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Full Length Research Paper

Saturation mapping of QTL region determining resistance specificity to bacterial leaf blight pathogen in rice with molecular markers, ESTs and genes on sequences *in silico*

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Genetic improvement for biotic resistance in rice involves the quantitative nature of inheritance, which reflects the additive effects of several genetic loci throughout the genome. To *in silico* identify putative candidate genes involved in defense response, we performed *in silico* anchoring of the QTL genetic marker data to the rice physical map. The QTLs responsible for resistance was analyzed on CT 9993-5-10-1-M / IR 62266-42-6-2 double haploid population. In total there are 77 markers, 53 ESTs, and 32 genes on sequences were localized in the QTL region RG1028-ME7_4 on chromosome 1. The genes on sequences were classified based on their function in the resistance pathway. Our functional categorized results are clearly showing that there are many defense related proteins which express during the disease resistance against the bacterial leaf blight and the generated data can further be used for the validation of the QTL and further fine dissection of the same for the development of the blight resistant variety.

Key words: QTL, quatitative disease resistance, target region amplified polymorphism, candidate genes, DR-genes.

INTRODUCTION

Xanthomonas oryzae pv *oryzae*, is one of the most devastating pest of rice pest line causing bacterial leaf blight (BLB), (Srivastava et al., 1967; Wen et al., 2003) which act as notoriously “shifty enemies” 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 host plant resistance. Two types of resistance against *X. oryzae* pv *oryzae*, vertical and horizontal resistance have been recognized in rice (Zhang and Mew, 1985). Vertical resistance, governed by the single major gene, is race specific and can be broken down easily (Mew, 1987; Eamchit and Mew, 1982; Mew and Vera Curz, 1979; Mew et al., 1992). Horizontal resistance is quantitative, presumably non-race specific and controlled by polygenes (Nelson, 1972). Achieving/or developing durable resistant plant variety needs deeper understanding of molecular principles underlining the character of interest, so that the understood molecular principles can be very well employed for the development of long durable disease resistant variety.

An effective approach for studying complex and polygenic forms of disease resistance is known as QTL mapping. QTL mapping can provide paved starting points for identifying such candidate genes/ alleles, which can further be used for the introgression-breeding program. QTLs responsible for the BLB have been identified using different mapping populations (Causse et al., 1994; Li et al., 1999). Integration of QTL mapping with genomic sequence data and information on allelic differences will provide the basis for candidate gene approaches to clone

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Table 1. Putative QTLs detected using interval mapping associated with bacterial blight resistance against isolate 4 (IS4).

S/N	Molecular Marker Interval	Weight	Variance explained (%)	LOD
Chromosome No.1				
1	RG1028-ME7_4	-0.3874	13.2%	3.53
Chromosome No.3				
2.	C 63-RG 369	-0.316	7.60	1.957
Chromosome No.7				
3.	ME6_8-RG678	-0.3268	8.00	2.03
4.	EAAM17_5 - ME 10_6	-0.330	6.7	1.55
5.	EM 11_1-BCD 855	-0.420	9.90	1.684
Chromosome No.8				
6.	ME9_1 - ME2_1	-0.285	6.90	1.776
Chromosome No.9				
7.	G103-ME5_9	0.3711	8.50	2.20
8.	RM 219-RG553	0.365	8.0	2.004
9.	RG 141-RM 205	-0.349	7.0	1.806

the QTLs (Tuberosa and Salvi, 2006) for disease resistance. Cloning a blight resistant QTL substantially contributes toward a better understanding of the genetic and functional basis of the response of a plant to blight resistance. Furthermore, it makes available the sequence responsible for the QTL, and can be used for genetic engineering for the development of blight resistant variety and/ or mining for the most desirable alleles within germplasm collections. However, positional cloning of a QTL gene locus normally requires fine scale mapping with large mapping populations across many seasons (Wang et al., 2005; Tuberosa and Salvi, 2006). Availability of the whole rice genome sequences (Goff et al., 2002; Yu et al., 2002; Sasaki et al., 2002; Feng et al., 2002) provides a new tool for this task, along with a means of characterizing their associated molecular functions. In this study, we exploited this new source of data by anchoring the QTL regions responsible for the resistance against the different isolates of BLB with rice physical map to construct the high resolution map with molecular markers, ESTs, and genes on sequences and then we functionally categorized the identified putative candidate genes aligned in the region for further research work.

