A Major Quantitative Trait Locus for Rice Yellow Mottle Virus Resistance Maps to a Cluster of Blast Resistance Genes on Chromosome 12

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ABSTRACT

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Two doubled-haploid rice populations, IR64/Azucena and IRAT177/Apura, were used to identify markers linked to rice yellow mottle virus (RYMV) resistance using core restriction fragment length polymorphism (RFLP) maps. Resistance was measured by mechanical inoculation of 19-day-old seedlings followed by assessment of virus content by enzyme-linked immunosorbent assay tests 15 days after inoculation. IR64/Azucena and IRAT177/Apura populations, 72 and 43 lines, respectively, were evaluated, and resistance was found to be polygenic. Resistance was expressed as a slower virus multiplication, low symptom expression, and limited yield loss when assessed at the field level. Bulked segregant analysis using the IR64/Azucena population identified a single random amplified polymorphic DNA marker that mapped on chromosome 12 and corresponded to a major quantitative trait locus (QTL) evidenced by interval mapping. When pooling RFLP data, integrated mapping of this chromosome revealed that the QTL was common to the two populations and corresponded to a small chromosomal segment known to contain a cluster of major blast resistance genes. This region of the genome also reflected the differentiation observed at the RFLP level between the subspecies *indica* and *japonica* of *Oryza sativa*. This is consistent with the observation that most sources of RYMV resistance used in rice breeding are found in upland rice varieties that typically belong to the *japonica* subspecies.

Rice yellow mottle virus (RYMV) is the most rapidly spreading disease of rice (*Oryza sativa* L.) in Africa. This virus was first reported in Kenya by Bakker (5) and is now prevalent throughout Africa including Madagascar (3,10). RYMV is a sobemovirus (17, 28). In the field, RYMV is essentially transmitted by chrysomelid beetles (*Chaetocnema* spp., *Sesselia pusilla*) (4). Infected plants are characterized by mottle and yellowing symptoms; stunting, malformation, and partial emergence of panicles; and sterility. Yield losses of near 100% were reported in highly susceptible varieties (11). Since many grasses and African wild rice species like *O. longistaminata* A. Chev. et Roehr. are natural reservoirs of RYMV (5), this virus, which is absent from Asia, is thought to have originated in Africa (38). During the last decades, the introduction of high-yielding rice varieties from Asia and the intensification of rice cultivation, particularly through extended irrigation, may have favored RYMV spread. Most lowland varieties now grown in West Africa are susceptible to infection. As RYMV is highly infectious, vector control by chemical treatments and cultural practices are likely to be of limited use (11). Selection of resistant varieties seems to be the most promising way to prevent RYMV spread under irrigated conditions.

The recent development of saturated rice genetic linkage maps has provided the basis to localize single genes and quantitative trait locus (QTL) conditioning important agronomic traits via linkage to restriction fragment length polymorphism (RFLP) markers (6,20). Several genes conferring resistance to rice blast (*Magnaporthe grisea*), bacterial blight (*Xanthomonas oryzae*), and several insects (gall midge, green leafhopper [GLH], and whiteback planthopper) have already been tagged in rice (6). This can improve the efficiency of conventional breeding in transferring resistance in rice varieties by marker-aided selection. Moreover, identification and fine mapping of resistance is a prerequisite for the map-based cloning of single genes (32,36). In this study, we report the first observations on the inheritance of RYMV resistance derived from the analysis of two doubled-haploid (DH) populations, and we attempt to identify and map RFLP and random amplified polymorphic DNA (RAPD) markers linked to RYMV resistance.

MATERIALS AND METHODS

Plant material. Two DH populations derived from F_1 anther culture were analyzed to characterize the inheritance of resistance to RYMV. The IR64/Azucena cross was developed at the International Rice Research Institute (IRRI) and involved the elite *indica* line IR64 and the traditional *japonica* variety Azucena from the Philippines (14). The second population, IRAT177/Apura (13), was generated between the upland variety IRAT177, released by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), and Apura, a variety with exceptional long grain quality. The parents of these two crosses gave contrasting patterns of RYMV resistance in earlier evaluations by enzyme-linked immunosorbent assay (ELISA) tests and symptom scoring (31). Azucena and IRAT177 showed the highest resistance level observed in *O. sativa*, while IR64 and Apura were very susceptible. Therefore, the two DH populations provided the opportunity to perform a multiple evaluation of resistance at different levels and assess the genetic basis of RYMV resistance. IR64/Azucena (74 DH lines) and IRAT177/Apura (48 DH lines) were

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tested for RYMV resistance. Additionally, a core RFLP map on 135 IR64/Azucena DH lines was developed by Huang et al. (16). For the IRAT177/Apura population, RFLP data were available from Cornell University (Ithaca, NY), but limited polymorphism between parents, small population size, and strong segregation distortions hampered the construction of a reliable map (23).

