Theor Appl Genet (2001) 103:1084–1092

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Genetic basis and mapping of the resistance to *Rice yellow mottle virus*. III. Analysis of QTL efficiency in introgressed progenies confirmed the hypothesis of complementary epistasis between two resistance QTLs

Received: 11 August 2000 / Accepted: 20 March 2001

Abstract Our previous studies have hypothesised that a complementary epistasis between a QTL located on chromosome 12 and a QTL located on chromosome 7 was one of the major genetic factors controlling partial resistance to *Rice yellow mottle virus* (RYMV). We report research undertaken to verify this hypothesis and to introgress the resistant allele of these two QTLs from an upland resistant *japonica* variety, Azucena, into a lowland susceptible *indica* variety IR64. Three cycles of molecular marker-assisted back cross breeding were performed using RFLP and microsatellite markers. Resistance to RYMV was evaluated in F_2 and F_3 offspring of the BC_1 and BC_2 generations. Marker-assisted introgression (MAI) was very efficient: in the selected BC_3 progeny the proportion of the recipient genome was close to 95% for the ten non-carrier chromosomes, and the length of the donor chromosome segment surrounding the two QTLs was less than 20 cM. The relevancy of the complementary epistasis genetic model proposed previously was confirmed experimentally: in BC_1 and BC_2 generations only F3 lines having the allele of the resistant parent on QTL_{12} and QTL_7 show partial resistance to RYMV. Comparison of our experimental process of MAI with the recommendations of analytic and simulation studies pointed out the methodological flexibility of MAI. Our results also confirmed the widely admitted, but rarely verified, assumption that QTL-alleles detected in segregating populations could be treated as units of Mendelian inheritance and that the incorporation of these alleles into elite lines would result in an enhanced performance. The next step will be the design of tools for the routine use of molecular markers in breeding for partial resistance to RYMV and the development of material for the analysis of resistance mechanisms and the structure of a virus resistance gene in rice.

Keywords Rice yellow mottle virus · Partial resistance · QTL · Marker assisted selection · Complementary epistasis

Communicated by H.C. Becker

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Introduction

At least 15 viruses affect rice crops. Among these viruses, three, each of them endemic in one of the three major rice-growing continents frequently reach epidemic proportions: the Rice Tungro in Asia, the *Rice hoja blanca virus* (RHBV) in America and the *Rice yellow mottle virus* (RYMV) in Africa (Hibino 1996).

In Africa, RYMV was observed for the first time in Kenya (Bakker 1974), and later, during the seventies, has been found in many rice-growing countries (Fauquet et Thouvenel 1977; Raymundo et Konteh 1980; John et al. 1984). The disease is now present in all rice-growing ecosystems, but its incidence is particularly high in the irrigated paddy fields of the Sudanean area where losses can reach 95% of the expected harvest in double rice cropping systems (Awoderu 1991; Abo et al. 1998). Infected rice plants show mottling or yellow discoloration of leaves, stunting, a reduced number of fertile tillers and sterility (Bakker 1974).

RYMV is a *sobemovirus* whose genome, of 4450 nucleotides, is organised into four open reading frames (ORF) (Ngon A. Yassi et al. 1994). The sequence diver-

sity of the coat protein encoded by ORF4 and its serological proprieties allow distinction between five major strains with different geographical distributions (N'Guessan et al. 2000; Pinel et al. 2000). Different levels of pathogeny between isolates from Ivory Coast have also been found (N'Guessan,1999). The virus replication takes place preferentially in the mesophyl cells and xylem parenchyma tissue. Cell-to-cell movement occurs through the plasmodesmes (Bonneau et al. 1998) and long distance movement through the xylem vessels (Opalka et al. 1998).

The recent increase in the RYMV epidemic is commonly attributed to crop intensification and the use of improved but susceptible photo-insensitive cultivars of short growth duration, introduced from Asia during the last 30 years (Abo et al. 1998). Nevertheless, varietal resistance remains the backbone of all strategies of RYMV control.

Today, three types of varietal resistance to RYMV are known: a resistance obtained through genetic transformation (Pinto et al. 1999), a natural high resistance (Thottappilly and Rossel 1993; Ndjiondjop et al. 1999), and a natural partial resistance (Ghesquière et al. 1997; Albar et al. 1998).

