Prunus serotina* amygdalin hydrolase isoform AH I precursor (AH1) mRNA, complete cds.|PLN
AK068772	Os04g0513400	5.88	-0.67	0.72	-0.65	0.69	-0.72	Pinus contorta beta-glucosidase mRNA, complete cds.|PLN
AK107142	Os04g0522500	3.09	-0.69	0.76	-0.68	0.75	-0.80	*Oryza sativa* gibberellin C-20 oxidase mRNA, complete cds.|PLN

AK071069	Os04g0531900	2.94	-0.78	0.85	-0.83	0.76	-0.86	*Arabidopsis thaliana* clone U10011 putative carbonyl reductase (At1g01800) mRNA, complete cds.|PLN
AK065130	Os04g0535600	2.15	-0.10	0.18	-0.35	0.23	-0.15	*Allium cepa* mRNA for invertase.|PLN
AK100658	Os04g0595300	3.73	-0.53	0.54	-0.48	0.59	-0.59	*Homo sapiens* mRNA for KIAA1038 protein, partial cds.|PRI
AK066668	Os04g0607400	6.77	0.33	-0.41	0.46	-0.42	0.48	Unknown expressed protein
AK069804	Os04g0613000	10.07	-0.75	0.80	-0.73	0.73	-0.81	*Arabidopsis thaliana* putative zinc transporter (ZIP1) mRNA, complete cds.|PLN
AK102306	Os04g0614600	2.48	-0.71	0.77	-0.70	0.71	-0.80	*Oryza sativa* putative aminotransferase mRNA, partial cds.|PLN
AK066343	Os04g0616900	5.04	0.22	-0.32	0.53	-0.39	0.40	Unknown expressed protein
AK063017	Os04g0621800	6.03	0.58	-0.53	0.48	-0.55	0.54	Unknown expressed protein
AK105171	Os04g0623200	2.77	0.24	-0.34	0.35	-0.25	0.33	Unknown expressed protein
AK072654	Os04g0630400	5.52	-0.75	0.81	-0.80	0.75	-0.80	*Medicago truncatula* anthocyanidin reductase (BAN) mRNA, complete cds.|PLN
AK107902	Os04g0666800	5.36	-0.72	0.77	-0.68	0.79	-0.76	*Arabidopsis thaliana* unknown protein (At5g42500) mRNA, complete cds.|PLN
AK062996	Os05g0103300	2.04	-0.54	0.65	-0.62	0.64	-0.68	*Arabidopsis thaliana* clone 29744 mRNA, complete sequence.|PLN
AK072967	Os05g0113000	2.23	-0.75	0.79	-0.66	0.78	-0.83	*Arabidopsis thaliana* unknown protein (At2g39570/F12L6.23) mRNA, complete cds.|PLN
AK069868	Os05g0136900	2.13	-0.63	0.69	-0.64	0.68	-0.69	*Musa acuminata* putative 0-deacetylbaccatin III-10-O-acetyl transferase-like protein mRNA, partial cds.|PLN
AK065206	Os05g0137400	2.63	-0.71	0.77	-0.64	0.76	-0.79	*Oryza sativa* (japonica cultivar-group) mRNA for aspartic proteinase, complete cds.|PLN
AK066037	Os05g0148000	9.45	0.53	-0.57	0.61	-0.59	0.62	Unknown expressed protein
AK066168	Os05g0148600	2.19	-0.61	0.66	-0.52	0.64	-0.68	*Triticum aestivum* Na+/H+ antiporter (NHX1) mRNA, complete cds.|PLN
AK067837	Os05g0159200	2.59	-0.63	0.65	-0.45	0.69	-0.69	*Arabidopsis thaliana* clone RAFL15-05-K10 (R20381) putative lipase (At1g09390) mRNA, complete cds.|PLN
AK100711	Os05g0168500	2.53	-0.53	0.63	-0.53	0.64	-0.64	*Arabidopsis thaliana* putative protein (At5g35160) mRNA, complete cds.|PLN
AK064110	Os05g0369900	4.70	0.37	-0.31	0.27	-0.34	0.30	Unknown expressed protein