MATERIALS AND METHODS

Molecular linkage map

Published genetic mapping data from a double haploid mapping population, CT 9993-5-10-1-M / IR 62266-42-6-2 was used to find out putative QTLs molecular marker intervals.

QTLs chose for construction of high resolution map

Database of the putative QTLs associated with bacterial blight resistance used in the present investigation is given in Table 1. The continuity and distribution of lesion length variance data assessed graphically and by interval mapping (locating the QTLs between flanking molecular markers by maximum-likely-hood estimation) (Lander et al., 1987) using Mapmaker / QTL 1.1 identified putative QTL associated with bacterial blight resistance. A threshold of LOD > 1.5 was used per test to claim the presence of a QTL. Among mapped eight QTLs, we chose only one QTL region that showed highest LOD value that is, RG1028 - ME7_4 on chromosome 1 for *in silico* analysis.

In silico data retrieved from the public data bases for the construction of consensus map

For the construction of consensus map the following publicly available databases used are as follows. ESTs, HD 00, RM 00 and GoS were derived 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 by making the HD 00 map as a master map, and then placing the cM distance of the flanking marker of the QTL region in the option given side by. We also used the SSR marker data available in Gramineae database that is, SSRs developed by Mathias Lorieux and his group at CIAT (16 February 2006). The data was co-localized according to the corresponding cM in relation to the identified QTL.

We have adopted another alternative method for downloading the centimorgan corresponding sequence information data. Annotation of QTL region of rice genome to identify genes was performed by aligning the marker sequence with the BAC end sequence-using BLASTN, a rapid sequence comparison program. FASTA concatenated marker query sequence (of markers flanking QTL regions) with a non-alphanumeric spacing character was used to search against the BAC/ PACs end sequence data base to identify the high

