Physical mapping, expression analysis and polymorphism survey of resistance gene analogues on chromosome 11 of rice

Irfan A Ghazi[1,2,4], Prem S Srivastava[2], Vivek Dalal[1], Kishor Gaikwad[1], Ashok K Singh[3], Tilak R Sharma[1], Nagendra K Singh[1] and Trilochan Mohapatra[1,*]

[1]*National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012, India*
[2]*Department of Biotechnology, Faculty of Science, Jamia Hamdard, New Delhi 110 062, India*
[3]*Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012, India*
[4]*Present address: Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India*

**Corresponding author (Fax, 91-11-25843984; Email, tm@nrcpb.org)*

Rice is the first cereal genome with a finished sequence and a model crop that has important syntenic relationships with other cereal species. The objectives of our study were to identify resistance gene analogue (RGA) sequences from chromosome 11 of rice, understand their expression in other cereals and dicots by *in silico* analysis, determine their presence on other rice chromosomes, and evaluate the extent of polymorphism and actual expression in a set of rice genotypes. A total of 195 RGAs were predicted and physically localised. Of these, 91.79% expressed in rice, and 51.28% expressed in wheat, which was the highest among other cereals. Among monocots, sugarcane showed the highest (78.92%) expression, while among dicots, RGAs were maximally expressed in *Arabidopsis* (11.79%). Interestingly, two of the chromosome 11-specific RGAs were found to be expressing in all the organisms studied. Eighty RGAs of chromosome 11 had significant homology with chromosome 12, which was the maximum among all the rice chromosomes. Thirty-one per cent of the RGAs used in polymerase chain reaction (PCR) amplification showed polymorphism in a set of rice genotypes. Actual gene expression analysis revealed post-inoculation induction of one RGA in the rice line IRBB-4 carrying the bacterial blight resistance gene *Xa-4*. Our results have implications for the development of sequence-based markers and functional validation of specific RGAs in rice.

[Ghazi I A, Srivastava P S, Dalal V, Gaikwad K, Singh A K , Sharma T R, Singh N K and Mohapatra T 2009 Physical mapping, expression analysis and polymorphism survey of resistance gene analogues on chromosome 11 of rice; *J. Biosci.* **34** 251–261]

1. Introduction

Rice (*Oryza sativa* L.) is a model agronomically important crop species whose complete genome sequence is now available in the public domain. This has enabled identification and isolation of important genes that are essential to meet the growing demands of food production. Since rice has a syntenic relationship with other cereal species, information obtained from the rice genome will also impact the biological understanding of other cereals. One of

Keywords. Bacterial blight resistance; DNA polymorphism; gene expression analysis; physical mapping; resistance gene analogues; rice

Abbreviations used: BB, bacterial blight; dNTP, deoxyribonucleotide triphosphate; EST, expressed sequence tag; GAI, gibberellic acid-insensitive; InDel, insertion–deletion; IRGSP, International Rice Genome Sequencing Project; NBS-LRR, nucleotide-binding site leucine-rich repeat; NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction; PGIP, polygalacturonase-inhibiting protein; RGA, resistance gene analogue; R-gene, resistance gene; SNP, single-nucleotide polymorphism; TBE, tris-borate-EDTA; TIR, toll/interleukin-1 receptor; TNL, toll/interleukin-1 receptor NBS-LRR

Supplementary tables pertaining to this article are available on the *Journal of Biosciences* Website at *http://www.ias.ac.in/jbiosci/june09/pp251-261-suppl.pdf*

the areas that has benefited most from complete sequencing of the rice genome is cloning of disease-resistance genes and their analogues. Several disease-resistance genes have been cloned from different plant species. Analyses of these genes reveal that they share various similar sequences and common structural motifs, suggesting a common defence signal transduction pathway in different plant–pathogen interaction systems (Martin *et al.* 2003).

The International Rice Genome Sequencing Project (IRGSP) reported a total of 37 544 non-transposable element protein-coding genes in the whole rice genome (International Rice Genome Sequencing Project 2005). These included disease-resistance (R) genes, which are also called resistance gene analogues (RGAs) due to their sequence homology to cloned resistance genes or to those involved in defence-related mechanisms. Products of R-genes have been classified into five groups: detoxifying enzymes (e.g. *Hm1* gene in maize), kinases (e.g. *Pto* gene in tomato), NBS/LRR proteins (the largest group, e.g. *Arabidopsis RPS2* and *RPM1* genes, tobacco *N* gene, tomato *Prf* gene, flax *L6* gene), extracellular receptors (e.g. *Cf* gene of tomato) and receptor kinases (rice *Xa 21* gene, Song *et al.* 1995).

Using the draft sequence of the rice genome, which was made available in the public domain earlier, Monosi *et al.* (2004) predicted more than 500 R-genes of which about 25% were on chromosome 11. The richness of rice chromosome 11 for RGAs was further reported based on the finished quality genome sequence (Rice Chromosome 11 and 12 Sequencing Consortia 2005). These observations were, however, based on *in silico* predictions, which need to be refined to improve their accuracy. Moreover, it is not known what proportion of these predicted genes express in rice and other plant species. The relationship of the abundant RGA sequences on chromosome 11 with those located on other chromosomes has not been examined as yet. Many genes for resistance to different biotic stresses, particularly those due to fungal and bacterial pathogens, have been genetically mapped on rice chromosome 11. For instance, the genes for resistance against bacterial leaf blight caused by *Xanthomonas oryzae* such as *Xa3*, *Xa4* and *Xa21* (Song *et al.* 1995; Yoshimura *et al.* 1995), as well as genes for resistance against the fungal pathogen *Pyricularia grisea* such as *Pi1(t), Pi7(t)* and *Pik*^{m} (Wang *et al.* 1994; Yu *et al.* 1996; Li *et al.* 2007) are reported to be located on the long arm of this chromosome. Of these genetically mapped loci, *Xa21* has been cloned and characterised in great detail (Song *et al.* 1995). Therefore, there is an immediate need to establish the functional significance of RGAs predicted from the DNA sequence in relation to genetically mapped genes and their phenotypic effects. One of the approaches could be to understand the expression pattern of RGAs under specific artificial selection environments favouring expression of a disease. Besides, RGA-based molecular markers can be designed and used to establish their association with genetic resistance.

This study was undertaken to construct a physical map of all the predicted RGAs on rice chromosome 11, study their relationship with RGAs on other rice chromosomes and analyse their expression *in silico* in rice, other cereals and dicot species including *Arabidopsis, Brassica* and tomato. Efforts were also made to establish a correlation between RGAs and bacterial blight resistance through gene expression analysis. Further, polymerase chain reaction (PCR) amplification and sequencing of a subset of RGAs was carried out to develop candidate gene-based markers for possible use in marker-assisted breeding.

2. Materials and methods

2.1 *Gene prediction and* in silico *physical mapping of RGAs*

Our study was performed on the sequences of 217 ordered BAC/PAC clones identified in the minimum tiling path for rice chromosome 11, which were available to the public (*http://www.rgp.affrc.dna.go.jp/IRGSP/index.html*) under the IRGSP. The FASTA form of each BAC/PAC clone sequence was downloaded as a text file one by one. Gene prediction was performed for each clone using FGENESH (*www.softberry.com*) trained for monocot species (Salamov and Solovyer 2000). The FGENESH output was stored as text files and subjected to the BLASTX program (*www.genome.ad.jp*) with the nr-protein database to check the similarity with known proteins. After removing the overlaps, the genes were classified into different categories of RGAs and physically located on the chromosome 11 pseudomolecule by BLASTN (*http://www.gramene.org/multi/blastview*). After gene prediction from the BAC/PAC sequence, manual editing was carried out and the positions of all predicted RGAs were seen on the finished quality sequence of rice. A map was drawn manually and the position of each RGA was shown on a megabase scale.

2.2 *Analyses of sequence similarity*

In order to compare RGAs on rice chromosome 11 with those present in the other chromosomes of rice, all the predicted RGAs were BLAST-searched against the other rice chromosome pseudomolecules (*www.gramene.org* and *http://www.tigr.org*). Simultaneously, the Unigene databases of rice, wheat, maize, barley, sorghum, *Arabidopsis, Brassica*, tomato and sugarcane were also downloaded, configured as a local database and used to determine the extent of representation of rice chromosome 11 RGAs in the transcriptomes of these plant species. Experiments were

designed to optimise the setting of the BLASTN search option and reporting parameters in order to detect similarities with rice RGAs. The optimised search parameters were: *G*=10, *E*=1, *q*=–1, *r*=1, a word size of 11 and an expect value of 10 (Singh *et al.* 2004), without the low complexity filter. Sets of individual RGAs were searched using the MEGABLAST tool against the cereal and other plant UniGene databases of the National Center for Biotechnology Information (NCBI) (*http://www.ncbi.nlm.nih.gov/dbUniGene/index.html*). Details of the top hits of BLASTN search results with each chromosome 11 RGA were tabulated in an Excel file and all those genes showing a bit score of 100 or more were extracted into a separate file.

2.3 *Analyses of protein motif structure*

The structure of a few RGAs was deduced based on the information obtained from the FGENESH software. Protein sequences of selected RGAs were subjected to a BLASTP search of the NCBI (*http://www.ncbi.nlm.nih.gov/blast/Blast.cgi*) and the structure of the protein was deduced. The protein structure was also confirmed by the Pfam (*www.sanger.ac.uk/Software/Pfam/search.shtml*) and STRING softwares (*http://string.embl.de*).

2.4 *Primer design*

A pair of primers was designed from the exon sequences of each RGA (supplementary table 1) using the Primer3 software (*http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi*) and custom synthesised. These were based on the conserved motifs of nucleotide-binding site leucine-rich repeat (NBS-LRR), *Cf2/Cf5*, serine/threonine protein kinase and putative disease-resistance genes. By BLASTN analysis against the whole rice genome, the uniqueness of the primers was examined.

2.5 *Plant materials*

Fifteen rice varieties with differential reaction to bacterial blight (BB) disease caused by *Xanthomonas oryzae* were used: Pusa Basmati 1 (PB-1), Pusa Sugandha 2 (PS-2), IRBB-3 (containing *Xa3* BB resistance gene), IRBB-4 (containing *Xa4*), IRBB-13 (containing *xa13*), IRBB-21 (containing *Xa21*), IR-24, IRBB-55 (containing *xa13* and *Xa21*), IRBB-59 (containing *xa5, xa13* and *Xa21*), IRBB-60 (containing *Xa4, xa5, xa13* and *Xa21*), IR-64, Pusa-1121, Basmati 370 (B-370), Swarna and Bindli. The rice lines were germinated in pots in a phytotron at a night temperature of 25°C and day temperature of 32°C. Young leaves were collected and the genomic DNA was isolated following the standard plant DNA isolation protocol.