Evaluation of resistance through serological tests. As the resistance level is estimated by the content of virus in plants, which is measured by a serological test, the terms 'resistance' and 'susceptibility' reflect the ability to reduce the multiplication and spread of the virus. The dynamics of RYMV titer was studied in IR64 and Azucena between 14 and 87 days postinoculation. This preliminary experiment permitted us to determine the optimal reading time to get contrasting results between the parents.

Plants were grown in a glasshouse under controlled conditions (28 to 32°C, 13 h of light per 24 h). They were mechanically inoculated 19 days after sowing. The virus isolate used in this experiment was initially collected in Mali, propagated on the variety BG90-2, and then stored at –80°C. One gram of infected leaves was ground in 10 ml of phosphate buffer (0.1 M, pH 7.2) containing Carborundum. Experiments included two replicates of each DH line (10 to 15 plants per replicate), the parents of the respective crosses, and the highly susceptible line BG90-2 as a control. Following the preliminary experiment, 14 days after inoculation was the reading time adopted for the virus concentration assessment. At that time, the new emerging leaves were ground in 0.05 M carbonate buffer at pH 9.6 at different dilutions (1 g of leaves per 10 ml of buffer to 1 g per 1,000 ml).

The isolate of RYMV also was purified to prepare a polyclonal antiserum following the protocol of Fauquet and Thouvenel (10). The antigen-coated plate ELISA method was performed essentially as described by Clark and Adams (8). A volume of 100- or 200-µl samples was incubated in microtiter plates. Except for "saturation" and substrate incubation, incubations were 2 or 4 h at 37°C or overnight at 4°C. Between steps, microplates were washed three times with 0.15 M phosphate buffer (pH 7.2) containing 0.05% Tween (PBS-T). Wells were saturated with milk solution (3 g of powdered milk per 100 ml of PBS-T) and then incubated 1 h at 37°C. Antibodies from polyclonal serum were diluted 1 in 10,000 or 1 in 65,000 in PBS-T and added to the plate. Bound antibodies were detected with antiglobulin conjugate (goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase, 1/2,000; Sigma Chemical Co., St. Louis). Specific antigen-antibody reactions were assessed by adding *p*-nitrophenyl phosphate (Sigma Chemical Co.) at 1 mg/ml in diethanolamine buffer (pH 9.8). Absorbance at 405 nm was measured after 15 to 30 min of incubation.

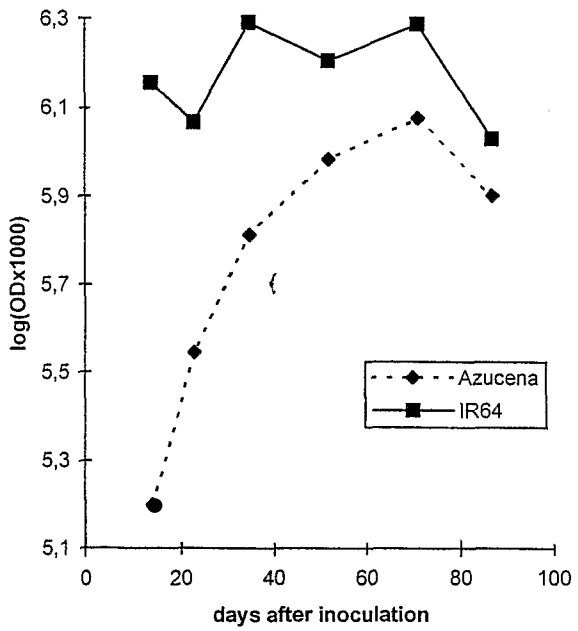

Fig. 1. Dynamics of the rice yellow mottle virus titer as evaluated by enzyme-linked immunosorbent assay and expressed in log(optical density × 1,000) in the rice varieties IR64 and Azucena. Plants were inoculated 19 days after sowing, and new emerging leaves were harvested 14, 23, 35, 50, 71, and 87 days after inoculation.

Field evaluation of resistance. IR64/Azucena and IRAT177/Apura, 28 and 13 DH lines, respectively, were analyzed in a replicated field trial at the Sikasso Research Station (Mali) of the Institut d'Economie Rurale (IER). This subset involved resistant and susceptible DH lines selected for bulked segregant analysis (26), as well as other DH lines showing contrasting patterns of virus titer. The experimental design included three replicates, and each DH line was represented by two rows with five plants per row. The first row was mechanically inoculated with a local RYMV isolate 10 days after transplanting, while the second row served as a control. A local isolate was used instead of the reference isolate to avoid introduction of an isolate possibly different from the one present in the experimental area. Nevertheless, preliminary experiments using this local isolate on parents and other varieties indicated a pattern of resistance similar to the reference isolate. The following observations were made during the experimentation: plant height and number of tillers at two growing stages (40 days post-inoculation and plant maturity), heading date, number of grains per panicle, fertility, and grain weight. For each parameter, DH lines were characterized by the ratio between the data obtained with inoculated plants and with noninoculated plants. The resulting data were subjected to principal component analysis. In addition, symptoms were noted 20 and 40 days after inoculation following the rating scale used for RYMV resistance assessment in international testing nurseries (19). This scale ranged from 0 to 9 and is based on the degree of foliar discoloration and on the alteration of plant development. A rating of 0 corresponds to healthy plants, 1 to the presence of virus but absence of symptoms, and 3 to 9 to increasing yellowing and stunting. Ratings of 0 and 1 were indistinguishable without an ELISA test. As the virus multiplied in all the DH lines, only scores 1 to 9 were given. The five plants of each row were noted independently, and an average value was calculated.

