Theor Appl Genet (2007) 116:53–62
DOI 10.1007/s00122-007-0646-6

ORIGINAL PAPER

Evaluation of genes from *eIF4E* and *eIF4G* multigenic families as potential candidates for partial resistance QTLs to *Rice yellow mottle virus* in rice

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Received: 11 May 2007 / Accepted: 9 September 2007 / Published online: 27 September 2007

Abstract QTLs for partial resistance to *Rice yellow mottle virus* (RYMV) in rice were mapped in two populations of doubled-haploid lines (DHLs) and recombinant inbred lines (RILs) derived from the same cross but evaluated for different resistance criteria (virus content and symptom severity). An integrative map was used to compare the two genetic maps and a global analysis of both populations was performed. Most of the QTLs previously identified in DHL population were confirmed with increased significance and precision. As many recent studies evidenced the role of eukaryotic translation initiation factors (eIF) of 4E and 4G families in plant susceptibility to RNA viruses, we checked if these genes co-locate with QTLs of resistance to RYMV. Their systematic in silico identification was carried out on the rice genome and their physical locations were compared to QTL positions on the integrative map. In order to confirm or not the co-locations observed, the analysis was completed by evaluation of near-isogenic lines, QTL fine mapping and sequencing of candidate genes. Three members from *eIF4G* family could be retained as reliable candidates whereas *eIF4E* genes, commonly found to govern resistances in other plant/virus interactions, were discarded. Together with the recent identification of an *eIF(iso)4G* as a major resistance gene, data suggests an important role of genes from *eIF4G* family in rice resistance to RYMV but does not exclude the contribution of factors different from the translation initiation complex.

Communicated by Y. Xue.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-007-0646-6) contains supplementary material, which is available to authorized users.

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Introduction

Viruses are among the most agriculturally important and biologically intriguing groups of plant pathogens and genetic resistance can be used efficiently to protect crops from virus infection and to identify key factors in plant/virus interactions. RYMV is a Sobemovirus which causes a highly damaging rice disease in Africa (Kouassi et al. 2005). Whereas high resistance is very rare and monogenic, partial resistance is widespread in *Oryza sativa* upland *japonica* varieties and involves several quantitative trait loci (QTLs) (Ghesquière et al. 1997; Albar et al. 1998). In particular, two major QTLs mapped on chromosomes 1 and 12 were detected in different environments and for different resistance parameters. The effect of the QTL of chromosome 12 has been confirmed in the susceptible genetic background using a near-isogenic line (NIL) (Ahmadi et al. 2001).

Unlike major resistance genes, the factors that govern quantitative resistance of plants to viruses remain unknown and relatively few of them have been genetically mapped (Kang et al. 2005). Identification of the genes behind these QTLs has been described as the greatest challenge for geneticists in this century (Luo et al. 2002). In plant virus resistance, QTLs can be used alone or in combination with major genes and may improve resistance durability. In addition, their characterization will identify key genes for plant/virus interactions and, in particular, genes which would be difficult to identify by other approaches, like mutant screening. QTL positional cloning is of course

possible using NILs. However, this approach is often an expensive and long-term work due to the moderate effect of QTLs on phenotypic variation (Salvi and Tuberosa 2005). For this reason, the candidate gene approach may be the most powerful way to characterise QTLs (Pflieger et al. 2001). Some obvious candidates are the genes involved in major monogenic resistance. Indeed, qualitative and quantitative resistance may be governed by different allelic forms of the same factors and the effect of a factor on resistance may depend on the genetic background. For instance, different alleles of the *pvr2* resistance gene either lead to a major resistance or play a role in quantitative resistance to PVY in pepper (Ruffel and Caranta, unpublished). Moreover, the *pvr2*^{3} allele may trigger either complete or partial resistance to PVY depending on the genetic background (Ruffel and Caranta, unpublished). Thus, as more and more is known about genes conferring qualitative resistance to virus infections, the candidate gene approach has emerged as a promising method for identifying genes associated with virus resistance QTLs.

In recent years, the outstanding role of eucaryotic translation initiation factors (eIF) of 4E or 4G families has been demonstrated in several plant/virus interactions (for review, see Robaglia and Caranta 2006). Knockout mutants in these factors, as well as naturally occurring short deletions or amino-acids substitutions, lead to recessive resistance. Whereas the exact resistance mechanism remains unknown, it probably relies on a defective interaction between these host factors and viral components, such as the VPg (genome-linked viral protein). The recent characterization of a gene encoding an eukaryotic translation initiation factor eIF(iso)4G as the only gene, *Rymv*1, known to confer high resistance to *Rice yellow mottle virus* (RYMV) and the identification of three different resistance alleles underline the key role of such factors in resistance to RYMV (Albar et al. 2006). A part of our objective was to check if these factors could also be involved in partial resistance to RYMV in rice.

We refined the resistance QTL identification and we exploited the rice genomic sequence to identify in silico all the *eIF4E* and *eIF4G* multigenic family members. Then, the combination of different genetic and candidate gene approaches enabled us to evaluate the co-locations of some of those genes with QTLs and discuss about their potential status of candidate genes.

Materials and methods

Plant materials

RYMV partial resistance was analysed in two populations derived from the cross IR64 (*O. sativa indica*, susceptible) × Azucena (*O. sativa japonica*, partially resistant). The first population comprised 174 doubled-haploid lines (DHLs) (Guiderdoni et al. 1992). A genetic map of 210 RFLP markers has been developed on this population (Huang et al. 1997) and QTLs of partial resistance to RYMV have already been mapped (Albar et al. 1998; Pressoir et al. 1998). The second population comprised 178 recombinant inbred lines (RILs) and a genetic map of 226 single sequence repeat (SSR) markers was available (Dubreuil-Tranchant et al. 2005).

