Theor Appl Genet (1998) 97: 1155–1161

G. Pressoir · L. Albar · N. Ahmadi · I. Rimbault
M. Lorieux · D. Fargette · A. Ghesquière

Genetic basis and mapping of the resistance to rice yellow mottle virus. II. Evidence of a complementary epistasis between two QTLs

Received: 20 April 1990 / Accepted: 30 April 1998

Abstract The genetic basis of resistance to rice yellow mottle virus (RYMV) was studied in a doubled-haploid (DH) population derived from a cross between the very susceptible *indica* variety 'IR64' and the resistant upland *japonica* variety Azucena. As a quantitative trait locus (QTL) involved in virus content estimated with an ELISA test has been previously identified on chromosome 12, we performed a wide search for interactions between this QTL and the rest of the genome, and between this QTL and morphological traits segregating in the population. Multiple regression with all identified genetic factors was used to validate the interactions. Significant epistasis accounting for a major part of the total genetic variation was observed. A complementary epistasis between the QTL located on chromosome 12 and a QTL located on chromosome 7 could be the major genetic factor controlling the virus content. Resistance was also affected by a morphology-dependent mechanism since tillering was interfering with the resistance mechanism conditioned by the epistasis between the two QTLs. Marker-assisted backcross breeding was developed to introgress the QTLs of chromosome 7 and chromosome 12 in the susceptible 'IR64' genetic background. First results confirmed that if both QTLs do not segregate in a backcross-derived F_2 population, then the QTL of chromosome 12 cannot explain differences in virus content. A near-isogenic line (NIL) approach is currently being developed to confirm the proposed genetic model of resistance to RYMV.

Key words QTL · Complementary epistasis · Marker-assisted selection · Quantitative resistance · Rice yellow mottle virus

Communicated by H. F. Linskens

G. Pressoir · L. Albar (✉) · I. Rimbault
M. Lorieux · A. Ghesquière
Unité de Génétique, GeneTrop, ORSTOM, BP5045,
34032 Montpellier cedex 1, France

N. Ahmadi[1]
IER/CIRAD, BP183, Sikasso, Mali

D. Fargette
LPRC, ORSTOM/CIRAD, BP5035, 34032 Montpellier
cedex 1, France

Present address:
[1] CIRAD-CA/CALIM, BP5035, 34032 Montpellier cedex 1, France

Introduction

Rice yellow mottle virus (RYMV) is a highly damaging rice pest in Africa (Awoderu 1991; Fomba 1988). Most varieties adapted to lowland or intensive irrigated culture are highly susceptible, while resistance is usually associated to the upland *japonica* rice varieties (Thottappilly and Rossel 1993; Rasaonary 1990). During the last decade, breeders have tried to introduce resistance into *indica* varieties, but no variety with both a high level of resistance and adaptation to irrigated cultivation has yet been released (Singh 1995).

In order to better understand the genetic basis of RYMV resistance, a wide search for quantitative trait loci (QTLs) has been performed in the population 'IR64/Azucena' using various approaches including morphological traits, symptom expression, incidence of artificial inoculation on morphological traits and virus content estimated with an ELISA test. Some RYMV resistance QTLs and plant architecture QTLs mapped on the same chromosomal fragments, suggesting a possible implication of morphology on resistance (Albar et al. 1998). A QTL mapped on chromosome 12 was found to be more specifically involved in virus content and symptom expression, and it mapped in a region where no morphological QTL had been localised. This QTL was also evidenced in another DH population derived from a 'Irat177 × Apura' cross (Ghesquière et al. 1997).

When QTLs show interaction with unlinked genes, their effects may be considerably altered when attempts are made to incorporate them into another genetic background. In maize, for instance, some QTLs did not appear in absolute levels (Stuber 1989). The importance of epistasis is also supported by the frequent observation that the total genotypic variation explained by all of the detected QTLs is generally much smaller than the sum of genotypic variances explained by each QTL separately. For this reason, the search for epistasis between a characterised QTL and other loci is a prerequisite before the potential utilisation of this QTL in plant breeding applications can be envisaged.

In our previous analyses (Ghesquière et al. 1997; Albar et al. 1998), the QTL mapped on chromosome 12 seemed to be a major genetic factor of resistance, independent of plant morphology. In the study presented here, interactions between this QTL and genetic background were tested, and the effect was checked during the first steps of a backcross introgression process monitored by mapped molecular markers.