AK111590	Os05g0414700	2.67	-0.64	0.71	-0.68	0.62	-0.76	*Arabidopsis thaliana* putative receptor protein kinase (At5g48380) mRNA, complete cds.|PLN

AK064877	Os05g0426400	3.83	-0.69	0.77	-0.73	0.68	-0.82	*Arabidopsis thaliana* unknown protein (At3g18660) mRNA, complete cds.|PLN
AK069208	Os05g0439200	2.22	0.61	-0.64	0.67	-0.66	0.68	Unknown expressed protein
AK100916	Os05g0455200	2.59	-0.62	0.70	-0.69	0.62	-0.74	*Oryza sativa* (japonica cultivar-group) mRNA for qSH-1, complete cds.|PLN
AK060834	Os05g0507300	2.37	-0.62	0.71	-0.70	0.66	-0.72	*Arabidopsis thaliana* calreticulin, putative (At1g08450) mRNA, complete cds.|PLN
AK062488	Os05g0542300	3.47	-0.82	0.85	-0.78	0.86	-0.87	Unknown expressed protein
AK069230	Os05g0555700	3.07	-0.70	0.78	-0.71	0.75	-0.80	Unknown expressed protein
AK100983	Os05g0563000	2.46	-0.61	0.71	-0.66	0.69	-0.75	*Arabidopsis thaliana* clone 25342 mRNA, complete sequence.|PLN
AK069724	Os05g0568100	2.35	-0.59	0.65	-0.64	0.65	-0.69	*Arabidopsis thaliana* clone 8156 mRNA, complete sequence.|PLN
AK058833	Os06g0115300	3.42	-0.62	0.74	-0.86	0.69	-0.77	Panax ginseng ACBP mRNA for Acyl-CoA-binding protein, complete cds.|PLN
AK066196	Os06g0120200	9.28	-0.63	0.60	-0.45	0.67	-0.64	Unknown expressed protein
AK063905	Os06g0202300	6.38	-0.71	0.73	-0.63	0.71	-0.79	Unknown expressed protein
AK100029	Os06g0323100	5.35	0.43	-0.37	0.35	-0.40	0.36	*Arabidopsis thaliana* clone 37493 mRNA, complete sequence.|PLN
AK067262	Os06g0338200	3.98	-0.70	0.74	-0.63	0.69	-0.78	*Cicer arietinum* mRNA for copper containing amine oxidase (DAO).|PLN
AK108507	Os06g0487300	7.38	0.40	-0.45	0.47	-0.46	0.47	Unknown expressed protein
AK064475	Os06g0491800	7.82	0.59	-0.60	0.63	-0.65	0.62	*Arabidopsis thaliana* unknown protein (At3g14800) mRNA, complete cds.|PLN
AK064561	Os06g0499200	2.23	0.48	-0.56	0.63	-0.56	0.61	Unknown expressed protein
AK111851	Os06g0557100	2.28	-0.60	0.67	-0.73	0.61	-0.73	*A.thaliana* receptor-like protein kinase mRNA, complete cds.|PLN
AK111567	Os06g0557700	2.68	-0.75	0.79	-0.75	0.72	-0.85	*Malus domestica* leucine-rich receptor-like protein kinase (LRPKm1) mRNA, complete cds.|PLN
AK111737	Os06g0602500	4.15	-0.69	0.73	-0.63	0.69	-0.79	Maize mRNA for putative protein kinase.|PLN
AK065620	Os06g0624900	2.94	-0.75	0.82	-0.78	0.76	-0.85	*Arabidopsis thaliana* At1g21000/F9H16_1 mRNA, complete cds.|PLN
AK060486	Os06g0639800	3.81	-0.49	0.58	-0.68	0.57	-0.55	*Solanum tuberosum* mRNA for cytochrome P450 (CYP71D4

								gene).|PLN
AK063834	Os06g0651100	3.09	-0.67	0.71	-0.53	0.72	-0.71	*Zea mays* NADPH HC toxin reductase (hm1) mRNA, hm1-W22 allele, complete cds.|PLN
AK058439	Os06g0653200	2.77	0.46	-0.47	0.44	-0.45	0.52	Unknown expressed protein
AK103523	Os06g0676600	2.37	-0.72	0.76	-0.66	0.72	-0.82	*Arabidopsis thaliana* Unknown protein (At1g16670) mRNA, complete cds.|PLN
