Theor Appl Genet (1998) 97: 1145–1154

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Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology

Received: 20 April 1998 / Accepted: 30 April 1998

Abstract Rice yellow mottle virus (RYMV) resistance QTLs were mapped in a doubled-haploid population of rice, 'IR64/Azucena'. Disease impact on plant morphology and development, expression of symptoms and virus content were evaluated in field conditions. Virus content was also assessed in a growth chamber. RYMV resistance was found to be under a polygenic determinism, and 15 QTLs were detected on seven chromosomal fragments. For all of the resistance QTLs detected, the favourable allele was provided by the resistant parent 'Azucena'. Three regions were determined using different resistance parameters and in two environments. On chromosome 12, a QTL of resistance that had already been detected in this population and another *indica/japonica* population was confirmed both in the field and under controlled conditions. Significant correlations were observed between resistance and tillering ability, as measured on control non-inoculated plants. In addition, the three genomic fragments involved in resistance were also involved in plant architecture and development. In particular, the semi-dwarfing gene *sd-1*, on chromosome 1, provided by the susceptible parent, 'IR64', mapped in a region where resistance QTLs were detected with most of the resistance parameters. In contrast, the QTL of resistance mapped on chromosome 12 was found to be independent of plant morphology.

Key words Rice yellow mottle virus · Disease resistance · Plant morphology · Quantitative trait locus · Mapping

Communicated by H. F. Linskens

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Introduction

First described in 1974 (Bakker 1974) rice yellow mottle virus (RYMV) is now, with blast, the most damaging pest of rice, *Oryza sativa* L., in Africa. The main symptoms are yellowing and mottling, with susceptible varieties showing stunting and sterility. With early infection, a yield loss of nearly 100% has been reported (Fomba 1988). RYMV is a sobemovirus (Hull 1988; Ngon A Yassi et al. 1994), and under natural field conditions it seems to be essentially transmitted by chrysomelid beetles. As RYMV is also found in African wild rice species and in many grasses, there are ample natural reservoirs (Bakker 1974). RYMV causes the most severe damage on high-yielding varieties from Asia that were introduced into Africa with the intensification of rice culture. Vector control by chemical treatments and cultural practices are of limited use against RYMV spread, and the most promising method of control is the selection of resistant varieties.

In *O. sativa* species, *indica* varieties adapted to irrigated cultivation are very susceptible, whereas partial resistance is generally found in upland *japonica* varieties (Thottappilly and Rossel 1993; Rasaonary 1990). During the last decade, breeders have tried to introduce resistance into *indica* varieties, but as yet no variety with both a high level of resistance and adaptation to irrigated cultivation has been released (Singh 1995). Resistance is found in traditional upland varieties in

Africa and in Asia where RYMV is not present. As resistance does not result from a co-evolution between host and pathogen, some resistance components may be associated with developmental and morphological traits specific to upland *japonica* varieties, such as great height and little tillering.

We previously mapped a quantitative trait locus (QTL) involved in virus accumulation assessed by the ELISA test (Ghesquière et al. 1997). This QTL was detected in two populations of doubled-haploid (DH) lines derived from crosses between *indica* and upland *japonica* varieties, 'IR64/Azucena' and 'IRAT177/Apura'. It mapped on chromosome 12 in a region containing a cluster of blast resistance genes (Yu et al. 1996) and close to a marker differentiating *indica* and *japonica* subspecies (Qian et al. 1995). However, the distribution of RYMV resistance in Ghesquière et al. (1997) suggested a polygenic control, and probably QTLs with minor effects were not detected because of the low number of DH lines used in our earlier study. Besides, it was of interest to study the genetic basis of RYMV resistance, in particular the effect of the QTL of chromosome 12, at the field level.

The purpose of the study presented here was: (1) mapping resistance QTLs in a larger part of the 'IR64/Azucena' population in order to detect minor QTLs, (2) comparing the ELISA test performed under controlled conditions and evaluating resistance at the field level and (3) assessing the impact of plant morphology on RYMV resistance.

Materials and methods

Plant material

The population 'IR64/Azucena' was used in this study. 'IR64' is an elite *indica* line susceptible to RYMV and 'Azucena' is a traditional upland variety from the Philippines with partial resistance (mild symptoms, limited impact on plant development and reduced virus content). One hundred and eighty three DH lines were derived from F_1 anther culture by Guiderdoni et al. (1992).

Evaluation of resistance at the field level

One hundred and fifteen DH lines were evaluated in 1996 at the IER/CIRAD Research Station of Sikasso, Mali, for their resistance under field conditions. The experimental design included two replicates. DH lines were disposed according to their line number inside each replicate. Each line was represented by two rows of 1 m facing each other with 5 plants per row. The first row was mechanically inoculated, while the second row served as a control. Inoculation was performed 4 weeks after sowing using leaves infected with RYMV ground in water and abrasive. A local isolate was used to avoid the introduction of another isolate into the experimental area. The following observations were made during the trial: plant height 8 weeks after sowing and at maturity, number of tillers 8 weeks after sowing, number of fertile tillers at maturity (number of panicles), heading date and grain weight. Eight weeks after sowing corresponded approximately to the end of the vegetative phase and to panicle initiation for DH lines having a medium cycle duration. Control non-inoculated lines were used to characterise plant morphology. As the morphology of the control plants showed great variation, we evaluated field resistance using the ratio (score obtained on inoculated line)/(score obtained on controls). In addition, leaf discolouration on inoculated plants was noted 3 weeks after inoculation according to a rating scale ranging from 1 to 9: 1 corresponded to infected but symptomless plants and 3–9 to increasing yellowing. For all these parameters, the 5 plants of each row were noted independently and an average value was calculated. The different traits noted and their corresponding symbols are summarised in Table 1.