DNA extraction and RFLP analyses. DNA extraction was based on the procedure of Dellaporta et al. (9) using freshly harvested leaves ground in liquid nitrogen or following laboratory protocols of the International Maize and Wheat Improvement Center (CIMMYT) using ground lyophilized leaves (15). The RFLP map of the IR64/Azucena population, based on 135 lines, served as a reference for marker analysis, and additional marker data were generated for all 183 DH lines available. Seven restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Sca*I, *Xba*I, and *Hae*III [Boehringer GmbH, Mannheim, Germany]) were used to test polymorphism between parental lines. Five micrograms of DNA was digested with the appropriate enzyme, and fragments were separated on 0.8% agarose gels in a 89 mM Tris, 89 mM borate, and 2 mM EDTA buffer at pH 8.0. DNA was transferred to nylon membranes (Boehringer GmbH) in either 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) or 0.4 M NaOH following the procedure described by Southern (37). Filters were baked for 20 to 30 min at 120°C to ensure complete fixation of transferred DNA on the membranes. Probes were obtained from Cornell University (6). "RG" clones were from a rice genomic library, and "RZ" and "CDO" clones were from rice and oat cDNA libraries, respectively. Inserts were amplified and labeled with digoxigenin-labeled dUTP (Boehringer GmbH) using the polymerase chain reaction (PCR) and standard M13 F+R primers (Stratagene Inc., La Jolla, CA). Prehybridization for 4 to 6 h and overnight hybridization were performed at 65°C in an oven. Filters were washed twice with 2× SSC and 0.1% sodium dodecyl sulfate (SDS) at room temperature for 5 min, and then with 0.5× SSC and 0.1% SDS at 65°C for 15 min. Signal

detection with AMPPD (3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane disodium salt [Tropix, Inc., Bedford, MA]) or an equivalent luminogen compound was performed following the procedures recommended by Boehringer GmbH. Filters were exposed to X-ray films at room temperature for 3 to 6 h. Probes were removed from the membranes by washing in 0.1% SDS at 80°C for 20 min.

RAPD/bulked segregant analysis procedure. RAPD analysis was performed with commercially available 10-base pair primers (Operon Technologies Inc., Alameda, CA) (42). Amplification reactions were in a volume of 25 µl with 1× PCR buffer, 150 µM dNTP, 0.5 unit of *Taq* polymerase (Appligene, Pleasanton, CA), 0.2 µM primer, and 15 to 20 ng of genomic DNA. PCR was performed using a TRIO Thermoblock (Biometra, Göttingen, Germany) or in a DNA thermal cycler PTC-100 (MJ Research Inc., Watertown, MA) with the following conditions for 45 cycles: 95°C for 1 min (4 min for first cycle), 35°C for 1 min, 72°C for 2 min, followed by a final extension for 6 min at 72°C. A RAPD survey was carried out to refine the mapping of QTLs using bulked segregant analysis methodology (26). The most resistant and susceptible DH lines from the IR64/Azucena DH population were selected for the bulks based on the ELISA test. Each bulk consisted of five or seven DH lines, and the DNA concentration of each was adjusted to 10 ng/µl prior to combining. Dilutions were made to obtain a final concentration of 3 to 4 ng/µl for the primer survey.

Linkage analysis. The mapping of RAPD and RFLP markers in the IR64/Azucena DH population (183 lines) was made with Mapmaker 3.0 (22), and markers were ordered using two-point (log of the likelihood ratio [LOD] = 3) and multipoint analysis. With the IRAT177/Apura population, mapping was based on RFLP data from Cornell University (180 markers tested on 75 DH lines) (23; S. R. McCouch, *unpublished data*). A subset of these lines consisting of 72 IR64/Azucena DH lines and 43 IRAT177/Apura DH lines were evaluated for RYMV resistance and analyzed to detect putative QTLs using optical density (OD) values obtained with ELISA as the quantitative trait. Interval mapping (21) was performed and confirmed by the Kruskal & Wallis test with MapQTL version 2.4 (40).

RESULTS

Kinetics of multiplication in IR64 and Azucena. Virus content and symptom expression varied with time and varieties. Severity of symptoms was correlated with OD obtained in ELISA. The first symptoms were observed in IR64 8 days after inoculation. Two weeks after inoculation, high virus concentration and clear mottle symptoms were observed in IR64, whereas, at the same time, Azucena showed the beginning of symptoms and a low titer of virus (Fig. 1). After 35 days, virus titer stabilized in IR64 and was still increasing in Azucena. The difference between the two varieties decreased with time, and 15 days after inoculation was considered optimal for assessing the response to infection with the ELISA test in the two progenies.