AK105513	Os06g0697000	2.99	-0.70	0.77	-0.68	0.72	-0.77	*H.vulgare* mRNA for xyloglucan endotransglycosylase-like protein (XEA).|PLN
AK062493	Os06g0705400	4.78	-0.70	0.72	-0.60	0.80	-0.69	Unknown expressed protein
AK107043	Os06g0714300	2.05	-0.57	0.65	-0.64	0.58	-0.70	*Arabidopsis thaliana* unknown protein (At4g00880) mRNA, complete cds.|PLN
AK062594	Os07g0187900	2.61	-0.71	0.76	-0.75	0.70	-0.83	*Arabidopsis thaliana* At2g39210/T16B24.15 mRNA, complete cds.|PLN
AK064606	Os07g0273000	2.09	0.41	-0.53	0.63	-0.52	0.54	Unknown expressed protein
AK106593	Os07g0521300	13.38	-0.82	0.84	-0.77	0.79	-0.90	Unknown expressed protein
AK067405	Os07g0522600	4.10	0.69	-0.68	0.56	-0.69	0.72	*Arabidopsis thaliana* putative glutamate receptor protein GLR3.4b (GLR3.4) mRNA, GLR3.4-2 allele, complete cds; alternatively spliced.|PLN
AK105211	Os07g0664600	11.66	-0.83	0.87	-0.71	0.87	-0.87	*Arabidopsis thaliana* At3g26770/MDJ14_21 mRNA, complete cds.|PLN
AK073202	Os07g0677200	3.12	-0.69	0.71	-0.59	0.77	-0.71	*Oryza sativa* peroxidase (POX22.3) mRNA, complete cds.|PLN
AK102372	Os08g0100600	3.45	0.58	-0.68	0.72	-0.63	0.68	Unknown expressed protein
AK069503	Os08g0113000	4.58	-0.80	0.83	-0.69	0.84	-0.84	*Arabidopsis thaliana* class III peroxidase ATP32 mRNA, complete cds.|PLN
AK065217	Os08g0127100	4.94	-0.77	0.83	-0.82	0.79	-0.85	*Arabidopsis thaliana* At5g40780/K1B16_3 mRNA, complete cds.|PLN
AK109659	Os08g0150700	6.59	-0.78	0.83	-0.74	0.81	-0.84	*Arabidopsis thaliana* unknown protein (At1g80110) mRNA, complete cds.|PLN
AK065548	Os08g0203300	2.92	-0.54	0.57	-0.63	0.57	-0.57	*Arabidopsis thaliana* putative receptor serine/threonine kinase (At1g29750) mRNA, complete cds.|PLN
AK066265	Os08g0208300	8.78	0.68	-0.68	0.59	-0.67	0.70	*Daucus carota* transposable element TdcA1-ORF2 mRNA, partial cds.|PLN
AK072666	Os08g0244400	3.74	0.56	-0.61	0.52	-0.59	0.64	Unknown expressed protein

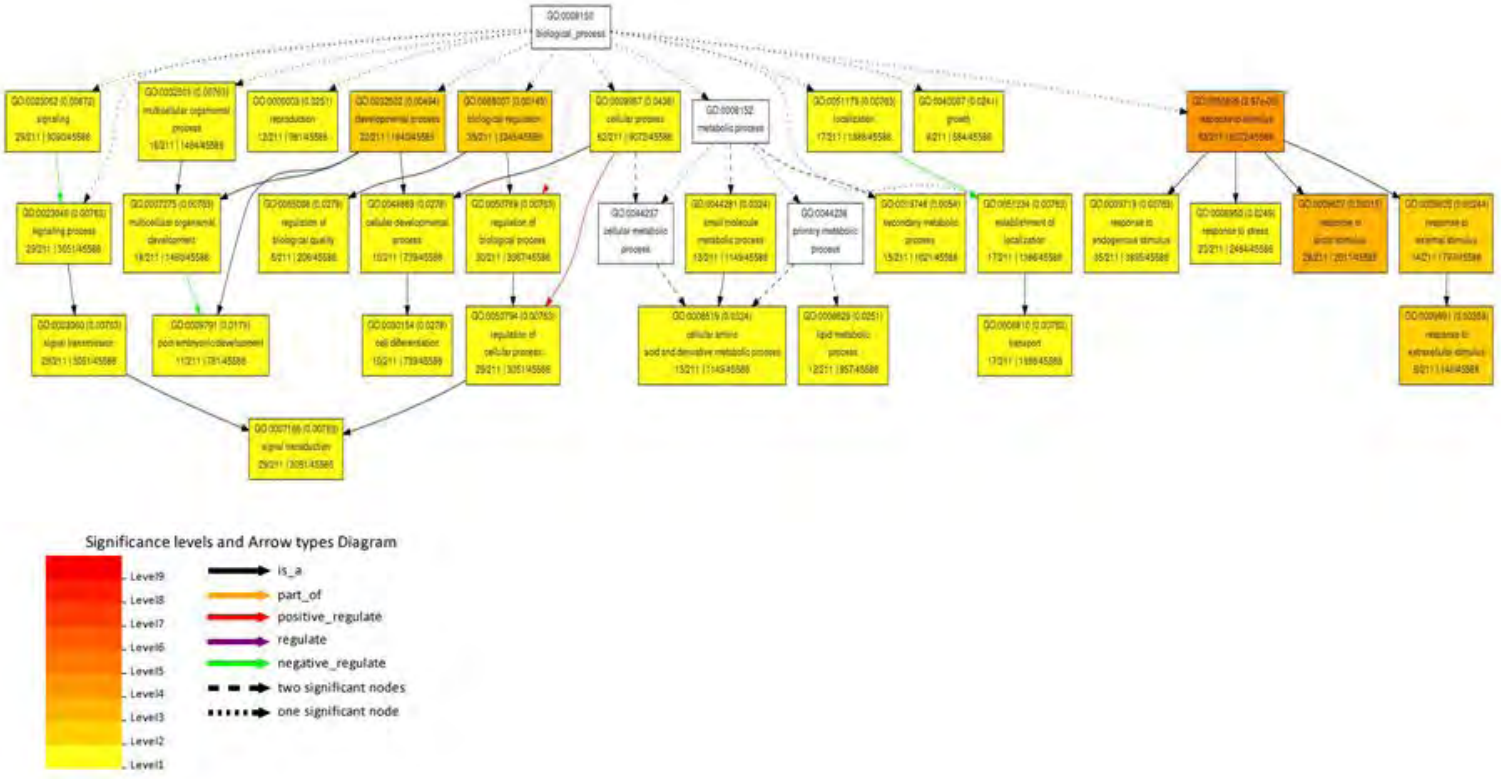
AK068999	Os08g0246700	2.59	0.66	-0.72	0.69	-0.65	0.73	Unknown expressed protein
AK073806	Os08g0272800	5.33	0.61	-0.59	0.57	-0.62	0.62	Unknown expressed protein
AK072190	Os08g0301600	4.33	0.40	-0.50	0.58	-0.51	0.55	Unknown expressed protein
AK068483	Os08g0338000	5.97	0.22	-0.32	0.48	-0.38	0.40	*Arabidopsis thaliana* unknown protein (At4g02210) mRNA, complete cds.|PLN
AK101110	Os08g0356500	2.84	-0.71	0.77	-0.67	0.71	-0.83	*Arabidopsis thaliana* unknown protein (At2g36430) mRNA, complete cds.|PLN
AK058502	Os08g0434100	4.20	-0.80	0.87	-0.82	0.87	-0.88	*Oryza sativa* (japonica cultivar-group) mRNA for ribonuclease, complete cds, clone:C30227.|PLN
AK105927	Os08g0440100	5.67	0.37	-0.45	0.51	-0.46	0.51	*Avena sativa* fructose 1,6-bisphosphate aldolase precursor, mRNA, complete cds; nuclear gene for chloroplast product.|PLN