Materials and methods

Mapping of a doubled-haploid (DH) population

A DH population 'IR64/Azucena' (Guiderdoni et al. 1992) was analysed to characterise the inheritance of resistance to RYMV (Albar et al. 1998). A restriction fragment length polymorphism (RFLP) map population had earlier been developed by Huang et al. (1997). This map served as the reference for marker analysis, and additional markers were generated (Albar et al. 1998).

DNA extraction, RFLP and sequence-tagged sites-polymerase chain reaction (STS-PCR) assays

DNA extraction was based on the method of Hoisington et al. (1994). For RFLP, the preparation of filters, probes and hybridisations were performed as described in Ghesquière et al. (1997). Signals were detected with anti-digoxygenine antibody (dilution 1/10 000) and CSPD™ or CDPStar™ (1/500) using the procedures recommended by Boehringer. Probes were removed from the membranes by washing in 0.1% SDS at 80°C for 20 min. Microsatellite marking was carried out according to Chen et al. (1997). PCR products were run on 2.5% agarose gels, and marker bands were revealed in ethydium bromide.

Phenotypic evaluation of resistance through serological tests of the DH population

Eighty-nine DH lines were considered for resistance. Sixty lines were evaluated in 1993 (Ghesquière et al. 1997), and 29 complementary lines were evaluated in 1997. For the 1997 evaluation, plants were grown in a growth chamber in parcels of 10 plants, replicated in two complete randomised blocs. The third leaf of each plant was mechanically inoculated 19 days after germination with an isolate of virus from Burkina-Faso as in Albar et al. (1998). The last leaf to emerge was assayed for virus content 15 days after inoculation.

Virus content assessment in plants was measured using the Double Antibody Sandwich-ELISA method, as described in Albar et al. (1998). The antibody was prepared against a virus isolate from Madagascar. Leaf extracts were ground in phosphate buffer at a dilution of 1 g for 20 000 ml. This dilution was chosen as it gave a good proportionality between optical density and virus concentration in preliminary experiments.

Field evaluation data

The set of data studied by Albar et al. (1998) was used. One hundred and fifteen DH lines were evaluated in 1996 at the IER/CIRAD research station in Sikasso, Mali, for field resistance. The experimental design included two replicates; each DH line was represented by two rows of plants facing each other with 5 plants per row. DH lines were disposed according to their line numbers inside each replicate. The first row was mechanically inoculated, while the second row served as the control. Inoculation was performed 4 weeks after sowing using infected leaves ground in water and abrasive. The impact of the disease on plant growth was evaluated by the ratio between inoculated and non-inoculated plants. HMr is the ratio for height at maturity, and HDr is the ratio for heading date. Tillering was measured on non-inoculated plants and refers here to the number of tillers 8 weeks after sowing.

Data analysis

A three-step method was used for the identification and validation of QTLs. First, conventional QTL mapping (as in Albar et al. 1998) was performed with a single-QTL model using simple interval mapping with MAPMAKER/QTL (Lander and Botstein 1989). A LOD ≥ 2.6 was used as a threshold for claiming the presence of a putative QTL. The QTLs detected were fixed, and additional QTLs were claimed if they contributed to the multi-QTL model by an additional LOD ≥ 2.6. For virus content in the last emerging leaf 15 days after inoculation, two independent tests were conducted at different times (in 1993 and 1997) and with different DH lines. A hierarchical design with the year as the hierarchical factor was adopted to perform regression. A probability of $P \leq 0.001$ was used as a threshold for claiming the presence of a putative QTL using SAS Proc GLM (Statistical Analysis System 1990) and Type-III sums of squares.

The second step consisted of testing two-way analysis of variance with interaction between RG869, mapped on the QTL12, and all markers from the linkage map. This step was realised using SAS Proc GLM (Statistical Analysis System 1990) and Type-III sums of squares. All epistasis between RG869 and any other marker significant at $P \leq 0.001$ were used in further model building steps.

The third step consisted of testing all identified genetic factors including epistasis in a multiple regression model and proceeding to a backward stepwise regression technique as suggested by Jansen (1993). A probability of $P < 0.05$ was used as a threshold. Interactions between the QTLs identified in step 1 and RG869 were added even if they did not show up in step 2. All these models were then treated as linear additive models using SAS Proc GLM (Statistical Analysis System 1990) and Type-III sums of square.