NILs were developed using two different strategies in order to analyse specific QTLs in more detail. First, NILs were derived from heterogeneous inbred families (HIFs), i.e. families still segregating for a target locus, selected from the F_7–F_9 generations of RILs screened with SSR markers, as described by Tuinstra et al. (1997). The second strategy consisted in a classical back-crossing process in order to introgress a target region from the donor parent into the recurrent parent. This process was previously used to develop a pair of NILs with IR64 versus Azucena alleles for the RYMV resistance QTL of chromosome 12 (IR64 versus NIL-QTL_{12}) in the IR64 background (BC_4 level) by Ahmadi et al. (2001). A population of 649 F_2 was derived from the cross NIL-QTL_{12} × IR64 in order to fine-map this QTL. A second introgressed line was developed in an Azucena resistant background to analyse a region of chromosome 1 which was thought to contain a resistance QTL.

Phenotypic evaluations

The DHL population has already been evaluated for partial resistance to RYMV (Albar et al. 1998). The virus content in plants measured 7 days after mechanical inoculation in a growth chamber by ELISA (VC2) was retained as the resistance criterion in the present study.

The other resistance evaluations performed in this study were based on symptom intensity. Plants cultivated in greenhouse were mechanically inoculated 10–15 days after sowing, as described in Ndjiondjop et al. (1999). Symptoms were observed between 7 and 35 days after inoculation, using a symptom severity scale. The Area Under Symptoms Progression Curve (AUSPC) was calculated as $AUSPC = \sum((S_i + S_{(i+1)})(T_{(i+1)}–T_i))/2$, where S_i corresponds to the symptom score at T_i date.

For the RIL population, the resistance evaluation assay consisted in two randomized complete trials. Each trial contained the 178 RILs, with six inoculated plants per line. As no trial effect was detected for controls, the AUSPC mean of the two tests was used for the analysis.

For HIF analysis, a single trial comprising six randomly laid out repetitions of four plants for each progeny was performed to compare the different NIL pairs.

For fine mapping of QTL_{12}, only the progenies of individuals recombined in the target region were evaluated to

determine their allelic status on the QTL. Evaluations were divided into five independent trials, each one containing two repetitions of ten plants of progenies to be tested and four repetitions of each control (IR64, NIL-QTL_{12} and the progeny of a non-recombinant heterozygous F_2). For each test, the most discriminating date between controls (depending on the test, between 7 and 16 days after inoculation) was retained for analysis. Given the expected heterogeneity of heterozygous plant progenies, the non-parametric Kolmogorov–Smirnov test was used to test the homogeneity of the score distribution obtained for different samples: first, the homogeneity between lines of the same genotype was confirmed ($P = 0.01$) and then each genotype to be tested was compared to the controls ($P = 0.001$). Statistical results were compared with the individual genotype to determine allelic status at QTL_{12}.

For introgressed line segregating for the QTL_1 region, progenies were compared in a single trial comprising four randomly laid out repetitions of six plants for each progeny.

DNA extractions and molecular markers

The DNA of DHLs has already been extracted by Albar et al. (1998). DNA of additional material was extracted from fresh leaves using the protocol described in Edwards et al. (1991). SSR markers were amplified and detected on LI-COR Genotyper as described by Albar et al. (2006).

Thirty-one SSR markers were used to complete available genetic maps (on QTLs or candidate gene regions for both DHL and RIL populations and on four marker-poor regions of the genetic map for the DHL population) and in the course of NIL development (Table S1). These markers are either published markers that have been experimentally tested (Albar et al. 2006; Temnykh et al. 2000, 2001; Panaud et al. 1996) or in silico designed markers (McCouch et al. 2002), or markers that have been newly designed on the Nipponbare sequence, as described by McCouch et al. (2002). In the latter case, primers were designed on SSR flanking regions by Primer3 (Rozen and Skaletsky 2000). Results obtained on ten markers designed by McCouch et al. (2002) and 50 markers newly designed in this study were very consistent, except for the QTL_{12} region: in both cases 80% gave an amplification product and respectively, 50 and 64% of them revealed polymorphism between Azucena and IR64. In the specific QTL_{12} region, only seven polymorphic markers were obtained from 26 markers which gave an amplification product.

Genetic map, QTLs and statistical analysis

An integrative map was used to compare and analyse both DHL and RIL populations. The map was based on the RIL genetic map and on different versions of the DHL genetic map (http://www.gramene.org/db/cmap/viewer): Cornell SSR 2001 (Temnyckh et al. 2000), IRRI IR64/Azu DH QTL 2003 (Ramalingam et al. 2003), Cornell IR64/Azu DH QTL 2001 (Temnykh et al. 2000) and CIRAD IR64/Azu DH QTL 2003 (Sallaud et al. 2003). SSR markers of the RIL genetic map were present on the rice Gramene and TIGR assembly of pseudo-molecules and served as framework markers to integrate other markers. As sequence information was available for most of the markers, they were mapped in silico and interpolated in their respective intervals spanned by the framework SSR markers. The markers that were not physically mapped were genetically interpolated. Mapping errors and slight order discrepancy could then be solved. More than 95% of the markers gave consistent projections on the integrative map and accurate positions between framework markers. The resulting genetic integrative map was therefore enriched by a considerable number of markers (1,729) in a physically correct order and with exact genetic correspondence (Dubreuil-Tranchant et al. 2005). Candidate genes and markers added in the current study were placed on this integrative map according to both their position on rice pseudo-molecules and to their genetic mapping using Mapmaker/Exp v3.0. In the DHL population, data from 17 markers, including ten markers of chromosome 6, did not fit with the integrative map and these markers had to be removed from the analysis. The resulting maps included 197 markers for the DHL population and 231 for the RIL population.

