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# Differential gene expression in response to brown planthopper feeding in rice

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## Summary

Plant responses to herbivores are complex. 108 cDNA clones representing genes relating to plant responses to chewing insect-feeding, pathogen infection, wounding and other stresses were collected. Northern blot and cDNA array analysis were employed to investigate gene expression regulated by piercing-sucking insect, brown planthopper (BPH), *Nilaparvata lugens* (Homoptera: Dephacidae) on both the resistant and susceptible rice genotypes. After BPH feeding in rice for 72 h, the expression of most tested genes was affected. 14 genes in resistant rice variety B5 and 44 genes in susceptible MH63 were significantly up- or down-regulated. Most of the well-regulated genes were grouped in the categories of signaling pathways, oxidative stress/apoptosis, wound-response, drought-inducible and pathogen-related proteins. Those related to the flavonoid pathway, aromatic metabolidsm and the octadecanoid pathway were mostly kept unchanged or down-regulated. Our results indicate that BPH feeding induces plant responses which would take part in a jasmonic acid-independent pathway and crosstalk with those related to abiotic stress, pathogen invasion and phytohormone signaling pathways.

**Key words:** brown planthopper (*Nilaparvata lugens*) – cDNA array – gene expression – Northern blot analysis – rice (*Oryza sativa*)

**Abbreviations:** ABA = abscisic acid. – BPH = brown planthopper. – CyP = cyclophilin. – ET = ethylene. – HR = hypersensitive response. – JA = jasmonic acid. – OPDA = oxophytodienoic acid. – PCR = polymerase chain reaction. – PI = protease inhibitor. – PR = pathogenesis-related. – SA = salicylic acid. – SABP = salicylic acid binding protein. – SAR = systemic acquired resistance. – SSC = standard saline citrate buffer

## Introduction

Much progress on plant responses to herbivores has been made during the last decade (Walling 2000, Baldwin et al. 2001, Kessler and Baldwin 2002). The knowledge has been mostly based on the interactions of plant-chewing insects (Mattiacci et al. 1995, Alborn et al. 1997, Korth and Dixon 1997, Paré and Tumlinson 1998, Bouwmeester et al. 1999). In the plant-herbivore interaction, direct and indirect defenses in plants are triggered by herbivore feeding to prevent the

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insect's sustaining attack. The former refers to the induced defense signaling pathways by herbivores' damage and the production of secondary chemicals such as phenolics, terpenoids, and nitrogen containing compounds that act as toxins or feeding deterrents to the herbivore. The latter consists of the production of volatile blends induced by kinds of elicitors (Mattiacci et al. 1995, Alborn et al. 1997) to attract the natural enemies of the herbivore (De Moraes et al. 1998, Paré and Tumlinson 1999, Thaler 1999, Baldwin et al. 2001). Several elicitors have been discovered. Mattiacci et al. (1995) discovered that β-glucosidase in caterpillar regurgitant is an elicitor. Another elicitor, volicitin, was extracted from beet armyworm oral secretion (Alhorn et al. 1997). The emergence of *SLW3* indicates the existence of a novel plant-derived elicitor (Van de Ven et al. 2000). These elicitors activate the jasmonic acid (JA)-, salicylic acid (SA)-, and/or ethylene (ET)-dependent/independent signaling pathways, which crosstalk with each other and form a complex signaling transduction network in the plants damaged by chewing herbivores (Reymond and Farmer 1998, Schenk et al. 2000, Kessler and Baldwin 2002). The chewing insects cause extensive plant tissue damage during their feeding and thus activate the wound-signaling pathway, which is a part of the induced defense reactions (Walling 2000). Wounding of potato leaf tissues can induce a local and systemic response, reflected by the accumulation of protease inhibitor (PI), pathogenesis-related (PR) protein RNAs or proteins (Korth and Dixon 1997). In *Arabidopsis*, many genes induced by wounding are regulated by JA and its precursor oxophytodienoic acid (OPDA) (Reymond and Farmer 1998).

Little is known about the interaction between plants and piercing-sucking insects, another kind of herbivore that uses the stylet mouthparts to imbibe the liquids from phloem of plants. Contrary to the chewing insects, the sucking ones produce little injury to plant foliage. Limited evidence shows that sucking insects are perceived as pathogens and activate the corresponding signaling pathways (Walling 2000). Using several critical genes as markers, Moran and Thompson (2001) found that phloem feedings by aphids on *Arabidopsis* lead to stimulation of response pathways that associate with both pathogen infection and wounding. It is quite clear now that when pathogens attack the plants, they cause a series of alterations within the plants. Oxidative burst occurs and hypersensitive reaction (HR) appears, PR-proteins are synthesized and the defense-associated genes express (Lamb and Dixon 1997). After pathogen invasion, plants can obtain the systemic acquired resistance (SAR) in which SA is the key signal molecular. When the plants respond to pathogen infection as well as other stresses, the plant hormones SA, JA and ET are the major players in the network of defense signaling pathways (Chen et al. 1993, Constabel et al. 1995, O'Donnell et al. 1996, Creelman and Mullet 1997, Korth and Dixon 1997, Lamb and Dixon 1997, Reymond and Farmer 1998). Interestingly, Stintzi et al. (2001) found out that resistance to insect and fungal attack can be observed in the absence of JA. Meanwhile, auxin, abscisic acid (ABA) and flavonoids also take part in the signaling pathways (Murphy et al. 2000, Leyser 2001, Shen et al. 2001, Winkel-Shirley 2002).

