Int.J.Curr.Microbiol.App.Sci (2017) *6*(12): 782-788

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 12 (2017) pp. 782-788**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.612.083

Identifying Candidate Genes Based on Linked Markers for Brown Planthopper [*Nilaparvata lugens* (Stål)] Resistance in Rice (*Oryza sativa* L.)

S.P. Timmanagouda[1*] and M. Maheswaran[2]

[1]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Bengaluru, Karnataka, India
[2]Director of Research, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India
**Corresponding author*

Keywords

Rice, Brown planthopper, *In-silico*, Locus, SSR Markers.

Article Info

Accepted:
07 October 2017
Available Online:
10 December 2017

A B S T R A C T

An *in silico* analysis was carried out on the brown planthopper resistant region of chromosome 6 (Chr6s: BPH-RR) in rice using the SSR markers. The study resulted in the identification of 748 gene loci/protein coding genes. Out of 748 loci analyzed 40% (301) genes are hypothetical /unknown expressed proteins and 60% of the loci are well annotated genes. Chr6s: BPH-RR (Resistance region) indicates that the majority of the genes were related to stress tolerances.

Introduction

Rice is the staple food for over half the world's population. It provides 27 *percent* of dietary energy and 20 *percent* of dietary protein in the developing world. The crop is cultivated in at least 114, mostly developing countries and is the primary source of income and employment for more than 100 million households in Asia and Africa (FAO, 2004). Many diseases and insects harass the rice plant, among these insect pests, planthoppers, stemborers and gallmidges are the most serious pests of rice (Joshi *et al.*, 2000). Sporadic but catastrophic outbreaks of the BPH have been recorded throughout the history of rice cultivation in Japan (Suenaga and Nakatsuka, 1958; Miyashita, 1963). The manifestation of yield loss due to planthoppers varies by 80 or 90, 50 and 10 *percent* respectively depending upon the occurrence of hopperburn within 30, 40 and 50 days after heading (Kisimoto, 1976). Besides the yield loss, higher percentages of dead, immature and broken grains have been recorded in the infected plants (Chou, 1969; Hisano, 1964).

In recent years, BPH has caused devastating damages to rice crop in China, Japan, Korea and Vietnam. In 2005 and 2008, China reported a combined yield loss of 2.7 million

tonnes of rice due to direct damage by BPH, while a yield loss of 0.4 million tonnes in Vietnam was mainly due to two virus diseases, rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV), transmitted by BPH (Brar *et al.,* 2010).

The advent of molecular marker technology and the construction of first genetic map of rice using molecular marker by McCouch *et al.,* (1988) led to the mapping of genes for various agronomic traits. Mapping genes associated with resistance to insect pests and diseases were targeted first considering the possibilities of doing phenotypic screening under controlled conditions. Identifying genes conferring resistance to BPH was in a bigway along with other diseases *viz*, blast and bacterial leaf blight following different strategies. Classical linkage analysis between markers and phenotypes were exploited for identifying quantitative trait loci (QTL).

Phenotypes to genes and their sequences

The development of basic tools such as a fine molecular genetic map with 2,300 markers (Harushima *et al.*, 1998), a physical map with YACs (yeast artificial chromosomes) covering 60 *percent* of the rice genome (Saji *et al.,* 2001) and 10,000 independent expressed sequence tags (EST) (Yamamoto and Sasaki, 1997) has greatly facilitated the launching of the whole-genome sequencing of rice.

During the 4th International Symposium of Plant Molecular Biology in 1997 in Singapore, a workshop on rice genome sequencing was held to organize the foundation for an international collaboration, the International Rice Genome Sequencing Project (IRGSP), aimed at accelerating completion of the sequencing of the entire rice genome. This resulted in the publication of 389 Mb genome of rice which can be exploited for the identification of genes associated with various agronomic traits (International Rice Genome Sequencing Project, 2005). The rice genome sequence will facilitate the identification of genes associated with the trait of interest using markers tightly linked to the phenotype of interest.

This study was taken to gain appreciation of the kind of candidate genes responsible for the underlying mechanistic basis of BPH resistance. An extensive *in-silico* data mining was undertaken to highlight the characteristics of this 4.9 Mb region in search of candidate gene. It must be noted that the reference genome for the data mining was Nipponbare.