QTL analyses of single populations were performed using composite interval mapping with the Windows QTL Cartographer v2.5 program (http://statgen.ncsu.edu/~shchwang/).

An additional QTL analysis was performed in a multicross design on the data sets of both DHL and RIL populations and is henceforth referred to as the multicross analysis. The phenotypic data, AUSPC and VC2 in RIL and DHL populations respectively, were independently centred and normalised. Data were then analysed with the MCQTL software package (Jourjon et al. 2005), using the iterative QTL mapping method. The two populations were considered as derived from different parents.

The LOD threshold was set by calculating 1,000 permutations and an overall Type I error of 5%. Confidence intervals were delimited by the difference between 2-LOD and the maximum LODscores.

In silico analysis of genes from *eIF4E* and *eIF4G* families

Rice (*O. sativa*, Nipponbare) and Arabidopsis (*Arabidopsis thaliana*) genomes were screened in silico for genes from *eIF4E* and *eIF4G* families. An initial search was carried out on TIGR (http://www.tigr.org/tdb/e2k1/osa1/domain_search.shtml) and riceGAAS websites (http://ricegaas.

dna.affrc.go.jp/rgadb/) for rice, and on TAIR website (http://www.arabidopsis.org/) for *Arabidopsis*, using keywords (IF4E and MIF4G) or PFAM accessions (PF01652 and PF02854) corresponding to eIF4E and eIF4G central domains, respectively. In rice, TIGR annotations (release 4) prevailed over riceGAAS ones when two different annotations were found for the same locus. All the annotations we found were then used as queries for the TBLASTN search on TIGR and TAIR gene annotations. The annotations obtained were challenged using the SMART program (Letunic et al. 2006) and only those corresponding to predicted protein sequences with eIF4E and MIF4G domains were retained for further analysis. This data set was completed with protein sequences from other plants found by BLASTP searches on the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/) using the predicted protein sequences obtained in rice and *Arabidopsis* as queries.

Alignments of amino acid sequences were performed using the CLUSTALX program (Thompson et al. 1997). Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987) in CLUSTALX. The robustness of the nodes were assessed by bootstrap proportion analysis (Felsenstein 1985) computed after 1,000 replicates. The phylogenetic trees were edited using the TreeView v1.6.6 program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) and manually optimized for better viewing clarity.

Sequence analyses of the *eIF4E* and *eIF4G-like* (chr. 12) candidate genes

The cDNA of the *eIF4E* (chr. 1) and *eIF4G-like* (chr. 12) candidate genes were sequenced in IR64 and Azucena varieties. Total RNA was isolated from leaf tissue with TRIzol reagent (Invitrogen, Paisley, UK). Retrotranscription was performed with an oligo(dT) primer, using ImproII retrotranscriptase (Promega) and the protocol recommended by the manufacturer. Sequencing reactions were carried out with Applied Biosystems BigDye terminator and analysed on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). The sequences are registered in the EMBL data library under accession numbers AM411440 (IR64) and AM411441 (Azucena) for *eIF4E* and AM778049 (IR64) and AM778048 (Azucena) for *eIF4G-like*.

Results

QTL analysis of partial resistance to RYMV

The genetic bases of partial resistance to RYMV were studied in the IR64 × Azucena DHL and RIL populations (Fig. 1, Table S2). Compared to the study of Albar et al. (1998), no additional QTL was detected in the DHL population despite the improved genetic map. QTLs for symptoms from the RIL population were located in the regions of QTLs for virus content from the DHL population, suggesting that the same factors underlie these two traits thus justifying the multicross analysis. However, new minor QTLs ($R^2 < 6\%$) were identified on chromosomes 6 and 7 in the RIL population and were confirmed in the multicross analysis. In addition, multicross analysis enabled the detection of a minor QTL ($R^2 = 2.7\%$) on chromosome 4. For all QTLs, the favourable allele for resistance came from the partially resistant parent Azucena.

The QTLs of chromosomes 1 (QTL_1), 2 and 12 (QTL_{12}) were confirmed as the most important ones and the multicross analysis enabled us to identify their locations more precisely (Fig. 1, Table S2). Whereas the addition of new

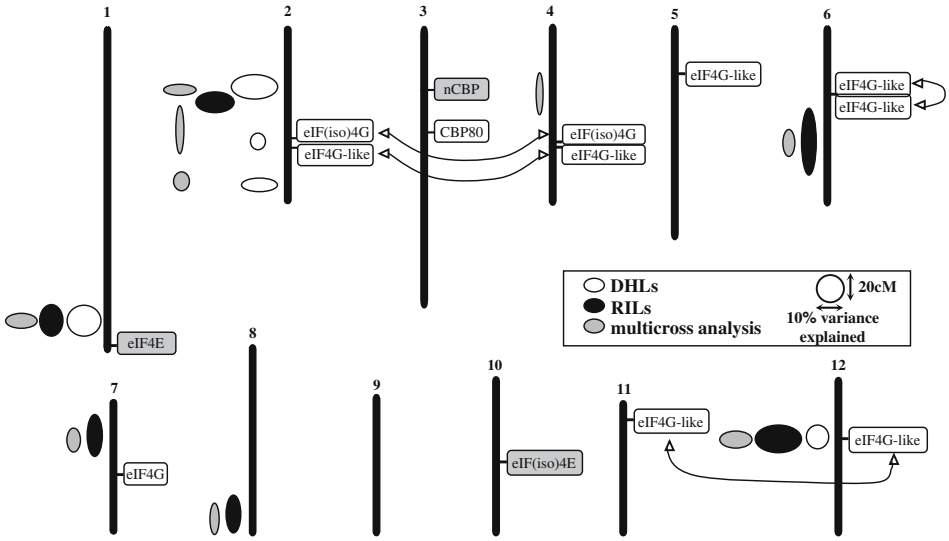

Fig. 1 Genetic locations of QTL positions (*left*) and gene annotations from *eIF4E* and *eIF4G* families (*right*) on the integrative map. QTLs of DHL, RIL and multicross analyses are represented by filled ovals covering a 2-LOD confidence interval in height and proportional to phenotypic variation explained in width. The *double-arrows* symbolise suspected duplications

