

How can planthopper genomics be useful for planthopper management?

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Recent developments in insect genomics, dealing with the entire genome of an insect, facilitate new approaches to study various aspects of fundamental and applied topics of the insect. To elucidate planthopper virulence against resistant rice varieties, pesticide resistance mechanisms, and virus transmission machinery, planthopper genomics will be powerful and useful. Expressed sequence tag (EST) analysis was performed on the brown planthopper *Nilaparvata lugens* (BPH), and a microarray was prepared as a functional study tool. Proteome studies also provide us with new information on molecules that take part in various biological phenomena of planthoppers. Molecular markers based on genetic information are becoming important as tools for monitoring and tracing planthopper migration routes. Planthopper genomics is expected to help in constructing a more powerful and sustainable planthopper management system, including developing novel insecticides.

Keywords: genomics, planthopper, EST, microarray, proteome, virulence, resistance, virus transmission, molecular markers

Planthoppers are a diverse group of phytophagous insects belonging to the superfamily Fulgoroidea, which is composed of approximately 20 described insect families (O'Brien and Wilson 1985). More than 9,200 species have been described (Woodward et al 1976, after O'Brien and Wilson 1985); some species attack important crops, such as maize, wheat, rice, and forage grasses. Among those, the most economically important species are doubtlessly three species of rice planthoppers: the brown planthopper *Nilaparvata lugens* (BPH), the whitebacked planthopper *Sogatella furcifera* (WBPH), and the small brown planthopper *Laodelphax striatellus* (SBPH). They belong to the family Delphacidae, which is a large group situated in a basal part of the phylogenetic tree of Fulgoroidea (Yeh et al 2005, Urban and Cryan 2007). BPH attacks only rice plants, WBPH mostly infests rice plants, and SBPH has a wider host range, including wheat and some grasses. BPH causes severe sucking damage and transmits virus diseases to rice plants, WBPH causes sucking damage on rice plants, and SBPH transmits a virus disease to rice plants.

BPH and WBPH are distributed in tropical Asia and have long-distance migration (Kisimoto 1976, 1979). In the temperate region, growing populations of these planthoppers start from the immigrants transported from the southern year-round planthopper area; they do not overwinter and die in cool and cold seasons. SBPH, on the other hand, has a wide range of distribution in the world. This species diapauses in cool regions. SBPH shows allozyme polymorphism among regional populations, suggesting that this species does not regularly show long-range dispersal (Hoshizaki 1997). The geographic variation of SBPH is also clearly supported by the trait of nymphal diapause; there is a geographic cline in the critical photoperiod for diapause (Noda 1992). Nevertheless, SBPH, as well as BPH and WBPH, shows wing dimorphism. Rice planthoppers show two wing patterns when they become adults: a long-wing (macropterous) form and a short-wing (brachypterous) form. Both males and females show wing dimorphism, but brachypterous males are rare in WBPH. The wing forms are closely related to population growth in the rice field and consequently to damage to rice. Macropterous planthoppers have wide distribution, and brachypterous ones deposit more eggs in rice plants, making their population density high. These traits or characteristics of planthoppers have been intensively studied from ecological and physiological viewpoints, and this has contributed to planthopper management.

Molecular studies in planthoppers, however, are still poor in spite of their economic importance. Recent developments in genomics in biology appear to enable us to form new approaches in studying, analyzing, and managing insect pests. Genomics is the field of study related to an organism's entire genome. Functional genomics studies, which deal with patterns of gene expression in various conditions, became popular in many organisms. Various fields, whose name has "-omics" or "-ome" in the suffix, have developed (http://omics.org/index.php/Main_Page). The suffix "-omics" usually means a field of study in biology, such as genomics, transcriptomics, proteomics, metabolomics, and so on. The suffix "-ome" indicates the objectives for study, such as transcriptome, proteome, metabolome, and so on. Molecular biology, which studies single genes and their functions, is also closely related to genomics. Genomics and related fields are surely changing biological studies, introducing computer science and bioinformatics into the life sciences.

In this chapter, various aspects of molecular studies and recent genomics studies of the brown planthopper are introduced, and what is required for planthopper management is discussed in terms of current biological and genomics trends. First, the present status of genomics studies in insects is reviewed. Second, a clear grasp of planthopper problems in rice production is attempted in order to know what we should study and how we could attack the problems using genomics and molecular approaches. Third, molecular information attributed to planthoppers is surveyed as statistical aspects of genome information. The information includes not only that on planthoppers themselves but also that on their associated microorganisms. Fourth, recent developments in genomics studies, especially EST studies and those using related tools, are presented. Finally, future studies on planthoppers in the next decade are discussed.