The heritability (h^2) on a plot basis was estimated from the following equation: $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_e/n)$ where σ^2_G and σ^2_e are the estimated genotypic and environmental variances, respectively, and n the number of repeats. Because of the non-total randomisation of the lines in each block, the estimators of the heritabilities calculated for HMr and HDr are biased. Nevertheless, as they give to some extent an approximation of the non biased heritabilities, they were used for further data analysis. Non-biased heritability estimators are referred to as h^2 and biased heritability estimators as h^{2*}.

Genotypic correlation was used to detect linkage and/or pleitropy between characters. If two characters are considered which are measured separately at two different locations, the phenotypic

correlation is due solely to the genotypic correlation because of the absence of environmental correlation. Therefore: $r_P = h_X h_Y r_G$ where r_P and r_G are the phenotypic and genotypic correlation and h_X^2 and h_Y^2 are the heritabilities of the two characters under consideration (Falconer and Mackay 1989). r_G can therefore be estimated : $r_G = r_P/\sqrt{(h_X^2 h_Y^2)}$ and $R^2_{Genotypic} = R^2_{Phenotypic}/(h_X^2 h_Y^2)$ where R^2 represents the percentage of variance explained by the regression.

Monitoring of the introgression process of the QTLs

In order to introgress two characterised QTLs (denoted by CDO418 for the QTL located on chromosome 7 and RG869 for the QTL located on chromosome 12), we conducted backcross breeding according to Hospital et al. (1992). The P303 DH line was crossed to IR64 to give a F_1. This F_1 was backcrossed to 'IR64' to give a BC_1 generation. Twenty-eight BC_1 plants were grown in the greenhouse. Plants were genotyped in order to select the QTLs to be introgressed and to select markers on non-carrier chromosomes, to shorten the backcross breeding process. Markers were chosen for their position on the genetic map. Seven RFLP markers, two microsatellites and the phenotypic marker C (chromogen for anthocyanin, on chromosome 6 ; Kinoshita 1995), were used. For QTL12, plants were genotyped using the RFLP marker RG869, but for QTL7 they were genotyped using the RFLP marker BNL16-06, because the marker CDO418 was not available in the laboratory. BNL16-06 is a genomic DNA probe of maize (Burr and Burr 1991). In this population BNL16-06 mapped at 5.3 cM from the marker CDO418. Two BC_1F_2 populations of 58 plants were evaluated for resistance to assure that they still segregated. One of these segregated for both BNL16-06 and RG869, while the other one segregated just for RG869. The evaluation of virus content was performed as previously described, except that inoculation took place 15 days after sowing. The most resistant plants, accumulating favourable alleles, were genotyped. This step was performed as a preliminary experiment in the monitoring of the introgression process.

Results

The two parents of the cross differed not only in resistance to rice yellow mottle virus but also in a wide range of adaptive and agronomic traits. The differences among DH lines for virus content (VC) and disease impact on plant height at maturity (HMr) and on heading date (HDr) were highly significant. HMr and HDr showed the highest heritabilities among all the traits used to estimate disease impact on agronomic characters (Albar et al. 1998). Variation among lines for viral concentration in the last emerging leaf showed a rough bi-modal distribution after log transformation, HMr and HDr showing continuous variation in the population. For these last two traits, transformation did not greatly improve normality of the distribution. All interactions between the candidate QTL located on chromosome 12 and all the other markers of the rice genome were tested for VC, HMr and HDr. The summary of all the loci identified to be associated with RYMV resistance is represented in Table 1.

Virus content in the last-emerging leaf (VC)

Virus content in the last emerging leaf, 15 days after inoculation, showed an estimated heritability of $h^2 = 0.92$ in the 1997 evaluation. A complementary epistasis was observed between RG869 (chromosome 12) and CDO418 (QTL7 : QTL located on chromosome 7). The percentage of variance explained by this interaction (QTL12*QTL7) (36.5%) was much higher then the one explained by RG869 alone (21%). A QTL located on chromosome 4 near marker RG214 was mapped. The parent contributing to an increase of resistance was the susceptible one, 'IR64'. This last QTL explained 10% of the phenotypic variance in 1993, but much less in 1997, which could suggest genotype × environment interactions.