markers only slightly reduced the QTL confidence interval in the DHL population, the multicross analysis appeared to reduce it greatly. For instance, the confidence interval of QTL_1 was reduced from 20 or 23 cM in the DHL and RIL populations, respectively, to 11 cM in the multicross analysis. A similar result was observed for QTL_{12}, with a confidence interval reduced from 17 or 20 to 12 cM. As previously observed (Albar et al. 1998), the role of chromosome 2 in resistance to RYMV appears to be complex. Three different intervals contain QTLs. In the first one, QTLs were found in DHL and RIL populations and by the multicross analysis. Whereas we cannot exclude that the QTLs identified in the RIL and in the DHL population are distinct, the partial overlapping of confidence intervals suggests a single QTL may be involved in both populations. According to this hypothesis, and as observed for QTL_1 and QTL_{12}, multicross analysis would reduce the confidence interval of this QTL. The last two QTLs were detected in the DHL population but not in the RIL population and were still significant in the multicross analysis. In these cases, the confidence intervals increased in the multicross analysis.

Identification of genes from *eIF4E* and *eIF4G* families in rice genome

Rice genomic sequence (*O. sativa* ssp. *japonica* cv. Nipponbare) was analysed to identify genes from *eIF4E* and *eIF4G* families and to compare them to their homologs in *Arabidopsis* and in other plants.

Three putative rice genes were identified in the *eIF4E* family. All were supported by cDNA or EST. Their predicted protein sequences were compared to those of five putative *Arabidopsis* genes and of 12 genes from other plants, including five *eIF4E* genes reported to be involved in virus resistance (Fig. 2a). Three groups corresponding to *eIF4E*, *eIF*(*iso*)*4E* and new cap binding protein genes were identified. One rice gene was identified in each group: rice *eIF4E* maps on chromosome 1, *eIF*(*iso*)*4E* on chromosome 10, and the novel cap-binding protein gene on chromosome 3.

Eleven putative rice genes were identified in the *eIF4G* family. All were supported by cDNA or EST. Their predicted protein sequences were compared to those of nine putative *Arabidopsis* genes and wheat *eIF*(*iso*)*4G* (Fig. 2b). Clustering with previously described genes enabled the identification of one rice *eIF4G* gene (Os07g36940) on chromosome 7, and two rice *eIF*(*iso*)*4G* genes (Os02g39840 and Os04g42140) on chromosomes 2 and 4, the latter corresponding to the major resistance gene *Rymv*1 (Albar et al. 2006). The eight remaining predicted rice proteins of the eIF4G family were distant from eIF4G and eIF(iso)4G groups. They are henceforth referred to as *eIF4G-like*. Although they appeared to be distributed in five groups, the trichotomy feature and a weak bootstrap value (476) impaired reliable clustering. *Arabidopsis* ortholog genes were found in all groups except one. eIF4G-like predicted proteins generally shared homology with proteins involved in RNA machinery or possessed domains characteristic of such proteins, as for instance MA3 domain (PF02847) from eIF4G family (Pontig 2000), or UPF2 domain (PF04050) involved in regulation of nonsense transcripts (Mendell et al. 2000) (Fig. 2b).

Genomic distribution of homologous *eIF*(*iso*)*4G* and *eIF4G-like* genes suggested ancestral duplications depending on the organisation of the rice genome (Fig. 1). First, a tandem duplication of an *eIF4G-like* gene might have occurred on chromosome 6. Second, another duplication was suspected between two homologous *eIF4G-like* genes on chromosomes 11 and 12. Third, regions of about 1.2 Mb containing both *eIF*(*iso*)*4G* and *eIF4G-like* genes were identified on chromosome 2 and on chromosome 4. They are the result of the extensive duplication between these chromosomes (Guyot and Keller 2004), which might have been preceded by an initial tandem duplication.

Positional relationships between RYMV resistance QTLs and genes from *eIF4E* and *eIF4G* families

The locations of the QTLs were compared to those of genes from *eIF4E* and *eIF4G* families. Three interesting co-locations were detected and concerned the QTLs of chromosomes 1, 2 and 12 (Fig. 1). First, QTL_1 mapped near the translation initiation factor gene *eIF4E*, largely involved in plant/virus interactions, and near the semi-dwarfing gene *sd-1*, previously reported as a possible candidate (Albar et al. 1998) (Fig. 4a). Second, two candidate genes, *eIF*(*iso*)*4G* and *eIF4G-like*, mapped in the confidence interval of a minor QTL of chromosome 2. Finally, another *eIF4G-like* gene was located inside the confidence interval of QTL_{12}.

NIL-based verification of QTL positions and co-locations with candidate genes

In order to confirm the co-locations observed between QTLs and candidate genes or to obtain more precise QTL positions, we developed NILs differentiated by QTL flanking regions or, more specifically, by candidate gene alleles using HIFs or a classical backcross process.