Genome sequencing in insects

Genomics in insects has been actively studied in the last decade. First, the entire genome was determined in the fruit fly *Drosophila melanogaster* (Adams et al 2000). The first whole-genome shotgun (WGS) sequencing method was applied to the higher eukaryotes in *D. melanogaster*, which definitely influenced the genome sequencing of higher eukaryotes thereafter. A whole-genome sequence of the mosquito *Anopheles gambiae* was reported in 2002 (Holt et al 2002); the genome of malaria *Plasmodium falciparum*, which is transmitted by *A. gambiae*, was also published simultaneously (Gardner et al 2002). The silkworm *Bombyx mori* genome was sequenced independently in Japan and China (Mita et al 2004, Xia et al 2004). The WGS sequence data from both countries were merged and reassembled; high-quality sequence data in the first lepidopteran species are released (The International Silkworm Genome Consortium 2008). Genome sequencing was followed by the honeybee, *Apis mellifera* (The Honeybee Genome Sequencing Consortium 2006); a virus-vector mosquito, *Aedes aegypti* (Nene et al 2007); and the red flour beetle, *Tribolium castaneum* (Tribolium Genome Sequencing Consortium 2008). Genome sequencing projects are carried out for many insects; Table 1 summarizes current genome sequencing projects in insects, ticks, and mites. Most of the genome sequences were determined by the whole-genome shotgun sequencing method, but clone-based sequencing was also performed in some species. Next-generation sequencing technologies have also been used recently.

Expressed sequence tag (EST) analyses were also performed in many insect species because EST analysis can be done at much lower cost than entire genome sequencing. EST analyses are also extensively done in the above-described insect species in which the genome sequence is mostly determined because the expressed gene sequences are useful information for genome annotation and analyses. Table 2 shows the insects or ticks in which a high number of ESTs are deposited in DNA databases.

What are the planthopper problems?

More than 30 years ago, in May 1977, a symposium was held at the International Rice Research Institute to discuss research results and to develop plans for brown planthopper control. The contents of talks of the symposium were published in the book *Brown planthopper: threat to rice production in Asia* (IRRI 1979). In this book, BPH was clearly regarded as the number-one insect pest in rice in Asia (Dyck and Thomas 1979) and some of the problems, which we still face, were already obvious. Breeding of various resistant rice varieties began in the late 1960s and the use of plant resistance for controlling pests was thought to be a simplistic solution. However, the first BPH-resistant rice variety, IR26, became susceptible in several years and a BPH biotype capable of destroying IR26 became abundant (Brady 1979). Resurgence of BPH also occurred where insecticides were used in the 1970s (Heinrichs 1979, 1994).

What are the problems or important topics in rice planthoppers now?

Table 1. Whole-genome sequencing projects in Insecta and Acari.^a

Order/species	Genome size, Mb (no. of chromosomes)	Method	Sequence depth and status
Diptera			
<i>Drosophila melanogaster</i>	180 (4)	WGS, clone-based	–, complete
<i>Anopheles gambiae</i>	– (3)	WGS	10X, assembly
<i>Culex pipiens</i>			
<i>quinquefasciatus</i>	– (–)	WGS	–, assembly
<i>Aedes aegypti</i>	800 (3)	WGS	8X, assembly
<i>Cochliomyia hominivorax</i>	– (–)		–, in progress
<i>Haematobia irritance</i>	– (–)		–, in progress
Lepidoptera			
<i>Bombyx mori</i>	530 (28)	WGS	10X, assembly
<i>Bicyclus anynana</i>	490 (–)		–, in progress
Coleoptera			
<i>Tribolium castaneum</i>	200 (10)	WGS	7.3X, assembly
Hymenoptera			
<i>Apis mellifera</i>	200 (16)	WGS, clone-based	7-8X, assembly
<i>Nasonia vitripennis</i>	– (5)	WGS	6.2X, assembly
<i>N. giraulti</i>	– (5)	WGS	13X, in progress
<i>N. longicornis</i>	– (5)	WGS	13X, in progress
Hemiptera			
<i>Acyrtosiphon pisum</i>	525 (4)	WGS	6X, assembly
<i>Rhodnius prolixus</i>	670 (11)	WGS	8X, in progress
<i>Diaphorina citri</i>	– (–)		–, in progress
Isoptera			
<i>Psammotermes</i>	– (–)		–, in progress
Anoplura			
<i>Pediculus humanus corporis</i>	– (–)	WGS	8X, assembly
Acari			
<i>Ixodes scapularis</i>	– (–)	WGS, clone-based	6X, assembly
<i>Tetranychus urticae</i>	– (–)	WGS	–, in progress
<i>Vorroa destructor</i>	– (–)	WGS	8X, in progress