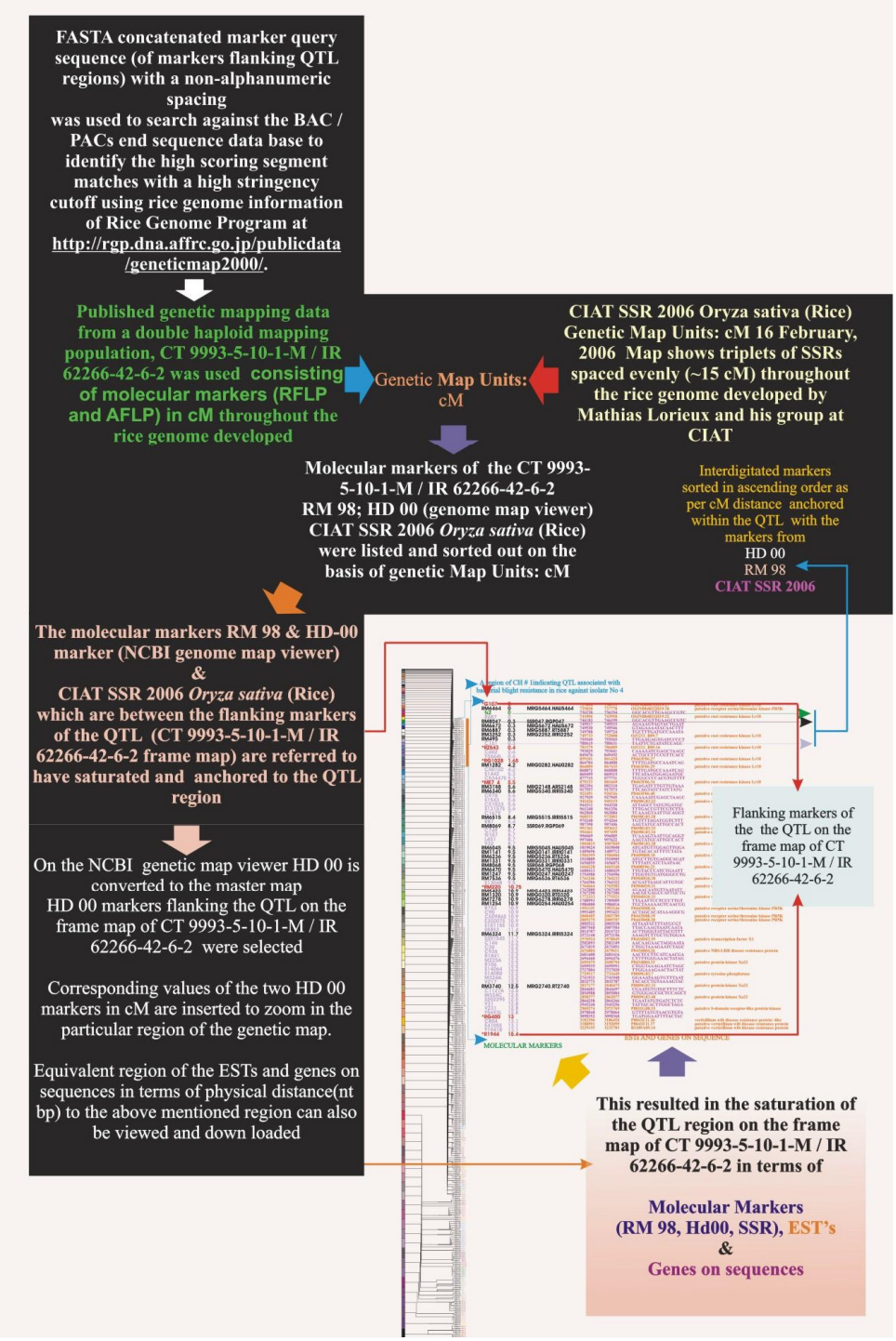

Figure 1. Strategy for generating high resolution map of the QTL region associated with bacterial blight resistance on different chromosomes.

scoring segment matches with a high stringency cutoff using rice genome information of Rice Genome Program at http://rgp.dna.affrc.go.jp/publicdata/geneticmap2000/. The map information within the region of the aligned marker sequence indicated the contig, total models in the region and total genes in region. The method followed was briefly illustrated in the Figure 1.

RESULTS AND DISCUSSION

Despite the importance of quantitative disease resistance in plants, relatively little is known about the genetic basis of this trait or the mechanism of action of the genes con-

Table 2. Identified disease response putative candidate genes localizing the saturated QTL regions.

S/N	Name of functional category	Gene ID	No. of genes
1	Rust resistance kinase Lr10	OSJNBb0032H19.27, OSJNBb0032H19.32, OJ1212_B09.7, OJ1212_B09.16, P0463F06.27, P0463F06.29, P0463F06.34, P0463F06.48, P0698G03.23, P0698G03.28, P0698G03.33, P0698G03.34, P0698G03.38	13
2	Receptor serine/threonine kinase PR5K	OSJNBb0032H19.30	1
3	Signal peptidase	P0504H10.30	1
4	Wound-induced protease inhibitor	P0504H10.31	1
5	Verticillium wilt disease resistance protein Ve2	P0504H10.33, P0041E11.36, P0041E11.37, B1189A09.14	4
6	Receptor serine/threonine kinase PR5K:	P0443D08.16, P0443D08.19, P0443D08.20	3
7	Transcription factor X1	P0416D03.39	1
8	NBS-LRR disease resistance protein	P0434B04.26	1
9	Protein kinase Xa21	P0434B04.32, P0009G03.33, P0009G03.40	3
10	Tyrosine phosphatase	P0009G03.7	1
11	*S*-domain receptor-like protein kinase	P0011G08.15	1

trolling it. Insights in to Quantitative Disease Resistance (QDR) have implications both for understanding host-pathogen interactions and for improving crop production. 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 nearly 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). Wang et al. (2005) has analyzed yield components and related traits under stressed and well water conditions using a mapping populations derived from Azucena x IR64 and Azucena x Bala. They conducted *in silico* analysis for the QTL flanked by the genetic markers RM212 and RM319 on chromosome 1, proximal to the semi-dwarf (sd 1) locus. Among 175 identified annotated genes, they identified 16 as a putative candidate genes involved in the drought tolerance. They further suggested that identified putative candidate genes can further be used for the confirmation of the QTL. Our current investigation that is, saturation of the QTL region specifying resistance towards bacterial leaf blight with molecular markers, ESTs and genes on sequences, helps to test this hypothesis. The results obtained were presented in the Figure 2.