Evaluation of virus titre

During field evaluation of resistance, samples of inoculated plants were collected 11 weeks after inoculation to evaluate virus titre using an ELISA test. The samples consisted of one piece of the last emerging leaf of the 5 inoculated plants. The leaves were ground in 0.15 *M* phosphate buffer (pH 7.2) containing 0.05% Tween 20 (PBS-T). The tests were performed at WARDA, Bouake, Ivory Coast.

Another evaluation of virus content in the plants was conducted under controlled conditions at Montpellier, France. One hundred and sixty-eight DH lines were evaluated in seven experiments. Each experiment involved 24 DH lines and vars 'IR64', 'Azucena' and 'Tox3219', used as controls. DH lines were repeated twice and the controls were repeated four times. 'Tox3219' was chosen as it had a level of resistance intermediate between that of 'IR64' and 'Azucena' (Rasaonary 1990). Plants were sown in lines of 12–15 plants, which constituted the elementary sample. They were grown in a growth chamber under controlled conditions (24°–26°C for 12 h in the dark and 28°–30°C for 12 h in the light) and mechanically inoculated with an isolate of virus from Burkina Faso 10 days after sowing. This isolate was found to be the most aggressive among the isolates available in the laboratory and was used in order to obtain a rapid multiplication of virus. The inoculum was prepared with 1 g of infected leaves ground in 30 ml of phosphate buffer (0.1 *M*, pH 7.2) containing carborundum. One week after inoculation, the whole plants were cut and ground in PBS-T. The Double Antibody Sandwich ELISA test was performed essentially as described by Clark and Adams (1977). Two polyclonal antisera were prepared following the protocol of Fauquet and Thouvenel (1977). For practical reasons, the antiserum used for the growth chamber test was directed against a RYMV isolate from Mali, and the one used for the field

Table 1 Traits observed to characterise morphology and RYMV resistance and corresponding symbols. Date of inoculation and scoring are indicated in weeks after sowing (was) or days after sowing (das)

Trait	Date	Morphology	Resistance
Field evaluation (inoculation 4 was)			
Height (cm)	8 was	H8c	H8r
Height (cm)	Maturity	HMc	HMr
Number of tillers	8 was	T8c	T8r
Number of fertile tillers	Maturity	TMc	TMr
Heading date (days)		HDc	HDr
Grain weight (g/plant)		GWc	GWr
Symptoms	7 was		S
Virus content (ELISA)	11 was		VC1
Evaluation in growth chamber (inoculation 10 das)			
Virus content (ELISA)	17 das		VC2

trials was directed against an isolate from Madagascar. However, these two antisera recognised the two isolates. A volume of 100 µl of antiserum was incubated in microtitre plates for 2 h at 37°C. After each step, the plates were washed three times with PBS-T. Wells were saturated with 200 µl of milk solution (3 g of powdered milk in 100 ml PBS-T) for 1 h at 37°C. One gram of leaf extract was diluted in 5000 ml and 10 000 ml of buffer in the field trial and growth chamber tests, respectively, as these dilutions gave the most reliable results in the preliminary experiments. Then, 100 µl of antibody conjugated to alkaline phosphatase (Boehringer Manheim) was added to the wells. After 1 night of incubation at 4°C, the specific antigen-antibody reaction was assessed by adding *p*-nitrophenyl phosphate (Sigma Chemical) at 1 mg/ml in diethanolamine buffer (pH 9.8). Absorbance at 405 nm was measured after varying lengths of incubation at 37°C.

For tests performed on plants grown in the field, the optical density obtained after 30 min was retained for analysis (VC1). For the evaluation of plants cultivated in the growth chamber, optical density read after 2 h of incubation was retained and transformed to correct for plate and experiment effects. Firstly, on each plate a scale of diluted virus, purified following the protocol of Fauquet and Thouvenel (1977), was used to correct for plate effect, and results were expressed as virus titre. Secondly, in order to correct for experimental effect, we adjusted virus titre to $X_2 = (X_1.X_{ct}/X_{cm})$, where X_1 was the titre of the sample, X_{ct} the average titre of the controls of the seven experiments and X_{cm} the average titre of the controls of the experiment. The results were then expressed as $VC2 = \log_{10}(X_2 + 100)$ to normalise the distribution and to obtain a variance independent of the mean.

Statistical analysis

Heritability for each trait was calculated using the equation $h^{2*} = \sigma^2_G/(\sigma^2_G + \sigma^2_e/2)$ in which σ^2_G and σ^2_e were estimates of genetic and residual variances as given by STATISTICA (V5.1) software. However, as the lines were not randomly distributed in the trials, these estimators of heritabilities were biased. Nevertheless, they gave an approximation of the non-biased heritabilities.