Materials and Methods

In-silico data mining to identify the candidate genes

The SSR markers associated with QTL identified for BPH resistance by Jegadeeswaran (2014) were used to trace the candidate genes for BPH resistance using RAP-DB database. The primers used for *in-silico* data mining is given in Table 1.

The SSR markers information like map position and base sequences was collected from the Gramene data base (http://www.gramene.org/). RM589 marker being closely associated with BPH resistance is present on the chromosome 6. Its flanking markers were chosen and the gene locus that are present in between the flanking markers were retrieved and analysed from the Nipponbare genome sequences using RAP-DB (http://rapdb.dna.affrc.go.jp/). The selected flanking markers cover the region on chromosome No. 6 from 537,430 bp to 5,425,408 bp which accounts to 4.9 Mb region. This region covers the 17 SSR markers including RM589 on Chromosome 6.

Results and Discussion

Tacking of candidate genes

In-silico data mining

A region on chromosome 6 covering RM589 and other 16 SSR markers was analysed to identify the genomic regions for candidate gene identification. Analysis indicated that Chr6s: BPH-RR contains a 748 gene locus/protein coding genes. A large number of these are hypothetical/unknown expressed proteins. Out of 748 loci analyzed 40 *percent* (301) genes are hypothetical/unknown expressed proteins and 60 *percent* of the loci are well annotated genes and could be tabulated. Each of these 447 genes was then categorized according to their biological function. The details of the major candidate genes identified and their frequency are furnished in Table 2.

The analysis of gene locus information from Chr6s: BPH-RR (Resistance region) indicates that the majority of the genes were related to stress tolerances. More specifically genes were belonged to the following classes: Pathogenesis- related transcriptional factor/ERF, Leucine- rich repeat (LRR), Nucleotide binding-APAF1, R proteins and CED-4(NB-ARC), Nucleotide binding site-Leucine rich repeats (NBS-LRR), Late embryogenesis abundant (LEA) protein, zinc finger, Homeodomain Leucine zipper protein, DUFs, F-box domain, Acyl-CoA binding protein, Guanine nucleotide binding protein (G- protein), ATOZI 1 protein (Stress induced protein), Pentatricopeptide repeat domain containing protein, Repair protein Rad1/Rec1 domain containing protein (Figure 1).

Certain harmone synthesis and harmone response factor genes were also included i.e., Auxin–responsive protein IAA20 (Indoleacetic acid–induced protein 20), Ethylene biosynthesis and cytokinin signalling. Typical wound response genes associated with pest attack were also present. i.e., Nodulins etc. Large number of genes was present in the class of protein degradation, sorting and modifications. Many changes associated with protein sorting and modification form the basis of signaling cascades. For examples, transferases, kinases, peptidases, hydrolases, peroxidases, phospho-glucomutases, esterases, monooxygenase, synthases, superoxide dismutase, acid phosphatase, decarboxylases, protien inhibitor, etc. Interestingly, a number of proteasome genes were present. These are typical plant cell response processes to pathogen and pest attack.

Genes were also present for calcium and G-protein-Mediated signaling cascades typical of immediate responses by cells to stress condition in general. Under such circumstances protein modification through phosphorylation, glycosylation or Ubiquitination are well documented (Zeng *et al.,* 2006). It was found genes responsible for these processes for example, protein kinases, glycosyltransferases and as well as ubiquitination related genes were noticed. Similarly, stress response associated chaperone activity for both protein and RNA stability is also well documented (Boston *et al.,* 1996; Wang *et al.,* 2004). A number of heat shock protein genes, Chaperonins, Penta- and tetratricopeptide genes were also present.

A limited number of primary and secondary metabolism genes were also noticed. However, these were not necessarily housekeeping genes and could be easily associated with stress responses. For example, a number of transferases, transporters, permeases and importins were noticed as especially related to amino acid and nucleotide remobilization and metabolism.