Inoculation impact on plant height (HMr)

HMr showed an estimated heritability of $h^2* = 0.57$. Two major interactions between RG869 and other

Table 1 Loci associated with resistance considered for multiple regression model

Character	Loci	Parent contributing higher allele value	Single-factor model		Multiple-locus model		R^2% all locus
			Significance	R^2 %	Significance	R^2 %	
VC[a]	RG869(12[b])*CDO418(7)	Azu*Azu	< 0.0001	36.5[a]	< 0.0001	37.5[a]	
	RG214(4)	IR64	0.0131[c]	10[a]	0.0046	11[a]	47.5
HMr	RG869(12)*RZ801(1)	Azu*Azu	0.0079[d]	14.5	0.0011	16	
	RG869(12)*Pgi-2(6)	Azu*IR64	0.0035[d]	16.5	0.0061	12	31
HDr	RG869(12)*RZ801(1)	Azu*Azu	< 0.0001	36.5	< 0.0001	18.5	
	RZ801(1)	Azu	< 0.0001	17	0.0003	12	36

[a] For VC, R^2 is given for the 1993 evaluation
[b] The number in parentheses refers to the chromosome on which the marker is mapped
[c] RG620 closely linked to RG214 is significant at $P \leq 0.001$, but RG214 fits better in multi-regression model and is at the LOD peak in interval mapping
[d] The interactions are significant at $P = 0.001$ level in two-way analysis of variance with interaction

Table 2 Regression of virus content (VC), on tillering with respect to the allelic state at the RG869 locus (given for the data obtained in 1997)

RG869[a]	Significance	h^2_{VC}	$h^{*2}_{tillering}$	$R^2_{Phenotype}$ %	$R^2_{Genotype}$ %
–	0.0093	0.88	0.72	23	36
IR64	0.0378	0.83[c]	0.76[c]	26	41
Azucena	NS[b]	0.87[c]	0.64[c]	–	–

[a] Nature of the allele at the RG869 locus
[b] Non-significant, $P > 0.15$
[c] Partial heritabilities

markers of the genome were characterised. The interaction between locus RG869 and locus RZ801 (chromosome 1) showed apparent characteristics of duplicate epistasis. RZ801 mapped near a plant height QTL which corresponds to *sd-1*, a semi-dwarf gene carried by the susceptible parent, 'IR64'. The interaction between RG869 and Pgi-2 was characteristic of a complementary epistasis which does not result from co-adapted genes ; the favourable sets of alleles showed the presence of the allele coming from 'Azucena' at one locus and of the allele from 'IR64' at the other, in any combination.

Table 3 Multi-factor regression model for virus content (given for the data obtained in 1993)

Factor	Significance	R^2%	R^2% all factors
RG869*Tillering	0.0233	10	
RG869*CDO418	0.0232	13	61
RG214	0.0183	8	

The sum of the percentage of variance explained in the model by its different components is much lower than the percentage of the variance explained by the model itself; this observation strongly suggests the existence of a hidden variable in the model

Inoculation impact on heading date (HDr)

HDr showed the highest estimated heritability of all the parameters measuring disease impact on agronomic traits in the field experiment ($h^{2*} = 0.62$). A duplicate epistasis between RG869, on chromosome 12, and RZ801, on chromosome 1, was also characterised for this trait. A QTL was mapped in the RZ801 region in a single-QTL model and remained significant in the multiple regression.

Tillering as an explanatory variable of resistance

A positive genotypic correlation between VC and tillering was characterised. Tillering showed an interaction with the allelic state at the RG869 locus (Table 2). The regression of viral concentration on tillering proved to be significant for plants with the allele from 'IR64' at the RG869 locus and non-significant for the ones with the allele provided by 'Azucena'. This interaction between RG869 and tillering was significant in both the 1993 and 1997 evaluations, but it was not significant in the joint analysis. The regression model with all identified genetic factors explained 61% of the phenotypic variance for the 1993 evaluation and 67% for the 1997 evaluation. In contrast with the model which did not include tillering (Table 1), this model (Table 3) was not linear additive: the sum of the percentages of variance explained by individual factors in the multi-factor regression model was much lower than the percentage of variance explained by the model. This suggests the presence of a hidden variable brought about by the introduction of the interaction of tillering and RG869 in the model.