Rice is a model organism and one of the most important food crops in the world, providing a food staple for more than one quarter of the world population. Brown planthopper (BPH), is the major pest to rice crops all over the world. When BPH feeds on rice, it pierces into the phloem of rice and sucks out the nutritive liquids. Meanwhile, it leaves saliva sheaths in the plant. This phloem-feeding insect is also a vector of virus diseases of rice. BPH feeding causes a decrease in leaf area, photosynthetic rate, plant height, leaf and stem nitrogen concentration, chlorophyll contents and organ dry weight, but an increase in free amino acids, sucrose and leaf ferri ion content (Rubia-Sanchez et al. 1999, Watanabe and Kitagawa 2000). Feeding by a large number of BPH insects may result in drying of the leaves, wilting of the tillers, dying of the whole plant and even no harvest of the whole crop, such condition is called hopperburn. In the susceptible rice varieties, BPH insects have ample food, causing a high inhabiting ratio, egg amount, and survival ratio of eggs and nymphs, and generally leads to hopperburn. While in the resistant varieties, the survival rate of nymphs is significantly lower, nymphal development is generally retarded, the oviposition is severely inhibited, and population growth is effectively suppressed (Lin et al. 1995, Hao et al. 2000, Wang et al. 2000).

Researchers have succeeded in identifying the resistance genes against BPH and locating them on the genetic map of rice. As to the molecular mechanism of BPH-rice interaction, there is no report as of yet.

In this study, we collected 108 cDNA clones representing genes associated with plant responses to chewing insect-feeding, pathogen infection, wounding and other stresses, and used them to detect how rice plants respond to BPH feeding. By utilizing both resistant rice variety 'B5' and susceptible genotype 'MH63' (Chen et al. 2002), we demonstrated that the host tissue preference of BPH varied with the resistance levels of the plants. By means of cDNA array and Northern blot analysis, we illustrated that molecular reactions in rice in response to BPH feeding differentiated between two genotypes of the host plants.

## Materials and Methods

### Plant growth, BPH feeding and the observation of saliva sheaths

The seeds of BPH-resistant rice (*Oryza sativa* L.) variety B5 and BPH-susceptible variety MH63 were germinated, and then grown in the pots (8 cm in diameter and 15 cm in height). When the rice seedlings were in the third leaf stage, the second- to third-instar nymphs of BPH *Nilaparvata lugens* Stål. (Homoptera: Delphacidae) were put in at 10 insects per seedling. A control experiment without insects feeding was also conducted. The plants were harvested after 72 h treatment. Rice plants for RNA extraction were collected, immediately frozen in

liquid nitrogen and then stored at –70 ˚C. Stems of seven plants of each treatment and control were soaked in 1 % (W/V) Eosin Y. The saliva sheaths in the stems were counted under stereo-microscope (Du and Ding 1988). The experiments were repeated for three times.

### cDNA array preparation

105 cDNA clones were obtained from the Japanese Rice Genome Research Program of the National Institute of Agrobiological Resources (NIAR) and the Institute of the Society of Techno-Innovation in Agriculture, Forestry and Fisheries (STAFF). The *Arabidopsis* CHS, CHI and DFR clones are kind gifts from Dr. B. Winkel-Shirley (Virginia Polytechnic Institute and State University, USA). All the clones are classified into seven possible groups (Table 2). These clones were first transformed to the host strain *E. coli* DH5a. Then the cDNAs were amplified by polymerase chain reaction (PCR) in 25 μL volume by using primers M13: 5′-GAAACAGCTATGACCATG-3′ and T7: 5′-TAATACGACTCACTATAGGG-3′. The concentrated purified products were quantified on the Hoefer DyNA Quant® 200 Fluorometer (Amersham Pharmacia Biotech, USA) and adjusted to 33 ng/μL. One μL of each product was loaded in duplication on a 10×5.5 $cm^2$ Hybond $N^+$ membrane (Amersham Pharmacia Biotech, USA). After being dried naturally, the membranes were baked at 80 ˚C. Before hybridization, the membranes were soaked in 2×standard saline citrate buffer (SSC) for several minutes.

### RNA extraction and cDNA array assay

Total RNA was extracted from rice seedlings with the TRIzol® Reagent (GIBCO, USA) according to the manufacturer's instructions. After electrophoresis to confirm the quality, RNA was used for probe labeling by [$^{32}$P]dCTP (Perkin Elmer Life Sciences, USA) with reverse transcriptase. The cDNA array filters were hybridized with the prepared probes, then washed with 1×SSC, 0.2 % (W/V) SDS and 0.5×SSC, 0.1 % SDS at 65 ˚C, and exposed to the X-ray films (FUJI medical X-ray film, Japan) for one week at –20 ˚C. The data were collected and calculated (Hu et al. 1999).

### Northern blot analysis

25 μg RNAs from plants of B5 and MH63 were eletrophoresed on 1 % formaldehyde denatured agarose gel containing ethidium bromide. After taken a photo, the RNA was transferred onto nylon membranes (Amersham Pharmacia Biotech, USA). PCR products of the clones which had been selected by the cDNA array assay were labeled by [$^{32}$P]dCTP with Prime-a-gene® labeling system (Promega, USA), and hybridized to the membranes. The hybridization was carried out as usual. The blots were exposed to the X-ray films.

## Results

### Feeding behaviors of BPH nymphs on rice plants with different genotypes

Rice variety B5 is resistant to BPH but MH63 is susceptible (Chen et al. 2002). After being caged on rice plants for 72 h, most of the BPH nymphs on the susceptible rice MH63 survived. In contrast, 67.1 % of the insects on the plants of resistant rice B5 were dead (Table 1), confirming the resistance of B5. Saliva sheaths left by BPH probing and feeding on rice plants allowed us to figure out the feeding behavior of the insects. More saliva sheaths remained in B5 plants than those in the susceptible MH63 plants, implying that BPH probing was more frequent on the resistant rice plants (Table 1). Distribution of saliva sheaths also revealed that there was a tissue preference of BPH insects on rice plants. More saliva sheaths were found on the upper part of stems of B5 plants, while those left in MH63 plants were mainly on the lower part of the stems.