The transgenic resistance involves a transgene encoding a RNA-dependent RNA polymerase of RYMV acting through a RNA-based mechanism associated with post-transcriptional gene silencing (Pinto et al. 1999). The natural high resistance to RYMV is a rare phenomenon observed in a few number of varieties of the African cultivated rice species *Oryza glaberrima* (Thottappilly and Rossel 1993), and in only one variety of *Oryza sativa* (Ndjiondjop et al. 1999). Within the two species, this resistance is controlled by the recessive allele of the same single gene mapped on chromosome 4 (Ndjiondjop 1999). It has been proposed that resistance is derived from an impaired cell-to-cell movement of the virus (C. Brugidou, personal communication).

The partial resistance to RYMV is more widely distributed and is closely related to the *O. sativa* subdivision into two sub-species: *indica* and *japonica*. Varieties with a significant partial resistance to RYMV all belong to the tropical *japonica* sub-species and are not adapted to lowland cultivation. The genetic determinism of this resistance is polygenic (Ahmadi and Singh 1995). Resistance quantitative trait loci (QTL) have been mapped in a doubled-haploid population of a cross between a susceptible lowland *indica* variety, IR64, and a resistant upland *japonica* variety, Azucena (Ghesquière et al. 1997; Albar et al. 1998). Fifteen QTLs have been detected on seven chromosomal fragments. For 14 of these the favourable allele was provided by the resistant parent Azucena.

Significant correlations were observed between resistance and tillering ability and four chromosomal segments associated with resistance were also involved in plant architecture and development. Among the resistance QTLs, the one mapped on chromosome 12 has an important effect on leaf virus content and did not colocate with any plant aerial-part morphology QTL. The search for interactions between this QTL and the rest of the genome provided evidence that a complementary epistasis between the QTL located on chromosome 12 (QTL_{12}) and a RFLP marker close to a tillering QTL located on chromosome 7 (QTL_7) could be the major genetic factor controlling the virus content (Pressoir et al. 1998).

The creation of lowland rice varieties resistant to RYMV is an important stake in the rice breeding programs in Africa. One possible answer to this problem is the transfer of the partial resistance of the upland *japonica* rice varieties into the susceptible lowland *indica* varieties by backcross breeding. The potential use of molecular markers in such programs has received considerable attention. Markers can be used not only to assess the presence of the introgressed genes or QTLs (Tanksley 1983; Melchinger 1990; Hospital et al. 1997), but also to accelerate the return to the recurrent parent genotype (Hillel et al. 1990; Hospital et al. 1992, 1997).

The potential benefit of QTL mapping is based on the assumption that QTL-alleles detected in segregating populations could be treated as units of Mendelian inheritance and that the incorporation of these alleles into elite lines would result in an enhanced performance. However, in most instances, these assumptions have not yet been experimentally confirmed (Mohan et al 1997). We report here: (1) the molecular-marker assisted simultaneous introgression (MAI) of the resistant allele of QTL_{12} and QTL_7 from the upland *japonica* variety, Azucena, into the lowland susceptible *indica* variety, IR64, (2) the test of the relevancy of the genetic model proposed by Pressoir et al. (1998) to assume the action of these QTLs, and (3) methodological considerations on the practical management of MAI and on breeding strategy for resistance to RYMV.

Material and methods

Monitoring of the introgression process of two QTLs

In order to introgress QTL_{12} and QTL_7, molecular-markers assisted backcross breeding was carried out (Fig. 1) according to Hospital et al. (1992). The first step of the backcross process was described by Pressoir et al. (1998). Briefly, the doubled-haploid line DH P303 was crossed to the recipient variety IR64 to give an F_1. This F_1 was backcrossed to IR64 to give a BC_1 generation. Twenty eight BC_1 plants were first submitted to selection for the resistance allele on the two QTLs. Then, the remaining plants were screened for genotypic similarity with the recipient parent on the non-carrier chromosomes. The selected BC_1 (P303.17) was heterozygous for the two QTL markers (Fig. 1). In BC_2, selection was carried out only for the resistance allele on the two QTLs. A first screening for heterozygosity on QTL_{12} and QTL_7 led us to select 17 plants among 58 BC_2. Each of these BC_2 plants were selfed in order to obtain 17 BC_2–F_2 populations. An individual homozygote for the resistance allele of the two QTLs was selected after a second screening of 60 plants of one of these BC_2–F_2 populations. The selected plant, P303.17.18.8, was backcrossed to IR64 to give the BC_3 generation. In BC_3, as all 54 plants were heterozygous for the two QTLs, selection was directed toward the IR64 background. The individual closest to the recurrent parent IR64 was selfed in order to produce a large BC_3 F_2 population for selection on the carrier chromosome around the two QTLs, for fine