^aData are based on the Entrez Genome Project in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/genomes/leuks.cgi, as of 4 November 2009). *Drosophila* species are not listed except *D. melanogaster*. WGS = whole-genome shotgun sequencing method.

Table 2. Species in which a high number of ESTs are deposited in DNA databases.

Species	Number of ESTs ^a
<i>Drosophila melanogaster</i>	821,005
<i>Aedes aegypti</i>	301,596
<i>Bombyx mori</i>	245,761
<i>Culex quinquefasciatus</i>	205,274
<i>Ixodes scapularis</i>	193,773
<i>Acyrtosiphon pisum</i>	169,928
<i>Anopheles gambiae</i>	153,273
<i>Nasonia vitripennis</i>	145,793
<i>Drosophila simulans</i>	118,742
<i>Glossina morsitans morsitans</i>	79,292
<i>Apis mellifera</i>	78,191
<i>Tribolium castaneum</i>	64,571
<i>Locusta migratoria</i>	45,708
<i>Drosophila sechellia</i>	38,257
<i>Drosophila auraria</i>	38,110
<i>Nilaparvata lugens</i>	37,312
<i>Drosophila pseudoobscura</i>	35,042
<i>Spodoptera frugiperda</i>	32,255
<i>Nasonia giraulti</i>	30,060
<i>Myzus persicae</i>	27,686
<i>Drosophila willistoni</i>	26,751

^aNCBI (www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html), as of 4 November 2009.

The following seem to be those we have to pay attention to:

1. Insecticide resistance
2. Occurrence of biotypes (virulent strains) of BPH
3. Virus transmission by BPH and SBPH
4. Monitoring and occurrence forecasting of planthoppers
5. Wing dimorphism (macropterous and brachypterous forms)
6. Finding new targets for novel insecticides

Insecticide resistance

Various insecticides have been used for controlling planthoppers, especially for BPH. Organophosphorus insecticides were first used in the 1950s and carbamate compounds were first used in the 1960s (Heinrichs 1979). Pyrethroid compounds were then

introduced and neonicotinoid compounds are now popular for planthopper control. Insecticide resistance became obvious in BPH in the late 1960s. BPH insecticide resistance has been monitored in Japan since the late 1960s (Fukuda and Nagata 1969) and the recent trend of insecticide resistance status in BPH and WBPH is summarized by Matsumura et al (2007). The susceptibility of BPH to neonicotinoid insecticides and that of WBPH to fipronil decreased. The development of a neonicotinoid-resistant strain of BPH was observed in a laboratory colony, which showed mutation in genes of acetylcholine receptor subunits, the target molecules of neonicotinoid insecticides (Liu et al 2005). It is problematic that these lately introduced compounds have been showing lower effectiveness against planthopper populations.

Virulent strains of BPH

In these 30 years, the presence of many resistance genes against BPH was reported from rice plants; 21 resistance genes are so far known (Zhang 2007, Yasui et al 2007). New rice varieties that possess plural resistance genes have been developed and used successfully in some regions. However, it is generally admitted that virulent BPH strains capable of attacking resistant rice varieties appear when a BPH-resistant rice variety is cultivated in a wide area. Nevertheless, we do not know the mechanism of the resistance of rice against BPH and the virulence of BPH against resistant rice varieties. Molecular mapping of the genes with resistance to BPH is done for the rice plant; chromosomal positions of some genes are narrowed down by map-based approaches (Sharma et al 2004a, Chen et al 2006). These genes are expected to be isolated soon. Once the genes are isolated, functional analyses of them will be carried out. How virulent BPH make the work of the resistance genes ineffective is another problem to be solved by entomologists.