The summary of all identified genetic factors for resistance to the rice yellow mottle virus is presented in Fig. 1.

Monitoring of the QTL introgression process

Since our results suggested a complementary epistasis between RG869 (chromosome 12) and CDO418 (chromosome 7), these two QTLs were chosen for marker-assisted backcross breeding. The DH line P303 was selected because of: (1) its high level of resistance; (2) the presence of the alleles derived from 'Azucena' at the RG869 and CDO418 loci; (3) recombinations on each side of the QTL12, near the RG869 locus; (4) a predominant 'IR64' genetic background (60%). A non-carrier chromosome selection was performed using convenient markers (Fig. 2). No carrier chromosome selection took place on BC_1 plants because: (1) the selection for non-carrier chromosomes is the most effective at early stages of backcross breeding; (2) a small number of plants were obtained in the first backcross generation (28 plants); (3) the selection for recombinants around the QTLs to be introgressed had already been made in the choice of the P303 DH line.

Two BC_1 plants were selected to check, using their F_2 progenies, if the QTL located on chromosome 7 affects the expression of the QTL on chromosome 12. The first one, 303-17, selected to continue the QTL introgression process, was heterozygous for QTL7 and QTL12. The second one, 303-0, was heterozygous only

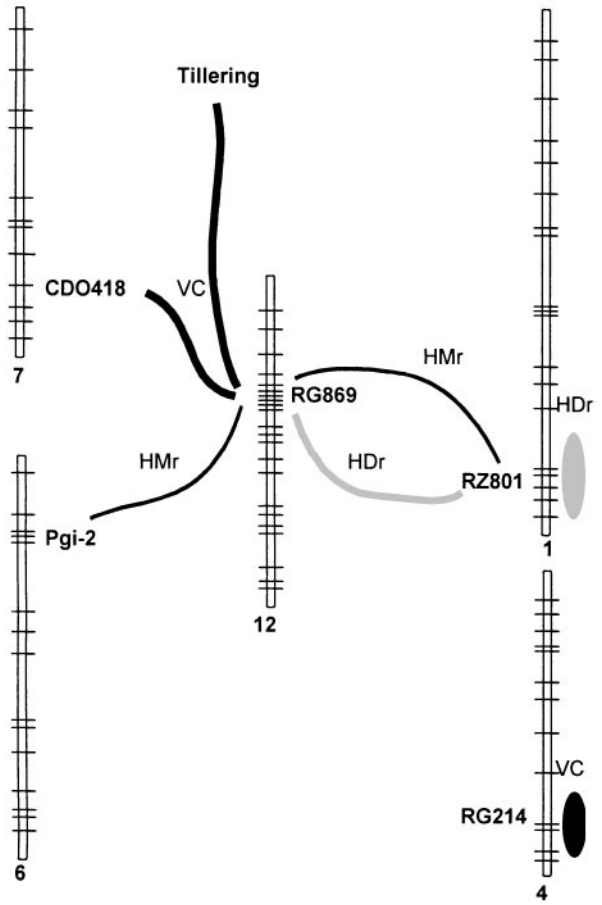

Fig. 1 QTLs and epistasis between QTLs involved in virus content (*VC*), inoculation impact on heading date (*HDr*) and inoculation impact on height at maturity (*HMr*)

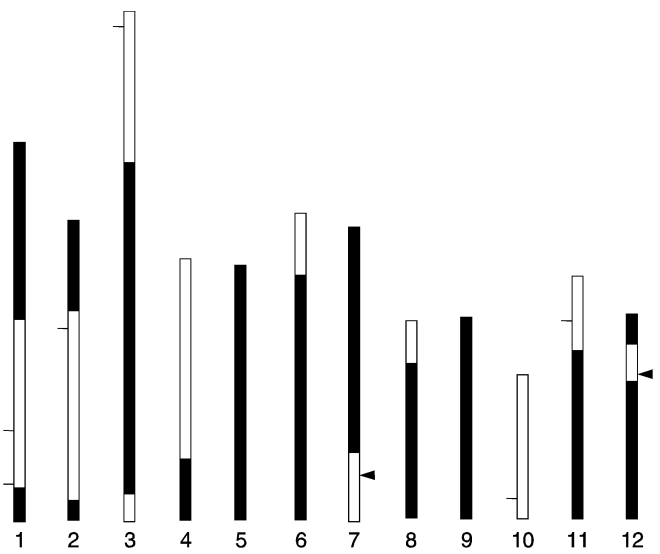

Fig. 2 Genetic conformation of the selected doubled-haploid line P303. In *black*, chromosomal segments with the 'IR64' allele; in *white*, segments with the 'Azucena' allele. Markers near selected QTLs are indicated by an *arrow*. *Horizontal bars* represent loci where the 'Azucena' allele has been counter-selected during the first backcross generation to give the next backcross generation