Inoculation effect and phenotypic correlations between characters were determined using STATISTICA (V5.1) software. Correlations involving resistance parameters expressed as ratios were calculated using the ratios or their opposite value so that an increasing value corresponded to an increasing level of susceptibility.

DNA extraction and restriction fragment length polymorphism (RFLP) procedure

Huang et al. (1997) developed a core RFLP map using 135 DH lines of the 'IR64/Azucena' population. This map served as our reference for marker analysis, and additional marker data were generated for all the 183 lines available. DNA extraction was based on the method of Hoisington (1994). RFLP probes were obtained from Cornell University, USA, or the MAFF DNA Bank of the Rice Genome Program, Japan. The preparation of filters and probes and hybridisations were performed as described by Ghesquière et al. (1997). The signal was detected with anti-digoxygenine antibody (dilution 1/10 000) and CSPD™ or CDPStar™ (1/500) using procedures recommended by Boehringer Manheim. Probes were removed from the membranes by washing in 0.1% SDS at 80°C for 20 min. Random amplified polymorphic DNA (RAPD) analyses were performed as described in Ghesquière et al. (1997).

Linkage analysis

The map of Huang et al. (1997) was used as a basis to map new RFLP and RAPD markers. The mapping of new RFLP markers was made with MAPMAKER 3.0 (Lander et al. 1987), and markers were ordered using two-point (LOD = 3) and multipoint analysis. Chromosomes were oriented as in Singh et al. (1996). QTL analysis was performed according to the method of interval mapping (Lander and Botstein 1989) using MAPMAKER/QTL (Lincoln et al. 1993). In the first step, a threshold of LOD = 2.6 was selected. In the second step, the QTLs detected were fixed to increase sensitivity, and a new analysis was performed (Lincoln et al. 1993). New QTLs were retained with a threshold of LOD score = (LOD score obtained with the fixed QTLs + 2.6). Simplified composite interval mapping using MQTL (Tinker and Mather 1995) was performed in order to localise more precisely some QTLs on chromosome 2. Markers regularly distributed along the chromosome and markers linked to QTLs on other chromosomes were selected as cofactors. The statistic test used by MQTL, in simple or composite interval mapping, is the statistic test described by Haley and Knots (1992). All the QTLs detected were then confirmed using a multi-QTLs model on MAPMAKER/QTL, as described in Li et al. (1997), and each QTL was confirmed if it contributed to the model by an additional LOD > 2. The percentage of variance explained by each QTL and by the multi-QTLs model was estimated with MAPMAKER/QTL.

Results

Morphological traits

As we were interested in studying relationships between resistance and plant morphology, we first studied height, tillering and heading date on non-inoculated control plants.

Segregation of morphological traits

Height, number of tillers and heading date showed great variation in the population (Table 2), reflecting the high differences observed for these characters between the parents. The variation between DH lines for grain weight depended on grain filling and sterility

Table 2 Phenotypic values of the traits noted to characterise morphology and resistance level. For each trait mean, standard deviation and heritabilities (h^{2*}) are indicated

Trait	Mean	SD	h^{2*}
H8c (cm)	56	8	0.81
HMc (cm)	86	16	0.88
T8c	10.0	3.0	0.70
TMc	4.6	1.52	0.45
HDc (days)	94	8	0.91
GWc (g/plant)	5.0	2.6	0.56
H8r	0.84	0.08	0.37
HMr	0.82	0.09	0.57
T8r	0.70	0.19	0.19
TMr	0.93	0.43	0.31
HDr	1.11	0.07	0.62
GWr	0.40	0.39	0.47
S	3.7	1.3	0.48
VC1	1014	287	0.60
VC2	6.51	0.61	0.92

level, since a high sterility is often observed in *indica* × *japonica* crosses. Under field conditions, however, outcrossing limited the sterility effect to some extent, and no DH line with high sterility was found. The semi-dwarfing gene *sd-1*, provided by 'IR64', segregated in this population, but the continuous distribution of plant height at maturity suggested that minor genes were also involved in this trait. The distribution of other characters suggested a polygenic determinism (for HDc see Fig. 1). Estimated heritabilities (h^2*) were high for tillering 8 weeks after sowing, height and heading date and were smaller for the number of fertile tillers and grain weight (Table 2), as was expected since number of fertile tillers and grain weight generally depend to a large extent on the environment. Plant height 8 weeks after sowing and plant height at maturity were highly correlated, as was tillering at those two stages (Table 3a). Plant height and tillering were correlated at maturity, but not 8 weeks after sowing. Heading date and grain weight were correlated both to height and tillering.

Mapping of QTLs controlling morphology

Results of QTLs analysis were summarised in Table 5 and Fig. 2.