Virus transmission by BPH and SBPH

BPH transmits the rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV) (Nault 1994, Hibino 1996). These virus diseases have occurred markedly since 2005 in the Mekong Delta in Vietnam, and are estimated to have caused a loss of 400,000 tons in two years (1.1% of Vietnam's total production) (Heong and Escalada 2008). SBPH transmits rice stripe virus, which belongs to the tenuivirus group. This persistently transmitted virus often prevails in some rice-growing areas; an outbreak in China was recently reported (Zhu 2006, Xiong et al 2008). Genomes of these viruses are determined and advanced detection methods for them are available. However, the interaction between the viruses and the host planthoppers is still poorly understood.

Monitoring and occurrence forecasting of planthoppers

Flight and migration of planthoppers are monitored by light or net traps. BPH and WBPH show long-distance flight; this is an important issue according to where the immigrants have flown from. The immigrants reflect the biological traits of planthoppers in the original region, for example, the level of insecticide resistance, the level of virulence against rice varieties, the infection rate of virus diseases, and wing dimorphic response under various conditions. Meteorological trajectory analysis is a

useful tool for estimating planthopper emigration area (Otuka et al 2005). However, we still do not have good molecular markers for distinguishing the local strains and for estimating the origin of immigrant planthoppers. We need to precisely evaluate molecular markers as classification tools of local populations because planthoppers fly a long distance and genetic variability seems to be small among geographical populations.

Wing dimorphism

Planthoppers show phase variation in wing form: some have a macropterous (long-wing) form for long-distance migration and others have a brachypterous (short-wing) form suitable for reproduction. This polymorphism is closely related to planthopper damage: macropterous planthoppers enlarge their territory and brachypterous ones increase their offspring. Hormonal studies suggest that juvenile hormone stimulated the brachypterous form (Ayoade et al 1999, Bertuso et al 2002). The molecular mechanisms, however, including related genes, are not elucidated.

New targets for novel insecticides

The occurrence of insecticide-resistant populations reduces options in selecting insecticides for controlling planthoppers. Resistant planthoppers often show cross resistance to insecticides with a similar mode of action. Therefore, a group of insecticides might lose its effectiveness. Every year, some new insecticides are placed on the market. The insecticides that have a novel mode of action are rare among them. New insecticides against a novel target are very promising compounds for insect control. Mining new target molecules and finding major compounds against the target is important work in molecular and genomics studies.

Statistical aspects of genetic and genomics information in planthoppers

The genetic background is quite poor in planthoppers. Here, we survey studies related to planthopper genetics. Genetic and genomics information seems to be useful to create molecular markers to discriminate local populations or colonies showing specific biological traits.

Chromosomes of planthoppers

Chromosomes of homopterans are holocentric and do not show a localized centromere during cell division. The chromosome number of BPH is 30: 14 autosomal bivalents and two sex chromosomes (Noda and Tatewaki 1990). Diploid chromosomes of males consist of 28 autosomes and each X and Y chromosome and those of females consist of 28 autosomes and two X chromosomes. In contrast, the chromosome number of WBPH and SBPH is 29 or 30: the male diploid number is 29 (28 + XO) and the female diploid number is 30 (28 + XX). In BPH, a Y chromosome-specific sequence can be used for sex discrimination since the Y chromosome is present only in males. PCR amplification using PCR primers corresponding to DNA sequences in the Y chromosome enables male detection in premature stages (Kobayashi and Noda 2007a).

Planthopper color mutants

Some mutants are observed when field populations are reared in the laboratory. Planthoppers with red eyes were often found in BPH, WBPH, and SBPH. One recessive gene is usually related to red-eyed phenotype: probably a gene that takes part in making black pigment in the eyes causes a mutation. A red-eyed mutant of BPH found by Mochida (1970) showed embryonic lethality in the eggs laid by homologous females. Red-eyed forms of SBPH, which were collected in Tokyo, led to the discovery of cytoplasmic incompatibility in SBPH through crossing experiments between a black-eyed western population and a red-eyed Tokyo colony (Noda 1984a, 1984b, 1987). Another example is body coloration in BPH. BPH usually shows brown body color; blackish planthoppers are sometimes found in the field and laboratory-reared colonies. Some genes might be involved in body color in BPH (Morooka et al 1988).

