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Identification of candidate genes for bacterial leaf blight resistance in rice by integration of genetic QTL map with the physical map

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To identify putative candidate genes involved in BLB disease resistance, we performed *in silico* anchoring of the generated QTL genetic marker data to the rice physical map. In total, one QTL responsible for resistance specificity to *Xanthomonas oryzae* pv. *oryzae* iso 6 was identified using CT 9993-5-10-1-M / IR 62266-42-6-2 double haploid population. The QTL qBB 10.1, on chromosome 10 RG 4561-EM 14-10 was saturated with 108 markers, 1097 ESTs, and 201 genes. In total, 28 putative positional candidate genes were identified and were classified based on their function. it is suggested that, the generated data can further be used for the dissection and validation of the QTL to understand the molecular mechanisms of the plant pathogen interaction and to develop a resistant variety.

Key words: QTL, Bacterial leaf blight, Expressed Sequence Tags, Candidate genes, Physical map.

INTRODUCTION

Xanthomonas oryzae pv. *oryzae* is one of the most devastating pest of rice which causes bacterial leaf blight BLB (Wen *et al*., 2003). It acts as notoriously "shifty enemy" through mutation, recombination, migration, complemented with random genetic drift and selection pressure and are often circumvented with disease management strategies. One among the best disease management strategy followed is, development of resistant varieties utilizing both horizontal and vertical resistances (Zhang and Mew, 1985). Vertical resistance, govern by the single major gene, is race specific and can be broken down easily (Mew *et al*., 1992). In contrast, horizontal resistance is govern by polygenes, presumably non-race specific and inherited quantitatively (Nelson, 1972). Achieving/or developing long durable resistant variety needs deeper understanding of molecular principles underlining the quantitative trait loci QTL. Several QTLs responsible for the BLB resistance have been identified using different mapping populations across the different environmental backgrounds, which provide paved starting for candidate gene mining. Cloning of a QTL is necessary for a better understanding of the genetic and functional basis of the plant responsible to BLB resistance (Causse *et al*., 1994; Li *et al*., 1999).

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However, positional cloning of a candidate gene normally requires fine scale mapping with large mapping population (Wang *et al*., 2005; Tuberosa and Salvi, 2006). Availability of the whole rice genome sequence (IRGSP, 2005) provide a new tool for this task, along with a means of characterizing the associated molecular function of candidate genes. In this study, we exploited this new source of data by anchoring the QTL regions responsible for the resistance against the BLB on rice physical map and construct a high resolution map with molecular markers, ESTs, and genes to identify candidate genes and linked markers.

MATERIALS AND METHODS

QTL identification and selection for construction of high resolution map :

The present study was conducted at the Research Farm of Indira Gandhi Krishi Vishwavidhyalaya, Raipur, India 21.15° N latitude, 81.86° E longitude and 289.6 m above MSL. The plant material used in the present study consisted of 154 double haploid lines derived from CT 9993-5-10-1-M *Japonica* cultivar X IR 62266-42-6-2 *Indica* cultivar, developed at Centro International de Agricultura Tropical CIAT, Columbia, and International Rice Research Institute IRRI, Philippines. 154 DH lines were planted along with parents in Randomized Block Design with two replications. The row to row and plant

to plant spacing of 20 cm was maintained. Each line was planted in two rows of 2 m length. All normal packages of practices were followed to raise a good crop.

A genetic linkage map consisting of 315 marker loci including 145 restriction fragment length polymorphisms RFLPs, 153 amplified fragment length polymorphism AFLPs, and 7 simple sequence repeats SSRs was previously constructed on the basis of the 154 DH lines was used for the QTLs analysis. MAPMAKER/ QTL 1.1 software was used for interval mapping (Lander *et al*., 1987), and to estimate the percentage of the total phenotypic variance explained by each QTL. A threshold of LOD >1.5 was used to claim the presence of QTL. The QTL with highest LOD and phenotypic variation was selected for the construction of high-resolution map and *in silico* analysis. The identified QTL was used for co-localization of genetic map to the physical map.

Assessment of available data bases for the construction of consensus map

For the construction of consensus map with genes, ESTs and molecular markers were downloaded from the National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/. The physical map of the corresponding chromosomal segment of the QTL region was downloaded. We have also used SSRs developed by Mathias Lorieux and his group at CIAT 16 February 2006 available in Gramene database http:// www.gramene.org for construction saturated consensus map. The data was co-localized along with NCBI marker data on to the physical map, and local database was created to identify candidate genes and linked markers.

RESULTS AND DISCUSSION

There is only one QTL has been identified against bacterial leaf blight resistance on chromosome 10. The LOD score and variance of the QTL are 1.62 and 10, respectively. As there is only one QTL is been identified, here we have no choice, except consider the QTL for construction of the high resolution physical map of the QTL. The details of the mapped QTL is described in Table 1.