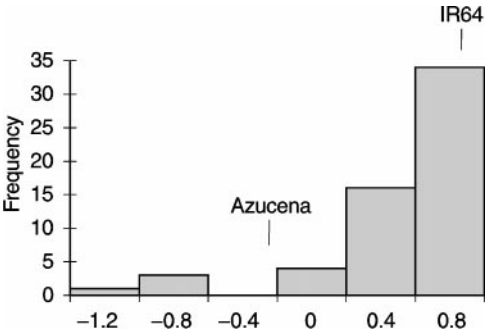

Fig. 3 BC_1-F_2 population from self-pollination of the 303-17. Virus content is expressed in log (absorbance measured by the DAS-ELISA method). Resistant plants show low virus content

for QTL12. Both F_2 populations showed a similar distribution of virus content, with a small proportion of plants exhibiting very low virus content (see Fig. 3 for the BC_1F_2 derived from 303-17). If we assume that the most resistant plants were supposed to accumulate all of the favourable factors for resistance, then the genotyping of resistant individuals can be considered to be appropriate to obtain a first validation of the proposed model. Four and 12 individuals from the BC_1F_2 populations derived from 303-17 and 303-0, respectively, were genotyped to check segregation at QTL12 and QTL7. The 4 most resistant plants derived from 303-17 were all homozygous for the allele coming from 'Azucena' ($P < 0.01$) at the RG869 locus, and none of these plants was homozygous for the allele coming from 'IR64' at locus BNL16-06. In the 12 most resistant plants from the second population, the segregation at the RG869 locus was not different from the segregation expected if RG869 and VC were independent. The totally different results obtained between the two populations suggest that the presence of the allele from 'Azucena' at QTL12 cannot explain the resistance without the presence of the allele from 'Azucena' at QTL7.

Discussion

Numerous authors (Edwards et al. 1987; Paterson et al. 1988; Fatokun et al. 1992; Stuber et al. 1992; DeVicente and Tanksley 1993; Schön et al. 1994) have discussed the low amount of epistasis found between markers with respect to quantitative trait variation. This is in opposition with the frequent observation of epistatic variation in classical quantitative genetic studies (Bauman 1959). Indeed, if all marker-marker interactions were to be tested, the amount of epistasis detected would not differ from that expected by chance only (Xiao et al. 1995). This is why, in this experiment, only interactions involving QTL12 were tested. We also performed a multiple regression with all of the identified genetic factors, which enabled us to remove

false-positive interactions due to the genetic background effects arising from segregating QTLs (Li et al. 1997).

QTL12 appears for a character for which the difference in resistance between the two parents of the cross would essentially be due to the movement and/or replication of the virus. It also appears for the disease impact on agronomic traits, but then it seems to be in a duplicate epistasis with a QTL on chromosome 1 co-located with *sd-1*, a semi-dwarf allele brought by the susceptible parent.

A very strong positive genetic correlation between virus content in the last-emerging leaf and tillering 8 weeks after sowing has been characterised. It points to the existence of pleiotropy and/or linkage between resistance and tillering QTLs. QTLs involved in the number of tillers at maturity were mapped on chromosome 1, near the semi-dwarf gene *sd-1*, and on chromosome 7 near CDO418; no QTL was detected for tillering 8 weeks after sowing, but the probability of a QTL controlling this trait on CDO418 is $P = 0.009$ (Albar et al. 1998). The importance of the genotypic R^2 and the fact that no major QTL has been identified for tillering 8 weeks after sowing support the idea of tillering QTLs having a pleiotropic effect on resistance to rice yellow mottle virus. The interaction of tillering and QTL12 shows characteristics of duplicate epistasis similar to the one between QTL12 and the region near the semi-dwarf gene, *sd-1*, carried by the elite *indica* parent. Tillering might therefore be a morphology-dependent resistance mechanism interfering with another mechanism of resistance for which QTL12 would be a major genetic factor.