Ribosomal RNA

Ribosomal RNA is a central component of ribosome. A ribosomal RNA gene (rDNA) sequence is often used for the identification of species or strains as well as various phylogenetic studies. A single transcription unit of eukaryotes (45S) includes 18S, 5.8S, and 28S rRNAs and two internally transcribed spaces (ITS) between 18S and 5.8S and between 5.8S and 28S rDNA. The sequences of the 18S and 28S rDNA are very conservative and are good markers for species identification. In contrast, those of ITS regions are less conservative and provide useful information for discriminating strains or populations in the same species. In planthoppers, the ITS sequence is a good candidate gene for detecting local populations. WBPH has size variation in the ITS1 region. Now, we do not have a correlation between size variation and the locality of WBPH in Asian countries (unpublished). BPH had an R2 retrotransposon in the 28S rRNA gene. This R2 retrotransposon appears to be similar to those found in various other insect species (Burke et al 1993).

Mitochondrial genome sequence

Mitochondrial genomes of arthropods are usually circular: 14–20 kb long, with 2 ribosomal RNAs, 22 tRNAs, and 13 protein-coding regions; they contain a small noncoding region (Boore 1999). The genetic orders in the mitochondrial genomes are similar, allowing the proposal of an ancestral gene order. Mitochondrial genome sequences are available in many hemipteran insects (www.ncbi.nlm.nih.gov/genomes/OrganelleResource.cgi?opt=organelle&taxid=6656). Hemipteran insects have mitochondria whose gene order resembles the proposed ancestral one. However, whitefly species show mitochondrial gene rearrangements; several genes have changed their position from the ancestral gene order (Thao et al 2004). BPH had mitochondria of ancestral gene order; the genome size was about 17,250 (unpublished).

Sequence variation in the mitochondrial genome within species is useful information for elucidating geographic structure among rice planthopper populations in Southeast and East Asia. Mun et al (1999) reported sequence variation in the *cytochrome oxidase-I* gene (COI) in BPH and WBPH, showing the first geographic molecular variation, except allozyme analyses. Genetic variation of this gene, however,

is small among local populations in Asia; a much varied region or larger region in the mitochondrial genome might be more informative for this purpose.

Genes characterized from planthoppers

Sequences of some genes are determined and deposited in DNA databases. In BPH, nucleotide sequences of some enzyme genes involved in detoxifying insecticides, such as the genes of cytochrome P450, glutathione S-transferase (Yang et al 2005), and carboxylesterase (Small and Hemingway 2000), are available, as well as other enzyme genes, those of trypsin-like protease, cathepsin B-like protease (Foissac et al 2002), and NADH-quinone oxidoreductase (Yang et al 2005). Genes of nicotinic acetylcholine receptors (Liu et al 2005), a hexose transporter (Price et al 2007), diuretic hormone receptors (Price et al 2004), and ferritin (Du et al 2000) are determined in their nucleotide sequences. More than 37,000 ESTs were recently deposited in the database, which enable us to get partial sequences of various genes from BPH (Noda et al 2008).

Endosymbiotes of planthoppers

Planthoppers harbor various intracellular microorganisms. The habituation or infection of microorganisms in planthoppers might provide us with discriminative information from other strains or local populations. An indispensable microorganism for planthoppers is yeastlike symbiote (YLS). YLS resides in the fat body cells and is transmitted to the next generation through the female ovary (Noda 1974, 1977, Chen et al 1981, Suh et al 2001). *Wolbachia* are found from almost all WBPH and SBPH; *Wolbachia* in these two species were indistinguishable as far as the sequences of several *Wolbachia* genes are concerned (Noda et al 2001). Some BPH are infected with *Wolbachia* and other microorganisms, for example, rickettsia and spiroplasma (unpublished). Among eukaryotic and prokaryotic endosymbiotes of planthoppers, *Wolbachia* can be cultured in insect cell lines (Noda et al 2002). No other microorganisms in planthoppers were successful in cultivation in vitro.

BPH transmits two virus diseases as described above. Three other viruses are characterized from BPH. *Nilaparvata lugens* reovirus, NLRV, is infected with some populations of BPH (Noda et al 1991, Nakashima et al 1996). Himetobi P virus, HiPV, which was first found in SBPH, propagates often and highly in the midgut of BPH. *Nilaparvata lugens* commensal X virus, NLCXV, seems to be a satellite virus (Nakashima et al 2006).