Concerning the co-location of chromosome 2, one HIF segregating for *eIF4G-like* but not for *eIF*(*iso*)*4G* was obtained but evaluation of this HIF for RYMV symptom development did not reveal significant difference between parental alleles (Student's *t* test $P > 0.05$).

Concerning the co-location of chromosome 12, two HIFs segregating for *eIF4G-like* were obtained. Signifi-

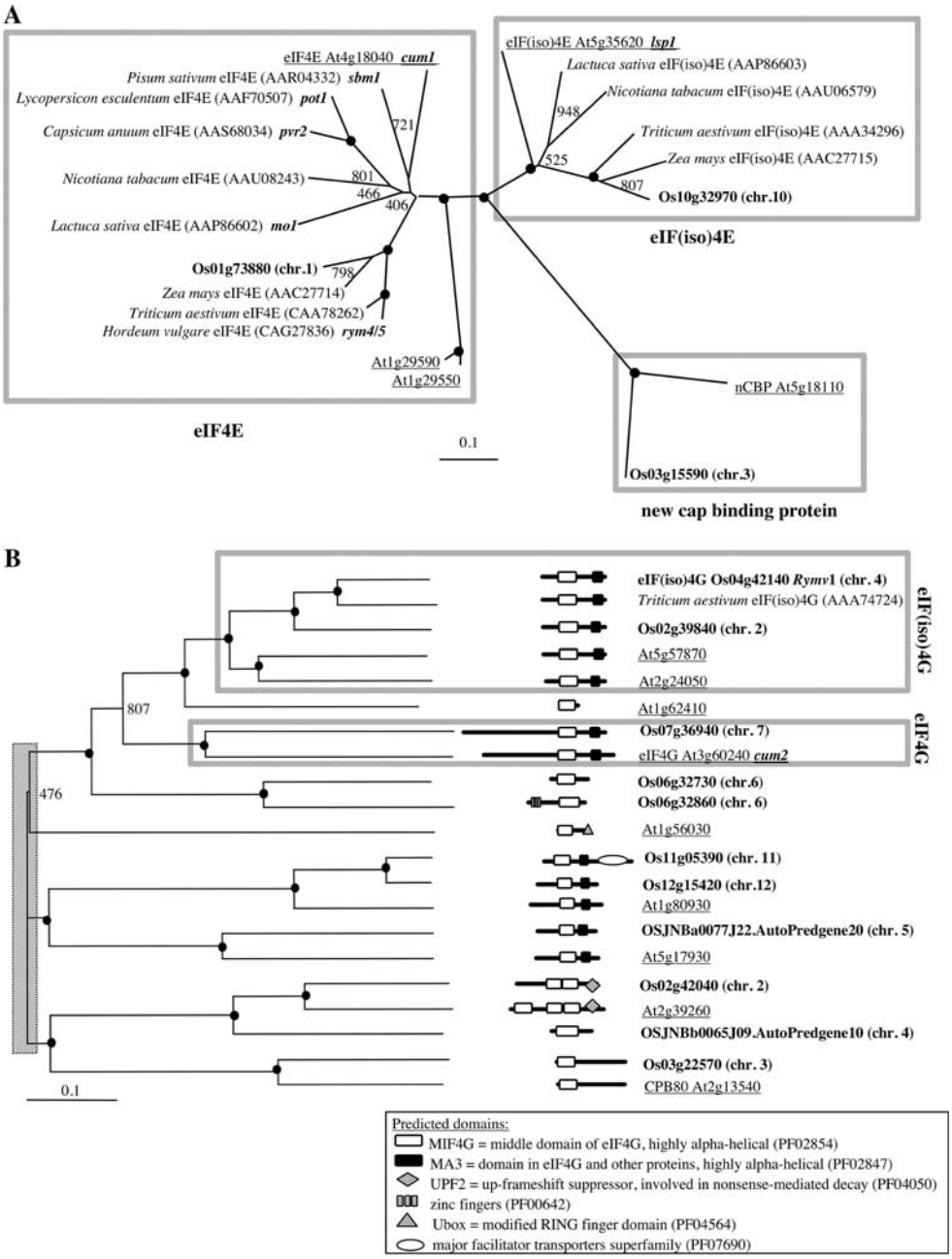

Fig. 2 Relationships between genes from *eIF4E* (**a**) and *eIF4G* (**b**) families in plants. The neighbor-joining unrooted tree and bootstrap proportions using 1,000 replicates were obtained from a total protein alignment. Bootstrap values higher than 950 are represented as *black dots*. Accession numbers are given and virus resistance genes or mutant names are in *bold*. Rice and *Arabidopsis* gene annotations are respectively in *bold* and *underlined*. The rice chromosome that carries the gene annotation is also indicated. Concerning the *eIF4G* family (**b**), predicted protein sequences and domains are represented. The *grey* zone on the phylogenetic tree indicates architecture with a trichotomy and a low boostrap value (476)

cant differences (Student's t test $P < 0.05$) for RYMV symptoms evaluation were observed in these families (Fig. 3a), confirming the co-location of *eIF4G-like* and QTL_{12}. A NIL of the IR64 variety introgressed with the Azucena allele in the QTL_{12} region (NIL-QTL_{12}) has already been developed and confirmed the effect of QTL_{12} in the IR64 background (Ahmadi et al. 2001). This line was used to fine-map QTL_{12}. Marker data indicated that Azucena introgression was contained in an 8.2 Mb interval, between markers RZ816 and AB25 (Fig. 3b). Then 649 F_2 derived from the cross IR64 × NIL-QTL_{12} were screened with five SSR markers defined in the introgression. Forty individuals recombined between markers AB1 and AB28 located at the extremities of the target interval. Their progenies were evaluated for resistance to RYMV. Phenotype was clearly established for 23 progenies which indicated that QTL_{12} was located in a 2.23 Mb interval between markers MV4 and AB28 (Fig. 3b). The maximum LODscore observed in the multicross analysis and the *eIF4G-like* candidate gene still mapped within this candidate region. The mapping strategy thus confirmed this *eIF4G-like* gene as a candidate for QTL_{12}. The coding region of *eIF4G-like* gene was sequenced on the cDNA of IR64 and Azucena. It corresponded to 2,364 nucleotides in Azucena and to 2,304 nucleotides in IR64. Five InDels and four substitutions, located in the first exon before the