AK072930	Os08g0460700	2.14	0.52	-0.57	0.53	-0.57	0.61	Unknown expressed protein
AK065693	Os08g0539700	2.33	0.60	-0.66	0.64	-0.64	0.70	*Oryza sativa* PibH8 mRNA, complete cds.|PLN
AK102459	Os08g0557600	3.19	-0.64	0.71	-0.69	0.66	-0.77	*Oryza sativa* (japonica cultivar-group) mRNA for monodehydroascorbate reductase, partial cds.|PLN
AK059180	Os09g0116600	20.02	0.49	-0.51	0.54	-0.54	0.57	Unknown expressed protein
AK069183	Os09g0129400	8.10	0.55	-0.54	0.54	-0.57	0.59	Unknown expressed protein
AK104742	Os09g0133600	2.91	-0.55	0.59	-0.44	0.58	-0.64	Solanum tuberosum mRNA for CDSP34 protein.|PLN
AK067408	Os09g0248900	8.83	0.51	-0.50	0.51	-0.53	0.57	*Euphorbia esula* putative flavonol synthase-like protein mRNA, complete cds.|PLN
AK100058	Os09g0271100	6.94	-0.72	0.81	-0.77	0.77	-0.83	Unknown expressed protein
AK068208	Os09g0272000	2.75	-0.36	0.49	-0.58	0.43	-0.51	Unknown expressed protein
AK107818	Os09g0290401	2.78	0.37	-0.32	0.25	-0.32	0.32	Unknown expressed protein
AK073821	Os09g0321900	2.24	-0.47	0.56	-0.48	0.56	-0.56	*Olea europaea* RUB1 conjugating enzyme (ORCE) mRNA, complete cds.|PLN
AK099766	Os09g0376900	2.81	-0.49	0.55	-0.44	0.57	-0.55	*Vicia faba* mRNA for putative potassium transporter (hak1 gene).|PLN
AK065194	Os09g0413600	2.38	-0.45	0.51	-0.41	0.51	-0.54	*Arabidopsis thaliana* unknown protein (At5g39780) mRNA, complete cds.|PLN
AK099489	Os09g0467200	4.88	-0.57	0.61	-0.59	0.54	-0.69	*Oryza sativa* subsp. japonica putative glutathione S-transferase OsGSTU17 mRNA, complete cds.|PLN
AK069281	Os09g0471100	2.44	-0.64	0.70	-0.69	0.68	-0.74	*Gossypium hirsutum* gaiacol peroxidase (pod5) mRNA, complete

								cds.|PLN
AK063132	Os09g0472900	2.04	-0.56	0.55	-0.51	0.59	-0.61	*Citrus jambhiri* blight-associated protein p12 precursor mRNA, complete cds.|PLN
AK064395	Os09g0518200	5.50	-0.77	0.81	-0.73	0.78	-0.82	*Nicotiana tabacum* UDP-glucose:salicylic acid glucosyltransferase (SA-GTase) mRNA, complete cds.|PLN
AK066238	Os09g0525400	4.15	-0.70	0.74	-0.56	0.76	-0.77	*Arabidopsis thaliana* clone 108568 mRNA, complete sequence.|PLN
AK065464	Os09g0563200	2.54	-0.68	0.71	-0.53	0.73	-0.75	*Arabidopsis thaliana* putative potassium transporter (At2g35060) mRNA, complete cds.|PLN
AK071860	Os09g0569100	2.10	-0.62	0.67	-0.51	0.67	-0.70	*Arabidopsis thaliana* clone C105073 unknown protein (At4g11570) mRNA, complete cds.|PLN
AK104790	Os10g0130500	6.18	0.49	-0.49	0.52	-0.53	0.50	Unknown expressed protein
AK106884	Os10g0158700	7.71	0.50	-0.60	0.63	-0.61	0.66	Unknown expressed protein
AK060781	Os10g0159800	2.16	-0.42	0.53	-0.52	0.53	-0.56	*Arabidopsis thaliana* clone 30996 mRNA, complete sequence.|PLN