2.6 *PCR amplification*

PCR was performed in a total volume of 25 μl containing 20 pmol each of forward and reverse primers, 2.5 mM of $MgCl_2$, 200 μM each of the four deoxyribonucleotide triphosphates (dNTPs), 0.5 U of Taq DNA polymerase, 1x concentration of PCR buffer (Invitrogen, Life Technologies, Brazil) and 50 ng of rice genomic DNA. The annealing temperature was different for different sets of primers as shown in supplementary table 1. The template was denatured by heating at 94°C for 5 min. This was followed by 35 cycles of 1 min at 94°C, 1 min annealing and 2 min elongation at 72°C, with a final extension of 10 min at 72°C. The Perkin Elmer GenAmp PCR system 9600 was used for PCR amplification. The amplicons were resolved in 1.6% agarose gel using 0.5x tris-borate-EDTA (TBE) buffer.

2.7 *PCR product sequencing and sequence analysis*

A few of the PCR products were purified by Amicon Micron-PCR Centrifugal Filter devices (Millipore, USA), and sequenced using the ET Dye Terminator Chemistry and capillary-based sequencer, MegaBACE 1000 or MegaBACE 4000 (Amersham Bioscience, NJ, USA). All the sequences were copied and saved in a notepad file. Sequence alignment was done using the ClustalW multiple alignment software.

2.8 *Bacterial inoculation and disease evaluation*

Inoculation of resistant and susceptible rice varieties was done using *Xanthomonas oryzae* (Xoo-4 strain) obtained from the Division of Plant Pathology, IARI, New Delhi. For preparation of the inoculum, the strain was grown on slants of yeast extract, glucose and $CaCO_3$ in a semi-synthetic agar medium for 3–4 days at 28°C. The inoculum was prepared by suspending the bacterial mass in sterilised water. Plants were inoculated after 55 days of sowing, using the leaf clipping method (Kauffman *et al.* 1973) on five of the uppermost fully expanded leaves of 10 plants of each variety in a disease nursery. Control plants were treated under the same conditions except that the pathogen inoculum was replaced by water. Reaction to the pathogen was evaluated 14 days after inoculation by visual assessment and by measuring the lesion length.

2.9 *RNA isolation and expression analysis*

Inoculated leaf tips (5 g) were harvested at 1, 2, 3, 4 and 5 days after inoculation. RNA was isolated using the TRIzol Reagent (Invitrogen, CA, USA) method according to the manufacturer's guidelines. Total RNA was treated with 1 μl DNaseI at 65°C for 10 min before reverse transcriptase

(RT)-PCR to remove contamination by genomic DNA. The reaction was stopped by adding 25 mM EDTA. RT-PCR was performed with the one-step RT-PCR Kit (QIAGEN). The amplified products were loaded in 1.8% agarose gel and electrophoresed in 0.5x TBE buffer at 60 V for 2 h.

3. Results

3.1 *Nature, distribution and physical mapping of RGAs*

A total of 195 RGAs were predicted on rice chromosome 11. These belonged to different categories based on sequence motifs/domains such as NBS-LRR, *Cf2/Cf5* disease resistance, LRR, serine/threonine protein kinase, receptor kinase, etc. These were unevenly distributed on the chromosome with a higher frequency of occurrence at certain physical positions. One hundred and forty RGAs were present on the long arm and the others on the short arm (figure 1). Most RGAs present on the short arm of the chromosome were of the NBS-LRR type. For instance, a large cluster of 16 miscellaneous RGAs was found between positions 6.35 Mb and 6.80 Mb on this arm, in which 12 were NBS-LRR-like genes. There were three putative CC-NBS-LRR type RGAs, all of which were on the short arm. There was no N-terminal toll/interleukin-1 receptor (TIR) NBS-LRR (TNL) homology region on chromosome 11. A total of 11 disease-resistance proteins with *RPM1* homology was found on this chromosome, of which 8 were on the short arm (supplementary table 2). No RGA was found in the vicinity of the centromere. The clusters of RGAs started from the 16 Mb position onwards on the long arm of the chromosome. Four large clusters of miscellaneous RGAs were found on the long arm of the chromosome between positions 16.46 and 16.63 Mb (10 RGAs), 19.18 and 20.03 Mb (30 RGAs, in which 15 were *Cf2/Cf5* disease-resistance protein homologues), 24 and 24.99 Mb (21 RGAs, in which 16 were putative NBS-LRR-like genes), and 26 and 26.65 Mb (18 RGAs). A small cluster of 8 NBS-LRR-like RGAs was found on the long arm between positions 25.97 and 26.07 Mb. Several other RGAs were also arranged in similar but smaller clusters. In the vicinity of the 20 Mb region of chromosome 11, 6 *Xa21*-like RGAs were found on the long arm.

3.2 *DNA-level homology of chromosome 11 RGAs with other rice chromosomes*

The overall DNA sequence homology of the 195 RGAs on chromosome 11 with other chromosomes was estimated to be 19.34%. Approximately 90% (175 genes) of all predicted RGAs showed significant homology with the *indica* 93-11 chromosome 11 pseudomolecule (bit score ≥100). Eighty RGAs (41.03%) of chromosome 11 had significant homology with chromosome 12, which was the maximum among all the chromosomes, whereas 14 RGAs (7.17%) had significant homology with chromosome 9, which was the lowest among all the rice chromosomes (figure 2). Putative leucine-rich repeat-containing protein was found to be more conserved among the chromosomes, followed by NBS-LRR protein. No specific order of the conserved genes was observed among the chromosomes. Eight RGAs, namely, RRGA11-185 to RRGA11-191 and RRGA11-193, were found to be conserved among six different chromosomes–chromosome 1, 2, 4, 6, 7 and 10.

It was interesting to note that the RGA RRGA11-090 was present in all the 12 chromosomes. This gene, coding for a putative *Cf2/Cf5* disease-resistance protein, showed significant homology (99%) with more than 1000 bit scores and zero e-value. It was found that this gene contained 4 exons (3918 bp) encoding 1305 amino acids. Seventy-six copies of this gene were present in the rice genome. When the protein sequence of this gene was subjected to a motif search, it was ascertained that it belonged to the LRR domain family.

3.2.1 In silico *expression analyses of chromosome 11 RGAs in rice, other monocots and dicots:* BLASTN search analyses of 195 RGAs performed against the rice Unigene database revealed that 91.79% (179 genes) expressed in rice. There were 82 RGAs with bit scores between 1000 and 6000, and zero e-value. The remaining 97 RGAs had bit scores between 100 and 1000. It was significant that 8.21% (16 genes) had no significant homology with rice unigenes. One hundred RGAs of chromosome 11 (51.28%) expressed in wheat, which was the highest among the cereals, followed by 94 (48.21%) that expressed in barley. Among cereals, sorghum showed the lowest frequency with 11.28% (22 RGAs) of the rice RGAs being represented in the unigenes (figure 3). Among monocots, sugarcane showed the highest expression; 154 genes (78.92%) expressed out of 195. Twenty-three RGAs (11.79%) expressed in *Arabidopsis*, which was the highest among the dicot species. Only 2 genes (1.03%) expressed in tomato, which was the lowest among the dicots (figure 3). Interestingly, two of the chromosome 11-specific RGAs, RRGA11-002 and RRGA11-082, encoding serine/threonine protein kinase, were found to be expressing in all the organisms under study.

3.3 *Development of RGA-specific markers*

RGAs were characterised by PCR amplification, actual expression analysis and sequencing of amplified products. A set of 15 resistant and susceptible rice genotypes were used in this study. These genotypes were first evaluated for their disease reaction. After 14 days of inoculation, disease reaction was recorded. Those genotypes with a lesion length

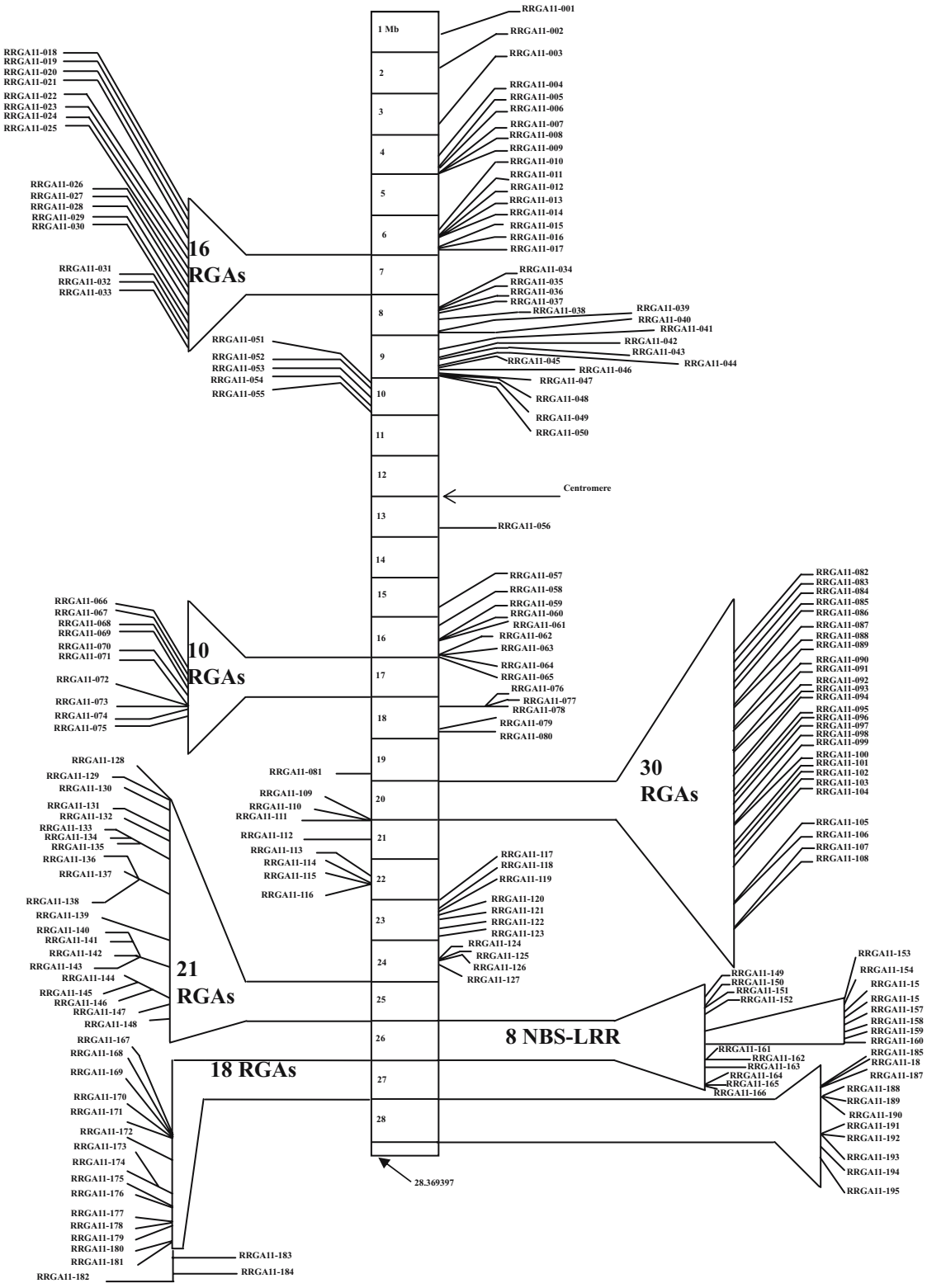

Figure 1. Physical map of 195 resistance gene analogues (RGAs) on the chromosome 11 of rice. RRGA11 stands for rice resistance gene analogue located on rice chromosome 11. The numbers 1–28 denote physical position in million base pairs. The centromere is located at the 12 Mb position.