A major QTL on chromosome 1 explained 38% of the variation in plant height at maturity (Table 5). We tried to map it as a Mendelian marker using the 20 taller and the 20 smaller DH lines: it mapped between RG109, and RZ14, at 5 cM from RG109, and corresponded to the semi-dwarfing gene *sd-1* previously mapped very close to RG109 (Cho et al. 1994). Three other QTLs involved in plant height at maturity were detected on chromosomes 3, 4 and 9. These 3 QTLs and *sd-1* explained 77% of the phenotypic variation. Four QTLs on chromosomes 2, 3, 9 and 12 explained 57% of the variation of plant height 8 weeks after sowing. QTLs of chromosomes 3 and 9 were co-located with QTLs explaining the height at maturity. No QTL

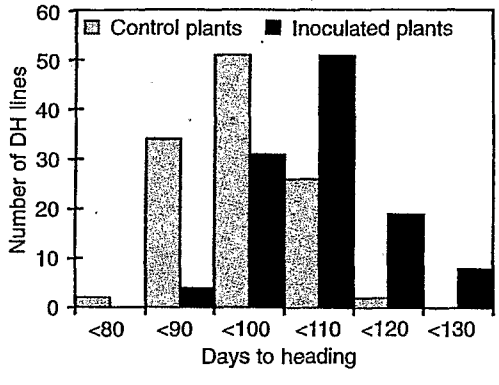

Fig. 1 Distribution of heading date for control and inoculated plants

Table 3 Phenotypic correlations (a) between the different components of plant morphology, (b) between the different parameters of field resistance and (c) between field resistance and morphology. Correlations involving resistance parameters expressed as ratios were calculated using the ratios or their opposite value so that an increase in the value corresponded to an increasing level of susceptibility

(a)	H8c	HMc	T8c	TMc	HDc
HMc	**0.66**[b]				
T8c	0.05	− 0.15			
TMc	0.04	− 0.28[b]	**0.70**[b]		
HDc	**− 0.21**[b]	**0.31**[b]	**− 0.21**[b]	**− 0.43**[b]	
GWc	**0.37**[b]	**0.27**[b]	**0.32**[b]	**0.47**[b]	− 0.08
(b)	**-H8r**	**-HMr**	**-T8r**	**-TMr**	**HDr**
-HMr	**0.44**[b]				
-WT8r	**0.57**[b]	**0.29**[b]			
-TMr	**0.22**[a]	**0.44**[b]	**0.43**[b]		
HDr	**0.47**[b]	**0.52**[b]	**0.53**[b]	**0.50**[b]	
-GWr	**0.28**[b]	**0.59**[b]	**0.24**[b]	**0.49**[b]	**0.36**[b]
(c)	H8c	HMc	T8c	TMc	HDc
-H8r	**0.23**[a]	− 0.04	**0.24**[a]	**0.25**[a]	**− 0.21**[a]
-HMr	− 0.03	− 0.17	− 0.02	**0.23**[a]	− 0.18
-T8r	0.06	− 0,10	**0.39**[b]	**0.45**[b]	**− 0.26**[b]
-TMr	0.08	0.01	0.03	**0.32**[b]	− 0.08
HDr	0.00	**− 0.22**[a]	**0.33**[a]	**0.49**[b]	**− 0.41**[b]
-GWr	**− 0.21**[a]	− 0.17	− 0.06	0.19	0.02

[a,b] Correlations were significant at $P < 0.05$ and $P < 0.01$, respectively

was detected in the region of *sd-1* at this growth stage. This was expected since the effect of *sd-1* on internode elongation becomes visible only after panicle initiation, and consequently later than H8c scoring. No QTL was detected for tillering 8 weeks after sowing. Two QTLs on chromosome 1 and 7 explained 31% of the phenotypic variation of fertile tillering. Three QTLs were detected for heading date on chromosomes 1, 3 and 4, explaining 46% of the phenotypic variation. The QTLs of chromosome 1, involved in the number of fertile tillers and heading date, mapped in the region of *sd-1*, close to marker RZ14. Two QTLs were detected for grain weight, on chromosome 9 and 12, and explained 26% of the variation. These QTLs could be implicated in grain filling or in sterility.

For most of these QTLs, the alleles coming from 'Azucena' were associated with an increase in height and grain weight, a smaller number of tillers and a delay in heading date. However, the alleles coming from 'Azucena' were correlated to a reduced height 8 weeks after sowing for the QTL of chromosome 2 and to an earlier heading date for the QTL of chromosome 4.

Field resistance

Virus resistance terminology is a contentious issue on which there is no general agreement. The term