AK065782	Os10g0389000	3.03	-0.70	0.77	-0.67	0.73	-0.79	*Nicotiana tabacum* centrin (CEN1) mRNA, complete cds.|PLN
AK069676	Os10g0415400	3.74	-0.49	0.49	-0.52	0.48	-0.55	Unknown expressed protein
AK069385	Os10g0417600	2.81	-0.59	0.63	-0.43	0.66	-0.63	*Arabidopsis thaliana* AT5g28840/F7P1_20 mRNA, complete cds.|PLN
AK066224	Os10g0420900	4.42	0.58	-0.60	0.63	-0.62	0.63	Unknown expressed protein
AK109893	Os10g0422600	3.79	-0.70	0.75	-0.68	0.73	-0.80	*Arabidopsis thaliana* unknown protein (At3g22550) mRNA, complete cds.|PLN
AK065193	Os10g0428200	9.90	0.45	-0.57	0.68	-0.57	0.62	Rice mRNA for aspartic protease, complete cds.|PLN
AK067591	Os10g0440000	3.52	-0.65	0.64	-0.40	0.70	-0.66	*Solanum chacoense* cytochrome P450 mRNA, complete cds.|PLN
AK105678	Os10g0515200	3.61	-0.69	0.74	-0.70	0.70	-0.81	*Arabidopsis thaliana* cytochrome p450, putative (At1g64900) mRNA, complete cds.|PLN
AK072155	Os10g0522500	3.68	0.51	-0.50	0.50	-0.52	0.52	Unknown expressed protein
AK068314	Os10g0522900	8.54	0.47	-0.48	0.48	-0.50	0.54	*Arabidopsis thaliana* partial mRNA for hypothetical protein, clone 105C20.|PLN
AK063773	Os10g0527800	3.21	0.33	-0.39	0.44	-0.41	0.44	*Oryza sativa* subsp. japonica putative glutathione S-transferase OsGSTU12 mRNA, complete cds.|PLN
AK102640	Os10g0539900	2.90	-0.66	0.71	-0.54	0.70	-0.73	*Hordeum vulgare* mRNA for hexose transporter (stp1 gene).|PLN

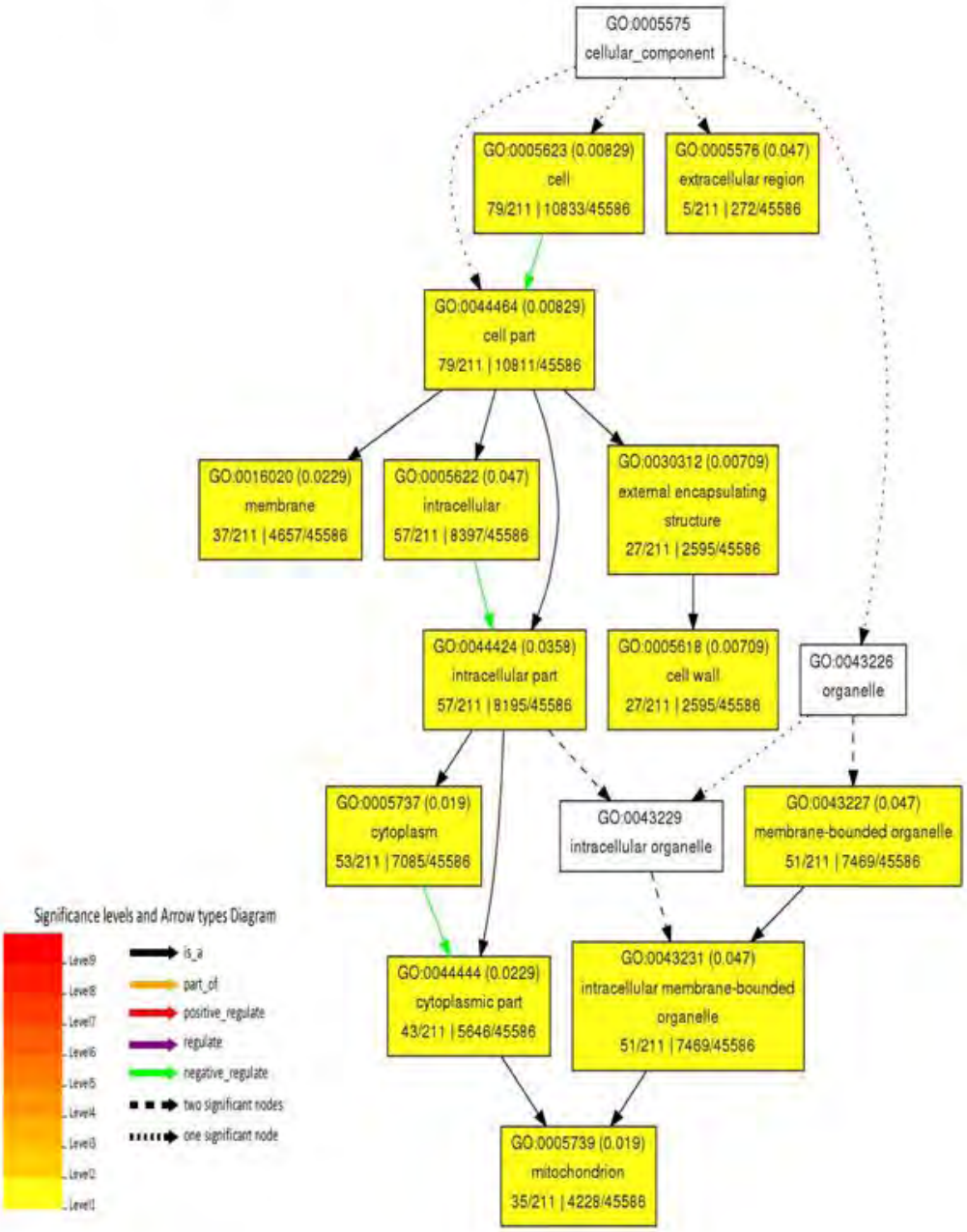
AK066334	Os10g0560000	2.53	-0.59	0.63	-0.41	0.67	-0.64	*Arabidopsis thaliana* unknown protein (At4g14740) mRNA, complete cds.|PLN
AK059449	Os11g0138900	4.60	-0.71	0.78	-0.75	0.71	-0.77	*Nicotiana tabacum* mRNA for hsr203J, complete cds.|PLN
AK101288	Os11g0210600	2.94	-0.58	0.65	-0.68	0.61	-0.65	Maize Adh2-N mRNA for alcohol dehydrogenase 2.|PLN
AK066271	Os11g0256300	12.80	0.49	-0.49	0.49	-0.52	0.53	Unknown expressed protein
AK109559	Os11g0294800	2.42	0.62	-0.61	0.58	-0.63	0.64	*Oryza sativa* receptor serine/threonine kinase mRNA, partial cds.|PLN
AK061209	Os11g0297000	12.15	0.45	-0.53	0.55	-0.49	0.56	Unknown expressed protein
AK099990	Os11g0308100	14.30	0.43	-0.52	0.63	-0.52	0.54	*Oryza sativa* OSKgamma mRNA for shaggy-related protein kinase gamma, complete cds.|PLN
AK069378	Os11g0432900	3.01	-0.75	0.81	-0.78	0.78	-0.85	*Arabidopsis thaliana* putative carboxypeptidase (At5g09640) mRNA, complete cds.|PLN
AK062908	Os11g0434800	3.28	0.39	-0.49	0.54	-0.49	0.52	Unknown expressed protein
AK068470	Os11g0439300	3.84	0.51	-0.46	0.42	-0.49	0.51	Unknown expressed protein
AK064092	Os11g0458100	2.54	0.76	-0.82	0.71	-0.75	0.83	Unknown expressed protein
AK106291	Os11g0550900	5.08	-0.71	0.69	-0.55	0.71	-0.75	Unknown expressed protein
AK067451	Os11g0562100	6.49	0.32	-0.37	0.43	-0.40	0.40	*Avena strigosa* mRNA for beta-amyrin synthase (bAS1 gene).|PLN