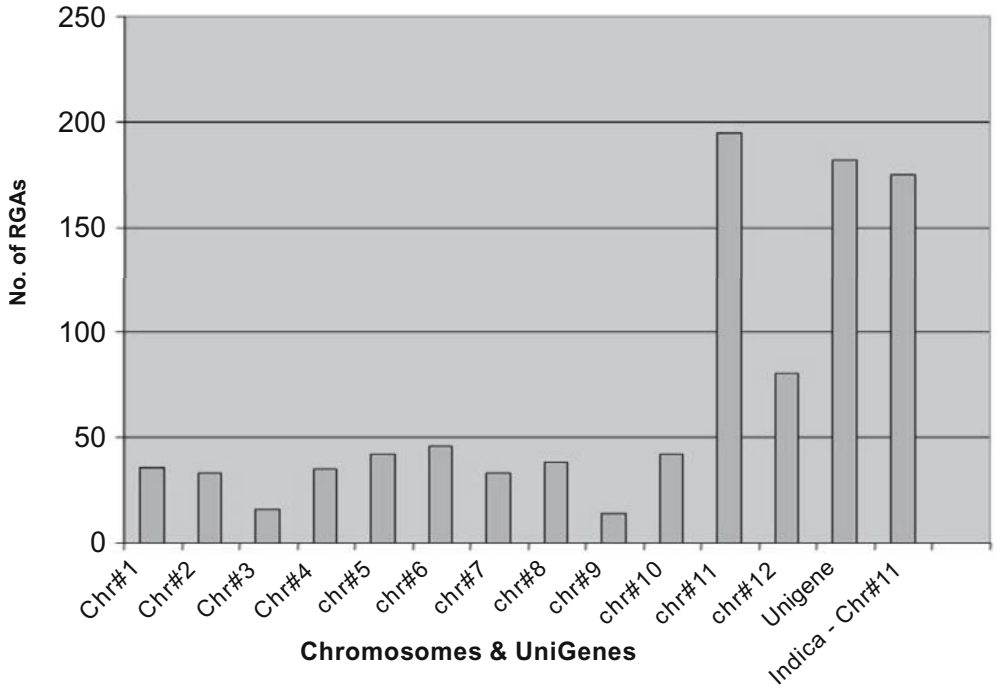

Figure 2. Presence of potential homologues of the resistance gene analogues (RGAs) of the rice chromosome 11 in other rice chromosomes of *japonica* cultivar Nipponbare, rice unigenes and *indica* chromosome 11

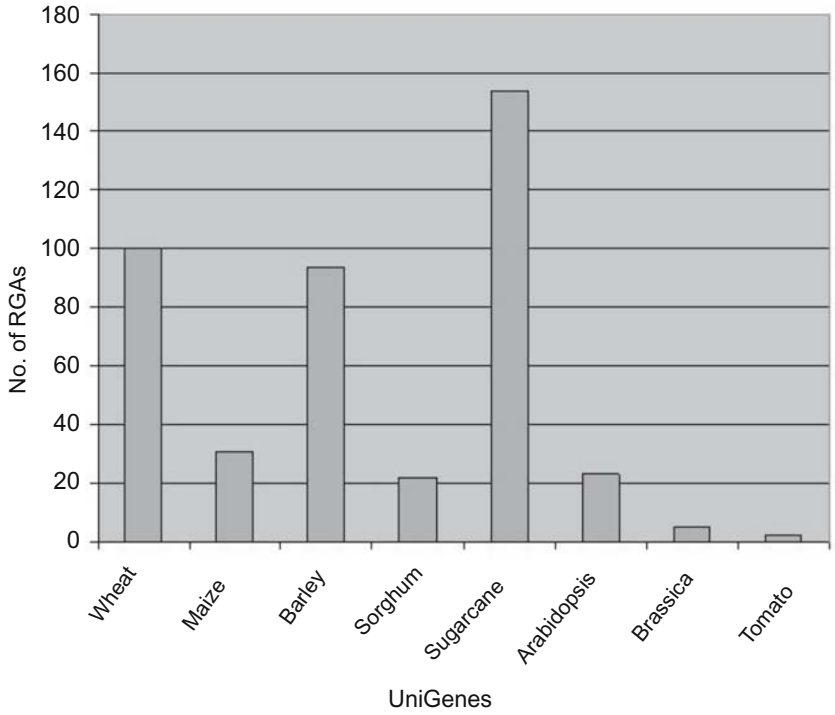

Figure 3. Extent of representation of resistance gene analogues (RGAs) of rice chromosome 11 in the transcriptomes (unigenes) of other monocots and dicots.

longer than 8 cm were classified as susceptible and those shorter than 8 cm were taken as resistant. The resistant genotypes were IRBB-3, IRBB-4, IRBB-13, IRBB-21, IRBB-55, IRBB-59 and IRBB-60, whereas PB-1, PS-2,

IR-24, IR-64, Pusa-1121, B-370, Swarna and Bindli were susceptible to Xoo. In the susceptible genotypes, the lesion was clearly visible in every inoculated leaf and covered large areas of the leaves. In our study, 100 exon-based PCR primers were used; in which only 88 gave amplification. Out of these, 57 produced a monomorphic band after amplification in all the rice varieties, whereas 31 produced polymorphic bands. As expected, most of the primers resulted in fragments of 300–800 bp in all the lines. It is interesting to note that 4 primers AC133216-11 (RRGA11-181), AC133216-16 (RRGA11-184), AC133216-15 (RRGA11-183) (figure 4) and AC134923-3 (RRGA11-172) amplified only in IRBB-4. These primers were designed from the BAC physically present in the *Xa4* gene region. Three other unique primers were also detected, which amplified in only one of the rice lines. Out of these primers, AC133216-13 (RRGA11-182) amplified only in Swarna, AC133291-1 (RRGA11-096) amplified only in Pusa-1121 and AC135121-8 amplified only in IRBB-55. Also, a few primers on amplification gave products that were larger than their expected size. On the long arm of the chromosome, 6 RGAs were found related to *Xa21*, and all were protein kinase genes. Primers designed from such genes (RRGA11-105, RRGA11-107, RRGA11-108, RRGA11-109, RRGA11-110 and RRGA11-111) were able to amplify in all the rice lines used in this study. This showed that analogues of the *Xa21* gene were common to all the rice varieties. There were clusters of *Cf2/Cf5* disease-resistance genes at the upstream region of the protein kinase *Xa21*-like gene and clusters of putative NBS-LRR resistance genes downstream of it.

To confirm that the amplification products did correspond to the target RGA sequence and to identify single-nucleotide polymorphism (SNP)/insertion–deletion (InDel) markers for the predicted genes, a few PCR products whose primers were designed from the predicted *Xa4* and *Xa21* gene regions were gel eluted and purified for direct sequencing. The sequence obtained for a set of 8 genotypes was compared; this revealed 2 SNPs as well as 3 InDels in a few genotypes. For instance, sequences of a 400 bp PCR product of IR-64, IR-24, IRBB-60, Swarna, PB-1, PS-2, B-370 and Bindli amplified by primer AC133216-1 matched with each other except for a few InDels/SNPs. Among these rice varieties, only IRBB-60 was a resistant variety. Sequence comparison did not reveal anything unique in the IRBB-60 sequence. At positions 31 and 38, single base insertions were found in IRBB-60, Swarna and PS-2 and, at position 111, a single base insertion was detected in PB-1 and PS-2. At position 60, C was replaced by T in genotypes 93-11, IRBB-60 and PS-2, whereas at position 186, G was replaced by A in IRBB-60 and PS-2.

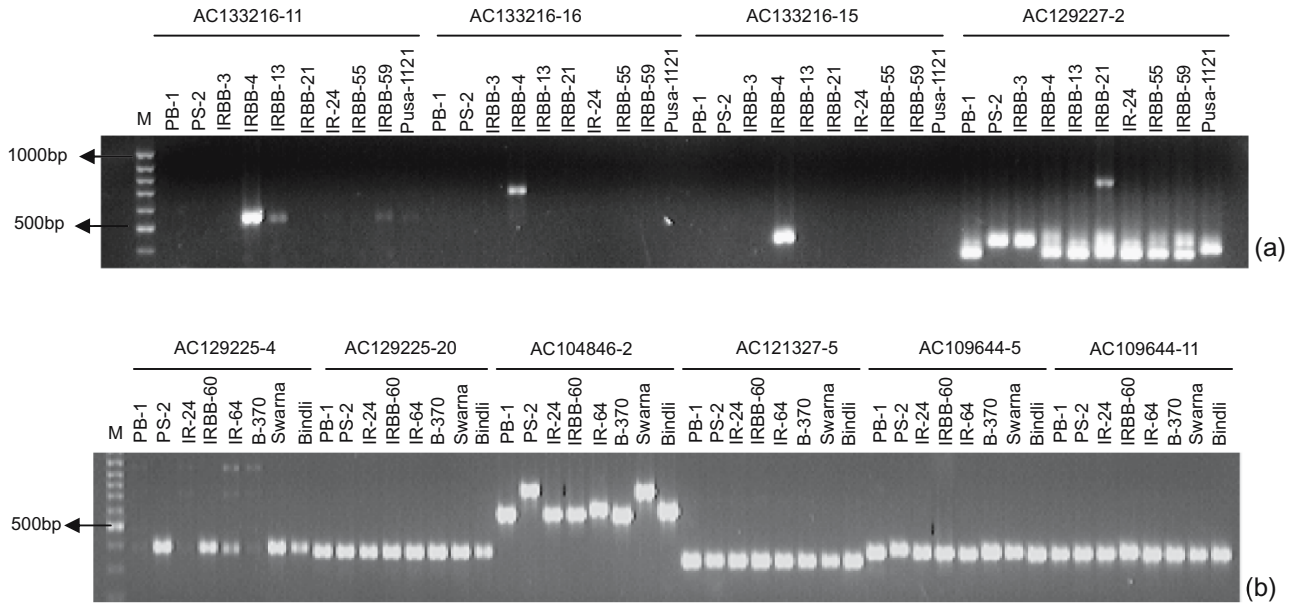

Figure 4. (**a**) PCR amplification of 10 different rice genotypes with different exon-based primers. The names of the rice genotypes and the primers used in this study are given on the top of the lanes. The primers AC133216-11 (RRGA11-181), AC133216-16 (RRGA11-184) and AC133216-15 (RRGA11-183) gave amplification only in IRBB-4 at an annealing temperature of 64˚C. These primers were designed from resistance gene analogue (RGA) sequences physically located in the *Xa4* gene region. The primer AC129227-2 (RRGA11-151) clearly distinguished PS-2 and IRBB-3 from the others. (**b**) PCR amplification of 8 different rice genotypes with different exon-based primers. The names of the rice genotypes and the primers used in this study are given on the top of the lanes. PS-2 and Swarna as well as IR64 and Bindli were found to be polymorphic as compared with the other genotypes with primer AC104846-2 (RRGA11-128). The primer AC109644-5 (RRGA11-104) also gave a polymorphic pattern in PS-2.