The QTL region on chromosome 10 RG 4561-EM 14-10 associated with BLB resistance against iso 6 was saturated *in silico* with 108 molecular markers and 1097 ESTs. Out of 201 total genes identified in the QTL region, we found 28 genes are positively involved in the disease resistance mechanism. They were classified in to ten different functional groups based on their functions viz. protein kinases, receptor-like protein kinases, serine carboxypeptidases, hsr201 hypersensitivity related protein, peroxidase, LeOPT oligopeptide transporter, cell wall associated protein kinase, NADPH dependent oxidoreductase, disease resistance protein Hcr2 and NPK1 related protein kinase.

Among all, the protein kinase functional category has comprised 14 genes, is highest in number of genes, followed by hsr201 hypersensitivity related protein and cell wall associated protein kinase comprises each category nine number of genes. A number of genes responsible for the initial recognition of the pathogen R-genes and down stream signaling molecules [Defense Response DR genes] were found to be localized in the QTL region. DR-genes generally considered downstream from the recognition step of the signal transduction pathway, include a wide variety of genes and are thought to enhance defense in a quantitative manner (Wisser *et al*., 2005; Young, 1996). An open-ended survey of QTL gene family associations revealed a few families that could be considered extreme with respect to the number of positive QTL-family member correspondences. These included the peroxidases, glutathionine *S*-transferease GST and UDP-glucosyltransferase gene families, whose members are known to play an important role in plant defense and stress responses (Chittoor *et al*., 1997; Marrs, 1996).

Table 1. QTLs detected against bacterial leaf blight Iso 6 resistance using the composite interval mapping.

QTL Name	Chromosome number	Flanking markers	Weight	Variance %	LOD
qBB 10.1*	10	RG 4561-EM 14-10	0.684	10.5	1.647

*QTLs are named by abbreviation using chromosome number and number of QTLs

Table 2. Identified putative candidate genes localizing the saturated QTL region

Sl. No	Name of functional category	Gene ID
1	Protein kinase	OSJNBa0046L02.4, OSJNBa0079B05.9, OSJNBa0079B05.11, OSJNBa0092N12.12, OSJNAa0049K09.12, OSJNBa0087H07.9, OSJNBa0034B05.21, OSJNBa0034B05.25, OSJNBa0034B05.26, OSJNBa0004E08.14, OSJNBa0004E08.15, OSJNBa0004E08.16, OSJNBa0036D19.2, OSJNBa0036D19.3
2	Receptor-like protein kinase	OSJNBa0046L02.6, OSJNBa0087H07.8, OSJNAa0029P06.10, OSJNBa0030B02.10, OSJNBa0004E08.12, OSJNBa0004E08.13, OSJNBa0056A15.4
3	Serine carboxypeptidase	OSJNBa0046L02.7
4	hsr201 hypersensitivity related protein	OSJNBa0065H03.3, OSJNBa0065H03.4, OSJNBa0065H03.6, OSJNBa0065H03.7, OSJNBa0065H03.8, OSJNBa0065H03.9, OSJNBa0013J21.6, OSJNBa0013J21.8, OSJNBa0013J21.9
5	Peroxidase	OSJNBa0065H03.11, OSJNBa0015O22.21
6	LeOPT1 oligopeptide transporter	OSJNBb0012A20.5, OSJNBb0012A20.6, OSJNBb0012A20.7, OSJNBb0012A20.8
7	Cell wall associated protein kinase	OSJNBb0012A20.9, OSJNBb0012A20.11, OSJNBb0012A20.12, OSJNBb0012A20.17, OSJNAa0029P06.11, OSJNBa0011A24.25, OSJNBa0011A24.26, OSJNBa0051J07.6, OSJNBa0051J07.7
8	NADPH dependent oxidoreductase	OSJNBb0012A20.18, OSJNBb0012A20.19, OJ1014H12.6, OSJNBa0092N12.14, OSJNBa0092N12.13
9	Disease resistance protein Hcr2 0B	OSJNBa0079B05.17, OSJNAb0072F04.15, OSJNBb0004A06.4, OSJNBb0004A06.5, OSJNBa0013J21.3, OSJNBa0087H07.5
10	NPK1 related protein kinase	OSJNBb0072F04.10, OSJNBb0072F04.11
11	NBS LRR type resistance protein	OSJNBb0072F04.19, OSJNAb0072F04.16

These identified various functional classes putative candidate genes involved in the defense response are the logical targets for further validation and functional analysis.