Toward functional genomics in planthoppers

Expressed sequence tag (EST) analysis

In order to study gene and protein functions, to elucidate molecular mechanisms of biological phenomena in planthoppers, and to find effective control means of planthoppers, genomic information is a useful resource. EST analysis, which is the creation of many short sequences of a transcribed spliced nucleotide sequence, is a good introduction for functional genomics (Nagaraj et al 2006).

More than 37,000 ESTs were created from 18 libraries of various BPH tissues and stages (Noda et al 2008). Ribosomal RNA sequences, mitochondrial genome sequences, and planthopper-infected virus genome sequences were eliminated from the EST database (<http://bphest.dna.affrc.go.jp/>). Their average size is 627 bp; 10,200 clusters were made from whole EST sequences. The actin gene was the most abundantly expressed and the myosin gene was also highly expressed in BPH whole ESTs. Actin and myosin were largely expressed in the thorax, where the flight muscle is located. Some enzyme genes, such as trypsin-like protease and enolase, showed high expression. The ESTs of the mucin-like protein gene and vitellogenin gene were often found in the salivary glands. EST libraries created from various tissues are useful for selecting tissue specifically expressed genes. Gonad specifically expressed genes, for example, were extracted (unpublished). The function of many of them has not been analyzed yet; they are considered to play important roles in reproduction.

The EST database would facilitate the cloning of important genes. However, many of the nominated ESTs are housekeeping genes, and the EST database might not contain genes of low expression level. In order to enrich BPH ESTs, further ESTs should be collected from the libraries made from small tissues or from planthoppers reared in unusual conditions. Another effort is to determine the sequence of clones from full-length cDNA libraries. Collection of the sequences of 5' and 3' regions of genes from the full-length cDNA would ease our tedious work to get whole cDNA sequences or the full sequence of a protein-coding region.

Proteomic analysis

Proteome studies, which deal with the entire complement of proteins expressed in tissues or organisms, are also an important approach for functional genomics. Proteomics, the study of the proteome, usually uses two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Protein spots on the gel, which have moved to a specific location, are given further analysis using a peptide sequencer or mass spectrometer. Recently, shotgun proteomics after proteolytic enzyme digestion of samples has been used as an alternative technology capable of identifying hundreds of proteins from single samples. When we use mass spectrometry for characterizing and identifying protein molecules, whole-genome sequences or well-accumulated genome information would be necessary. We do not have BPH genome sequences and have only some ESTs. Therefore, amino acid sequencing by a peptide sequencer is employed for BPH proteomics. The amino acid sequences obtained are then used in sequence similarity searches, FASTA or Basic Local Alignment Search Tool (BLAST), in public databases so as to annotate the protein molecules of interest. However, some proteins are difficult to annotate using public databases for the following two reasons. One is that many proteins in BPH are unique and we cannot find any similarity with the amino acid sequences in the databases. The other is the sequence length obtained from the peptide sequencer is not long enough to find similar proteins; usually, the obtained amino acid sequences are 20–30 residues. Moreover, N-terminal sequences often vary among homologous proteins among organisms. In these cases, the EST library is used to find the genes encoding the proteins. If we could find ESTs, the nucleotide

sequences and putative amino acid sequences of the peptide could be obtained, thus extending sequence information from small peptide fragments.

Proteomic analysis by insecticide application was performed in BPH. A carbamate insecticide, *o*-sec-butylphenyl methylcarbamate compound (BPMC), modulated 22 proteins at the expression level in 2D-PAGE compared with nontreated control BPH (Sharma et al 2004b). N-terminal and internal amino acid sequences were determined by a protein sequencer. The expression of putative serine/threonine protein kinase, paramyosin, heat shock protein (HSP) 90, beta-tubulin, calreticulin, ATP synthase, actin, and tropomyosin was elevated. That of beta-mitochondrial-processing peptidase, dihydrolipoamide dehydrogenase, enolase, and acyl-coA dehydrogenase was low. Cytoskeleton proteins were upregulated by BPMC treatment; an increased expression of cytoskeleton genes or proteins is often observed in response to toxic chemicals. Chaperone proteins HSP 90 and calreticulin increased after BPMC treatment, which might show insects' homeostasis maintenance under stress induced by insecticide exposure. Mitochondrial proteins showed different expression in response to BPMC, suggesting an overall change in mitochondrial response. A few enzymes, including enolase, one of the highly expressed genes in BPH (Noda et al 2008), decreased after treatment, suggesting a weakening of the usual metabolism.