Lark et al. (1995) discussed the possible role of autogamy in the fixation of unlinked pairs of interacting alleles. The type of interaction for which variation controlled by alleles at one locus is conditional upon alleles at another locus has been observed in rice (Li et al. 1997), soybean (Lark et al. 1995) and in capsicum (Caranta et al. 1997) for resistance to a virus. Here, we described the same type of interaction between the QTL located on chromosome 12 and the one located on chromosome 7 for virus content assessment in a newly emerging leaf.

Complementary epistasis and a similar type of action and interaction of two genes may suggest that, in an obligate inbreeding plant, pairs of selected alleles belonging to different linkage groups can be maintained (Lark et al. 1995). In the case of rice, most of the genetic diversity is supposed to be derived from two independant domestications, and the *japonica* and *indica* groups of varieties can be considered to be subspecies (Second 1991). The models of hybrid sterility assuming duplicate lethal gametophytic genes (Oka 1974) and restriction to recombination in remote crosses, thus accelerating parental phenotype recovery, can reflect co-adaptation between loci, for morphological and physiological traits, before and during the course of domestication. The complementary epistasis found for the viral concentration in the last emerging leaf between QTL12 and QTL7 strongly suggests just such a co-adaptative pattern. In this case, one should expect QTL7 to be involved in the same interaction as QTL12. Nevertheless, QTL7 was not significant, even in interaction with other QTLs, for inoculation impact on heading date and on height at maturity while QTL12 was. A tillering QTL has been identified near CDO418 (Albar et al. 1998) and, while CDO418 was significant to explain tillering, RG869 and the RG869*CDO418 interaction were not. Finally, we observed that the model built in Table 3 was not linear additive. In this model, the sum of the percentage of variance explained by the model's different components was much lower than the percentage of variance explained by the model. Therefore, we have to suppose the existence of a hidden variable inside the model. This could be due to *cis*-epistasis and/or interaction(s) of a rank superior than two. If QTL12 and QTL7 are co-adapted loci in terms of virus content and as they do not act similarly in terms of tillering, we have to suppose the existence of at least two distinct genes underlying the identification of QTLs and the interaction at the CDO418 marker: one being a gene involved in tillering and the other the co-adapted gene of QTL12. We would then expect an epistasis between these two putative genes similar to the one between QTL12 and tillering. An epistasis between these two *cis*-located QTLs close to the marker CD0418 would explain the hidden variable because the variable tillering would then contain other variables and CDO418 would be represented twice in the model. The fact that QTL7 is not significant even in interaction with other QTLs for disease impact on heading date and on height at maturity could well be explained by *cis*-epistasis that would alter the expression of the QTL detected at the CDO418 locus.

The first results during the monitoring of the introgression breeding process were in favour of complementary interaction between QTL12 and QTL7. However, in the population derived from plant 303-0, this interaction can not explain the segregation of virus content. In this case, resistance may be under the control of morphological components. In particular, the semi-dwarfing gene, *sd-1*, which plays an important role in resistance (Albar et al. 1998), segregated in the 303-0 F_2 population and not in the 303-17 population. The skewed distribution of virus content in the two populations may also indicate that other favourable interactions could have been lost during the backcross process.

The parents used in this study are representative of two highly differentiated gene pools. All of the upland *japonica* tested to this day have shown a good level of resistance. 'Azucena', like many traditional upland *japonica* varieties, was selected outside of the rice yellow mottle virus extension zone. The QTL12 locus was significant in explaining resistance in two distinct DH

populations between upland *japonica* and *indica* (Ghesquière et al. 1997). Therefore, it might be possible that this locus, whether or not in interaction with other loci, would be partly responsible for a non-specific adaptive trait not linked to an unfavourable morphologic trait.

Interactions between complementary resistance genes and morphology make it difficult to combine in one genotype the resistance level of the upland *japonica* varieties and the agro-morphological characters of *indica* varieties using only phenotypic selection. Marker-assisted backcross breeding could facilitate the introgression of resistance QTLs not linked to morphology and allow genetic background effects to be taken into consideration. The ongoing introgression process would allow selection on a larger BC_2 population. Available microsatellite markers (Chen et al. 1997) can be used for further non-carrier chromosome selection. Further evaluation of the constructed near-isogenic lines will either confirm or negate our genetic model for resistance to rice yellow mottle virus.