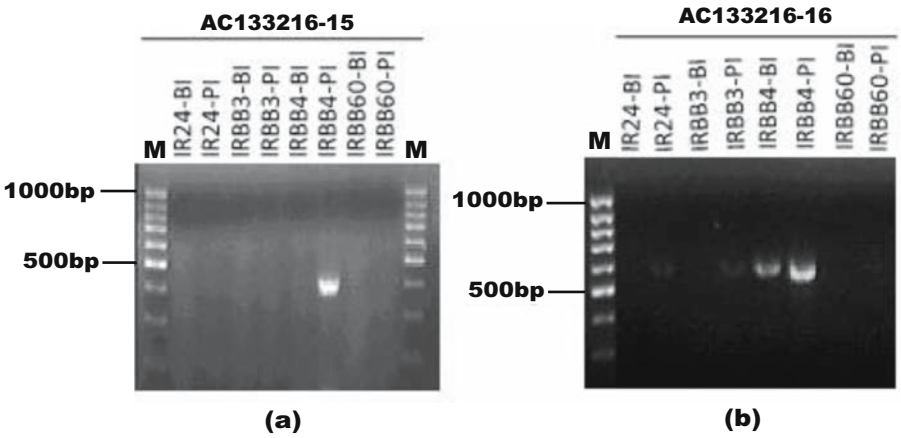

Figure 5. RT-PCR analysis with the resistance gene analogue (RGA)-based primers physically located in the *Xa-4* gene region. The RT-PCR products were resolved in 1.8% agarose gel. (**a**) The primer AC133216-15 gave amplification only in a post-inoculated (PI) sample of IRBB-4. No amplification was observed in any other sample. (**b**) The primer AC133216-16 produced a sharp band in a post-inoculated sample of IRBB-4 whereas a very faint band was observed in a pre-inoculated sample of IRBB-4. BI denotes sample collected before inoculation whereas PI denotes post-inoculated sample.

3.4 *Expression analyses of IRBB-4 specific genes under BB infection*

The primers AC133216-15 and AC133216-16, which amplified only in IRBB-4, were used for expression analysis. A single band of 455 bp size was obtained with AC133216-15 in IRBB-4 only after 24 h of inoculation. There was no amplification in the rest of the samples (figure 5). The primer AC133216-16 produced a faint band of 653 bp in uninfected IRBB-4, whereas a sharp band of the same size was obtained in the IRBB-4 sample after inoculation for 24 h. This showed that the specific gene was induced at a higher level in the resistant line than in the corresponding susceptible lines. The genes RRGA11-183 (AC133216-15) and RRGA11-184 (AC133216-16) were found to contain 317 and 584 amino acid residues, respectively. When the amino acid sequences of these two predicted genes were subjected to protein motif search analysis, the mechanism of resistance was predicted. It revealed that both RRGA11-183 and RRGA11-184 belonged to a single domain family of NB-ARC.

4. Discussion

We could identify 195 RGAs distributed in several large and small clusters on chromosome 11 of rice. Botella *et al.* (1997) reported earlier that clusters of disease-resistance and defence-response genes are found on the *Arabidopsis* chromosome. A few other groups have also reported that the majority of NBS-LRR genes are physically clustered in the genome of *A. thaliana* (Meyers *et al.* 2003). This could be due to tandem duplication followed by unequal crossing over. Interlocus recombination and diversifying selection might have played a major role in generating diversity within existing clusters (Leister *et al.* 1998). It has been reported that in barley additional genes are required for activation of an R-gene-mediated defence response upon pathogen infection. The presence of clusters of RGAs indicates that they might be involved in multiple signalling pathways to confer protection against a single pathogen.

A total of 69 RGAs encoding NBS-LRR-like proteins were found on chromosome 11 of rice in which no TIR–TNL homology was reported. Bai *et al.* (2002) reported a few genes with TIR in rice but none of them contained an LRR domain. Hence, these were divergent from NBS-LRR genes. NBS-LRR has been reported to recognise the pathogenic component of bacteria and fungi in plant species (Bai *et al.* 2002). Besides, they also recognise viral, nematode and insect species that parasitise dicot plants. Hence, the identified genes should serve as possible candidates and then help in establishing an association with the resistance reaction. Six *Xa21*-like RGAs were found on the long arm of chromosome 11. Although *Xa21* itself does not encode for NBS-LRR, a cluster of NBS-LRR genes was found at a distance of 0.6 Mb on the physical map (supplementary table 2). This also indicated the possibility of additional NBS-LRR-type genes for activation and expression of the *Xa21* gene. In tomato, a similar situation exists: the *Pto* gene, encoding a serine/threonine protein kinase, confers resistance against the pathogen *Pseudomonas syringae* (Martin *et al.* 1993) but additionally requires an NBS-LRR gene, *Prf*, for defence activation (Salmeron *et al.* 1996).

Of the 195 RGAs predicted in rice chromosome 11, 41.03% showed significant homology with chromosome 12. It might be because chromosomes 11 and 12 harbour duplicated regions at the distal ends of their short arms as

determined by physical and genetic mapping (Wu *et al.* 1998). Based on the genome sequence, it became evident that segmental duplications occur throughout the rice genome (Yu *et al.* 2005). Earlier, we had identified 546 gene models, which were duplicated between chromosomes 11 and 12. Among them, the maximum duplication occurred within the first 3 Mb in both the chromosomes. Of the duplicated gene models, 98% were found in the same orientation on both the chromosomes (Rice Chromosome 11 and 12 Sequencing Consortia 2005). Approximately 90% (175 genes) of all predicted RGAs showed significant homology with *indica* chromosome 11 pseudomolecules. However, 10% of the 195 RGAs of the *japonica* variety did not match with those of the *indica* variety 93-11. During sequencing and comparative analysis of chromosome 1, it was found that nearly 22% of the *japonica* sequence could not be recognised in the *indica* draft (Sasaki *et al.* 2002). These observations show that there are differences in these two subspecies, which have evolved independently subsequent to the *indica–japonica* divergence.

One of the objectives of our study was to investigate the extent of conservation of chromosome 11 RGAs in other rice chromosomes. Interestingly, the gene RRGA11-090 was present in all the 12 chromosomes of rice. The protein motif search revealed that the gene belongs to the LRR domain family. This protein has the crystal structure of polygalacturonase-inhibiting protein (PGIP) containing 7 LRRs. Its motif structure revealed that apart from PGIP, 3 sets of 72 different amino acids are responsible for the LRR. A few amino acids also code for a tyrosine kinase phosphorylation site as well as an N-glycosylation site. These sites are attached to the cell wall peptidoglycan by an amide bond which may be involved in plant defence.

We found that 8.21% (16 genes) RGAs of rice chromosome 11 did not show significant homology with rice unigenes. Hence, these 16 RGAs might either be wrong predictions or rarely transcribed genes. Some might represent genes that are difficult to clone from RNA. It is possible that these genes express for a brief period only under specific conditions and environments, and have not been used so far to generate expressed sequence tag (EST) libraries. In this study, *Arabidopsis* showed the highest similarity with chromosome 11 RGAs among the dicot species. Peng *et al.* (1999) have used rice EST information and cloned a gene, which is a homologue of a gibberellic acid-insensitive (GAI) gene involved in dwarfism in *A. thaliana*. Mapping of these homologues also confirms the same location as the *Rht-1* semi-dwarfing genes in wheat and the *d8* dwarf mutation in maize. This analysis highlights the powerful use of the EST database to establish a relationship between monocot and dicot species. Among cereals, wheat and barley showed the highest expression. Our earlier investigation based on comparative analysis between the long arm of chromosome 11 and the wheat EST database showed significant conservation of individual gene sequences between rice and wheat (Singh *et al.* 2004). Leister *et al.* (1998) reported that the RGA clones isolated from rice and barley have also been found to hybridise to the DNA of a number of grass species. RGAs described in this study can thus be used as probes to identify and map RGA loci in different cereals.

Interestingly, two RGAs, RRGA11-002 and RRGA11-082, had a homologous sequence in unigenes of all the plant species under study, suggesting that these two represent real, commonly expressed genes. The motif structure revealed that both the genes have a set of 23 amino acids, which constitute the protein kinase ATP-binding region and a few other amino acids responsible for the protein kinase C phosphorylation site, as well as cAMP- and cGMP-dependent protein kinase phosphorylation sites. These predicted results suggest that these protein kinases have enzymatic activity that is capable of autophosphorylation on multiple sites. Phosphorylated residues may bind different ligand molecules, which can initiate a downstream plant defence response. Catalytic kinase domains are highly conserved and this supports our results. These findings can help in developing molecular breeding approaches for identification of regions that are highly conserved or rapidly evolving genes for disease resistance using rice as a model genome.

Amplification of RGAs of rice chromosome 11 was carried out to study the extent of polymorphism and thus develop RGA-based markers. Amplification was obtained with 45.12% of the genes, of which 15.89% showed polymorphism. Interestingly, a few primers designed from the *Xa4* gene region gave amplification only in IRBB-4 but not in IRBB-60, both of which contain *Xa4*. This could be due to the presence of different recombination breakpoints and thus different regions of introgression around the BB resistance genes from the donor in the background of IR24. This has implications for the development of RGA-based markers for specific disease-resistance genes in rice. Mapping studies have shown that, in many cases, different families of NBS-LRR genes tend to cluster and are often genetically linked to known disease-resistance loci. In some cases, these amplification products have facilitated the isolation of functional disease-resistance genes, such as maize *Rp1-D* rust resistance gene (Collins *et al.* 1999), while in other cases they serve as valuable markers for disease-resistance breeding (de Majnik *et al.* 2003). Those RGAs whose primers were able to amplify only IRBB-4 in the present study, may serve as landmarks for the isolation and cloning of IRBB-4 line-specific genes. Previously, several groups have used a PCR-based strategy to clone RGAs from *Arabidopsis*, maize, rice and soybean, and several genes have also been mapped onto their respective chromosomes (Aarts *et al.* 1998; Leister *et al.* 1998). Such approaches may help in isolating major disease-resistance genes and

understanding their role in the expression of a defence response in rice.