### Differential gene expression profiles in response to BPH feeding

We collected cDNA clones of 108 genes to investigate the molecular mechanism in rice responding to BPH feeding. These genes were grouped into seven categories (Table 2). The signal intensity for each gene was recorded on X-ray films and read into computer by scanner. The expression of each gene was calculated and analyzed based on the signal intensity (Hu et al. 1999). The changing tendencies of all genes after infestation by BPH were described in Table 2. We found that in the total 108 defense-related genes, the expressions of 90 clones were affected by BPH feeding, among which 69 were in B5 and 78 in MH63.

According to the experience of Schenk et al. (2000) and Voiblet et al. (2001), a two-fold or even higher difference in signal intensity between the treatment and the control was treated as significant in terms of transcript concentrations. Based on the criterion of two-fold difference, it was found that

**Table 1.** Characterization of rice plants responding to BPH feeding. Data are mean ± s. d.

| Varieties of rice | Response to BPH insect | The number of saliva sheaths left per plant | Survival ratio of BPH insect (%) | The ratio of saliva sheaths (%) left in the rice stem | |
|---|---|---|---|---|---|
| | | | | Upper | Lower |
| B5 | resistant | 9.20±0.52 | 32.90±3.12 | 54.60±1.85 | 45.40±1.85 |
| MH63 | susceptible | 5.24±1.05 | 74.30±2.05 | 30.00±1.31 | 70.00±1.31 |

**Table 2.** The 108 cDNA clones and their characterizations. The changes of gene expression in B5 or MH63 after BPH feeding are indicated as D (down-regulated), U (up-regulated) or '–' (no difference). The number indicates the magnitude of the regulation.

| Groups | Clone name | Accession No. | Function or product description | Change in B5 | Change in MH63 |
|---|---|---|---|---|---|
| Flavonoid pathway | C53151 | C27821 | UTP-glucose glucosyltransferase | D | D2 |
| | E20134 | C73679 | Cinnamate-4-hydroxylase | – | D2 |
| | E21179 | C99729 | Chalcone synthase (CHS) | – | – |
| | S742 | D39427 | Dihydroflavonol reductase (DFR) | U | D |
| | S1919 | D40147 | Flavonol-sulphotransferase-like protein | – | D |
| | S2293 | D40365 | Phenylalanine ammonia lyase | D | D |
| | S5085 | D42007 | Flavonol sulphotransferase-like protein | – | D |
| | S10303 | D45974 | Leucoanthocyanidin dioxygenase | – | – |
| | S10456 | D46055 | UDP-glucose flavonoid 3-O-glucosyltransferase | D2 | D2 |
| | S15513 | D48933 | Isoflavone reductase | U | U |
| | S15580 | D48979 | Flavonol synthase | D4 | D |
| | S16470 | AU032923 | Chalcone isomerase (CHI) | – | – |
| | S20542 | AU056394 | Flavanone 3-hydroxylase (*Solanum tuberosum*) | U | D2 |
| | R2933 | D25009 | Flavanone 3-hydroxylase (*Bromheadia finlaysoniana*) | U | D2 |
| | CHI | see Materials and Methods | Chalcone isomerase | – | – |
| | CHS | see Materials and Methods | Chalcone synthase | – | – |
| | DFR | see Materials and Methods | Dihydroflavonol reductase | – | – |
| Octadecanoid pathway | C50151 | AU068705 | Lipoxygenase | – | – |
| | S1722 | D40007 | Allene oxide synthase | D | D4 |
| | S10929 | D46326 | OPDA reductase (*Arabidopsis thaliana*) | U | D |
| | S15760 | D49097 | Jasmonate-induced protein | D | D |
| Signaling pathways | C12220 | AU068157 | Transcription initiation factor IIB | D | D |
| | E11086 | C73586 | Acyl-CoA-oxidase | – | – |
| | E60493 | AU083004 | Auxin-responsive protein IAA1 | D | D2 |
| | E61487 | AU083008 | Auxin-induced protein | U | U |
| | S1792 | D40060 | Transcription factor BTF3 | D2 | U2 |
| | S1988 | D40194 | Abscisic acid ABA- and stress-inducible protein | D2 | D3 |
| | S2324 | D40381 | Endotransglycosylase | – | U3 |
| | S2392 | D40423 | Glutamine synthetase | U4 | D3 |
| | S2554 | D40519 | Ethylene-responding factor | D | D2 |
| | S3707 | AU077899 | Acid phosphatase precursor 1 | D | D |
| | S3985 | AU070561 | Ubiquitin conjugating enzyme | – | D2 |
| | S6413 | AU070640 | Ethylene-forming enzyme | D | – |
| | S10161 | AU083534 | Phenoloxidase | – | – |
| | S10421 | D46029 | Acid phosphatase precursor 1 | D | D |
| | S10981 | D46362 | Calmodulin | – | – |
| | S11067 | D46407 | Sucrose synthase | D | D2 |
| | S11190 | D46487 | ACC oxidase | D | D |
| | S11722 | D46812 | ACC synthase | D | – |
| | S13809 | D47977 | Fatty acid Hydroperoxide lyase | – | U |
| | S16157 | AU065955 | S-adenosylmethionine synthase 2 (*Lycopersicon esculentum*) | U2 | D3 |
| | R826 | D39032 | Acetoacetyl-coenzyme A thiolase | – | D2 |
| Oxidative stress/ | C60626 | AU063307 | Glutamate-cysteine ligase | – | – |
| Apoptosis | C61433 | C97685 | Cellulase precursor (*Lycopersicon esculentum*) | U | D2 |
| | E3527 | C73250 | Copper/Zinc superoxide dismutase | U | – |
| | E20851 | AU077623 | SAG12 | – | – |
| | S1808 | D40069 | L-ascorbate peroxidase | D3 | D2 |
| | S2092 | D40254 | Germin-like protein | – | – |
| | S2148 | D40283 | Glutathione transferase | – | D |
| | S3217 | D40989 | Salicylic acid binding protein (SABP) | U | U |
| | S3645 | D41273 | NADP-dependent D-sorbitol–6-phosphate dehydrogenase | D | D3 |
| | S10306 | D45977 | Glycolate oxidase | – | D4 |
| | S10450 | D46051 | Catalase | D | D |
| | S11047 | D46392 | Thioredoxin 1 | U | U2 |