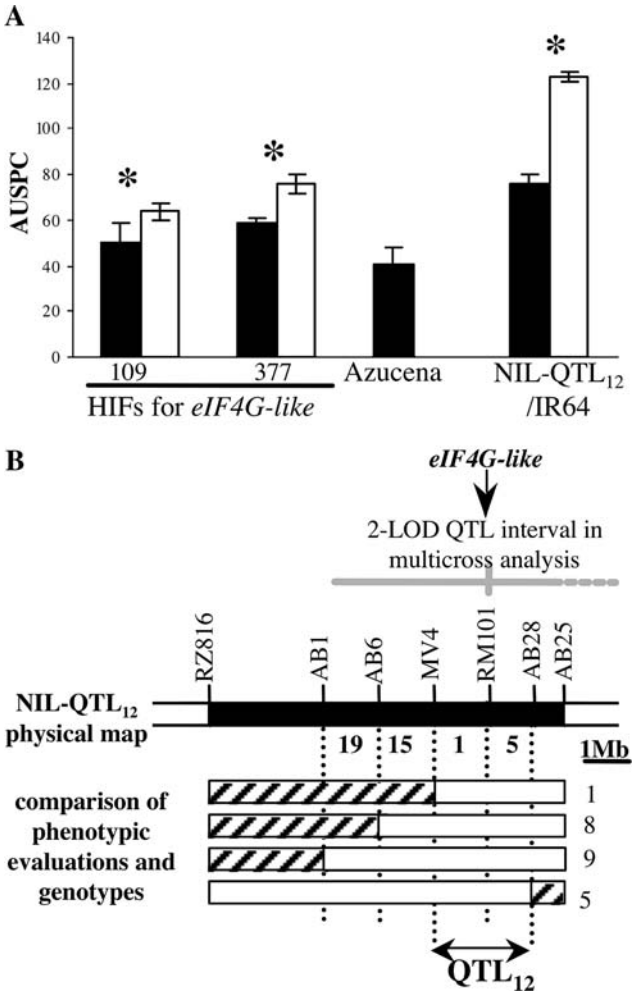

Fig. 3 QTL_{12} characterization **a** RYMV symptom evaluations of HIFs developed for *eIF4G-like* and introgressed line for the QTL_{12} region (NIL-QTL_{12}). *Black and white bars* show Azucena and IR64 alleles at the target locus, respectively. Confidence intervals are given. *Asterisks* indicate a significant difference (Student's *t* test, $P < 0.05$) between lines of Azucena versus IR64 alleles at the candidate gene or locus for QTL_{12}. **b** Fine genetic and physical mapping of the QTL_{12} region. The upper part of the figure is a physical representation of the QTL_{12} region in NIL-QTL_{12}, where the most informative markers are indicated and the Azucena introgressed fragment is coloured *black*. The positions of the *eIF4G-like* candidate and of the confidence interval for QTL_{12} obtained in the multicross analysis are shown. The number of recombinants observed in the NIL-QTL_{12} × IR64 population are indicated under the corresponding intervals. The lower part of the figure represents the physical fragments accepted (in *white*) or rejected (*hatched*), as QTL_{12} containing fragments, based on the comparison of phenotype and genotype for one to nine recombinants per interval, as indicated on the right. All data are in agreement with the location of QTL_{12} between MV4 and AB28 markers

MIF4G domain, distinguished Azucena from IR64 predicted amino-acids sequences (data not shown).

Concerning the co-locations of chromosome 1, two HIFs were obtained for *sd-1* and two for *eIF4E* (Fig. 4a). Evaluation of RYMV symptoms revealed no significant differences between parental alleles for each of these HIFs (Student's *t* test $P > 0.05$) (Fig. 4b), which did not support the involvement of *eIF4E* and *sd-1* genes in the effect of QTL_1. In addition, sequencing of the coding region of the rice *eIF4E* gene did not reveal any nucleotidic polymorphism between Azucena and IR64, and no variation in transcript accumulation was detected by semi-quantitative RT-PCR for this gene (data not shown). These results do not support *eIF4E* gene as a factor governing QTL_1. As the above results led us to think that QTL_1 was located between *sd-1* and *eIF4E* genes, we performed two successive backcrosses from a RIL with Azucena recurrent parent to introgress this candidate region from IR64 in an Azucena background. A preliminary experiment managed directly on BC_2F_2 individuals suggested an effect of QTL_1 on resistance to RYMV only in interaction with QTL_{12} (data not shown). Next we selected a plant (BC_2F_1-hQTL_1) heterozygous both for QTL_1 and QTL_{12} regions (Fig. 4a) to evaluate the respective effect of both QTLs on resistance. Two to four BC_2F_3 progenies were tested for each category of the BC_2F_2 genotype (Fig. 4b). On the one hand, as expected, the effect of QTL_{12} was significant whatever the allelic status on QTL_1 region (Fig. 4b) (Student's *t* test $P = 0.007$ for IR64 allele on QTL_1 and $P = 0.013$ for Azucena allele). On the other hand, the effect of QTL_1 was significant in progenies homozygous for the IR64 allele on QTL_{12} ($P = 0.007$) but not for progenies homozygous for the Azucena allele on this QTL ($P = 0.427$) (Fig. 4b), confirming the previous observation. These results are in agreement with the duplicate epistasis observed by Pressoir et al. (1998) between QTL_1 and QTL_{12} regions for the impact of RYMV inoculation on plant height and heading date. Thus, our results suggest that QTL_1 is controlled by a genetic factor located between *sd-1* and *eIF4E* genes, which could be evidenced only when the susceptible allele was present on QTL_{12}.