Whenever any resistance gene occurs in a cluster of a multigene family, isolation of the functional RGA becomes a tedious task (Song *et al.* 1995; Botella *et al.* 1997). Identification of the gene-based markers would be difficult, unless specific structural features are targeted to design gene-specific primers. Deng and Gmitter (2003) aligned the kinase domain of the rice *Xa21* and tomato *Pto* gene, and identified two well-conserved regions. They designed degenerate primers from those regions and amplified citrus DNA fragments that are most similar to the rice *Xa21* gene. Disease-resistance genes isolated from rice include *Xa21* (Song *et al.* 1995), *Xa1* (Yoshimura *et al.* 1998), *Pi-b* (Wang *et al.* 1999) and *Pi-ta* (Bryan *et al.* 2000). Several R-genes have been genetically mapped on chromosome 11. Sequence comparison of one of the RGAs revealed the presence of two SNPs and three single-base InDels in a 400 bp fragment. Once associated with traits, such SNPs in RGAs would be attractive tools for marker-assisted breeding and map-based cloning.

Expression analysis of the IRBB-4 specific gene using the primer AC133216-15 (RRGA11-183) for RT-PCR gave a single fragment only in the post-inoculated sample of IRBB-4, suggesting that this gene is induced by pathogen infection. Since IRBB-60 having *Xa-4* gave no amplification either on PCR or RT-PCR with this primer, this gene was not the *Xa4* gene but an IRBB-4 line-specific gene. When the exon sequence of this gene (RRGA11-183) was subjected to protein prediction analysis, it was found that the gene encodes a putative stripe rust resistance protein Yr10. Another primer, AC133216-16 (RRGA11-184), expressed slightly lower in a pre-inoculated IRBB-4 sample than a post-inoculated sample, which indicated that the gene gets induced on pathogen inoculation, similar to *Xa1* (Yoshimura *et al.* 1998) and *Pi-b* (Wang *et al.* 1999). This is similar to the previous observation by Ward *et al.* (1991) that the expression of different defence-related genes gets upregulated in plants by pathogen infection. The differentially upregulated gene, RRGA11-184 in IRBB-4, also encodes a protein similar to the putative stripe rust resistance protein Yr10.

Motif structure analysis of RRGA11-183 showed that this protein contains 317 amino acids of which 25 amino acids are responsible for the p-loop nucleotide-binding motif whereas a few amino acids code for the tyrosine kinase phosphorylation site as well as ATP/GTP binding-site motif. These motifs may be involved in the binding of pathogen components such as elicitor molecules that lead to the activation of a full defence response, which may be sufficient to inhibit pathogen advance. Apart from elicitor molecules, R-genes may recognise other pathogen components such as an *AVR* gene product, structural components from bacteria such as toxins, flagellin, cell wall components such as chitin and melanin, enzymes that degrade plant polymers such as pectate lyase and cutinase, various peptides such as *AVR-Pita* or elicitins. The gene RRGA11-184 also belongs to the single-domain family of NB-ARC but a few residues of LRR were also found. LRR residues of this gene may participate in protein–protein interaction and ligand binding. It was also found that the protein sequence of RRGA11-183 had similarity to the DRL4-ARATH protein in *A. thaliana* and codes for putative disease-resistance protein, and RRGA11-184 had similarity to the putative disease-resistance RPP13-like protein of *A. thaliana.* The information on the physical location and amplification of the candidate RGAs analysed in this study is expected to facilitate cloning of other genes for disease resistance in rice.

Acknowledgements

We thank Dr Jayshree Gopalkrishnan, Division of Plant Pathology, IARI for kindly providing the bacterial strain Xoo-4. We also thank Dr Sheetal Yadav, Swaroop Parida and Anand for disease evaluation; Sambit and Chitra for technical assistance. Support from the Department of Biotechnology, New Delhi is gratefully acknowledged.

References

Aarts M G M, Hekkert B L, Holub E B, Beyon J L, Stiekema W J and Pereria A 1998 Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana; Mol. Plant Microbe Interact.* **11** 251–258

Bai J, Pennill L A, Ning J, Lee S W, Ramalingam J, Webb C A, Zhao B, Sun Q, Nelson J C, Leach J E and Hulbert S H 2002 Diversity in nucleotide binding site-leucine-rich repeat genes in cereals; *Genome Res*. **12** 1871–1884

Botella M A, Coleman M J, Hughes D E, Nishimura M T and Jones J D G 1997 Map position of 47 *Arabidopsis* sequences similarity to disease genes; *Plant J.* **12** 1197–1211

Bryan G T, Wu K S, Farall L, Jia Y, Hershey H P, Mc Adams S A, Falk K N, Donaldson G K, Tarchini R and Valent B 2000 *t/A* single amino acid difference distinguishes resistant and susceptible alleles of the rice blast gene *Pi-ta*; *Plant Cell* **12** 2033–2046

Collins N, Drake J, Ayliffe M, Sun Q, Ellis J, Hulbert S and Pryor T 1999 Molecular characterization of the maize *Rp1-D* rust resistance haplotype and its mutants; *Plant Cell* **11** 1356–1376

de Majnik J, Ogbonnaya F C, Moullet O and Lagudah E S 2003 The *cre1* and *cre3* nematode resistance genes are located at homeologous loci in the wheat genome; *Mol. Plant Microbe Interact*. **16** 1129–1134

Deng Z and Gmitter F G Jr 2003 Cloning and characterization of receptor kinase class disease resistance gene candidates in citrus; *Theor. Appl. Genet*. **108** 53–61

International Rice Genome Sequencing Project 2005 The map-based sequence of the rice genome; *Nature (London)* **436** 793–800

Kauffman H E, Reddy A P K, Hsieh S P Y, Merca S D 1973 An improved technique for evaluating resistance to rice varieties of *Xanthomonas oryzae*; *Plant Dis. Rep*. **57** 537–541

Leister D, Kurth J, Laurie D A, Yano M, Sasaki T, Devos K, Graner A and Schulze-Lefert P 1998 Rapid reorganization of resistance gene homologues in cereal genomes; *Proc. Natl. Acad. Sci. USA* **95** 370–375

Li L Y, Ling W, Jing J X, Li Z Q, Lin F, Huang L F and Pan Q H 2007 The *Pik*^{m} gene, conferring stable resistance to isolate of *Magnaporthe oryzae*, was finely mapped in a crossover-cold region on rice chromosome 11; *Mol. Breed.* **20** 179–188

Martin G B, Bogdanove A J and Sessa G 2003 Understanding the functions of plant disease resistance protein; *Annu. Rev. Plant Biol*. **54** 23–61

Martin G B, Brommonschenkel S H, Chunwongse J, Frary A, Ganal M W, Spivey R, Wu T, Earle E D and Tanksley S D 1993 Map-based cloning of a protein kinase gene conferring disease resistance in tomato; *Science* **262** 1432–1436

Meyers B C, Kozik A, Griego A, Kuang H and Michelmore R W 2003 Genome-wide analysis of NBS-LRR proteins encoded in *Arabidopsis* and other plant genomes; *Plant J.* **32** 77–92

Monosi B, Wisser R J, Pennill L and Hulbert S H 2004 Full-genome analysis of resistance gene homologous in rice; *Theor. Appl. Genet*. **109** 1434–1447

Peng J, Richards D E, Hartley N M, Murphy G P, Devos K M, Flintham J E, Beales J, Fish. *et al*. 1999 'Green revolution' genes encode mutant gibberellin response modulators; *Nature (London)* **400** 256–261

Rice Chromosome 11 and 12 Sequencing Consortia 2005 The sequence of rice chromosome 11 and 12, rich in disease resistance genes and recent gene duplications; *BMC Biol.* **3** 20

Salamov A A and Solovyer V V 2000. *Ab initio* gene finding in *Drosophila* genomic DNA; *Genome Res*. **10** 516–522

Salmeron J M, Oldroyd G E, Rommens C M, Scofield S R, Kim H S, Lavelle D T, Dahlbeck D and Staskawicz B J 1996 Tomato *Prf* is a member of leucine rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster; *Cell* **86** 123–133

Sasaki T, Maatasumoto T, Yamamoto Baba T, Katayose Y, Wu J, Niimura Y, Cheng Z and Nagamura Y 2002 The genome sequence and structure of rice chromosome 1; *Nature (London)* **402** 312–316

Singh N K, Raghuvanshi S, Srivastava S K, Gaur A, Pal A K, Dalal V, Singh A, Ghazi I A, *et al.* 2004 Sequence analysis of long arm of rice chromosome 11 for rice–wheat synteny; *Funct. Integr. Genomics* **4** 102–117

Song W Y, Wang G L, Chen L L, Kim H S, Pi L Y, Gardner Wang B, Holsten T, Zhai W X, Zhu L H, Fauquet C and Ronald P C 1995 A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*; *Science* **270** 1804–1806

Wang G L, Mackill D J, Bonman J M, McCouch S R, Champoux M C and Nelson R J 1994 RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistance rice cultivar; *Genetics* **136** 1421–1434

Wang Z X, Yano M, Yamanouchi U, Iwamoto M, Monna L, Havasaka, Katavose Y and Sasaki T 1999 The *Pib* for rice blast resistance belong to nucleotide binding and leucine rich repeat class of plant disease resistance genes; *Plant J.* **19** 55–64

Ward E R, Uknes S J, Williams S C, Dincher S S, Wiederhold D L, Alexander D C, Ahl-Gov P, Metraux J P and Ryals J A 1991 Coordinate gene activity in response to agents that induce systemic acquired resistance; *Plant Cell* **3** 1085–1094

Wu J, Kurata N, Tanoue H, Shimokawa T, Umehara Y, Yano M and Sasaki T 1998 Physical mapping of duplicated genomic regions of two chromosomes ends in rice; *Genetics* **150** 1595–1603

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang S X, Kono I, Kurata N, Yano M, Iwata N and Sasaki T 1998 Expression of *Xa1,* a bacterial blight-resistance gene in rice is induced by bacterial inoculation; *Proc. Natl. Acad. Sci. USA* **95** 1663–1668

Yoshimura S, Yoshimura A, Iwata N, McCouch S R, Abenes M L, Baraoidan M R, Mew T W and Nelson R J 1995 Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers; *Mol. Breed*. **1** 375–387