The saturated QTLs were found to posses the different functional categories of genes involved in defense response (Table 2). A number of proteins responsible for the initial recognition of the pathogen (R-genes) and down stream signaling molecules [Defense Response genes (DR)] were found to localize the QTL region. In consistent with the previous reports, the QTL regions are found to positively associate with the NBS-LRR classes of R-genes. Nonetheless, the genes resembling other classes of putative R genes that is, protein kinases namely array clusters of putative rust resistance kinase Lr10, putative verticillum wilt disease resistance protein Ve2, putative protein kinase Xa21, putative *S*-domain receptor like-protein kinases and an LRR transmembrane protein kinases were found to localize in the QTL regions, indicating the presence of other R gene classes in QTL region devoid of sequences resembling the NBS-LRR class. However, these different classes of R-genes are specific for pathogen races and have limited lifetime in any particular specie. due to rapid evolution of the pathogen (Wen et al., 2003). 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 (Young, 1996). DR genes found in saturated QTL region are signal peptidase, wound-induced protease inhibitor, isoflavone reductase, pathogenesis related protein, chitinases, peroxides 1 precursor-like protein, cellulose synthase-like protein, zinc finger protein, and DNA-binding protein WRKY2. Some of the DR genes are members of gene families, with only one or a few of the family members likely participating in defense. 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 (Chittor et al., 1997), glutathionine *S*-transferase (GST) and UDP-glucosyltransferase gene families, whose members are known to play an important role in plant defense and stress responses (Marrs, 1996; Li et al., 2001). The significant QTL-GST relationship could be deceptive; several members were arranged in tandem arrays, and one such array co-localized with a single bacterial blight QTL (Wisser et al., 2005). These members were found to