Genetics of resistance to RYMV in two populations of rice. Results were expressed in log(OD × 1,000). In the IR64/Azucena population, the parent Azucena was in the most resistant class with a very low virus titer, while about 10% of DH lines had a higher virus titer than IR64. A low frequency of intermediate values gave a rough bimodal pattern of the resistance distribution in the population (Fig. 2A). The distribution of virus titer in the IRAT177/Apura population was skewed towards the highly susceptible category, with almost 25% of the lines being as susceptible as Apura (Fig. 2B). Moreover, 12% of plants exhibited lower log(OD × 1,000) values than the resistant parent IRAT177. This pattern suggested that a major QTL and several minor genetic determinants were involved in controlling virus content in these populations and that an approach based on RFLP maps was an appropriate way to identify QTLs for RYMV resistance.

Identification and mapping of RYMV resistance markers. As a first step, QTL analysis was performed using ELISA values on the RFLP data set from Huang et al. (16). This analysis revealed a single significant QTL (LOD = 2.4) close to the RFLP marker RG341 on chromosome 12. Complementary approaches were then applied to refine the identification of this QTL: (i) more DH lines were analyzed, (ii) other mapped RFLP markers were added on chromosome 12, (iii) bulked segregant analysis was undertaken to saturate the target chromosome region, and (iv) integrative mapping was performed combining the two DH populations segregating for RYMV resistance.

Four probes (CDO344, RG341, RZ816, and RG574) already mapped by Huang et al. (16) on the core set of DH lines were analyzed on the entire DH population (183 lines). Referring to the localization of RFLP markers on the interspecific map (6), four RFLP markers were added (CDO459, RG869, RZ76, and RZ257). RG869 cosegregated with RG341, and RZ257 filled a large gap in the chromosome. The resulting map measured 115 cM and was composed of 16 markers showing excellent collinearity with the interspecific map (Fig. 3).

In bulked segregant analysis, 101 RAPD primers revealing a clear polymorphism between the parents IR64 and Azucena for one or several bands ranging from 0.6 to 2 kb were tested on the susceptible and resistant bulks. Ten polymorphic bands were identified and then verified by testing separately each individual of the bulks. Bands were considered as false positives when a bulk characterized by an absence of band contained some individuals dis-

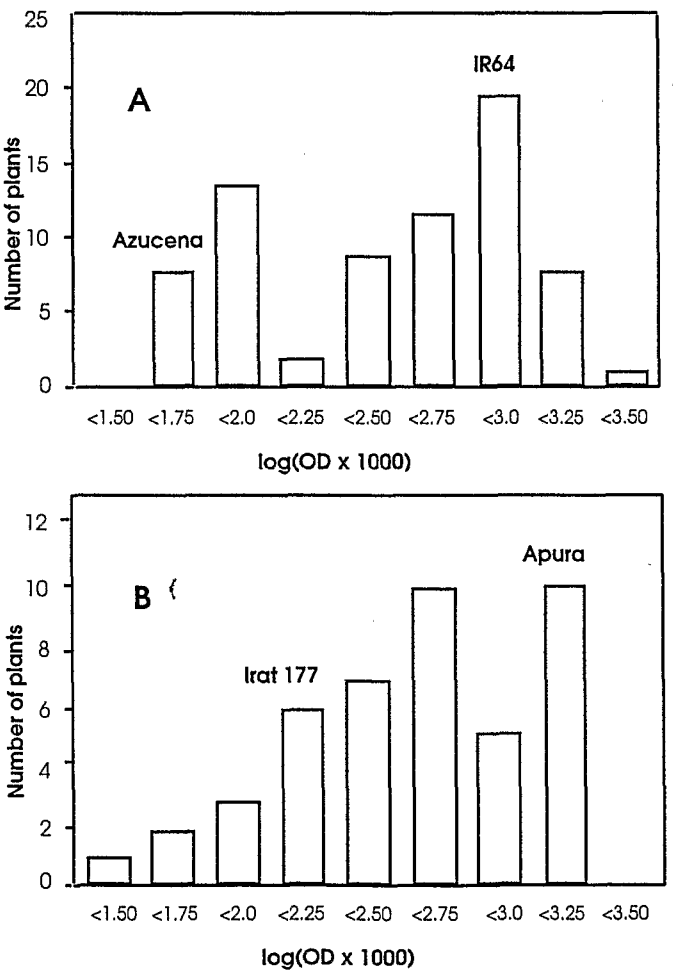

Fig. 2. Distribution of rice yellow mottle virus titer phenotypes as estimated by enzyme-linked immunosorbent assay and expressed in log(optical density × 1,000) in two doubled-haploid (DH) populations of rice: **A**, IR64/Azucena (72 DH lines) and **B**, IRAT177/Apura (43 DH lines).

playing a weak signal or when segregation distortion occurred for the marker. A 800-bp band from primer O10 was observed in the susceptible parent IR64 and in all five individuals of the susceptible bulk, while no amplification was found in the resistant parent or the resistant bulk (Fig. 4A). Finally, $O10_{800}$ was kept as the only putative marker of RYMV resistance. The analysis of the complete IR64/Azucena DH population showed that this marker was closely linked to RG341 on chromosome 12 (2.9 cM). In addition, the 800-bp band was isolated, reamplified, and labeled to use as a probe on progeny filters. The RFLP showed two copies that cosegregated with PCR-based detection of $O10_{800}$ (Fig. 4B). The new map reinforced the significance of QTL detection on chromosome 12 with a LOD of 3.45 at the RG341 marker (R^2 = 21%), which was confirmed by the Kruskal & Wallis test (K = 13.4) (Fig. 5).