Yu J, Wanj J, Lin W, Li S, Li H, Zhou J, Ni P, Dong W, *et al.* 2005 The genomes of *Oryza sativa*: A history of duplications; *PloS Biol*. **3** e38

Yu Z H, Mackill D J, Bonman J M, McCouch S R, Guiderdoni E, Notteghem J L and Tanksley S D 1996 Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea* Sacc.); *Theor. Appl. Genet.* **93** 859–863

MS received 20 May 2008; accepted 11 March 2009

*e*Publication: 23 May 2009

Corresponding editor: Imran Siddiqi

Physical mapping, expression analysis and polymorphism survey of resistance gene analogues on chromosome 11 of rice

Irfan A Ghazi, Prem S Srivastava, Vivek Dalal, Kishor Gaikwad, Ashok K Singh, Tilak R Sharma, Nagendra K Singh and Trilochan Mohapatra

J. Biosci. 34(2), June 2009, 251–261 © Indian Academy of Sciences

Supplementary tables

Table 1. List of specific primers designed for the resistance gene analogues (RGAs) predicted in the BAC/PACs of rice chromosome 11

S. no.	Primer name	RGA no.	BAC/PAC	Forward primer	Reverse primer	Tm	Expected product size (bp)
1	AC133216-1	RRGA11-179	b0049B20	CACTTCACTTGGAGCCATGC	AGTCACGTCAGAGTCAATCACG	60	428
2	AC133216-10	RRGA11-180	do	TTCTTGAAGGAGGAGGAAGG	GAGTTATGGAGCGAGCAAGC	60	397
3	AC133216-11	RRGA11-181	do	GTCCAGGGTGTACGAGATCC	GCATGAGAGGATGCAAGTAGC	64	535
4	AC133216-13	RRGA11-182	do	CGATCACTGACCATGTTTGG	CCTTCTGCAGTCGATTCTCC	60	461
5	AC133216-16	RRGA11-184	do	AGGTCGGTGACAGAGACTCG	CTCAGAGACCGCAAGAAAGC	62	653
6	AC133216-15	RRGA11-183	do	AGCATAATCTGCCGAAGAGC	CTGGTCAATCCCAACTAGGC	64	455
7	AC135643-1	RRGA11-168	a0023F01	AGTATGTGGGTGGCTGAAGG	CAAGTGTGCCAGTCAAATCC	60	412
8	AC135643-4	RRGA11-164	do	AGAAGAGCTCGCAGAGTTGC	TGCAGGCATGTATGATCTCC	60	446
9	AC135643-6	RRGA11-165	do	CAGCCTAGTTGAACCCAACC	TCCACACATCATCCAACACC	60	362
10	AC135643-7	RRGA11-166	do	GCCAGGTTCTTACACACAAGG	GAGGTTGCAGCATACAGTGG	60	352
11	AC129227-2	RRGA11-151	b0099J16	CAGTTTCGGTGTTGTGATGC	CTAGATTGGCCACGACTTCC	66	395
12	AC129224-1	RRGA11-143	a0029P13	CTGCACGGTACTTGACTTGG	CAGCTTCTCCATTTCCTTCG	60	352
13	AC129224-8	RRGA11-144	do	ACCTCACCAAGTCCAAGACG	GGAATTCGTCCCTCTTCTCC	60	351
14	AC129224-10	RRGA11-146	a0029P13	ACCAGCACTCCTCTGTCACC	ACATAACCGGGGCTATCTGG	60	370
15	AC129224-11	RRGA11-147	do	GTGGATGAACCAGGTCAAGG	CTGATCTGCCATCTCCTTCC	60	434
16	AC129224-20	RRGA11-148	do	TGGAGACATTGGACATCAGG	GGTTGATCTGGATCGACAGG	60	375
17	AC129225-3	RRGA11-140	a0051D10	GGCATTCCAAGAAGGAAGG	GAGACGCGACATGAGAAAGC	58	540
18	AC129225-4	RRGA11-141	do	AAAGCATCAGCAGGACAAGC	TCTTGGAACCCTGCAGTAGC	60	407
19	AC129225-11	RRGA11-142	do	CAGCCAACCTGAAGAAGAGC	GGACAATCATCTGGGAAAGC	64	406
20	AC129225-20	RRGA11-145	do	CTCGACCACCACTACCACAAC	CGTTCACAGTGTTCTTCAGGTC	60	388
21	AC122143-3	RRGA11-131	a0090F16	TCCTCCTCAAAGACGACTACG	AAGATGGTTGGTTGGTAGCC	56	357
22	AC104846-2	RRGA11-128	P0004C07	CCAGTGCCTGAAAACTTTGG	GAGGAAAGATGCTGCAAACC	60	438
23	AC104846-5	RRGA11-129	do	AAGCACGGAAACTAGGATGC	AAGATGGGTTTTCCCTGACC	56	383
24	AC104846-10	RRGA11-130	do	TTGGTCGATAGGAAGGATGG	TCCACCGGATCAGAGTTAGC	54	409
25	AC121327-1	RRGA11-125	a0016O23	TCAAATGCAGCAGACTACGG	GCATTCATCCTGGAAGTTGG	54	434
26	AC121327-5	RRGA11-126	do	ATCCTCGCCGTCTTATCTCC	GGCACATGCATACTTTGTGG	60	355
27	AC109832-22	RRGA11-124	P0038B07	GAATGGCATCATGGTGAGC	CCTTGAGCTCCTGAATCTGC	56	395
28	AF161269-1	RRGA11-123	a0034K22	GAGAGGTGGTTATGGCATCG	TTCCTGTGTCGTTTGTCTGC	54	443
29	AC109644-3	RRGA11-103	P0459F09	TGACCTCAGCAACAACAACC	TTACGTGTTTGAGCGAGTGG	56	356
30	AC116367-6	RRGA11-185	a0059H21	ACATTACCATGCTGGCTTCC	CAGCGTGCTTGATAGATTGC	56	383
31	AC116367-7	RRGA11-186	do	ATTGCAAGGCTGCTGTTAGG	CGACAACATCACTCATCACC	56	500
32	AC116367-8	RRGA11-187	do	GGGCAACACAAGTTTCAACC	ACCAAGAGATGCAGGAATGG	56	450
33	AC116367-15	RRGA11-188	do	TATGGCAACCACATCTCAGG	GACTCGGGATTGTACCAACG	56	550

S. no.	Primer name	RGA no.	BAC/PAC	Forward primer	Reverse primer	Tm	Expected product size (bp)
34	AC116367-18	RRGA11-189	do	CCTCCAAGTTATTGCCATGC	TGCCGTTAGTGAGTTCATGC	56	372
35	AC116367-19	RRGA11-190	do	TGCCCTGTCAGGTAACATCC	TCAGCTGGGTATGGGATAGG	56	396
36	AC116367-20	RRGA11-191	do	GCTGCTTCCACAAAACTGC	GCAGCCTCCCAAGATTAGC	56	380
37	AC116367-21	RRGA11-193	do	GGGTCTTGTTCTGACCTTGC	TATATCACCCGGGAGTGTCC	56	361
38	AC109644-5	RRGA11-104	P0459F09	TTCCTCCTCGTTGGAGTAGC	CCTGAGAGAGCTGACGAACC	60	390
39	AC109644-11	RRGA11-105	P0459F09	ATTGCTCTGCTCCTCACTGC	CGAGCTTAGGTTCAGCATCC	60	385
40	AC109644-14	RRGA11-106	do	TCACCTGTAGCAACCTCACC	ACGTAAGGCCAATCAGATGC	60	415
41	AC109644-16	RRGA11-107	do	ACTCCGGACGATATCAATGG	ACCCAAGGATGAAGGTAGGG	60	442
42	AC109644-17	RRGA11-108	do	CAATCTCTGGCCGAGTTACC	GAATTACCCAGCGAGACAGG	60	380
43	AC109644-18	RRGA11-109	do	GATGGACCTCACCATCAACC	ACAGAGCAAGCTCCAAGAGG	60	374
44	AC109644-20	RRGA11-110	do	TTTGGGCAATCTCACTAGCC	AACCCCGAGGTGATAATTCC	60	369
45	AC109644-21	RRGA11-111	do	CACTAATGGCGTTCACTGTCC	CAGGAAGAACTCCTCCAAAGC	60	360
46	AC133291-1	RRGA11-096	P0452B04	ATGCAATACCCCTCATCAGC	AGCTTGGAGGTTGTTCATGC	54	408
47	AC133291-3	RRGA11-097	do	GGGGCAGTTGACTAGTTTGG	AGCCATGCAGGAAACATAGG	60	353
48	AC133291-6	RRGA11-098	do	CGTGGTCCTTACTTCCATCG	GGCCATCTGAACCACTTAGG	60	365
49	AC133291-7	RRGA11-100	do	GATTGTTGCCAATGGAGAGG	AGGCCAATCGAGTACTGTGC	60	438
50	AC133291-14	RRGA11-101	do	CAAGTTACCGCTGCTCTTCC	TGTTGTTGCTGAGGTCAAGG	60	359
51	AC133291-16	RRGA11-102	do	AGACTGCCAAGTTGGATTGG	TGGTCGCTAGTGATGTCTCG	60	383
52	AC136905-4	RRGA11-093	b0064E13	TGGGAGACGTCAACCTTAGC	ACCCTATGCACATTCCTTGG	60	382
53	AC136905-7	RRGA11-094	do	AATCCTCCGGTAAACCTTGG	TCTTGTCACGCACAAAGAGG	58	409
54	AC136956-24	RRGA11-090	A0038B22	CTCAGCTCGGTAACCTTTCG	CAAGAACTTGGAGGGACAGC	60	429
55	AC136956-20	RRGA11-089	a0038B22	GGTGTTTTGCAGGTTTGAGC	GATTCGCAGATGGATTCAGG	60	715
56	AC134624-4	RRGA11-083	a0041I05	GGATGAAGCACAACTCATCG	GGCCAACATACTCGCTATGG	58	358
57	AC134624-7	RRGA11-084	do	AGCTGCATAAGACCGTCAGG	GGCCTGGCTACTGTAACACC	60	355
58	AC134624-12	RRGA11-085	do	AGCTGAGTTGGAGAGCATGG	ACAAAGCTGATAGGCGAAGG	60	380
59	AC134624-16	RRGA11-086	do	AGGTCAACTCATTCGCAAGC	TAAGCACCTTTTCCCACTGC	56	410
60	AC137751-7	RRGA11-138	a0014E11	CAGCAGCAATTCTGATCTCG	AGCTCTCCTTCTCCATGTCG	58	353
61	AC125781-4	RRGA11-136	a0058G18	CCTACGACTCCCAGAAATGC	ATCGTCGTTGTGTCCTTTGC	56	383
62	AC125781-6	RRGA11-137	do	GAGAAGATCACCTTGTCCTTCG	CTCGAGCTCCTTTAGCATCG	60	426
63	AC122143-4	RRGA11-132	a0090F16	TCTCGACCTCAGGAACAACC	GAACTTGTTCCCGTTCAGG	54	361
64	AC122143-15	RRGA11-133	a0090F16	TGGACTACGACGAGCTGAGG	CCAGTTCTTGGAGGTGAAGC	60	404
65	AC122143-16	RRGA11-134	do	CAATGCCACATGGAGAAGG	GAAATGGCATCCTGTCAACC	58	367
66	AC122143-17	RRGA11-135	do	AAAGTCGGTTGGCACTATGG	TTTCCATACGCAGCTTCTCC	60	408
67	AC125780-20	RRGA11-127	a0030I15	TGACTCTCCTGCTGCTTGC	CTTCTCCGTCGAGATGAAGC	56	382
68	AC120885-20	RRGA11-122	a0042J05	GCCAAACAAGAACCAACAGG	TGCCTGGATTAGTCCTCACC	58	374
69	AC120887-13	RRGA11-121	a0088K21	TCCTGGGCATGATCATAAGG	CCTCAGCTCTTCGATGTTCC	58	401
70	AC120884-1	RRGA11-119	a0017D19	TACACCCTCGAGCTTTCTGG	CCACCATTCTGAACCTCAGC	60	361
71	AC121328-7	RRGA11-117	b0030E22	CCACCACACACAGATTGTCC	CATCTGAGCATCTCGTTTGC	58	350
72	AC136148-15	RRGA11-113	a0029K08	GGGGGTAACATTTCTCATGG	CCTTGTCAATCCCAACAAGC	58	422
73	AC136148-17	RRGA11-114	a0029K08	CCGCTAGACCAACTTGACG	CCTTATCAACCCCAACAAGC	58	355
74	AC135121-1	RRGA11-069	b0089M05	TGCACTGTTTGCATCTCTGG	GCCAATTCATCTGGAACTGC	58	776
75	AC135121-2	RRGA11-070	do	TGCACAGGATGTGGTAGAGG	TGACTCGCAACATTGCTACC	60	384
76	AC135121-4	RRGA11-071	b0089M05	TGCCAGATCATGCAACTACC	GAAGGTCAGGCCTCTGTAACC	60	438
77	AC135121-8	RRGA11-072	do	AGGAGCACTGGATCATCAGG	TGGAGCTCTCCTTTGTCAGC	58	372
78	AC135121-9	RRGA11-073	do	AGAATGATGGGGACAGAAGG	CTGGCACTTCTTTGCAATCC	60	673