**Table 2.** Continued.

| Groups | Clone name | Accession No. | Function or product description | Change in B5 | Change in MH63 |
|---|---|---|---|---|---|
| | S11970 | D46973 | Cyclin | D | U |
| | S14319 | D48217 | Asparagine synthetase | D | U2 |
| | S14493 | D48344 | Peroxidase | D2 | D2 |
| | R596 | D28287 | Glutathione s-transferase PM24 | – | U2 |
| Wound-response, | C148 | D38785 | Myrosinase (*Brassica napus*) | – | U3 |
| drought-inducible and | C30344 | AU068483 | Glucosidase-like protein (*Arabidopsis thaliana*) | U | U |
| pathogen-related proteins | S2162 | AU070534 | Thioglucosidase (myrosinase precursor) | U | U |
| | C53725 | C28025 | Systemic wound response proteins (*Zea mays*) | U | – |
| | R10958 | AU078055 | Systemic wound response proteins (Basic) | U | – |
| | R3069 | D25064 | Cytochrome B/C1 | – | – |
| | S2160 | AU070533 | Cruciferin 1 precursor | – | – |
| | S10932 | D46329 | Myrosinase binding protein | D | – |
| | S13993 | D48061 | Hydroxynitrile lyase | D | – |
| | S14373 | D48250 | Cytochrome P450 | – | – |
| | S14639 | D48442 | Cyclophilin (*Zea mays*) | U4 | U2 |
| | C52004 | AU076010 | Pectin methylesterase-like protein | U | – |
| | C53229 | C27853 | Esterase | – | U3 |
| | E3667 | C98982 | Chitinase | U | – |
| | E10426 | C19444 | Putative cyt-p450 monooxygenase | D | – |
| | E30837 | C74300 | Meloidogyne incognita | – | U |
| | E31480 | AU029763 | Pectin methylesterase | U | U |
| | E61932 | AU031601 | Protease inhibitor (PI) | U8 | U14 |
| | S1788 | D40058 | Thionin | U | D |
| | S3206 | D40983 | Glucanase | D | U2 |
| | S12346 | AU070353 | Pectate lyase | D | U2 |
| | R3106 | D25070 | Metallothionein-like protein | – | D2 |
| | C12524 | C26524 | Cysteine protease (*Hordeum vulgare*) | – | U |
| | E1635 | C72446 | Sugar transporter (*Arabidopsis thaliana*) | U | U2 |
| | E31561 | C91795 | Osmotic stress-induced proline dehydrogenase | U | D |
| | E60018 | AU083000 | Aquaporin | D2 | D |
| | S1932 | D40157 | Cysteine protease inhibitor | U | U |
| | R3880 | AU065451 | Cysteine protease (*Zea mays*) | – | U |
| Aromatic metabolism | C10531 | C96653 | Citrate synthase | – | D3 |
| | C12187 | C26364 | D. discoideum gene for transfer RNA Ile | U | U |
| | C30692 | AU068686 | DAHP synthase precursor | U | D2 |
| | E143 | C71727 | Anthranilate synthase | – | U3 |
| | E742 | C71989 | β-fructofuranosidase precursor | D | – |
| | E1370 | C72289 | Ferulate–5-hydroxylase | D4 | D2 |
| | E2880 | C73083 | Lignin bispecific caffeic acid/5-hydroxyferulic acid o-methyl transferase | D2 | D |
| | E2937 | C73113 | Leu/Ile/Val-binding protein precursor | U | U |
| | E61006 | AU031151 | Cinnamoyl-CoA reductase (*Zea mays*) | D | D3 |
| | S1743 | D40024 | 4-coumarate-CoA ligase | D | U |
| | S4742 | AU070578 | ADP-ribosylation factor | – | U |
| | S4946 | AU070587 | Chorismate mutase (*Arabidopsis thaliana*) | – | D2 |
| Others | C259 | D22550 | Ubiquitin extension protein similar | – | D3 |
| | C10987 | D22416 | Cathepsin B precursor | U | D4 |
| | C11542 | C26051 | Epoxide hydrolase | – | – |
| | C60307 | C28190 | Osr40 g2 protein | D | D2 |
| | C60366 | C28210 | Phosphoglycerate mutase | – | – |
| | E3354 | AU081288 | Wheat cytosol 18S rRNA 3' terminus Cytosol aminopeptidase | U2 | U3 |
| | E4350 | C99032 | Chaperonin | U | D3 |
| | E10437 | C19453 | Globulin | D | – |
| | S21565 | AU057565 | Extensin precursor | U | U |
| | S2361 | D40407 | Ubiquitin | D | D2 |

**Table 3.** Number of genes whose expression is changed by at least two-fold after BPH feeding in rice.

| Groups | Number of clones in total | Number of clones regulated | | | |
|---|---|---|---|---|---|
| | | B5 | | MH63 | |
| | | Up-regulated | Down-regulated | Up-regulated | Down-regulated |
| Flavonoid pathway | 17 | 0 | 2 | 0 | 5 |
| Octadecanoid pathway | 4 | 0 | 0 | 0 | 1 |
| Signaling pathways | 21 | 2 | 2 | 2 | 8 |
| Oxidative stress/Apoptosis | 16 | 0 | 2 | 3 | 5 |
| Wound-response, drought-inducible and pathogen-related proteins | 28 | 2 | 1 | 7 | 1 |
| Aromatic metabolism | 12 | 0 | 2 | 1 | 5 |
| Others | 10 | 1 | 0 | 1 | 5 |
| Total | 108 | 5 | 9 | 14 | 30 |

after BPH feeding for 72 h, five clones were up-regulated while nine diminished in B5, and 14 increased while 30 decreased in MH63 (Table 3).