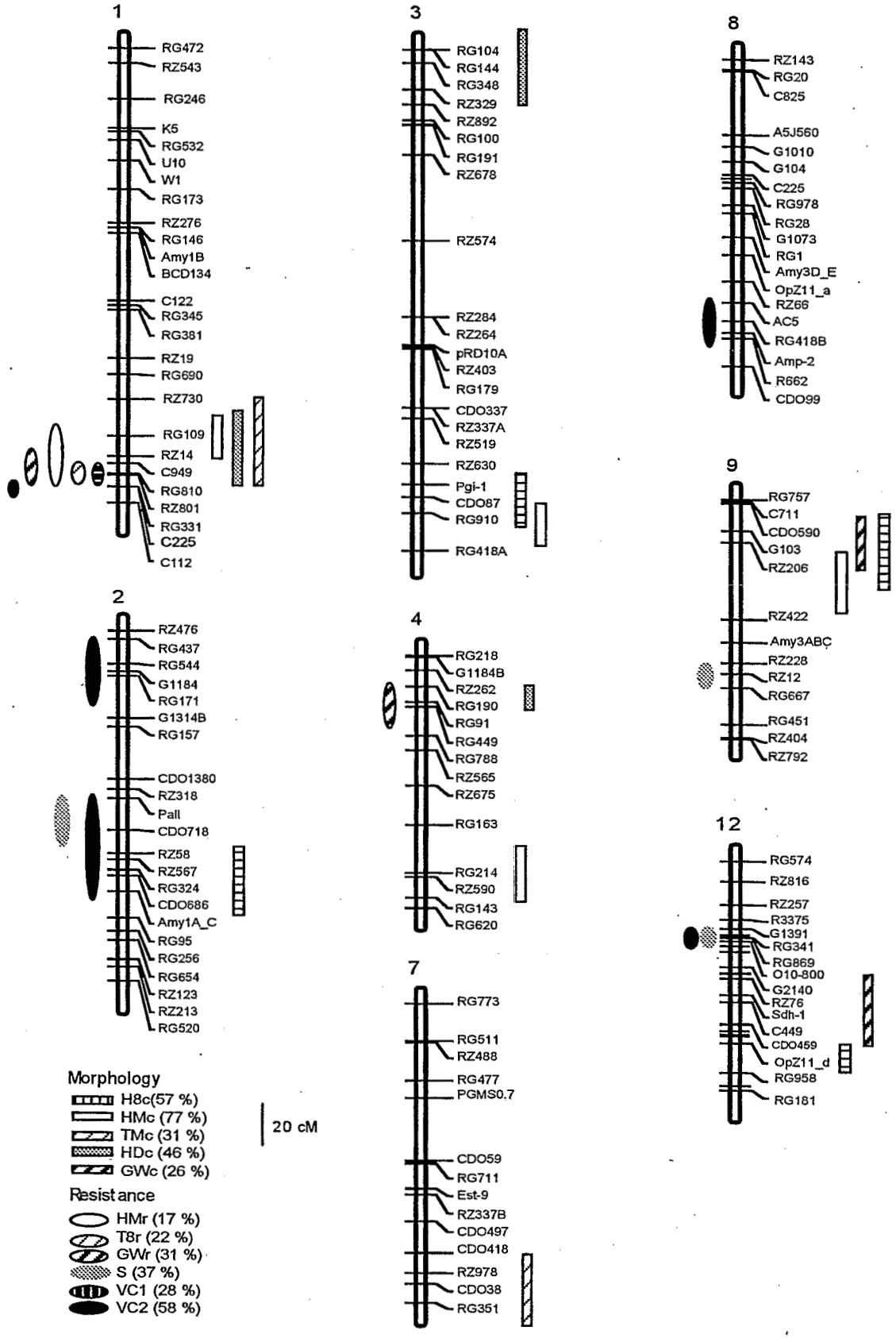

Fig. 2 Map position of QTLs involved in plant morphology (on the *right* of chromosomes) and RYMV resistance (on the *left*) in 'IR64/Azucena' DH population. QTLs are represented by *boxes* covering a 1-LOD confidence interval. Percentages of variance explained by the multi-QTL model are mentioned in the *legend* in front of each trait

"resistance" is used here in the general sense of Fraser (1986) for "any inhibition of virus multiplication or of its pathogenic effects on the host". When used in this way, we have followed different components of resistance: virus content, symptoms expression, impact on growth and yield. "Field resistance" refers here to disease impact on growth and yield under field conditions (H8r, HMr, T8r, TMr, HDr, GWr).

Characterisation of field resistance and correlations with plant morphology

RYMV was responsible for stunting, slow development and yield loss. For instance, on inoculated plants, heading occurred on average 10 days later than on controls (Fig. 1). The inoculation effect was highly significant ($P < 0.0001$) for height, heading date, grain weight and tillering 8 weeks after sowing. This is less clear for tillering at maturity ($P = 0.03$). Heritabilities were smaller for ratio than for measures on control plants and were particularly small for the tillering ratio (Table 2). This observation, combined with the small effect of disease on fertile tillering could explain that TMr was above 1 for 45% of DH lines. However, for most of the DH lines with TMr > 1, the number of fertile tillers was not significantly different between inoculated plants and controls. None of the inoculated DH lines was significantly higher than the control (ratio ≤ 1.05 for H8r and HMr, Table 2), and very few (<5%) inoculated DH lines headed earlier or had more tillers 8 weeks after sowing than the controls. According to the heritabilities and the effect of the disease on the different traits, ratio values for plant height at maturity, heading date and grain weight seemed to be the best estimators of field resistance. The distribution of these different traits suggested a polygenic determinism.

Significant correlations were observed between the ratios (Table 3b). The highest correlations were observed between the ratios scored at the same growth stage (H8r and T8r or HMr, TMr and GWr). This indicated the importance of growth stage in the evaluation of resistance. High correlations were also observed between ratios based on the same trait but scored at different stages (H8r and HMr or T8r and TMr). The impact on heading date was highly correlated to the impact on tillering and height. We studied the relationship between field resistance and plant morphology (Table 3c). Some correlations were expected between a ratio and the corresponding trait on control plants, since the two data were not independent, but correlations were also observed between independent traits. Particularly, all of the ratios except GWr were correlated to tillering at maturity (TMc), suggesting that tillering ability had an impact on field resistance to RYMV.