AK111536	Os11g0607200	3.49	-0.66	0.75	-0.77	0.68	-0.78	*Sorghum bicolor* mRNA for protein serine/threonine kinase RLK1.|PLN
AK062653	Os11g0704500	2.12	-0.46	0.49	-0.52	0.49	-0.52	*Oryza sativa* metallothionein-like protein mRNA, complete cds.|PLN
AK069456	Os12g0112000	7.02	-0.59	0.66	-0.64	0.66	-0.66	*Nicotiana tabacum* mRNA for peroxidase, complete cds, clone:tpoxC1.|PLN
AK063661	Os12g0117800	10.82	0.59	-0.52	0.46	-0.56	0.54	Unknown expressed protein
AK066991	Os12g0118200	14.93	0.62	-0.60	0.59	-0.64	0.63	*Arabidopsis thaliana* At2g30530/T6B20.12 mRNA, complete cds.|PLN
AK111804	Os12g0118400	17.40	0.56	-0.57	0.57	-0.61	0.61	*Arabidopsis thaliana* At3g10840 mRNA for putative alpha/beta hydrolase, complete cds, clone: RAFL17-30-F07.|PLN
AK068868	Os12g0121300	15.01	0.56	-0.59	0.60	-0.61	0.64	*Arabidopsis thaliana* putative phospholipid cytidylyltransferase (At2g38670) mRNA, complete cds.|PLN
AK068675	Os12g0129700	2.45	-0.56	0.60	-0.46	0.65	-0.57	*Arabidopsis thaliana* unknown protein (At1g80110) mRNA, complete cds.|PLN

AK066886	Os12g0132000	11.55	0.49	-0.48	0.49	-0.53	0.50	Unknown expressed protein
AK110747	Os12g0135000	2.50	0.62	-0.63	0.60	-0.65	0.63	Unknown expressed protein
AK107470	Os12g0146500	2.52	-0.62	0.63	-0.64	0.67	-0.68	*Arabidopsis thaliana* putative protein (At5g04550) mRNA, complete cds.|PLN
AK059860	Os12g0147800	3.14	-0.73	0.76	-0.74	0.74	-0.82	*Oryza sativa* putative phytosulfokine peptide precursor (PSK3) mRNA, complete cds.|PLN
AK062437	Os12g0148500	17.94	0.56	-0.56	0.56	-0.62	0.59	Unknown expressed protein
AK065623	Os12g0168000	8.35	0.55	-0.60	0.66	-0.63	0.62	*Arabidopsis thaliana* At1g76730 mRNA for unknown protein, complete cds, clone: RAFL21-46-B03.|PLN
AK059809	Os12g0170800	3.46	-0.50	0.50	-0.43	0.58	-0.49	*Solanum tuberosum* StCBP mRNA for citrate binding protein, complete cds.|PLN
AK067635	Os12g0182300	2.14	-0.76	0.82	-0.78	0.77	-0.86	*Arabidopsis thaliana* putative receptor protein kinase, ERECTA (At2g26330) mRNA, complete cds.|PLN
AK063668	Os12g0197200	2.93	0.60	-0.64	0.62	-0.65	0.66	Unknown expressed protein
AK100699	Os12g0197700	4.24	0.54	-0.55	0.57	-0.60	0.60	*Arabidopsis thaliana* clone RAFL14-93-K05 (R20243) unknown protein (At5g23570) mRNA, complete cds.|PLN
AK065572	Os12g0199100	6.87	0.62	-0.62	0.65	-0.64	0.64	*Oryza sativa* nucleotide-binding leucine-rich-repeat protein 1 mRNA, complete cds.|PLN
AK102672	Os12g0204600	20.83	0.55	-0.56	0.53	-0.57	0.61	*Phaseolus vulgaris* NBS-LRR resistance-like protein J78 (J78) mRNA, complete cds.|PLN
AK073343	Os12g0254400	9.15	0.54	-0.58	0.60	-0.57	0.61	*Oryza sativa* unknown mRNA.|PLN
AK072652	Os12g0254601	3.72	0.42	-0.51	0.62	-0.51	0.57	Unknown expressed protein