A total 17 genes in the flavonoid pathway were included in this experiment. Among those, two in B5 and five in MH63 showed down-regulated after insect damage. Genes in the octadecanoid pathway were almost all repressed in both rice varieties and one (S1722) of them was down-regulated for more than a four fold difference in MH63. Genes in the aromatic metabolism pathway were detected as mostly reduced in transcripts. The mRNA levels of genes for wound-response, drought-inducible and pathogen-related proteins were up-regulated except for R3106 and E60018 which were down-regulated. The genes in the remaining pathways mainly diminished upon BPH feeding. The variations are summarized in Table 3. It is found that there are more significant changes in gene expression in MH63 than in B5, and the dominant pattern of changes in MH63 is down-regulated by BPH feeding. These results suggest that normal physiological processes might be more affected after BPH feeding in MH63 than in B5.

Eighteen genes (e.g., S 742, S 20542, R 2933, S 2392, S 11970, S 14319, S 3206, S 1743) showing different patterns of expression in MH63 and B5 are likely to be BPH-resistance/-susceptibility related genes. These differences in gene regulation might account for the different degrees of resistance found in B5 and MH63.

## Verification of gene expression by Northern blot analysis

From the results of cDNA array assay, we randomly selected several clones that showed differences between the insect-feeding treatment and the control in both B5 and MH63 for further Northern blot analysis (Fig. 1).

The S10456 gene encodes an UDP-glucose flavonoid 3-O-glucosyltransferase, one enzyme involved in the flavonoid pathway (Dixon et al. 1989, Ford et al. 1998, Taguchi et al. 2000). Its expression was down-regulated both in B5 and MH63 after BPH infestation as detected in cDNA array. The Northern blot confirmed the variation. In comparison with the control, the signal was much weaker in the BPH-treated plants. The transcript concentration of another gene S15513 (isoflavone reductase) slightly increased in the BPH-fed rice plants.

The gene for an ABA- and stress-inducible protein (S1988) was repressed in both B5 and MH63 plants upon BPH sucking. The protein has been reported as being induced by ABA and plays a role in the stress response signal pathway (Shen et al. 2001). A gene (E61487) for another plant hormone-induced protein (Auxin-induced protein) had two transcripts. Responding to the insect attack, the major transcript decreased but the smaller one enhanced in the treatment.

Gene encoding cyclophilin (CyP) is inducible by abiotic stress (Marivet et al. 1992, 1994, Droual et al. 1997, Godoy et al. 2000). The gene (S14639) showed an enhanced expression profile and its transcript increased two- or four-fold after 72 h of BPH feeding. PI is an important element in the plant defense response to chewing insect damage (Pautot et al. 1991, Tamayo et al. 2000). The transcript level of a *PI* gene (E61932) increased dramatically in the BPH-fed plants, up to eight-fold in B5 and 14-fold in MH63.

E2880 (Lignin bispecific caffeic acid/5-hydroxyferulic acid o-methyl transferase) had two transcripts both in B5 and MH63. Upon BPH feeding the larger transcript repressed and the smaller one was enhanced.

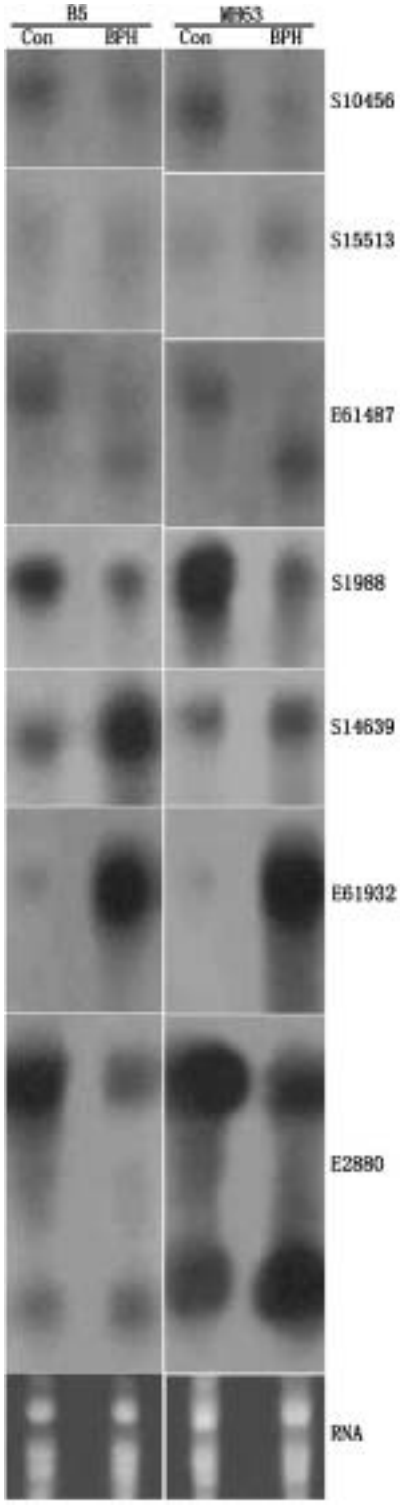


**Figure 1.** Northern blot analysis of selected clones (S10456, S15513, E61487, S1988, S14639, E61932 and E2880) in rice B5 and MH63. Total RNAs from the control and BPH-fed seedlings of B5 (left column) and MH63 (right column) were used for Northern blot analysis (25 μg/lane) following procedure described in Materials and Methods. Con, RNA extracted from the control plants; BPH, RNA from plants fed by BPH for 72 h. RNAs stained with ethidium bromide before membrane transfer are displayed in the bottom.