The IRAT177/Apura cross presented several disadvantages that made QTL analysis difficult and less informative, most notably a lack of polymorphism, small population size, and strong segregation distortion. As seven RFLP markers (RG181, RG958, RG901, CDO344, RG457, RG341, and RG869) and one isozyme marker (*Sdh*-1) were common to the two populations, construction of an integrated map was useful for providing additional evidence of the resistance QTL on chromosome 12. This integrated map (122 cM) of chromosome 12 was established using 258 individuals and exceeded only by 7% the genetic linkage map of the IR64/Azucena population alone. The consensus map was used for confirming the resistance QTL first on the IRAT177/Apura population only, and then on the two populations together. Quantitative data of RYMV resistance were thus expressed as standard deviation units to be analyzed simultaneously on the two populations. In a similar manner to IR64/Azucena, the IRAT177/Apura population displayed a QTL associated with RG341 (LOD = 3.27, K = 8.6, and R^2 = 32.5%) (Fig. 5). Pooled resistance data on this integrated map greatly improved the significance of the previously identified QTL, giving a LOD score of 6.3 (R^2 = 21%) and K of 22.4. Altogether, these results strongly supported the hypothesis that a single chromosomal region on chromosome 12, defined by RG341 and RG869, conferred RYMV resistance in the two resistant parents Azucena and IRAT177.

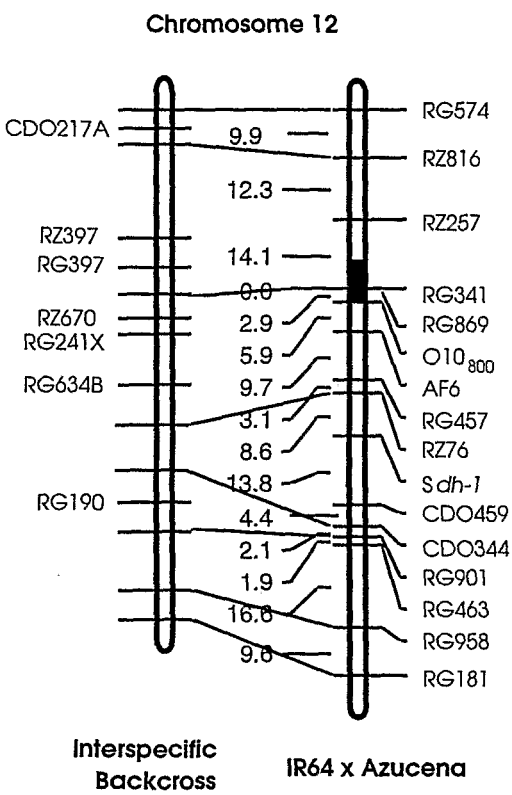

Fig. 3. Mapping of the random amplified polymorphic DNA marker $O10_{800}$ bp and the linked rice yellow mottle virus resistance quantitative trait locus (QTL) on rice chromosome 12. A black-filled bar indicates the QTL region with LOD > 5. Comparison of genetic linkage map is based on the interspecific backcross (6) and the IR64/Azucena doubled-haploid population used in this study. Genetic distances, in centimorgans, were calculated with the Kosambi function.

Evaluation of field resistance. During the field experiments, no symptoms were observed in the noninoculated control lines, which suggested that natural spread was very limited between adjacent inoculated and noninoculated lines. Susceptible lines were characterized by reduced tillering, lower plant height, and delayed panicle emergence. In addition, the effect of RYMV on fertility ranged from complete sterility to almost complete fertility, but with reduced grain weight. In principal component analysis, most of the traits (tillering, plant height 20 days after inoculation, heading date, weight of 1,000 grains, and number of grains) were highly correlated with the first axis, which explained 60% of the total variation The second axis was more closely related to sterility and plant height 40 days after inoculation (Fig. 6). The observation of symptoms using the field assessment scale showed homogenous patterns on the inoculated row in most of the DH lines, although, in some cases, different but consecutive rating scores were given to plants of the same row. No symptomless plant was observed in the inoculated rows. The observation of symptoms permitted the characterization of three reaction groups. The first group (score 3) consisted of DH lines showing very mild symptoms. The second group (scores 4 to 5) included moderately resistant lines. In these two groups, effect of infection on plant development was slight and the position of corresponding DH lines was restricted to the right part of Figure 6. The last group (scores 8 to 9) consisted of very susceptible DH lines with severe symptoms, sterility, stunting, and long cycle duration. Only DH lines with contrasting values for virus content in ELISA tests were selected for this experiment, which indicated that none of the tested lines gave intermediate scores of 6 to 7. Each DH line selected in the susceptible bulk and additional lines of either DH population with high virus content in ELISA tests belong to the highly susceptible field group (scores 8 to 9). Reciprocally, all DH lines of the resistant bulk and lines with