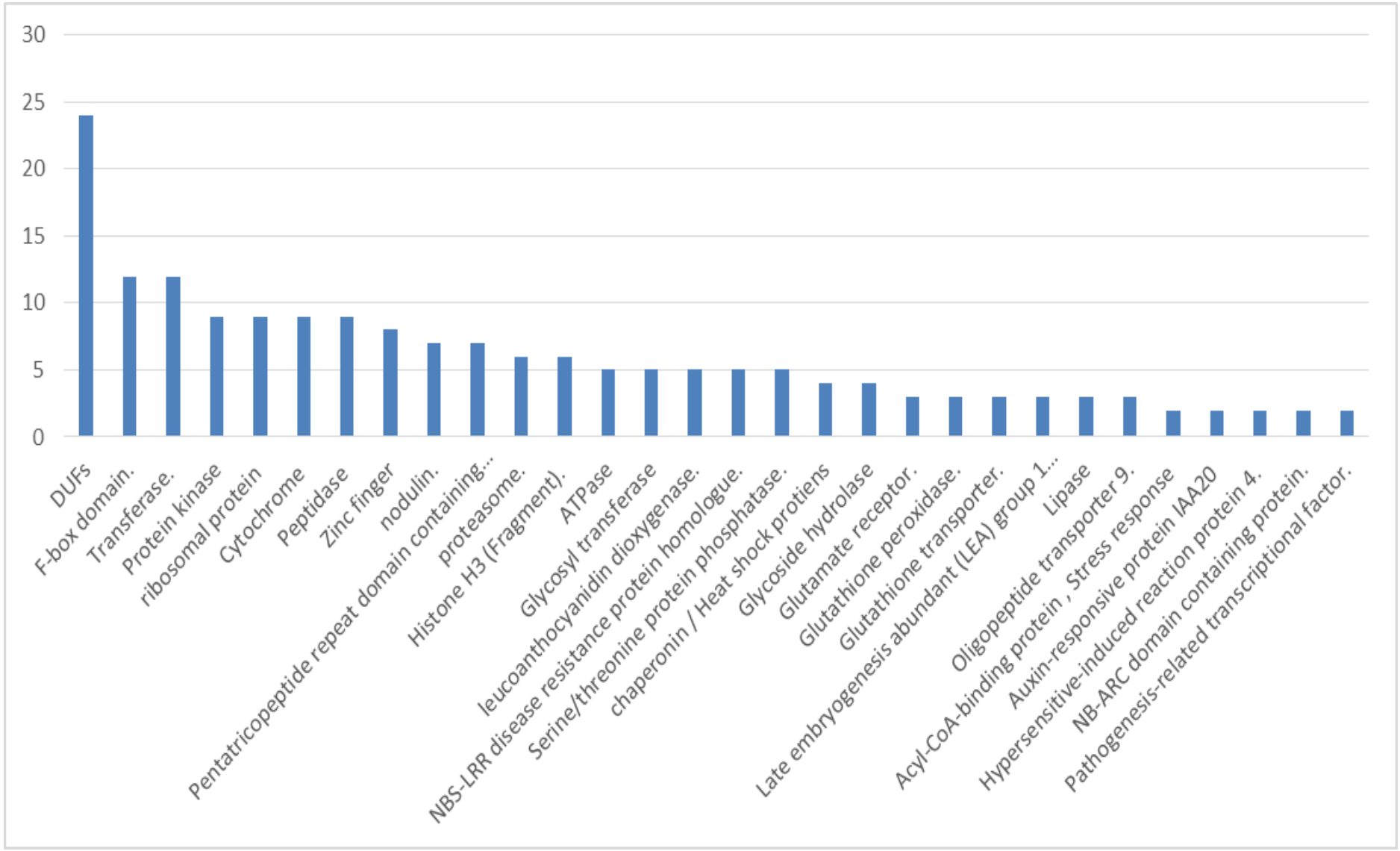
Table.1 The SSR primers on linkage group 6 used for *in-silico* data mining

S.No	Marker	Map Position
1	RM435	537320-537430 bp
2	RM469	564135-564314 bp
3	RM19291	1215884-1216030 bp
4	RM597	1368649-1368824 bp
5	RM589	1380876-1381023 bp
6	RM8072	1408336-1408481 bp
7	RM19311	1463393-1463487 bp
8	RM586	1476793-1477087 bp
9	RM588	1611398-1611524 bp
10	RM8101	1704743-1705015 bp
11	RM190	1764586-1764729 bp
12	RM510	2831443-2831635 bp
13	RM204	3168314-3168547 bp
14	RM225	3416533-3416728 bp
15	RM111	5096744-5096867 bp
16	RM310	5115740-5115915 bp
17	RM253	5425408-5425602 bp