## Discussion

### Plant responses induced by BPH feeding might differ from that by chewing insects

Herbivores induce several well-characterized plant defense- and wound-response pathways. The central role of octadecanoid pathway in plants responding to chewing insects has been demonstrated by Creelman and Mullet (1997) and Reymond and Farmer (1998). The lipid-based signaling cascade involving jasmonate production via the octadecanoid pathway and leading to direct and indirect defenses against chewing insect herbivores is activated (Bergey et al. 1996, Liechti and Farmer 2002). Four genes in the octadecanoid pathway were listed in this experiment (Table 2) and nearly none of these genes presented altered expression patterns except that the expression level of S1722 decreased four fold in MH63 (Table 2 and 3). Lipoxygenase (C50151), a key enzyme in the synthesis of jasmonates (Creelman and Mullet 1997), did not show any difference at all in expression profile (confirmed by Northern blot, data not shown). A similar result was obtained in aphid about *LOX1* by Moran and Thompson (2001). Our results suggest the octadecanoid pathway might be not so important in the signaling pathways against BPH feeding in rice. As far as Stintzi et al. (2001) are concerned, we deduce that the interaction of rice and BPH is probably involved in a JA-independent pathway.

PI is the first identified wound-inducible protein and an important defensive element in plants against chewing insects (Pautot et al. 1991, Koiwa et al. 1997, Tamayo et al. 2000). It may also act to restrict infection by some nematodes (Atkinson et al. 1996). PIs are antidigestive and antinutritive proteins, which can inhibit elastases in the larval midgut (Tamayo et al. 2000) and decrease herbivore performance on some plants. Production of these inhibitors is highly regulated by a signal transduction pathway that is initiated by insect damage and transduced as a wound response. The dramatic increase of *PI* (E61932) transcription level in the insect-treated plants in our work proved that piercing-sucking of BPH could induce its expression. However, the higher expression level in susceptible rice MH63 implies that the genes of this group participate in rice responses to the piercing/sucking insect.

Flavonoids are secondary metabolites derived from phenylalanine and acetate metabolism that perform a variety of essential functions in plant growth, reproduction and survival, and also serve as important micronutrients in human and animal diets (Winkel-Shirley 2002). Flavonoids also play an important role in resistance to herbivore and other biotic or abiotic stresses (Li et al. 1993, Lee et al. 1998, Murphy et al. 2000, Winkel-Shirley 2002). Seventeen clones of this pathway that we collected and are listed in Table 2, showed a down-regulated expression profile. More decreasing expression patterns were observed in MH63 than in B5 (five in MH63 and two in B5). The facts indicate that flavonoids in rice against BPH attack are not as important as in maize resistance to corn earworm (Lee et al. 1998).

## Plant responses to BPH damage crosstalked with those related to abiotic stress, pathogen invasion and phytohormone signaling pathways

In addition to being a sucking insect that causes direct damage to plant, BPH also transmits several viruses that cause viral diseases in rice. In this case, insect-plant interactions actually represent the interactions of three organisms: the plant, herbivore, and endo-microbes (Walling 2000). The wound created by insect provides access for pathogens and endosymbionts to invade. As a result, mechanisms against pathogen invasion and herbivore attack should co-exist in plants.

When plants defend against pathogens, reactive oxygen species (ROS) produced in the oxidative burst could serve not only as protectants against invading pathogen, but could also be the signals activating further plant defense reactions, including the HR of infected cells (Tenhaken et al. 1995). The gene for salicylic acid binding protein (SABP) (S3217) gave a slight enhancement response to BPH feeding in rice. However, ascorbate peroxidase (S1808), a key enzyme to detoxify $H_2O_2$ in the oxidative stress process (Wojtaszek 1997), gave an obvious decreasing expression profile both in B5 and in MH63. SABP is a soluble protein with the activity of peroxidase that combines SA strongly (Chen et al. 1993), affecting $H_2O_2$ concentration, resulting in inducing the oxidation/reduction pathway to alter the expressions of plant defense genes. We deduce that the slightly increasing level of SABP (S3217) and decreasing level of ascorbate peroxidase (S1808) in our work indicate the oxidation/reduction pathway and the following defense reactions are affected, and rice response to piercing-sucking insects' attack would coordinate with the expression of PR genes.

Many biotic and abiotic stresses can induce the expression of defense compounds such as pathogen-related proteins and/or wound-inducible proteins. These compounds protect the plants and minimize the damages caused by the stresses (Pautot et al. 1991, Droual et al. 1997, Koiwa et al. 1997, Godoy et al. 2000, Baldwin et al. 2001, Moran and Thompson 2001). CyPs are ubiquitous proteins with an intrinsic enzymatic activity of peptidyl-prolyl *cis-trans* isomerase that catalyzes the rotation of x-pro peptide bonds. These enzymes are believed to play a role in the folding of certain proteins. In addition, CyPs might be important in signal transduction processes (Godoy et al. 2000). Plant *CyP* genes are stress-responsive as their expression can be induced by abiotic stresses such as treatment with chemical agents, heatshock, salt stress, low temperature and wounding (Marivet et al. 1992, 1994, Droual et al. 1997). Godoy et al. (2000) discover that accumulation of *Solanum tuberosum CyP* (*StCyP*) mRNA in fungal infected potato tubers is dependent upon, and a response to the wound produced during the process of penetration of the pathogen in the host tissue. The expression level of *CyP* (S14639) increased in BPH-fed rice, which may also be a response to the damage caused by BPH.