S. no.	Primer name	RGA no.	BAC/PAC	Forward primer	Reverse primer	Tm	Expected product size (bp)
79	AC135121-13	RRGA11-074	do	CGGACTACTTGCCTCTCTGG	CTGGTACTTGCGCTTGTGG	64	352
80	AC135644-2	RRGA11-175	a0085H07	CGACCAGCTCCTTTCTATGG	CACGGAGGCAGAATTTATCC	60	413
81	AC135644-5	RRGA11-176	do	ATGACCCTCGCACAAAGG	GATCTGAGCCCTGAAAATGC	60	698
82	AC135644-17	RRGA11-177	do	GCAGATGCGAGTGATCTCC	GGTCCGCCCACATATTAGC	60	626
83	AC135644-22	RRGA11-178	do	AATGAGGGTCATCTCCATCG	TCCTTGCAGTCCTCTTCACC	58	444
84	AC135190-7	RRGA11-173	a0064H09	CTCACTGAGCTGACGCTACG	TCATCATGTTGGGACACTCC	56	445
85	AC135190-9	RRGA11-174	do	GAGAGGATGCACAGGAAAGC	GCCTGGACAAGCAACTAAGC	66	379
86	AC134923-3	RRGA11-172	b0030I07	GAGAAGTCCCGGCATCTAGC	GTGAGCAACACCATCAATGC	58	487
87	AC134924-14	RRGA11-163	b0076M06	GGGATAGCACAGGGTCTAGC	ACCATGATCTCACCCATGC	56	355
88	AC133609-1	RRGA11-160	a0002C14	ACCTGTCGTTGTCAATGTGG	AAAGCCGAGGTAGGAGAAGC	56	425
89	AC133609-8	RRGA11-161	do	AGCAGAAGAGTTGCCTTTGG	CAATGTAGACGGAGGGATGC	60	457
90	AC133609-12	RRGA11-162	a002C14	GACCCTGACATGTGGAAACC	AAGCACCTACACCGATTTGG	58	382
91	AC129226-1	RRGA11-152	b0024E08	GAGACGTACGGGACAACAGC	CACTTCTCGTCGAGTGATCG	60	409
92	AC129226-4	RRGA11-153	do	AGAACGGCTCCCTTAACAGC	ACCTCCAGCATCACAACACC	60	358
93	AC129226-5	RRGA11-154	do	CGTGTACAGGTTGGTGATGG	GAAGCCTTCCCATGTACACC	60	713
94	AC129226-12	RRGA11-155	do	GCAACAGCAACCTATCTCTGG	GCAACTCCGAGCTACACTCC	58	417
95	AC129226-14	RRGA11-156	do	CCTCTTCCCCTTCATCAGC	GCTTCTTTCTCACGCTGACC	60	419
96	AC129226-17	RRGA11-157	do	GAAGAACGGCTCACTTCACG	GCCCTTCTTCCTGTAATCACC	60	387
97	AC129226-18	RRGA11-158	do	CACATCATCCGTCTCTTTGG	ACACCCGAAGTCTGACAAGC	60	354
98	AC129226-20	RRGA11-159	do	GTCGTGCATTATCCCATTCC	TACTTCCTAAAGCCCCAACC	60	378
99	AC125783-3	RRGA11-149	b0039K08	GAGACGGCCAGATGTACAGG	CGGTGTTGATCTCACTCTGC	58	540
100	AC108872-13	RRGA11-139	b0079P23	CACAAAGAGGGGTTCAATGG	TCTTGTCCTTGATGCTGTCG	58	449

Table 2. List of different classes of resistance gene analogues (RGAs) found on the pseudomolecule of chromosome 11 of rice

Sl. no.	Starting position on pseudomolecule	Size of CDS	Dir	Predicted protein function
RRGA11-001	774340	192	+	Putative cyst nematode resistance protein
RRGA11-002	1606391	1326	+	Probable serine/threonine protein kinase
RRGA11-003	2868704	768	-	Serine/threonine protein kinase
RRGA11-004	3513774	330	+	Similar to serine/threonine protein kinase
RRGA11-005	3868311	402	-	Putative serine/threonine protein kinase
RRGA11-006	3899735	537	-	Disease resistance protein like
RRGA11-007	3913928	537	-	Putative disease resistance protein
RRGA11-008	3933226	549	-	Disease resistance protein
RRGA11-009	3939449	555	+	Disease resistance protein
RRGA11-010	5534409	3225	-	Leucine-rich repeat containing protein kinase
RRGA11-011	5719178	3645	-	NBS-LRR disease resistance protein homologue
RRGA11-012	5733182	2502	-	NBS-LRR disease resistance protein homologue
RRGA11-013	5758113	3468	-	NBS-LRR disease resistance protein homologue
RRGA11-014	5760050	321	-	NBS-LRR disease resistance protein homologue
RRGA11-015	5821044	2304	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-016	5843385	2748	-	NBS-LRR disease resistance protein homologue
RRGA11-017	5850314	1224	-	NBS-LRR disease resistance protein homologue
RRGA11-018	6350414	2931	+	Putative disease resistance protein
RRGA11-019	6462782	3066	-	NBS-LRR-like protein

Sl. no.	Starting position on pseudomolecule	Size of CDS	Dir	Predicted protein function
RRGA11-020	6472595	3102	-	Putative disease resistance protein
RRGA11-021	6524618	2178	+	Stem rust resistance protein
RRGA11-022	6532579	450	-	NBS-LRR-like protein
RRGA11-023	6548700	2931	+	Putative disease resistance protein
RRGA11-024	6561519	2325	-	NBS-LRR-like protein
RRGA11-025	6567984	2835	-	NBS-LRR disease resistance protein homologue
RRGA11-026	6582952	4416	+	NBS-LRR disease resistance protein homologue
RRGA11-027	6617393	270	+	NBS-LRR-like protein
RRGA11-028	6622543	2346	+	NBS-LRR-like protein
RRGA11-029	6632755	2742	+	NBS-LRR-like protein
RRGA11-030	6765355	1554	+	NBS-LRR disease resistance protein homologue
RRGA11-031	6772273	1536	+	NBS-LRR-like protein
RRGA11-032	6799005	1020	+	NBS-LRR-like protein
RRGA11-033	6802914	1473	+	NBS-LRR-like protein
RRGA11-034	7214691	3000	-	Putative disease resistance protein
RRGA11-035	7228755	2790	-	Putative disease resistance protein
RRGA11-036	7237687	2157	-	Putative disease resistance protein
RRGA11-037	7277433	2304	-	Putative disease resistance protein
RRGA11-038	7591140	3243	+	NBS-LRR-like protein
RRGA11-039	7731868	4302	-	Putative disease resistance protein
RRGA11-040	7802285	2757	-	Disease resistance protein *RPM1* homologue
RRGA11-041	8371546	2424	-	Disease resistance protein *RPM1* homologue
RRGA11-042	8580455	2820	+	Disease resistance protein *RPM1* homologue
RRGA11-043	8583168	390	+	Disease resistance protein *RPM1* homologue
RRGA11-044	8686155	2658	-	NBS-LRR disease resistance protein homologue
RRGA11-045	8699949	1227	-	Putative bacterial blight-resistance protein
RRGA11-046	8870685	378	-	Putative NBS-LRR resistance protein
RRGA11-047	8883944	2775	+	Putative CC-NBS-LRR resistance protein
RRGA11-048	8900055	579	-	Putative CC-NBS-LRR resistance protein
RRGA11-049	8906667	642	-	Disease resistance protein *RPM1* homologue
RRGA11-050	8915296	2883	+	Putative CC- NBS-LRR resistance protein
RRGA11-051	9250625	948	+	Putative disease resistance protein
RRGA11-052	9256607	2388	+	Putative disease resistance protein
RRGA11-053	9332878	870	-	Disease resistance protein *RPM1* homologue
RRGA11-054	9336482	801	-	Disease resistance protein *RPM1* homologue
RRGA11-055	9488195	2862	+	Disease resistance protein *RPM1* homologue
RRGA11-056	12931774	4248	-	NBS-LRR disease resistance protein homologue
RRGA11-057	14932935	3048	-	Putative disease resistance protein
RRGA11-058	15299580	750	-	Receptor-like serine/threonine protein kinase
RRGA11-059	15859839	3105	-	Putative NBS-LRR resistance protein
RRGA11-060	15884488	2550	-	Putative NBS-LRR resistance protein
RRGA11-061	15896777	987	-	Putative NBS-LRR resistance protein
RRGA11-062	15908033	915	+	Putative disease resistance protein
RRGA11-063	15921851	2289	+	Putative disease resistance protein
RRGA11-064	15952271	3153	-	*RPS2* (*Arabidopsis thaliana)*