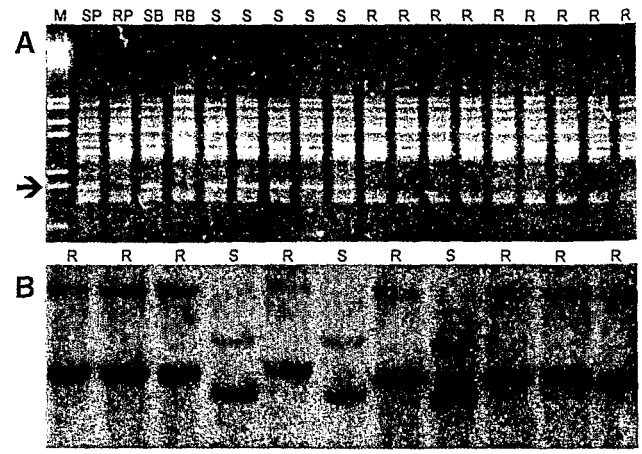

Fig. 4. A, Amplification of random amplified polymorphic DNA (RAPD) primer O10 selected by bulked segregant analysis. The $O10_{800}$-bp product, linked to rice yellow mottle virus resistance, is indicated by an arrow. Lane 1 (M), lambda-*Hin*dIII/*Eco*RI; lanes 2 and 3, susceptible parent IR64 (SP) and resistant parent Azucena (RP), respectively; lanes 3 and 4, susceptible bulk (SB) and resistant bulk (RB), respectively; and the other lanes are susceptible (S) or resistant (R) IR64/Azucena doubled-haploid (DH) lines. **B,** Hybridization of the 800-bp band after isolation, reamplification, and labeling to a Southern blot of a portion of the IR64/Azucena DH population digested by *Eco*RI. A two-band pattern is observed, and lanes 1 (R) and 4 (S) correspond to the restriction fragment length polymorphism (RFLP) found in Azucena and IR64, respectively. The RFLP pattern completely cosegregates with $O10_{800}$-pb RAPD product in the IR64/Azucena DH population.

low virus titer were included in the field resistant groups (scores 3 or 4 to 5) and none of them were found highly susceptible (Fig. 6). Therefore, the virus content measured by ELISA tests was highly correlated with field resistance.

DISCUSSION

Little is known about the genetic basis of naturally occurring resistance to viruses in rice, and no detailed genetic host-pathogen relationships have been reported so far. Analysis of resistance to rice hoja blanca virus based on a DH population was consistent with single Mendelian locus (39). Another analysis based on F_2 and near isogenic lines demonstrated that a dominant gene gives resistance to the GLH vector and rice tungro spherical virus (RTSV) (33). The mechanical inoculation used here for RYMV resistance assessment bypasses insect transmission used with other rice viruses (rice hoja blanca virus, tungro virus, and rice grassy stunt virus). Resistance and susceptibility in our experiments are estimated by the ability of DH lines to limit the multiplication and spread of the virus. The distribution of virus content in the IR64/Azucena progeny fits with the presence of a major gene possibly accompanied by other QTLs not detected in this study. The resistance pattern of the IRAT177/Apura DH population is more complex and possibly reflects the complex genetic background of IRAT177. This line was derived from an *indica* × *japonica* cross and might interact with the pathogen in a more complex fashion. Recently, a complete (6 × 6) diallele experiment of field resistance to RYMV was made using mechanical inoculation and included the four parents of the current study (2). This work showed that field resistance was polygenic with predominantly additive effects. Moreover, these results reinforce the view that IRAT177 differed from Azucena in the number and action of resistance genes. This could explain differences in resistance distribution of IRAT177/Apura DH lines. By contrast, mapping of RYMV resistance identified only a single QTL shared by the two DH populations. This suggested strongly that other QTLs, undetected in this analysis because of a low population size, may also contribute to RYMV resistance and have to be determined.

Bulked segregant analysis proved to be an efficient way to tag a major gene for disease resistance (26,27). Bulked segregant analysis and RAPD markers were useful for adding markers to a specific chromosomal segment. Though usually used to identify specific resistance based on hypersensitivity, our study showed that bulked segregant analysis could also be performed successfully to identify major QTLs. ELISA tests are mainly used to estimate virus content and assess resistance in varietal screening. In this study, ELISA tests were found to be precise enough to serve as a reliable measure of resistance for QTL identification. The evaluation of virus content at an early growth stage was a good predictor of field resistance, and the presence of alleles found in Azucena and IRAT177 on the $O10_{800}$ to RG869 chromosome segment is linked to a consistent level of field resistance. Saturation of the target chromosomal segment and conversion of RAPD markers to sequence characterized amplified region markers represents the next step for fine mapping and plant breeding applications (29). Identification of QTLs requires high polymorphism and complete linkage maps. The low polymorphism observed within *indica* or *japonica* crosses often limits mapping work to *indica* × *japonica* crosses (20) or interspecific cross (6). For example, the RFLP map of IRAT177/Apura had more than 12 linkage groups, and chromosome 12 was split into three different groups even at a low LOD threshold. In this work, integrative mapping allowed us to accumulate data from two populations expressing the same trait and to improve greatly the efficiency of QTL detection.