Mapping of QTLs involved in field resistance

Analysis of QTLs based on HMr, T8r, H8r and HDr detected QTLs on chromosome 1, in the region of *sd-1* (Table 5, Fig. 2). These QTLs explained 17%, 22%, 12% and 15% of the phenotypic variance observed on HMr, T8r, H8r and HDr, respectively. The allele conferring resistance was provided by 'Azucena'. In order to better understand Fig. 2, we have chosen to represent only QTLs detected for T8r and HMr. These two characters were chosen as they represent the effect of the disease on two different components of architecture and at two different growth stages. For GWr, QTLs were detected on chromosome 4 and chromosome 1, in the region of *sd-1*. These 2 QTLs explained 31% of the variation. The QTL mapped on chromosome 4 was co-located with a QTL implicated in heading date, and the favourable allele come from 'Azucena'.

Resistance estimated with symptoms and virus titre

Expression of resistance and correlation with plant morphology

The symptoms and virus content of the plants were also measured to evaluate resistance since they are independent from plant morphology. Symptoms ranged on a scale between 1 and 7 on the inoculated lines, with most of the DH lines rated 3 or 5. The infection was not very severe. However, a measure of the virus content confirmed that no plant had escaped the infection or was immune. The symptoms and the virus titre evaluated on the same DH lines at different growth stages were not correlated ($P > 0.05$). However they were correlated to the ratios, estimators of field resistance (Table 4). This suggested that the disease impact on plant morphology and development depended on virus accumulation in the plant.

An evaluation of virus titre was also performed on plants in the growth chamber. The conditions of that

Table 4 Correlations between symptoms and virus content with field resistance and plant morphology

	VC2	VC1	S
VC1	**0.24**[a]		
S	**0.38**[b]	**0.18**	
-HMr	**0.27**[b]	**0.31**[b]	**0.20**[a]
-T8r	**0.36**[b]	**0.41**[b]	**0.51**[b]
H8c	**−0.21**[a]	−0.05	−0.09
HMc	**−0.26**[b]	**−0.28**[b]	−0.17
T8c	**0.25**[b]	0.10	**0.23**[a]
TMc	**0.24**[a]	**0.29**[b]	**0.32**[b]
HDc	−0.13	**−0.35**[b]	−0.10
GWc	0.04	0.00	0.02

[a,b] Correlations were indicated for $P < 0.05$ and $P < 0.01$, respectively

test were much more controlled than in the field evaluation and heritability was far higher for this character than for any other estimator of resistance. (Table 2). 'IR64' and 'Azucena' were evaluated as controls and no DH line had a transgressive value. The virus content of plants in the growth chamber had a normal distribution in the population. It was correlated to symptoms, virus titre and ratios scored under field conditions (except TMr), eventhough the two tests were performed under highly different environmental conditions (Table 4).

As observed with field resistance, symptoms and virus titre were correlated to the number of fertile tillers (TMc). Moreover, the virus titre, both in the field and in the growth chamber, was correlated to plant height. As these estimators were measured independently of plant morphology scoring, especially for the growth chamber test, this confirmed the high relationship between resistance and morphology. Besides, virus titre evaluated in the growth chamber on young plants was correlated to the morphology of the 8-week-old plants, whereas virus titre evaluated later was not.

Mapping of QTLs controlling symptoms and virus titre

QTL analysis based on symptoms distinguished 3 QTLs on chromosomes 2, 9 and 12 which explained 37% of the phenotypic variation (Table 5, Fig. 2). For virus content assessed on plants grown in the field, only 1 QTL was detected. It mapped on chromosome 1, in the region where QTLs controlling field resistance had been detected, near *sd-1*. This QTL explained 28% of the phenotypic variation observed on this character. Three QTLs on chromosomes 1, 8 and 12, and 1 or more QTLs on chromosome 2 controlled virus content in plants grown in the growth chamber. As the LOD score never decreased under 2 on the major part of chromosome 2 (Fig. 3), it was not clear whether there were 1, 2 or 3 QTLs on this chromosome. Composite Interval Mapping was performed in selecting as cofactors 8 markers distributed along chromosome 2 each 25 cM and markers located on the QTLs of chromosomes 1, 8 and 12. The presence of at least 2 QTLs was confirmed (Fig. 3). The first one mapped near marker RG171. At least 1 QTL mapped between CDO1380 and RG256, but it was not clear if there were 1 or 2 closely linked QTLs in this region. One QTL involved in the expression of symptoms was also mapped on this fragment. The model explained 58% of the phenotypic variation observed for VC2. The QTL of chromosome 12 involved in symptoms in the field and virus content in the growth chamber corresponded to the QTL reported by Ghesquière et al. (1997) near RFLP markers RG341 and RG869.