Sl. no.	Starting position on pseudomolecule	Size of CDS	Dir	Predicted protein function
RRGA11-065	15960401	3393	+	Putative NBS-LRR resistance protein
RRGA11-066	16462329	4593	-	Putative NBS-LRR resistance protein
RRGA11-067	16483840	3555	-	Putative disease resistance complex protein
RRGA11-068	16495365	2733	-	Putative disease resistance protein
RRGA11-069	16495460	2397	-	Putative disease resistance protein
RRGA11-070	16504512	3033	-	Putative disease resistance protein
RRGA11-071	16519460	2172	-	Putative disease resistance protein
RRGA11-072	16548317	1806	-	Putative NBS-LRR resistance protein
RRGA11-073	16552181	2796	+	Putative disease resistance complex protein
RRGA11-074	16570760	1209	+	Putative disease resistance complex protein
RRGA11-075	16626201	3198	-	Putative disease resistance complex protein
RRGA11-076	17466543	753	-	Leucine-rich repeat protein LRP-sorghum
RRGA11-077	17472162	228	-	Leucine-rich repeat protein LRP-sorghum
RRGA11-078	17479664	756	-	Leucine-rich repeat protein LRP-sorghum
RRGA11-079	17959782	3930	-	Putative stripe rust resistance protein Yr10
RRGA11-080	17989889	3783	-	Putative stripe rust resistance protein Yr10
RRGA11-081	18949916	750	-	Putative disease resistance protein
RRGA11-082	19176713	1089	+	Putative serine/threonine protein kinase
RRGA11-083	19228992	436	-	Putative stripe rust resistance protein Yr10
RRGA11-084	19241577	2364	+	Putative stripe rust resistance protein Yr10
RRGA11-085	19277363	2775	+	Putative NBS-LRR resistance protein
RRGA11-086	19294315	2913	+	Putative disease resistance protein
RRGA11-087	19408735	1899	+	Putative rust resistance protein
RRGA11-088	19411907	552	+	Putative rust resistance protein
RRGA11-089	19515830	1731	-	*Cf2/Cf5* disease resistance protein homologue
RRGA11-090	19535936	3918	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-091	19561483	2766	+	*Cf2/Cf5* disease resistance protein
RRGA11-092	19567787	2019	+	Protein kinase *Xa-21*
RRGA11-093	19603009	1458	-	Putative *Cf2/Cf5* disease resistance protein
RRGA11-094	19626062	2739	-	Putative disease resistance protein
RRGA11-095	19774229	498	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-096	19775024	468	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-097	19782631	1761	+	*Cf2/Cf5* disease resistance protein homologue
RRGA11-098	19796470	4503	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-099	19806048	424	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-100	19806264	2850	+	*Cf2/Cf5* disease resistance protein homologue
RRGA11-101	19871478	486	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-102	19884037	3369	+	*Cf2/Cf5* disease resistance protein homologue
RRGA11-103	19896999	2022	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-104	19915541	1938	+	Putative *Cf2/Cf5* disease resistance protein
RRGA11-105	19956076	3402	-	Protein Kinase *Xa21*
RRGA11-106	19974836	2268	-	Putative *Cf2/Cf5* disease resistance protein
RRGA11-107	19985672	3207	-	Protein kinase *Xa21*
RRGA11-108	19993744	3108	-	Protein kinase *Xa21*
RRGA11-109	20005100	2937	-	Protein kinase *Xa21*

Sl. no.	Starting position on pseudomolecule	Size of CDS	Dir	Predicted protein function
RRGA11-110	20016606	3114	-	Protein kinase *Xa21*
RRGA11-111	20027483	2376	-	Protein kinase *Xa21*
RRGA11-112	20615804	4263	+	Putative NBS-LRR resistance protein
RRGA11-113	21494989	3888	+	Putative NBS-LRR resistance protein
RRGA11-114	21532416	1803	+	Putative NBS-LRR resistance protein
RRGA11-115	21560114	4269	-	Putative NBS-LRR resistance protein
RRGA11-116	21595129	3873	+	Putative NBS-LRR resistance protein
RRGA11-117	22067403	3039	+	Putative resistance protein
RRGA11-118	22112751	723	-	Putative disease resistance protein
RRGA11-119	22157986	861	+	Putative disease resistance protein
RRGA11-120	22302823	927	+	Putative resistance protein
RRGA11-121	22397381	2802	-	Putative NBS-LRR resistance protein
RRGA11-122	22689752	891	+	Putative disease resistance protein
RRGA11-123	22830110	1416	-	Putative serine/threonine protein kinase
RRGA11-124	23402171	2931	+	Disease resistance protein *RPM1* homologue
RRGA11-125	23404913	4254	+	Disease resistance protein *RPM1* homologue
RRGA11-126	23454064	1488	+	Disease resistance protein *RPM1* homologue
RRGA11-127	23548316	1899	+	Putative NBS-LRR resistance protein
RRGA11-128	24002653	810	-	L-zip NBS-LRR (Acc No. AB0179114)
RRGA11-129	24031460	2808	-	Putative NBS-LRR resistance protein
RRGA11-130	24084783	3186	-	L-zip NBS-LRR (Acc No. AB0179114)
RRGA11-131	24282364	1812	+	Serine/threonine protein kinase
RRGA11-132	24292210	1194	+	Leucine-rich repeat protein
RRGA11-133	24360270	429	+	Putative NBS-LRR resistance protein
RRGA11-134	24367200	4335		Putative NBS-LRR resistance protein
RRGA11-135	24373384	4458	+	Putative NBS-LRR resistance protein
RRGA11-136	24434797	4326	+	Putative NBS-LRR resistance protein
RRGA11-137	24448925	714	+	Putative NBS-LRR resistance protein
RRGA11-138	24486073	3054	-	Putative NBS-LRR resistance protein
RRGA11-139	24750519	4032	-	Putative NBS-LRR resistance protein
RRGA11-140	24803273	564	+	Putative NBS-LRR resistance protein
RRGA11-141	24811569	2547	-	Putative NBS-LRR resistance protein
RRGA11-142	24862150	2784	+	Putative NBS-LRR resistance protein
RRGA11-143	24863120	1965	+	Putative NBS-LRR resistance protein
RRGA11-144	24918929	2439	-	Putative NBS-LRR resistance protein
RRGA11-145	24934534	864	+	Putative NBS-LRR resistance protein
RRGA11-146	24935024	2559	+	Putative NBS-LRR resistance protein
RRGA11-147	24939342	2919	+	Putative NBS-LRR resistance protein
RRGA11-148	24987968	1275	-	Putative NBS-LRR resistance protein
RRGA11-149	25312043	1047	-	Putative disease resistance protein
RRGA11-150	25473232	1632	+	Receptor kinase-like protein
RRGA11-151	25483144	1086	+	Serine/threonine protein kinase
RRGA11-152	25579954	1638	-	Receptor kinase-like protein
RRGA11-153	25603889	1647	-	Serine/threonine protein kinase
RRGA11-154	25605415	1614	-	Receptor kinase-like protein

Sl. no.	Starting position on pseudomolecule	Size of CDS	Dir	Predicted protein function
RRGA11-155	25646617	1743	+	Receptor kinase-like protein
RRGA11-156	25653392	927	+	Receptor kinase-like protein
RRGA11-157	25664055	918	+	Receptor kinase-like protein (*Arabidopsis*)
RRGA11-158	25670226	2982	+	Receptor kinase-like protein
RRGA11-159	25684637	2445	-	NBS-LRR (cyst nematode resistance protein)
RRGA11-160	25692631	699	-	NBS-LRR (cyst nematode resistance protein)
RRGA11-161	25738270	1371	+	Putative receptor-like protein kinase
RRGA11-162	25758821	1440	+	Putative receptor-like protein kinase
RRGA11-163	25884853	1452	+	S-receptor kinase
RRGA11-164	25968841	1923	+	NBS-LRR-like protein
RRGA11-165	25984033	3096	+	NBS-LRR-like protein
RRGA11-166	25992585	3099	+	NBS-LRR-like protein
RRGA11-167	26004611	2394	+	NBS-LRR-like protein
RRGA11-168	26042998	2094	+	NBS-LRR-like protein
RRGA11-169	26057956	2898	+	NBS-LRR-like protein
RRGA11-170	26064770	2520	+	NBS-LRR-like protein
RRGA11-171	26072862	2823	+	NBS-LRR-like protein
RRGA11- 172	26151007	2718	+	Putative disease resistance protein
RRGA11-173	26320517	1068	-	Putative cyst nematode resistance protein
RRGA11-174	26328704	1758	-	Putative cyst nematode resistance protein
RRGA11-175	26412798	2853	+	Putative disease resistance protein
RRGA11-176	26428166	2997	-	Putative disease resistance protein
RRGA11-177	26516051	3288	+	Putative disease resistance protein
RRGA11-178	26542493	1362	+	Putative disease resistance protein
RRGA11-179	26545017	1197	+	Putative disease resistance protein
RRGA11-180	26608731	2463	+	Putative resistance protein
RRGA11-181	26616504	1917	+	Putative disease resistance protein
RRGA11-182	26629250	1584	+	Putative disease resistance protein
RRGA11-183	26641770	954	+	Putative stripe rust resistance protein Yr10
RRGA11-184	26650935	1755	+	Putative stripe rust resistance protein Yr10
RRGA11-185	27233113	2529	+	Putative leucine-rich repeat-containing protein
RRGA11-186	27235152	576	+	Putative leucine-rich repeat-containing protein
RRGA11-187	27237951	3309	+	Putative leucine-rich repeat-containing protein
RRGA11-188	27280838	3186	+	*Oryza sativa* leucine-rich repeat-containing protein
RRGA11-189	27291194	2643	-	Putative leucine-rich repeat-containing protein
RRGA11-190	27299459	2256	-	*Oryza sativa* leucine-rich repeat-containing protein
RRGA11-191	27305107	3324	+	*Oryza sativa* leucine-rich repeat-containing protein
RRGA11-192	27315485	1359	-	Putative leucine-rich repeat-containing protein
RRGA11-193	27315523	3177	-	Putative leucine-rich repeat-containing protein
RRGA11-194	27467261	2475	+	Stripe rust resistance protein Yr10
RRGA11-195	27622480	3408	-	NBS-LRR protein

CDS, complementary DNA sequence