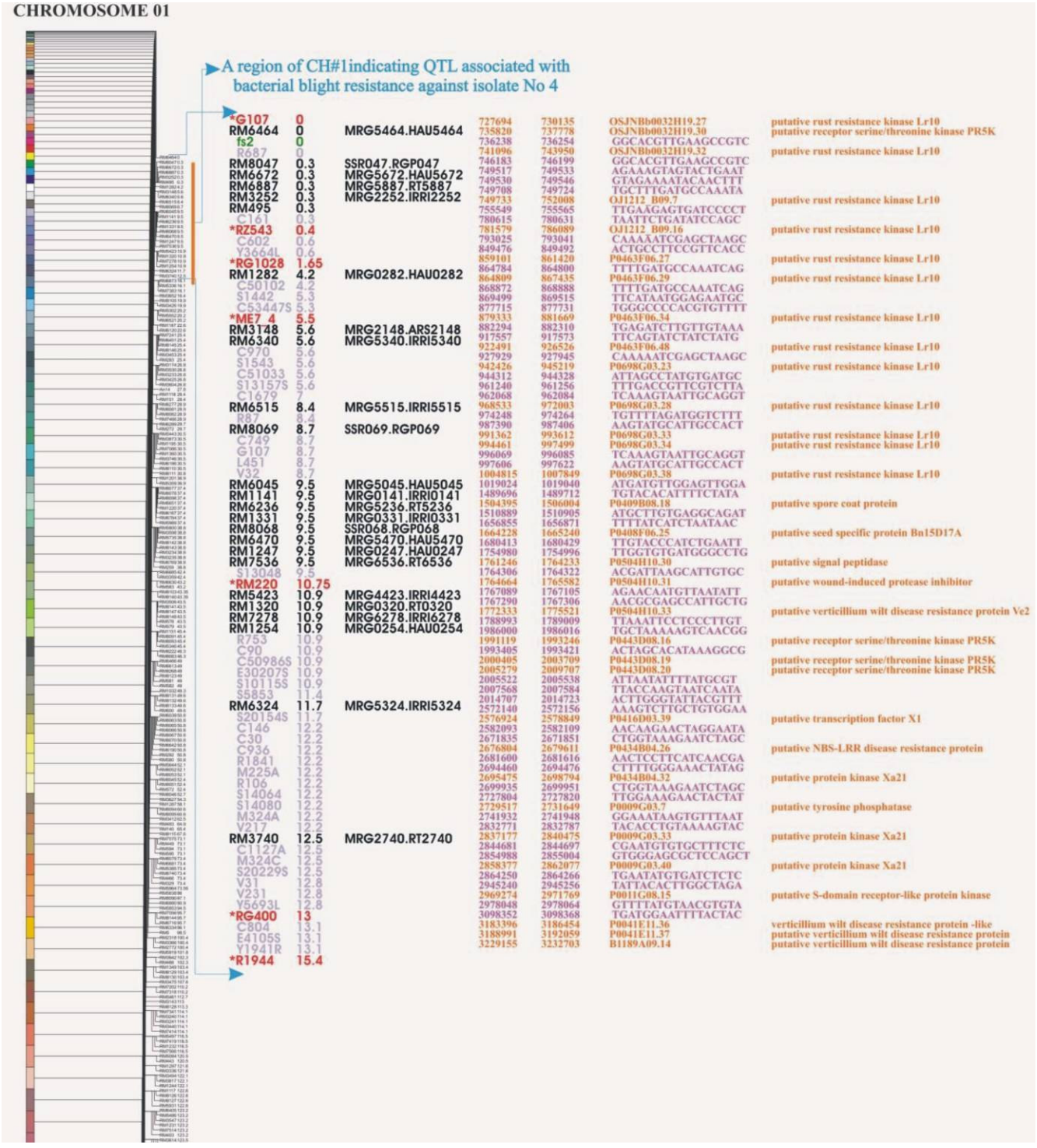

Figure 2. Saturation of QTL region (G107 – R1944 CT 9993-5-10-1-M / IR 62266-42-6-2 double haploids) associated with bacterial blight resistance against isolate no. 4 with molecular markers, ESTs and genes on sequence.

be involved in mediating the detoxification of electrophilic compounds (Marrs, 1996). Different putative candidate genes identified in the saturated QTL region are described in Table 2. These identified various functional classes putative candidate genes involved in the defense response are the logical targets for further validation and functional analysis. 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. R genes exhibit race specificity and QTL have been generally assumed to be race nonspecific. But, recently Talukder et al. (2004) performed QTL analyses of independent inoculation experiments using three pathogen isolates and found that most rice blast QTL also exhibit race specificity. Understanding by molecular dissection of the QTL facilitates us to give correct climax to this paradox. 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 data what we have shown can efficiently be used as the supplementary data for the co-localization and, subsequently repeatability analysis of QTL region across different population in various environmental conditions, which further helps to conclude about the phenotypic contribution of that QTL confining chromosomal segment.

In our study, we found that QTL region associated with many RGA's, 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 (Wang et al., 1994; Pflieger et al., 1999; Gebhardt and Valkonen, 2005; Quint et al., 2003; Ramalingam et al., 2003), 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 gene(s)]; and second, that functional gene clusters exist, which include genes conditioning qualitative and quantitative resistance. The existence of Broad Spectrum Resistance (BSR) is another issue for which a QTL summary could be illuminating. The concept of BSR can be used to refer to resistance to multiple strains of a pathogen or to multiple taxa. A number of evidences suggest that BSR exists in plats 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 Broad Spectrum-Quantitative Disease Resistance (BS-QDR), which allow identification of specific genomic regions that can be useful in crop improvement, and permit the identification of genes potentially contributing to BS-QDR. 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). Faris and associates (1999) demonstrated that several candidate DR genes, including oxalate oxidase, peroxidase, superoxide dismutase, chitinase, and thaumatin, from various cereals were associated with disease resistance QTL in wheat. To systematically associate function with available candidate gene sequences from multiple species, it would be useful to locate them onto a frame map with a maximal amount of phenotypic information. Simply locating candidate genes to chromosomal regions with mapped phenotypes does not confirm the function of the gene. However, this approach provides an efficient way to narrow down a few candidate sequences that can be tested by detailed genetic analyses using appropriate mapping populations and mutants (Collins et al., 1998). We have identified 32 putative candidate genes responsible for the blight disease resistance. We suggest that the generated data can efficiently use for the conformation and validation of the QTL for that particular character of interest by Quantitative PCR (Q-PCR) analysis of the putative candidate genes. 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; Kebelka et al., 2002). The well furnished EST and GoS data will help to develop new markers, which are sequence specific that is, 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 transfer of resistance genes that is, pyramiding of genes particularly with similar reaction to pathogen, requires the marker assisted selection for the gene(s) of interest. The use of MAS not only accelerate the breeding program but is the only way to transfer the multiple gene for resistance in one back ground (Huang et al., 1997; Kelly, 1995), particularly with the similar effect. 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 characte-

rized candidate genes are the indispensable keystones for transformation to construct resistant varieties against the bacterial leaf blight pathogen.

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