Mapping projects of resistance to several virus and vectors of viruses are in progress, and several resistance loci have been located on rice chromosomes (6): resistance to RTSV and its vector, GLH, on chromosome 4 (33); and resistance to the vector whiteback planthopper Wph-1 on chromosome 7 (25). Two other loci of

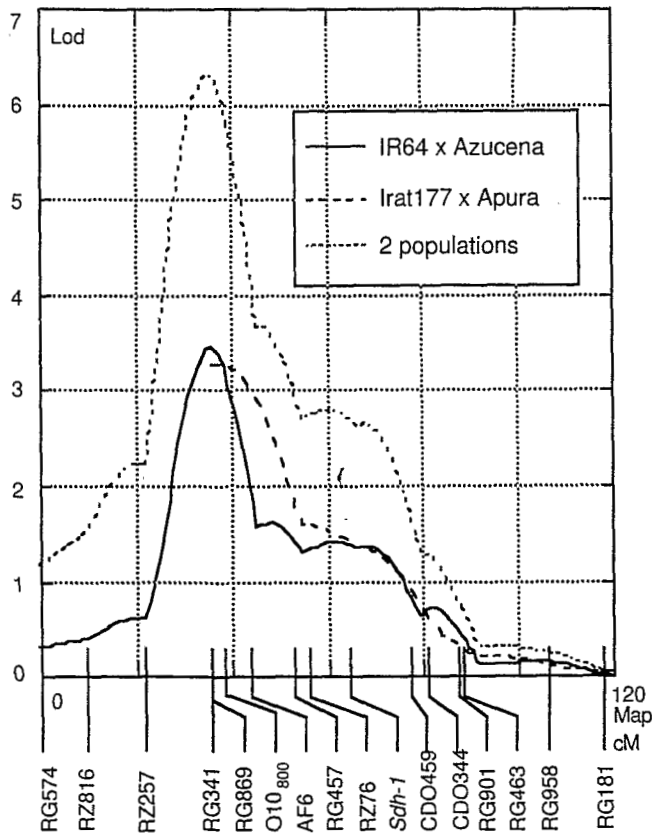

Fig. 5. Localization of rice yellow mottle virus resistance quantitative trait locus (QTL) on chromosome 12 based on interval mapping with log(optical density × 1,000) as the quantitative trait. Each population was first analyzed separately, and data was then pooled to establish a consensus map based on 258 individuals for improving the QTL detection.

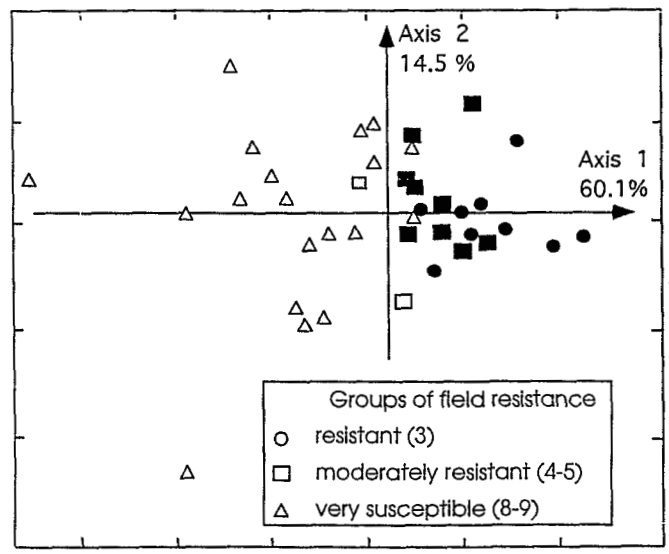

Fig. 6. Forty-one IR64/Azucena and IRAT177/Apura doubled-haploid (DH) lines plotted on the first plane of a principal component analysis computed with seven characters related to vegetative growth, development, and yield components. The first axis is mainly defined by tillering and plant height (20 days postinoculation), heading date, and grain weight. The second axis is linked to sterility and plant height 40 days after inoculation. DH lines are clustered in three resistance groups on the basis of symptom scoring: resistant (score 3), moderately resistant (scores 4 to 5), and very susceptible (scores 8 to 9). A black-filled symbol indicates the presence of Azucena or IRAT177 markers at the resistance quantitative trait locus identified on chromosome 12.

resistance have been tentatively identified on chromosome 12: *Bph*-10(t) (brown planthopper) and *Hbv* (hoja blanca virus). Mapping of genes for complete resistance to *M. grisea* is increasing rapidly, and most of them tend to occur in clusters on specific segments of rice chromosomes (24). On chromosome 12, Yu et al. (45) reported a cluster of resistant genes linked to RG869 and comprising three genes closely linked or allelic, Pi-4(t) (44), Pi-ta (18), and a resistance gene derived from the variety Apura. Other blast resistant genes (35,45) and a QTL for partial resistance (41) have also been identified near RG869. Consequently, our results clearly showed that the locus conferring RYMV resistance with both IR64/Azucena and IRAT177/Apura crosses is lying on a small chromosome region where several blast resistance genes exist.