Discussion

The two parents of the population used in this study present a very distinct pattern of response to RYMV

Table 5 QTLs controlling morphology and resistance in 'IR64/Azucena' DH population. For each QTL, the trait, the position of the LOD peak on the map, the LOD score and the percentage of variance explained (%) are indicated. The QTLs with LOD < 2.6 were detected when QTLS with LOD ⩾ 2.6 were fixed

Chromosome interval	Morphology				Resistance			
	Trait	Pos[a]	LOD	%	Trait	Pos	LOD	%
1 RZ730-C112	HMc	207	10.7	38	H30r	215	3.2	12
	TMc	213	4.2	17	HMr	210	4	17
	HDc	213	4.3	19	T30r	215	5.6	22
					HDr	215	3.7	15
					GWr	210	2.3	9
					VC1	211	7.4	28
					VC2	215	4.2	11
2 RG476H-G1314B					VC2	25	4.8	15
2 RZ318-RG95	H30c	135	1.1	5	S	97	2	9
					VC2	129	4.8	14
3 RG104-RG100	HDc	9	4.2	23				
3 RZ630-RG418A	H30c	226	4.6	24				
	HMc	231	1.9	11				
4 RG190-RG788	HDc	24	1.7	8	GWr	24	4.1	19
4 RG163-RG143	HMc	113	1.2	7				
7 CD0418-RG351	TMc	136	3.2	20				
8 RZ66-CD099					VC2	139	3.7	11
9 CD0590-RZ422	H30c	18	2.6	15				
	HMC	42	2.1	23				
	GWc	18	3	16				
9 RZ228-RG667					S	88	2.3	12
12 R617-Y6854R					S	41	3.7	15
					VC2	39	2.9	8
12 G2140-RG958	H30c	90	5.4	23				
	GWc	61	2.3	10				

[a] Pos, Distance in centiMorgans from the first marker of the chromosome

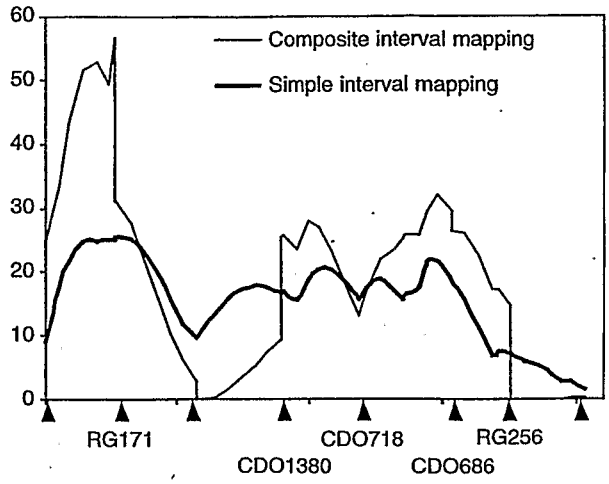

Fig. 3 Comparison of simple and composite interval mapping on chromosome 2 for virus content in growth chamber (VC2), using MQTL. In composite interval mapping, markers used as cofactors are represented by *black arrows*. The values of the statistic test obtained in simple interval mapping using MQTL are proportional to the LOD score calculated with MAPMAKER/QTL

and have a quite different morphology: 'Azucena' is a traditional upland *japonica* variety, and 'IR64' is a semi-dwarf *indica* adapted to irrigation. As RYMV resistance has often seemed to be linked to the *japonica* upland morphotype, we were interested in evaluating the relationship between resistance and morphology. We subsequently have studied the genetic basis of some morphological traits in the 'IR64/Azucena' population.

Nine chromosomal fragments involved in plant height, tillering, grain weight and heading date were detected. Five fragments were involved in at least two characters, which can be explained by linkage between QTLs or by pleiotropy. The hypothesis of pleiotropy is the most likely of the two to explain co-locations of QTLs involved in height at maturity and 8 weeks after sowing on chromosomes 3 and 9. Moreover, Yadav et al. (1997) reported that *sd-1* has an effect on tillering and, in this study, the QTL of chromosome 1 involved in tillering probably corresponded to a pleiotropic effect of *sd-1*. In this work, QTL mapping for morphological traits was highly consistent with other studies. Particularly, Huang et al. (1996) detected QTLs controlling plant height on the same regions of chromosomes 1, 2, 3 and 4 on the same population, and they also attributed the detection of a QTL on chromosome 1 to *sd-1*. As the threshold was less strict in their study than in ours they detected more QTLs. However, we detected an additionnal QTL on chromosome 9, probably explained by genotype × environment interactions (Paterson et al. 1991; Zhuang et al. 1997). In the cross *indica* × *japonica* 'Lemont' × 'Teqing' (Li et al. 1995b), QTLs involved in height and heading date on chromosomes 2, 3 and 9 were co-located with the QTLs detected here for the same traits.

In this work, RYMV resistance was estimated by the impact of the disease on morphological and agronomical traits, by the expression of symptoms and by virus content. These different estimators of resistance, were correlated, at the field level or in the growth chamber, suggesting that one or two estimators are sufficient to roughly evaluate the resistance level of the lines. In particular, virus titre evaluated on young plants in the growth chamber was correlated to characters measured during the field experiment, indicating that the ELISA test can be used successfully in quantitative analysis. As this character had a high heritability and was measured independently of plant morphology, it provides a very useful tool to assess RYMV resistance for plant breeding applications. When resistance was studied more precisely, growth stage seemed important in characterising the resistance level; higher correlations were noted between traits scored at the same date. This observation is also supported by another experiment conducted in ADRAO, Ivory Coast, on a subset of 48 DH lines of this population (our unpublished data). This suggests that some resistance components may be stage-dependent.