Discussion

This study refined the QTL analysis of partial resistance to RYMV in rice and investigated if genes from *eIF4E* and *eIF4G* families participate in the control of this resistance. For this purpose, we combined QTL analysis with a candidate gene approach. QTL analysis can only reveal loci and the underlying genes for which polymorphism between the parents of the population studied is linked to differential expression of the trait. For instance, *eIF(iso)4G* is known to be linked to resistance to RYMV in rice (Albar et al. 2006) but this gene does not co-locate with a QTL in IR64 × Azucena population. Similarly, other rare polymorphisms may be involved in partial resistance but may not exist between IR64 and Azucena parents. However, partial

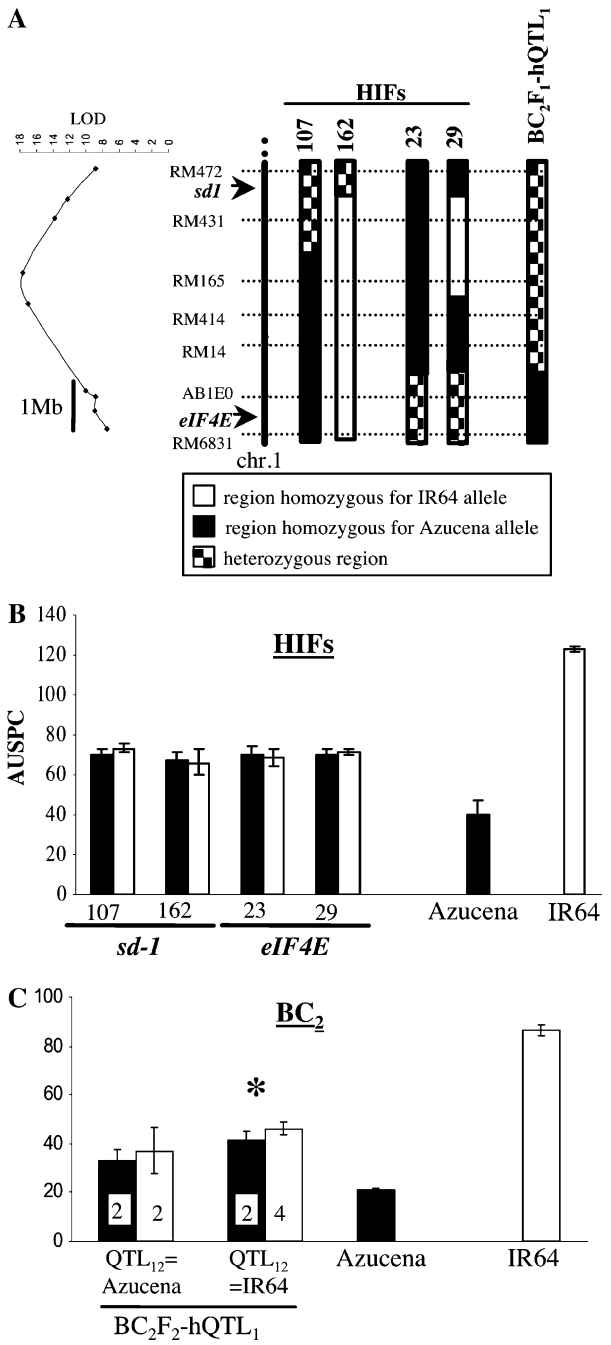

Fig. 4 Characterization of QTL_1. **a** Representation of the genotypes of lines developed for characterization of QTL_1. The LODscore curve of multicross analysis and the positions and the names of candidate genes and genetic markers are indicated. **b** Evaluation of RYMV symptoms in HIFs and in lines developed from BC_2F_1-$hQTL_1$. *Black and white bars* show Azucena and IR64 alleles at the target locus, respectively. Confidence intervals are given. *Asterisks* indicate a significant difference (Student's *t* test, $P < 0.05$). For BC_2F_1-$hQTL_1$ evaluation, both genotypes at QTL_1 and QTL_{12} were considered and two to four progenies were tested for each category of genotype, as indicated inside the bars

resistance to RYMV is characteristic of *O. sativa japonica* subspecies and considering the genetic structuration of *japonica* and *indica* subspecies into *O. sativa* (Glaszmann 1987), a single *indica* × *japonica* cross, such as IR64 × Azucena, is assumed to maximise polymorphism and to sample most of the genes underlying partial resistance to RYMV.