The plant hormones are central to the regulation of growth and development of plants and also additional regulators of the singling pathways responding to environmental conditions. The expression pattern of ABA- and stress-inducible protein (S1988) was suppressed in both rice varieties upon the BPH damage. We assume that when BPH pierces and sucks the rice, it might emit something to suppress the expression of ABA gene, or to combine the endogenous ABA, resulting in the declining level of ABA- and stress-inducible protein.

The smaller transcript of auxin-induced protein (E61487) appeared while the major one declined upon the damage of the BPH, and its expression level was enhanced (Fig. 1 and Table 2). The results not only strengthen the idea of Leyser that auxin signaling depends on targeted protein degradation (Leyser 2001) but also suggest that the rice-BPH interaction might be associated with the auxin signaling pathway.

Ethylene is also an important phytohormone that acts as a mediator of adaptation responses to stress and pathogen infection (Abeles 1992, O'Donnell et al. 1996). Clones for ACC oxidase (S11190), ACC synthase (S11722), S-adenosylmethione synthase (S16157) and two ethylene-related clones (S2554 and S6413) are the key genes in ethylene synthesis. In our study, the changes of transcripts of these genes reveal that the ethylene signaling pathway should be a part of the reaction of rice plant with BPH.

## The differences between rice genotypes resistant and susceptible varied from BPH-feeding preference behaviors to both spectra and levels of gene expression

Rice variety B5 has proven that it carries two major resistance genes against BPH (Huang et al. 2001, Wang et al. 2001). The insects of BPH prefer feeding on susceptible plants to feeding on resistant plants when different rice varieties placed together (Wang et al. 2000). Usually BPH insects gather on the lower part of rice stems, as indicated on MH63 in Table 1, and suck assimilates from the phloem of rice plants. In contrast, on B5 plants the surviving insects are mainly distributed on the upper part of plant, indicating there is something on the lower part of plants repulsing the insects. Meanwhile, more saliva sheaths were left but less BPH insects survived on B5 than on MH63, suggesting that some substances in B5 controlled the feeding behavior of BPH.

As to the gene expression profiles detected by cDNA array and Northern blot analysis, both the expression spectra and regulated levels differed between MH63 and B5. Expressions of 14 genes in B5 and 44 genes in MH63 were significantly regulated by the insect feeding (Table 3), indicating that more genes in MH63 were sensitive to BPH feeding. Some genes were regulated in the same direction both in B5 and MH63 after BPH feeding but with different expression levels. For instance, transcripts of *PI* (E61932) increased eight fold in B5 while increasing 14 fold in MH63 from the control to the BPH-

treated plants. Clones S1988, S2554, S3645, S14639, E3170, E60493 are the genes with differential degrees in the same direction regulated by BPH attack. Most transcripts were changed to the same tendency in both rice plants but the expression levels in B5 were always lower than in MH63. This phenomenon leads us to believe that these genes are potentially defensive genes against BPH. Contrary to resistant rice B5, in which the damage by BPH is healed easily by slight alterations in gene expressions, susceptible rice MH63 fights for its life against equal or even greater amounts of BPH insects by changing more in gene expression profiles. There were 12 genes that were up-regulated in B5 while being down-regulated in MH63 (Table 2), and 6 genes showed the reverse results (S1792, S11970, S14319, S3206, S12346 and S1743). The genes presenting conflicting regulation patterns in MH63 and B5 are likely to be BPH resistance/susceptibility genes and are being investigated further for their functions in resistance to BPH.

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## References

Abeles F (1992) Ethylene in plant biology. Academic Press, Inc, New York

Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH (1997) An elicitor of plant volatiles from beet armyworm oral secretion. Science 276: 945–949

Atkinson HJ, Urwin PE, Clarke MC, McPherson MJ (1996) Image analysis of the growth of *Globodera pallida* and *Meloidogyne incognita* on transgenic tomato roots expressing cystatins. J Nematol 28: 209–215

Baldwin IT, Halitschke R, Kessler A, Schittko U (2001) Merging molecular and ecological approaches in plant-insect interactions. Curr Opin Plant Biol 4: 351–358

Bergey DR, Howe GA, Ryan CA (1996) Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. Proc Natl Acad Sci USA 93: 12053–12058

Bouwmeester HJ, Verstappen FWA, Posthumus MA, Dicke M (1999) Spider mite-induced (3S)-(E)-Nerolidol synthase activity in cucumber and lima bean. The first dedicated step in acyclic C11-Homoterpene biosynthesis. Plant Physiol 121: 173–180

Chen RZ, Weng QM, Huang Z, Zhu LL, He GC (2002) Analysis of resistance-related proteins in rice against brown planthopper by two-dimensional electrophoresis. Acta Bot Sin 44: 427–432

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

Constabel CP, Bergey DR, Ryan CA (1995) Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. Proc Natl Acad Sci USA 92: 407–411

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48: 355–381

De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393: 570–572

Dixon SC, Martin RC, Mok MC, Shaw G, Mok DWS (1989) Zeatin glycosylation enzymes in *Phaseolus*. Isolation of O-glucosyltransferase from *P. lunatus* and comparison to O-xylosyltransferase from *P. vulgaris*. Plant Physiol 90: 1316–1321

Droual AM, Maaroufi H, Creche J, Che'nieux JC, Rideau M, Hamdi S (1997) Changes in the accumulation of cytosolic cyclophilin transcripts in cultured periwinkle cells following hormonal and stress treatments. J Plant Physiol 151: 142–150

Du J, Ding JH (1988) Review on feeding behavior and physiology of brown planthopper (*Nilaparvata lugens* Stål.). Entomol Knowl 25: 182–187 (in Chinese)

Ford CM, Boss PK, Hoj PB (1998) Cloning and characterization of Vitis vinifera UDP-glucose: flavonoid 3-O-glucosyltransferase, a homologue of the enzyme encoded by the maize Bronze-1 locus that may primarily serve to glucosylate anthocyanidins *in vivo*. J Biol Chem 273: 9224–9233