Microarray

Microarray was developed as a high-throughput tool used for transcriptome analyses. A BPH microarray was fabricated based on the EST data accumulated from various tissues of BPH. Approximately 17,000 sequences were selected from all ESTs (about 37,000) based on a clustering analysis. Probes of 60-mer were designed for each sequence. Agilent's 22K oligonucleotide microarray (oligoarray) was first made and used for some studies, including preliminary test analyses. The oligoarray showed quite stable results and revealed that a twofold change in gene expression level could be reliably distinguished between two samples based on the color-swap data of Cy3 and Cy5. Now, a 4X 44K oligoarray is used for microarray analyses.

The following studies are now being done using microarray. First, expression profiles between BPH sucking resistant and susceptible rice plants were compared. Some genes were up-regulated or down-regulated in BPH sucking a resistant variety. Some of them were related to starvation in resistant rice plants. Second, genes related to wing dimorphism are sought using planthopper nymphs reared under different conditions; one is suitable for brachypterous expression and the other for macropterous expression in the adult stage (Kobayashi and Noda 2007b). Prior to the array experiment, sex-discriminating methods in nymphs were developed (Kobayashi and Noda 2007a) because males and females show a different response in wing formation to environmental stimuli (Kisimoto 1956, 1965, Kisimoto and Rosenberg 1994). Third, the effect of *Wolbachia*, alpha-proteobacteria that manipulate the sex and reproduction of host arthropods, on gene expression is studied in the testes of BPH. The *Wolbachia* taken from *Laodelphax striatellus* were cultivated in vitro (Noda et al 2002) and then introduced into BPH by micro-injection. These *Wolbachia* cause cytoplasmic incompatibility and embryonic death of eggs deposited by *Wolbachia*-

infected males and uninfected females. Microarray could be used for various molecular studies in BPH.

Genomics studies of planthoppers in the next ten years

Whole-genome sequencing

The results of first-round EST analyses are open to the public (<http://bphest.dna.affrc.go.jp/>), with sequence and annotated information on transcribed genes. Now, full-length cDNAs of BPH are analyzed and a combined database with ESTs and full-length cDNA will be created as a more efficient and useful resource. The next step in genomics studies in BPH is sequencing of the whole genome. Whole-genome sequencing needs the cooperation of many scientists and technical assistants, including those in entomology, genome-biology, bioinformatics, and general biology. The recent development of new DNA sequencers based on next-generation sequencing technology would facilitate the determination of a large amount of sequence and decrease the cost of whole-genome sequencing.

Rice plant resistance and planthopper virulence

Many genes resistant to planthoppers, especially to BPH, have been reported (Zhang 2007) and some of these genes are mapped on the chromosomes of rice. Map-based cloning of resistance genes is performed based on rice genome sequence information (IRGSP 2005). Candidate genes involved in resistance are almost identified and await precise studies by plant physiologists and entomologists. In contrast, we do not have any clue to virulence against resistant rice varieties. Characterization of the resistance genes of rice might help to solve the virulence problem of planthoppers. Some approaches to elucidate the molecular basis of virulence have been developed (Hao et al 2008); this will be more actively studied using genome information and genomics tools.

Genome-based discovery of insecticide targets

Useful and excellent agricultural pesticides have been developed in the past 50 years. However, the efficiency in finding new major compounds through biological screening has decreased. Insecticidal target molecules are mostly related to the nerve or neuron and some to the respiratory machinery or cuticle formation. Chemicals acting on other targets are quite limited. Recent genome studies in insects appear to contribute to finding unexplored target molecules. To find the genes of target molecules, genome information-based and postgenome tools-based investigation, for example, microarrays or proteomic analyses, will be used as well as empirical investigation. When we find a novel target molecule, suitability and effectiveness of the candidate target molecule should be evaluated, namely, we should know whether insects are dead when we disturb the function of the molecule. RNA interference (RNAi) is convenient and is a simple method for target validation.

Once the target molecule is determined, an *in vitro* assay system will be constructed for high-throughput screening of chemical libraries. This kind of approach

has some more advantages. The mode of action of the newly developed pesticides is clear since the target molecules are already known. From the viewpoints of environmental problems and the insecticide resistance problem, it is highly desirable that the mode of action of the chemicals be fully clear. An insecticide resistance mechanism is also easy to study. Because of the recent trend of rising resistance in planthoppers against insecticides, novel compounds seem to be required in Asian rice production.

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Notes

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