AK064516	Os12g0256600	4.47	0.57	-0.63	0.66	-0.62	0.66	Unknown expressed protein
AK100592	Os12g0448900	4.94	-0.65	0.73	-0.71	0.69	-0.76	*Oryza sativa* fatty acid alpha-oxidase mRNA, complete cds.|PLN
AK105219	Os12g0571100	4.99	-0.73	0.76	-0.59	0.77	-0.77	*Oryza sativa* metallothionein-like protein mRNA, complete cds.|PLN
AK065760	Os12g0596800	2.49	-0.74	0.79	-0.76	0.73	-0.85	*Arabidopsis thaliana* unknown protein (At4g36860) mRNA, complete cds.|PLN
AK101710	Os12g0631200	2.54	-0.71	0.79	-0.78	0.73	-0.84	*Arabidopsis thaliana* clone 108517 mRNA, complete sequence.|PLN
AK106275	Unknown	2.77	0.67	-0.72	0.61	-0.66	0.70	*C.lacryma*-jobi mRNA for alpha-coixin 17kDa.|PLN
AK060115	Unknown	2.77	-0.67	0.74	-0.66	0.72	-0.74	*Callistephus chinensis* flavone synthase II (CYP93B5) mRNA, complete cds.|PLN

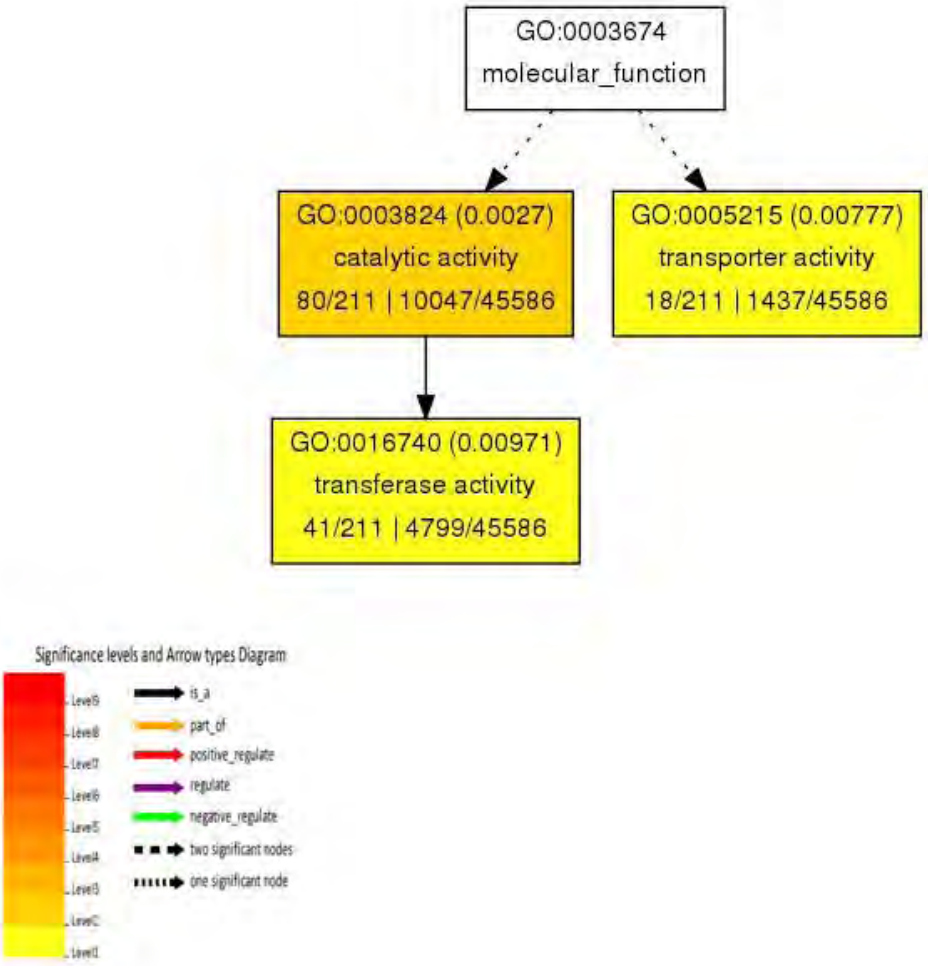
Appendix F. Figure shows results of GO analysis under biological process in graphical forms separated into 30 categories



Appendix G. Figure shows results of GO Analysis under cellular component in graphical forms separated into 13 categories



Appendix H. Figure shows results of GO Analysis under molecular function in graphical forms separated into 3 categories



Appendix I List of BPH marker used for mapping candidate genes

Gene	Chromosome	Donor	Marker	References
Bph1	12	IR64	CDO344	Huang et al.(2009)
Bph1	12	IR64	RG463	Huang et al.(2009)
Bph1	12	IR64	RG901	Huang et al. (2009)
Bph10	12L	IR65482-4-136-2-2	RM519	Ishii et al. (1994)
Bph3	6S	Rathu heenathi	RM589	Jairin et al. (2007)
Bph3	6s	PTB33/Rathu	RM190	Jairin et al. (2007)
Bph3	6s	PTB33/Rathu	RM204	Jairin et al. (2007)
Bph3	6s	PTB33/Rathu	RM469	Jairin et al. (2007)
Bph3	6s	PTB33/Rathu	RM3353	Jairin et al. (2007)
Bph3	6s	PTB33/Rathu	RM8101	Jairin et al. (2007)
Bph15	**8**	**Rathu heenathi**	**RG1**	**Jairin et al. (2007)**
Bph15	8	Rathu heenathi	RG2	Jairin et al. (2007)
Bph18	12L	IR65482-7-216-1-2	RM3331	Jena et al. (2006)
Bph18	12L	IR65482-7-216-1-2	RM3726	Jena et al. (2006)
Bph18	12L	IR65482-7-216-1-2	RM6217	Jena et al. (2006)
Bph18	12L	IR65482-7-216-1-2	RM7376	Jena et al. (2006)
Bph18	12L	IR65482-7-216-1-2	7312.T4A	Jena et al. (2006)
bph4	6s	Babawee	C531	Nagato and Yoshimura (1998)
bph4	6s	Babawee	C891	Nagato and Yoshimura (1998)
Bph1	**12**	**IR64**	**RM1103**	**Park et al. (2008)**
Bph1	5	IR64	RM163	Park et al. (2008)
Bph1	5	IR64	RM459	Park et al. (2008)
Bph1	12	IR64	RM28493	Park et al. (2008)
Bph1	12	IR64	RM6693	Park et al. (2008)
Bph1	12	IR64	RM5609	Park et al. (2008)
bph(21t)	12	IR71033-121-15	RM3726	Kumari et al. (2010)
bph(21t)	12	IR71033-121-15	RM5479	Kumari et al. (2010)
Bph1	12	Mudgo	RG901	Kumari et al. (2010)
Bph1	12	Norin-PL3	em2802 N	Kumari et al. (2010)