Table.2 The details of the major candidate genes identified

Function	No of genes
DUFs	24
F-box domain.	12
Transferase.	12
Protein kinase	9
Ribosomal protein	9
Cytochrome	9
Peptidase	9
Zinc finger	8
Nodulin.	7
Pentatricopeptide repeat domain containing protein.	7
Proteasome.	6
Histone H3 (Fragment).	6
ATPase	5
Glycosyl transferase	5
Leucoanthocyanidin dioxygenase.	5
NBS-LRR disease resistance protein homologue.	5
Serine/threonine protein phosphatase.	5
chaperonin / Heat shock proteins	4
Glycoside hydrolase	4
Glutamate receptor.	3
Glutathione peroxidase.	3
Glutathione transporter.	3
Late embryogenesis abundant (LEA) protein.	3
Lipase	3
Oligopeptide transporter 9.	3
Acyl-CoA-binding protein, Stress response	2
Auxin-responsive protein IAA20	2
Hypersensitive-induced reaction protein 4.	2
NB-ARC domain containing protein.	2
Pathogenesis-related transcriptional factor.	2

Fig.1 The candidate genes identified on chromosome 6 based on the SSR markers



Serine/threonine accumulation is known as a stress response. Glycerate modification genes (glycerol-3-phosphate acyltransferase, glycerophosphoryl diester phosphodiesterase, and bisphosphoglycerate-independent phosphoglyceratemutase) were also present and can affect the content of this organic acid and in turn sugar metabolism under stress. Similarly, the secondary metabolism genes present, such as the flavin monooxygenase and shikimate dehydrogenase, are associated with stress responses.

One of the quickest and lasting stress responses at the cellular level is the change in redox status, reactive oxygen species (ROS) and in the enzymes that scavenge ROS (Suzuki *et al.,* 2012). Redox genes such as stress responsive oxidoreductase aldo/keto reductase, peroxidase, ubiquinone oxidoreductase were all present. Some of the genes were involved in the synthesis of new compounds. For example, ACC synthase, ATP synthase, Sucrose synthase, Starch synthase, Chloroplast biogenesis, Ethylene biosynthesis, *etc*.

This Chr6s:Bph-RR also has important gene locuses such as: receptor-like kinase (RLK) (Os06g0130100) which is related to drought and salt stress tolerance, homolog of the *Arabidopsis* early flowering 3 protein (Os06g0142600) which is involved in short-day/long day promotion, florigen (Os06g0157500, Os06g0157700) which is involved in long day promotion, chloroplast biogenesis (Os06g0168600), rice wax synthesis regulatory gene 4 (Os06g0181700), RCI2 (Rare Cold- Inducible 2) family gene (Os06g0184800) and mature anther – preferentially expressed gene (Os06g0186000).

Fujita Daisuke *et al.,* (2013) reported the same kind of candidate genes in the Chr12L: Bph-RR. They undertook an extensive *in silico* data-mining exercise to highlight the characteristics of 10 Mb region. They indicated that Chr12L: *Bph*-RR contains nearly 1550 protein coding genes. A large number of these are either for transposons/ retrotransposons or hypothetical or unknown expressed proteins. Only 448 (33.5%) well annotated genes could be tabulated. Shalini *et al.,* (2013) reported that out of various candidate genes identified, three candidate genes *viz*. PIII-1 Proteinase inhibitor protein precursor gene, pentatricopeptide genes and NB-ARC domain containing protein genes were found to be associated with BPH resistance.

From the present study, it is clear that the markers in the vicinity of genomic regions associated with BPH resistance can be exploited to trace the candidate genes provided 1) suitable mapping population with right genetic architecture, 2) right screening methods to unravel the mechanisms of resistance, 3) genome sequence information and 4) results of expression analysis are made available.

Once all these components are available the QTL with the statistical associations can be made biologically meaningful with reference to BPH resistance in rice to exploit the results in practical rice breeding.

References

Boston, R. S., P. V. Viitanen and E. Vierling. 1996. Molecular chaperones and protein folding in plants. Plant Mol. Biol. 32:191–222.