The different estimators of resistance used here revealed seven chromosomal fragments. Three regions on chromosome 1, 2 and 12 were involved in several resistance parameters. In addition, the QTL of chromosome 12 has already been detected by Ghesquière et al. (1997) using an isolate from Mali and the ELISA test. For all these co-locations, the genetic effects of the alleles are in the same direction. While we cannot exclude the hypothesis of several linked QTLs being involved in different resistance mechanisms, on the basis of our data we consider that a single factor of resistance was probably underlying the detection of several of the resistance QTLs co-located. The detection of common QTLs in different experiments is often hampered by genotype × environment interactions (Paterson et al. 1991; Zhuang et al. 1997) or by genotype × isolate (or strain) interactions in the case of resistance QTLs (Caranta et al. 1997; Jung et al. 1996). Here, 4 QTLs, on chromosomes 2, 4, 8 and 9, were detected only for one trait and may depend on parameter, environment or/and isolate. In contrast, the QTLs mapped on chromosomes 1, 2 and 12 were detected for different resistance estimators, under two or three environmental conditions and with two or three RYMV isolates. These QTLs appear to be the most interesting ones for further studies and for commencing an introgression process. QTL analyses are a powerful tool by which to dissect the genetic basis of a complex resistance. For instance, Lefebvre and Palloix (1996) have identified different sets of resistance QTLs controlling the different interaction steps between pepper and *Phytophthora capsici*, and Danesh et al. (1994) have detected a QTL of tomato which participates in resistance to *Ralstonia solanacearum* only when a particular inoculation method is used. Here, the QTL of chromosome 1 was

involved both in field resistance and in resistance to virus accumulation and the expression of symptoms, whereas QTLs of chromosomes 2 and 12 might be more specific of mechanisms involved in virus accumulation and symptom expression. However, further studies are necessary to clarify the impact of these QTLs at the field level, since the results described in Ghesquière et al. (1997) suggest that the QTL of chromosome 12 could be also involved in field resistance.

The different parameters used here to evaluate RYMV resistance, except for disease impact on grain weight, were correlated with tillering: the more tillers the plants had, the less resistant they were. Symptoms and virus titre were also correlated with plant height. Dirlewanger et al. (1994) found a common genetic basis between components of pea morphology to explain correlations and, consequently, we expected co-located QTLs to explain the correlations between resistance and morphology that we observed. Indeed, 3 QTLs on chromosomes 1, 2 and 4 were implicated both in plant height, tillering or heading date and in resistance. In the middle of chromosome 2, Li et al. (1995a) have also found co-location of QTLs implicated in height and in resistance to *Rhizoctonia solani*, but they explained this co-location by their system of scoring and a direct effect of plant height on the epidemiology of the pathogen. Here, we ask ourselves if co-locations between QTLs involved in morphology and QTLs involved in RYMV resistance were due to (1) a direct effect of morphology on resistance, (2) the pleiotropy of one gene involved in morphology on resistance or (3) a linkage between QTLs. A direct impact of height or heading date on resistance is unlikely, as supported by the following observations. Firstly, heading date and the different resistance parameters were not significantly correlated, neither were plant height and field resistance. Secondly, favourable alleles for resistance were associated with the allele correlated to an increase in height on chromosome 1 and the allele correlated to a decrease in height on chromosome 2. Thirdly, the QTL of resistance on chromosome 1 was detected on very young plants, whereas the effect of *sd-1* on plant height was only detected at maturity. However, we can not determine if *sd-1* and the QTLs of morphology on chromosomes 2 and 4 have a pleiotropic effect on resistance or if they are linked to QTLs involved in resistance. In contrast, the co-location of a tillering QTL and a resistance QTL on chromosome 1 explained only partly the correlations between the two traits, and a direct effect of tillering on resistance was suspected. Indeed, for most of the field resistance parameters, correlations between tillering and resistance were still significant in the sub-population of lines with *sd-1* and the sub-population of lines without *sd-1* (results not shown).

RYMV resistance may be under a complex determinism involving plant morphology and, in particular, tillering, an important trait which strongly separates *indica* from *japonica* upland varieties. So, a diversity of QTLs explaining resistance is expected with respect to the plant developmental stage and the resistance parameters used. In our first study, only a single QTL on chromosome 12 was detected to explain virus content, while in the present analysis, additional QTLs were observed for this trait. These additional QTLs are unlikely to be due to more favourable experimental conditions for QTL detection (extended population size, more sensitive ELISA test), but they may depend on the morpho-physiological conditions of the plants at inoculation and test dates. Morpho-physiological differences between *indica* and *japonica* varieties can partly hide actual resistance mechanisms, independent of morphology. This may explain why conventional breeding has failed to transfer resistance from upland to irrigated varieties in combining in a single genotype a high level of RYMV resistance with adaptation to irrigated conditions. The specific selection of resistance factors independent of morphology may be very useful in breeding programmes. As the QTL of chromosome 12 was stable under different test conditions and did not appear to be co-located with morphological traits, it seems to be a particularly good candidate for introgression in *indica* varieties.

Acknowledgements The authors thank Cornell University, Ithaca, N.Y., and MAFF DNA Bank of the RGP, Japan, for providing DNA probes.

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THEORETICAL AND APPLIED GENETICS

International Journal of Plant Breeding Research

Volume 97 Number 7 November 1998

(Continued on page A3)