QTL mapping is often limited by the number of individuals analysed; it may impair the detection of minor QTLs and the precise location of major ones. For these reasons, we improved QTL mapping in DHL and RIL populations by analysing them together. This multicross analysis required a combination of both genetic and phenotypic data. Concerning the mapping strategy, problems were generally encountered when comparing QTLs obtained on different genetic maps due to the difficulties in aligning the maps. Recently several mapping tools were developed to compare genetic maps, such as Biomercator (Arcade et al. 2004), cMap (Fang et al. 2003) and CMTV (Sawkins et al. 2004). A similar approach was performed manually here to align the different rice genetic maps with pseudo-molecules and to construct an integrative map. This map was a quite powerful tool to compare the QTLs in our populations and to directly map candidate genes to identify co-locations with QTLs. Concerning the phenotypic data, resistance to RYMV was evaluated based on the virus content in the plant in the DHL population and on symptom development in the RIL population. The combination of these two criteria in the multicross analysis supposes that the same genetic factors control both of them. However, this is not always the case. For instance, the *Arabidopsis SBB1* locus is involved in symptom development after *Spring beauty latent virus* (SBLV) infection but has no effect on virus accumulation (Fujisaki et al. 2004). Few data are available on the comparison of QTLs of virus content and symptom development. Our previous data on a collection of rice varieties (unpublished results) and on the DHL population (Albar et al. 1998) strongly suggested these two traits are correlated. Moreover, in this study, QTL mapping in DHL and RIL populations on the integrative map pinpointed exactly the same loci on chromosomes 1 and 12. We thus considered that these resistance criteria were partially controlled by the same major QTLs. The multicross analysis enabled considerable more precision in the confidence intervals for major QTLs on chromosomes 1 and 12. Yet, results obtained in single populations suggest a complex role of chromosome 2 in resistance to RYMV and results of the multicross analysis on this chromosome should be interpreted with caution, as different QTLs might control the two resistance criteria. Precise dissection of the QTLs of this region would require the production of several set of NILs, so that the segregating regions successively overlap and cover the whole of chromosome 2. The detection in multicross analysis of minor QTLs that were not detected in the DHL population could also be explained by increased power of detection associated with the analysis of a larger

number of individuals. Multicross analysis may be thus an appropriate tool to detect QTLs with minor effects and to improve QTL confidence intervals.

It is generally believed that QTL mapping does not accurately locate genes that explain polygenic traits on the genome. However, based on several publications, Price (2006) recently reported that QTL position is accurate to within 2 cM or less for major QTLs and to within 3 cM or less for minor QTLs. So, we can be relatively confident about the positions of the QTLs, especially those obtained by multicross analysis, and on co-locations between QTLs and underlying candidate genes. In order to further verify (or not) the co-locations observed, the step following QTL mapping was the study of NILs which segregate for the target region. In this study, the target regions were either candidate QTL regions, candidate genes, or both. It should be noted that this is the first time that the development of HIFs is directly based on candidate genes. Introgressed lines and HIF strategies both proved to be reliable for QTL_{12} in this study. However, the absence of a significant difference in a family does not necessarily imply that the target region does not control a variation in the character. Indeed, as already highlighted by Tuinstra et al. (1997) and as suggested here by evaluation of BC_2F_2-$hQTL_1$, genetic background may influence the detection of a QTL effect. Thus, it would be better to analyse a candidate gene or region for a QTL in a relatively large set of HIFs, if available.

In this study, it is the relevancy of different approaches, namely, QTL detection in different mapping populations, NILs development and sequence data, which can converge to declare or not a peculiar gene as a reliable candidate gene. For QTL_1, the global information seems very consistent to discard *eIF4E* as a candidate gene. Combined with the absence of QTL detection on chromosomes that carry *eIF*(*iso*)*4E* and the new cap-binding protein gene, the results do not support a major involvement of genes from *eIF4E* family in QTLs of partial resistance to RYMV. It is probably more difficult to conclude at this stage for the chromosome 2 and the two candidate genes from *eIF4G* family, since the reproducibility of QTL detection between the two mapping populations was only partial. Nevertheless, because of the orthology (one of them at least) with *Rymv*1 gene, we can provisionally retain these genes as candidates, which will require further confirmation by classical NILs development and evaluation. Last, an *eIF4G*-like gene was confirmed as a candidate for QTL_{12} by HIF evaluation and by mapping to a candidate region of 2.23 Mb. This gene may have no function in translation initiation but the polymorphisms between the amino-acids sequences of the two parents are encouraging to consider this gene may be involved in RYMV partial resistance by its MIF4G or MA3 domain in a similar way as *Rymv*1 gene. The aim of our next study will be to further validate this co-location by continuing fine genetic mapping and by studying the *eIF4G-like* candidate gene.

Finally, in addition to the role of *eIF*(*iso*)*4G* (chr.4) in high resistance (Albar et al. 2006), we have identified three other members of the *eIF4G* family which can be reasonably considered as good candidates to focus the genetic studies later on, while members of the *eIF4E* family do not seem directly involved in QTLs of partial resistance to RYMV. Moreover, this analysis gives large room for other factors explaining partial resistance, in particular for QTL_1, and highlights the diversity of plant/virus interactions. It would be of great interest to identify these factors to see if, like other eIF or the poly-A binding protein, they play a role in the translation initiation complex or if they are involved in other functions. In all cases, characterization of the QTLs involved in partial resistance to RYMV would provide new insights in plant/virus interactions.

Acknowledgments This work was supported in part by the GENOPLANTE program TRANSVIR. Arnaud Boisnard was supported by a fellowship from the French Ministry of Research and Deless Thiémélé by a fellowship from the French-speaking University network (AUF). We gratefully acknowledge Thierry Mathieu and Sophie Perez for plant care and Nour Ahmadi for having provided us genotypes data of RIL population. Stéphane Jouannic's help with protein sequence alignment and phylogenetic tree was greatly appreciated. We thank Romain Guyot and Mathias Lorieux for critical reviews of the manuscript.

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