References

Albar L, Ahmadi N, Rimbault I, Pinel A, Lorieux M, Sy AA, Fargette D, Ghesquière A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology. Theor Appl Genet 97 : 1145–1154

Awoderu VA (1991) Rice yellow mottle virus in West Africa. Trop Pest Manage 37 : 356–362

Bauman (1959) Evidence of non allelic gene interaction in determining yield, ear height and kernel row number in corn. Agric J 51 : 531–534

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize. Trends Genet 7 : 55–60

Caranta C, Palloix A, Lefebvre V, Daubèze AM (1997) QTLs for a component of partial resistance to cucumber mosaic virus in pepper: restriction of virus installation in host-cells. Theor Appl Genet 94 : 431–438

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95 : 553–567

DeVicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134 : 585–596

Edwards MD, Stuber CW, Wendel JF (1987) Molecular marker facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116 : 113–125

Falconer DS, Mackay TFC (1989) Introduction to quantitative genetics, 4th edn. Longman Group, Harlow, UK

Fatokun CA, Menancio-Hautea DI, Danesh D, Young ND (1992) Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. Genetics 132 : 841–846

Fomba SN (1988) Screening for seedling resistance to rice yellow mottle virus in some rice cultivars in Sierra Leone. Plant Dis 72 : 641–642

Ghesquière A, Albar L, Lorieux M, Ahmadi N, Fargette D, Huang N, McCouch SR, Notteghem JL (1997) A major QTL for rice yellow mottle virus (RYMV) resistance maps to a cluster of blast resistance genes on chromosome 12. Phytopathology 87 : 1243–1249

Guiderdoni E, Galinato E, Luistro J, Vergara G (1992) Anther culture of tropical *japonica* × *indica* hybrids of rice (*Oryza sativa* L.). Euphytica 62 : 219–224

Hoisington D, Khairallah M, Gonzales-de-Léon D (1994) Laboratory protocols CIMMYT. Applied molecular genetics laboratory, 2nd edn. CIMMYT, El Batan, Mexico DF

Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. Genetics 132 : 1199–1210

Huang N, Parco A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu J, Subudhi P, Angeles ER, Kush GS (1997) RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a double haploid rice population. Mol Breed 3 : 105–113

Jansen RC (1993) Interval Mapping of multiple quantitative trait loci. Genetics 135 : 205–211

Kinoshita T (1995) Report of committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newsl 12 : 9–153

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121 : 185–199

Lark KG, Chase K, Adler F, Mansur LM, Orf JH (1995). Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA, 92 : 4656–4660

Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145 : 453–465

Oka HI (1974) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetics 77 : 521–534

Paterson AH, Lander SE, Hewitt JD, Peterson S, Lincoln HD, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335 : 721–726

Rasaonary M (1990) Application d'un test immunoenzymatique a l'étude de la résistance au rice yellow mottle virus. MSc thesis, CNEARC, Montpellier, France

Schön CS, Melchinger AE, Boppenmaier J, Jung EB, Herrmann RG, Seitzer JF (1994) RFLP mapping in maize: quantitative trait loci affecting testcross performance of elite european flint lines. Crop Sci 34 : 378–389

Second G (1991) Molecular markers in rice systematics and the evaluation of genetic resources. In: Bajaj YP (ed) Biotechnology in agriculture and forestry, vol 14: rice. Springer, Berlin Heidelberg New York, pp 468–494

Singh BN (1995) Breeding for RYMV resistance. In: First Int Symp Rice Yellow Mottle Virus (RYMV), WARDA, eds, Mbé/Bouaké, Ivory Coast

Statistical Analysis System (1990) SAS/STAT user's guide. version 6, 4th edn. SAS Institute, Cary, N.C.

Stuber CW, Lincoln SE, Wolf DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132 : 823–839

Stuber CW (1989) Marker based selection for quantitative traits In: Röbbelen G (ed) Science for plant breeding. Proc. XII Eucarpia Cong. Paul Paray, Berlin, pp 31–49

Thottappilly G, Rossel HW (1993) Evaluation of resistance to rice yellow mottle virus in *Oryza* species. Indian J Virol 9 : 65–73

Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is a major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140 : 745–754