Bph1	12	Norin-PL3	em5814 N	Kumari et al. (2010)
Bph1	12	Gayabyeo	OPD-07	Kumari et al, (2010)
Bph1	12	Mudgo	RG413	Kumari et al. (2010)
Bph10(t)	**12**	**IR 31917-45-3-2**	**RM17**	**Kumari et al. (2010)**
Bph10(t)	12	IR 31917-45-3-2	RM260	Kumari et al. (2010)
Bph10(t)	12	IR 31917-45-3-2	RM277	Kumari et al. (2010)
Bph10(t)	12	*O.australiensis*	RG457	Kumari et al. (2010)
bph11(t)	3	IR54742-1-11-17	G1318	Kumari et al., 2010)
Bph12(t)	**4**	**B14**	**RM185**	**Kumari et al., 2010)**
Bph12(t)	**4**	**B14**	**RM261**	**Kumari et al., 2010)**
Bph12(t)	4	B14	RM335	Kumari et al. (2010)
Bph12(t)	4	B14	C820	Kumari et al. (2010)
Bph12(t)	4	B14	C946	Kumari et al. (2010)
Bph12(t)	4	B14	R288	Kumari et al. (2010)
Bph13(t)	3	IR54745-21-12-17-6	RM22	Kumari et al. (2010)
Bph13(t)	3	IR54745-21-12-17-6	RM218	Kumari et al. (2010)
Bph13(t)	3	IR54745-21-12-17-6	RM231	Kumari et al. (2010)
Bph14	3	B5	R1925	Kumari et al. (2010)
Bph14	3	B5	R2443	Kumari et al. (2010)

Continue appendix table

Gene	Chromosome	Donor	Marker	References
Bph15	4	B5	S11182	Kumari et al. (2010)
Bph18(t)	12	*O.australiensis*	RM463	Kumari et al. (2010)
Bph18(t)	12	*O.australiensis*	RM6869	Kumari et al. (2010)
Bph18(t)	12	*O.australiensis*	R10289S	Kumari et al. (2010)
Bph18(t)	12	*O.australiensis*	S15552	Kumari et al. (2010)
Bph19(t)	3	AS201	RM3134	Kumari et al. (2010)
Bph19(t)	3	AS201	RM6308	Kumari et al. (2010)
bph2	12	Norin-PL4	G2140	Kumari et al. (2010)
bph2	12	Norin-PL4	KAM4	Kumari et al. (2010)
Bph20(t)	4	IR71033-121-15	RM5953	Kumari et al. (2010)
Bph20(t)	4	IR71033-121-15	MS10	Kumari et al. (2010)
Bph3	6	Rathu	RM588	Kumari et al. (2010)
Bph3	6	Rathu Heenathi	RM8072	Kumari et al. (2010)
Bph3	**6**	**Rathu Heenathi**	**RM19291**	**Kumari et al. (2010)**
bph4	6	Babawee	RM217	Kumari et al. (2010)
bph4	6	Babawee	RM225	Kumari et al. (2010)
Bph6	11	*O.officinalis*	RM209	Kumari et al. (2010)
Bph6	11	*O.officinalis*	OPA16938	Kumari et al. (2010)
Bph6	11	*O.officinalis*	RG167	Kumari et al. (2010)
Bph9	12	Kaharamana	RM5341	Kumari et al. (2010)
Bph3	3	PTB33	RM251	Santhanalakshmi et al. (2010)
Bph3	3	PTB33	RM3766	Santhanalakshmi et al. (2010)
Bph3	3	PTB33	RM14687	Santhanalakshmi et al. (2010)
Bph1	12L	Mudgo	em5814(AFLP)	Sharma et al. (2002)
Bph1	12	Mudgo	G148 (RFLP)	Sun et al. (2005)
Qbph10	**12L**	**Rathu Heenathi**	**RM484**	**Sun et al. (2005)**
Qbph10	12L	Rathu Heenathi	RM496	Sun et al. (2005)
Qbph3	3	Rathu Heenathi	RM7	Sun et al. (2005)
Qbph3	3	Rathu Heenathi	RM313	Sun et al. (2005)
Qbph3	4	Rathu Heenathi	RM8213	Sun et al. (2005)
bph2	2	ASD7	RM7102	Sun et al. (2005)
Qbph4	4S	Rathu Heenati	RM401	Sun et al. (2005)
Qbph4	4S	Rathu Heenati	RM518	Sun et al. (2005)
Qbph4	4S	Rathu Heenati	**RM5953**	Sun et al. (2005)