Godoy AV, Lazzaro AS, Casalongué CA, San Segundo B (2000) Expression of a *Solanum tuberosum* cyclophilin gene is regulated by fungal infection and abiotic stress conditions. Plant Sci 152: 123–134

Hao SG, Cheng XN, Zhang XX (2000) Effects of nine rice varieties on survival and oviposition of *Nilaparvata lugens* (Stål.). J Nanjing Agr U 23: 39–42 (in Chinese with English abstract)

Hu Y, Han C, Mou Z, Li J (1999) Monitoring gene expression by cDNA array. Chinese Sci Bull 44: 441–444

Huang Z, He GC, Shu LH, Li XH, Zhang QF (2001) Identification and mapping of two brown planthopper genes in rice. Theor Appl Genet 102: 929–934

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53: 299–328

Koiwa H, Bressan RA, Hasegawa PM (1997) Regulation of protease inhibitors and plant defense. Trends Plant Sci 2: 379–384

Korth KL, Dixon RA (1997) Evidence for chewing insect-specific molecular events distinct from a general wound response in leaves. Plant Physiol 115: 1299–1305

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol 48: 251–275

Lee EA, Byrne PF, McMullen MD, Snook ME, Wiseman BR, Widstrom NW, Coe EH (1998) Genetic mechanisms underlying apimaysin and maysin synthesis and corn earworm antibiosis in maize (*Zea mays* L.). Genetics 149: 1997–2006

Leyser O (2001) Auxin signalling: the beginning, the middle and the end. Curr Opin Plant Biol 4: 382–386

Li J, Ou-Lee TM, Raba R, Amundson RG, Last RL (1993) *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell 5: 171–179

Liechti R, Farmer EE (2002) The jasmonate pathway. Science 296: 1649–1650

Lin KM, He YX, Gan DY, Weng QY (1995) The effects of two rice resistant sources and their new breeding varieties on the biological characters of brown planthopper (*Nilaparvata lugens* Stål.). Entomol J East China 4: 99–102 (in Chinese with English abstract)

Marivet J, Frendo P, Burkard G (1992) Effects of abiotic stresses on cyclophilin gene expression in maize and bean and sequence analysis of bean cyclophilin cDNA. Plant Sci 84: 171–178

Marivet J, Margis-Pinheiro M, Frendo P, Burkard G (1994) Bean cyclophilin gene expression during plant development and stress conditions. Plant Mol Biol 26: 1181–1189

Mattiacci L, Dicke M, Posthumus MA (1995) β-Glucosidase: an elicitor of herbivore-induced plant odor that attacts host-searching parasitic wasps. Proc Natl Acad Sci USA 92: 2036–2040

Moran PJ, Thompson GA (2001) Molecular response to Aphid feeding in *Arabidopsis* in relation to plant defense pathways. Plant Physiol 125: 1074–1085

Murphy A, Peer WA, Taiz L (2000) Regulation of auxin transport by aminopeptidases and endogenous flavonoids. Planta 211: 315–324

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

Paré PW, Tumlinson JH (1998) Cotton volatiles synthesized and released distal to the site of insect damage. Phytochemistry 47: 521–526

Paré PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121: 325–331

Pautot V, Holzer FM, Walling LL (1991) Differential expression of tomato proteinase inhibitor I and II genes during bacterial pathogen invasion and wounding. Mol Plant-Microbe Interact 4: 284–292

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

Rubia-Sanchez E, Suzuki Y, Miyamoto K, Watanabe T (1999) The potential for compensation of the effects of the brown planthopper *Nilaparvata lugens* Stål. (Homoptera: Delphacidae) feeding on rice. Crop Prot 18: 39–45

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM (2000) Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc Natl Acad Sci USA 97: 11655–11660

Shen QX, Chen CN, Brands A, Pan SM, David Ho TH (2001) The stress- and abscisic acid-induced barley gene *HVA22*: developmental regulation and homologues in diverse organisms. Plant Mol Biol 45: 327–340

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE (2001) Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci USA 98: 12837–12842

Taguchi G, Imura H, Maeda Y, Kodaira R, Hayashida N, Shimosaka M, Okazaki M (2000) Purification and characterization of UDP-glucose: hydroxycoumarin 7-O-glycosyltransferase, with broad substrate specificity from tobacco cultured cells. Plant Sci 157: 105–112

Tamayo MC, Rufat M, Bravo JM, San Segundo B (2000) Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding, and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. Planta 211: 62–71

Tenhaken R, Levine A, Brisson LF, Dixon RA, Lamb C (1995) Function of the oxidative burst in hypersensitive disease resistance. Proc Natl Acad Sci USA 92: 4158–4163

Thaler JS (1999) Jasmonate-inducible plant defenses cause increased parasitism of herbivores. Nature 399: 686–688

Van de Ven WTG, Levesque CS, Perring TM, Walling LL (2000) Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding. Plant Cell 12: 1409–1423

Voiblet C, Duplessis S, Encelot N, 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

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

Wang BN, Huang Z, Shu LH, Ren X, Li XH, He GC (2001) Mapping of two new brown planthopper resistance genes from wild rice. Chinese Sci Bull 46: 1092–1095

Wang RF, Zhang CL, Zou YD, Lü L, Cheng XN (2000) Effect of rice variey resistance on population dynamics of *Nilaparvata lugens* and *Sogatella furcifera*. Chinese J Appl Ecol 11: 861–865 (in Chinese with English abstract)

Watanabe T, Kitagawa H (2000) Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). J Econ Entomol 93: 1192–1198

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol 5: 218–223

Wojtaszek P (1997) Oxidative burst: an early plant response to pathogen infection. Biochem J 322: 681–692