Rice genetic diversity at the molecular marker level and current evaluation of resistance for breeding in international rice testing nurseries provide additional information on the genetics of resistance of RYMV. Varieties such as Moroberekan, 63-83, OS6, Lac 23, and Dourado Precoce, mainly used as donors of RYMV resistance, are also found to be blast resistant under African growing conditions (1). All these varieties are typically upland varieties belonging to isozyme group VI, *japonica* (12). On the contrary, *indica* rice varieties (isozyme group I) are moderately to highly susceptible to RYMV. RFLP alleles can also be very discriminating markers to distinguish *indica* and *japonica* varieties. Among other mapped RFLP markers, the alleles observed at the RG869 locus gives a clear differentiation between isozyme group I and VI (30) and the specificity of RG869 is also confirmed in a representative rice collection of 150 varieties (34). The same pattern of variation is observed for RG81, which maps closely to RG869 (30). This may suggest that this chromosome segment is strongly conserved within each subspecies of Asian rice.

On an evolutionary time scale, *O. sativa* was only recently introduced in West Africa, so that long coevolution between host and pathogen is unlikely. Therefore, the different pattern of resistance between *indica* and *japonica* varieties may also be the consequence of the large differences in plant growth and development between irrigated varieties and *japonica* upland varieties, regardless of geographical origin; the traditional variety Azucena from the Philippines and other upland rice varieties from Southeast Asia had low virus concentration in ELISA tests despite the absence of RYMV in that continent (31). Resistance may arise indirectly from specific traits related to plant growth and development in upland varieties affecting the multiplication and migration of the virus in the plant. This idea is consistent with the observation that, in different mapping populations, several QTLs provided by the upland parent were mapped on chromosome 12 (RG457 to RG341 segment) and were supposed to contribute to drought resistance: root thickness for Moroberekan × Co39 cross (7), and root/shoot ratio shared by IR64/Azucena and Moroberekan × Co39 populations (43). Finally, our results emphasize that RFLP alleles associated with *japonica* varieties, RYMV resistance, blast resistance genes, and specific morphological traits of upland rice varieties are clustered on a small portion of chromosome 12. Future work on QTL dissection and fine mapping of blast and RYMV resistance will be focused on this segment.

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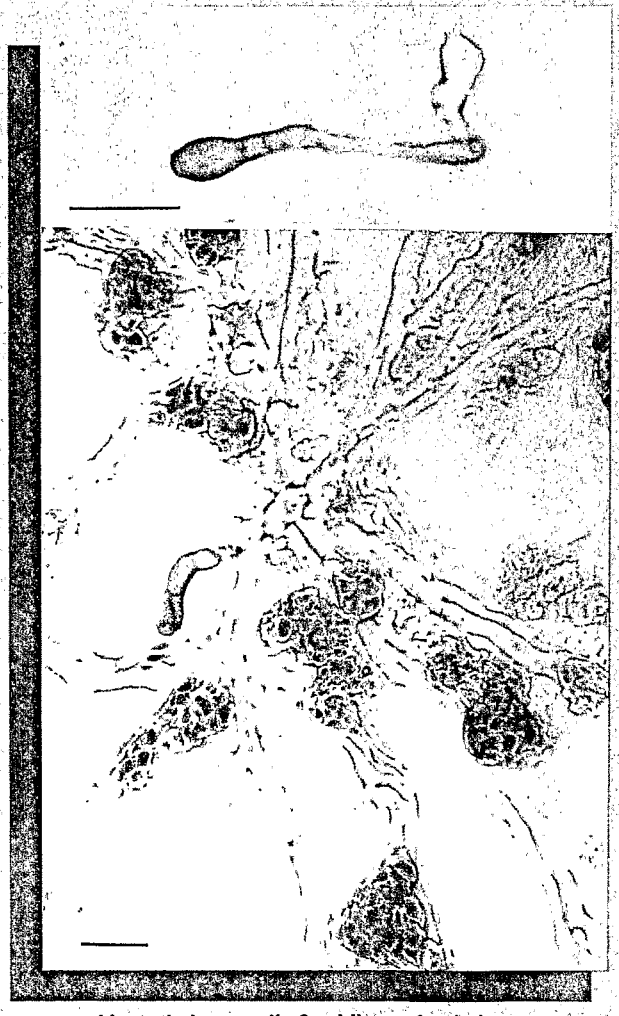

Venturia inaequalis Conidia on Apple Leaves