The obtained results indicted that the QTL associated with BLB resistance is not only associated with the blight resistance, but also contributing to the other disease resistances. The QTLs identified for sheath blight, bacterial leaf blight and blast rice shared common chromosomal region (Kotasthane *et al.*, unpublished data). The data is lucidly articulating the hypothesis of broad spectrum disease resistance BSDR, which states that the QTL loci may, contributes resistance to the wide spectrum of the pathogens. Loci associated with resistance to multiple diseases are of particular interest to pathologists and genetists as well as breeders as they may provides clues to plant mechanisms that decrease the virulence of or increase the plants ability to resist microbial pests. In recent years, much has been learned about the genes and pathways involved in the plant defense response,

and many studies have been done to identify chromosomal regions conditioning QDR. This information, coupled with availability of the full genomic sequences of rice, provides an opportunity to make inferences about QDR and to establish testable hypotheses for subsequent analysis Wisser *et al*., (2005). Our current investigation *i.e*., identification of candidate genes and linked markers to BLB helps to test this hypothesis. Wang *et al*. (2005) has analyzed yield components and related traits under stressed and well water conditions using the same approach.

Given the synteny among genomes in the grass family, which includes rice, wheat, barley, maize, sorghum, and the millets, positional candidates identified in rice may have utility into other cereal species (Wisser *et al*., 2005). This sentence can be inferred either way that the data generated in the current investigation can efficiently applicable for the related family members, after validation and conformation. An examination of the co-localization and distribution of QTL identified from multiple studies on a given disease and from a number of different diseases can shed light on a series of unresolved issues relating to QDR (Wisser *et al*., 2005). The presented QTL data can efficiently be used as the supplementary material for the QTL co-localization, to test the co-occurrence of the loci across different populations. In our study, we found that QTL region associated with many resistant gene analogs, R-genes, and putative disease resistance candidate genes.

The association of qualitative resistance genes or major genes, RGA's, in the QTL region has been widely noted (Quint *et al*., 2003; Ramalingam *et al*., 2003; Gebhardt and Valkonen, 2001), but current status of their coincidence in rice has not been systematically analyzed. We addressed this tendency across all available studies and found that their associations were significant. At present, the apparent clustering of R genes and QTL could be accounted for by either of two hypotheses: first, that R genes and /or QTL are allelic [e.g., that R genes function as QTL, and/or overlapping QTL are conditioned by the same genes]; and second, that functional gene clusters exist, which include genes conditioning qualitative and quantitative resistance. The existence of BSDR is another issue for which a QTL summary could be illuminating. The concept of BSDR can be used to refer to resistance to multiple strains of a pathogen or to multiple taxa. A number of evidences suggest that BSDR exists in plants and the first correlated resistances have been documented in monocot and dicot germplasm (Tapsoba *et al*., 1997; Fokunang *et al*., 2000). We hypothesized that a synthesis of QTL and genomic data would provide evidence regarding the existence of BSDR, which allow identification of specific genomic regions that can be useful in crop improvement, and permit the identification of genes potentially contributing to BSDR. Although QTLs are generally considered to be useful sources of wide-spectrum and durable resistance in breeding programs (Roumen, 1994), the genes underlying QTLs for resistance are still uncharacterized, which has hindered the use of QTLs for breeding purposes (Wen *et al*., 2003). Cloning of genes controlling quantitative traits is now a major research frontier in terms of understanding human disease (Katzov *et al*., 2004), livestock productivity (Grisart *et al*., 2004), and traits of agronomic importance in crops (Ishimaru *et al*., 2004). We have identified 28 putative candidate genes responsible for the blight disease resistance. We suggest that the generated data can efficiently used for the conformation and validation of the QTL. This can be assumed in support with the previous findings that over expression of some defense genes results in enhanced resistance (Zhu *et al*., 1994). Molecular genetists and breeders are understandably resistant to divert time and resources towards the use of QTL markers for crop improvement until the existence and effect of those QTLs are confirmed (Romagosa *et al*., 1999). The well furnished EST and genes data will help to develop new markers, which are sequence specific *i.e*., ESTs, Sequence Tagged Sites (STS) and Target Region Amplified Polymorphisms (TRAPs). Tightly linked with in gene molecular markers are inevitable in conducting efficient marker assisted selection (Sanchez *et al*., 2000). The pyramiding of genes particularly with dissimilar reaction to pathogen requires the marker assisted selection MAS for the genes of interest. The use of MAS not only accelerates the breeding program but is the only way to transfer the multiple genes for resistance in one back ground. Peters *et al*.(2001) mapped 500 AFLP markers by *in silico* analysis in *Arabidopsis*. They suggested that the conventional method of mapping of markers based on labor-intensive cumbersome segregation analysis could be pinned down physically by *in silico*. This integrated physical/genetic map will facilitate cross-referencing for positional cloning and fine mapping of genes or QTLs of interest. The next five years should see a burst in the number of QTLs cloned, thanks to advances in genomics and

bioinformatics. These QTLs will reveal new genes and alleles of known genes that have evolved in particular genetic backgrounds under specific environmental pressures. In final, the characterized candidate genes are the indispensable keystones for transformation to construct resistant varieties against the bacterial leaf blight pathogen.

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