Brar, D. S., P. S. Virk, K. K Jena, and G. S. Khush, 2010. Breeding for resistance to planthoppers in rice. Planthopper: new threats to the sustainability of intensive rice production systems in Asia. IRRI; p. 401-427

Chou, W. D. 1969. Ecological studies on brown planthopper and chemical control [in Chinese, English summary]. Taiwan Agric. 5:178–241.

Food and Agriculture Organisation (FAO). (2004). *Rice is life*:

Fujita Daisuke, Ajay Kohli, G. Finbarr and Horgan. 2013. Rice Resistance to Planthoppers and Leafhoppers. Critical Reviews in Plant Sciences. 32:162–191.

Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto,

S.Y. B. Lin, A. Antonio, A. Parco, H. Kajiya, N. Huang, K. Yamamoto, Y. Nagamura, N. Kurata, G. S. Khush, and T. Sasaki, 1998. A high-density rice genetic linkage map with 2275 markers using a single F2 population. Genetics 148:479-494.

Hisano, E. 1964. Occurrence and injury of white-backed and brown planthopper and control measures [in Japanese]. Agric. Hortic. 39: 141–141.

International Rice Genome Sequencing Project, 2005

Jagadeeswaran, M., C. R. Anandakumar and M. Maheswaran, 2014. Phenotypic Screening of Rice (*Oryza sativa* L.) Varieties for Brown Planthopper [*Nilaparvata lugens* (Stål)] Resistance. Indian Journal of Animal Nutrition, 7(16): 2257-2266.

Joshi, S. P., V. S. Gupta, R. K. Aggarwal, P. K. Ranjekar, and D. S. Brar. 2000. Genetic diversity and phylogenetic relationship as revealed by inter sample sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor. Appl. Genet. 100: 1311-1320.

Kisimoto, R. 1976. Bionomics, forecasting of outbreaks and injury caused by the rice brown planthopper. Paper presented at the International Seminar on the Rice Brown Planthopper. Asian and Pacific Council, Food and Fertilizer Technology Center, October 1976, Tokyo, Japan. 16 p.

McCouch, S.R., G. Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush, W.R. Coffman and S.D. Tanksley. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet., 76: 815-829.

Miyashita, K. 1963. Outbreaks and population fluctuation of insects, with special reference to agricultural insect pests in Japan. Bull. Natl. Inst.Agric. Sci. C., 15: 99-170.

Saji, S., Y. Umehara, B. A. Antonio, H. Yamane, H. Tanoue, T. Baba, H. Aoki, N. Ishige, J. Wu, K. Koike, T. Matsumoto and T. Sasaki. 2001. A physical map with yeast artificial chromosome (YAC) clones covering 63 percent of the 12 rice chromosomes. Genome 44:32-37.

Shalini, P and M. Maheswaran. 2013. Selective genotyping of recombinant inbred line population and mapping genes for Brown planthopper (*Nilaparvata lugens Stal)* resistance using simple sequence repeats.

Suenaga, H. and K. Nakatsuka. 1958. Studies on forecasting of the occurrence of leafhoppers in the paddy field. The first Special Research Report on the Disease and Insect Forecasting, Promotion Office, Ministry of Agriculture and Forestry, Japan. 468 p.

Suzuki, N., S. Koussevitzky, R. Mittler and G. Miller. 2012. ROS and redox signaling in the response of plants to abiotic stress. Plant Cell Environ, 35: 259–270.

Wang, C. M., C. C. Sui, X. L. Ding, H.Q. Zhai and J.M. Wan. 2004. QTLs linked to a gene cluster conferring resistance to sucking insect pests in rice (*Oryza sativa* L.) Rice Genet. Newsl., 21:56-59.

Yamamoto, K. and T. Sasaki. 1997. Large-scale EST sequencing in rice. Plant Mol. Biol. 35:135- 144.

Zeng, L. R., M. E. Vega-Sanchez, T. Zhu and G. L. Wang. 2006. Ubiquitination-mediated protein degradation and modification: an emerging theme in plant-microbe interactions. Cell Res. 16:413–426.

How to cite this article:

Timmanagouda, S.P. and Maheswaran, M. 2017. Identifying Candidate Genes Based on Linked Markers for Brown Planthopper [*Nilaparvata lugens* (Stål)] Resistance in Rice (*Oryza sativa* L.). *Int.J.Curr.Microbiol.App.Sci.* 6(12): 782-788. doi: https://doi.org/10.20546/ijcmas.2017.612.083