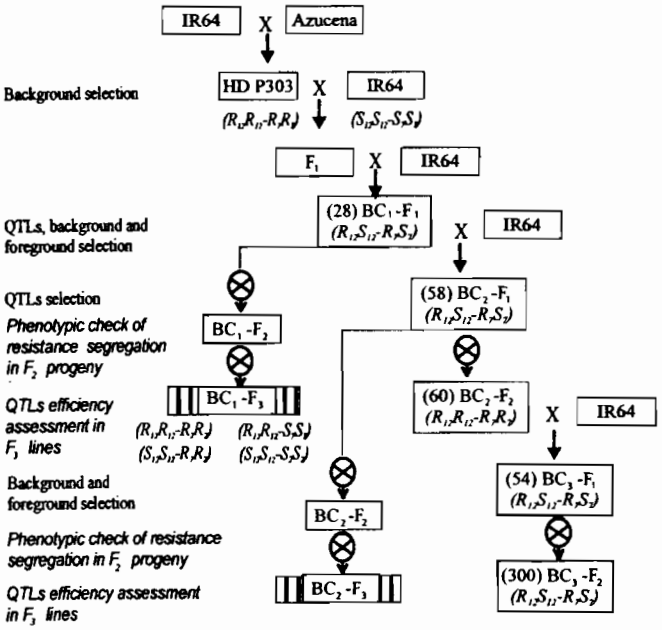

Fig. 1 General scheme of the marker-assisted introgression of two QTLs. *Figures in brackets* give the number of plants in each generation of selection. *Letters in brackets* give the allelic shape of the selected plant in each generation of selection

mapping of the QTLs and for the creation of a near-isogenic line (NIL). Concurrent to marker-assisted selection (MAS), phenotypic control was performed on approximately 60 F_2 plants of each backcross generation to check that they were still segregating for resistance.

Genotyping the individuals in the selection populations

Progenies were genotyped with the relevant RFLP markers, i.e. RG869 and BNL16-06, which are closely linked with QTL_{12} and QTL_7, respectively (Ghesquière et al. 1997; Albar et al. 1998; Pressoir et al. 1998). QTL genotypes were then inferred from the allelic shape of these flanking markers according to the following script conventions: resistant parent, Azucena genotype= $R_{12}R_{12}$–R_7R_7 and susceptible parent, IR64, genotype=$S_{12}S_{12}$–S_7S_7.

Genotyping on non-carrier chromosomes was made with seven RFLP markers, two microsatellites and the phenotypic marker C (chromogen for anthocyanin, on chromosome 6; Kinoshita 1995) in the BC_1 generation and with 32 microsatellite markers in the BC_3 generation. These markers were chosen for their position on the genetic map. DNA extraction, Southern blotting, and hybridisation with the probes selected were described by Albar et al. (1998). PCR-amplification of microsatellites was carried out according to Chen et al. (1997) The PCR products were run on 4% polyacrylamide denaturing gels and marker bands were revealed using labelling with α-^{33}P.

Analysis of the interaction between QTL_{12} and QTL_7

Sixty BC_1–F_2 and 60 BC_2–F_2 plants were genotyped for the two QTLs. In each F_2 population, 12 individuals homozygous for the two QTL markers were selected. Among them, three carried the resistance allele on both QTLs, three carried the resistance allele on QTL_{12} and the susceptibility allele on QTL_7, three others the reverse combination and the three last ones carried the susceptibility allele on both QTL_{12} and QTL_7. These 12 F_2 plants were selfed and the phenotypic evaluation of resistance was performed on their F_3 progeny. The experimental design was a randomised complete block with two replicates for BC_1–F_3 progenies and four replications for BC_2–F_3 progenies. Within a replicate, each F_3 progeny was represented by five plants. The data submitted to analysis of variance were the means of five individual scores. In addition to these two experiments, a third one was conducted with a BC_1–F_3 population within which the QTL_{12} marker locus was segregating and the QTL_7 marker locus was homozygous with the resistant parent allele.

Phenotypic evaluation of resistance to RYMV

Phenotypic evaluation of response to infection was performed in inoculated plants according to the symptom apparition date and the assessment of the virus titre through enzyme-linked immunosorbent assay (ELISA). Plants were grown in the growth chamber under controlled conditions (24–26°C for 12 h in the dark and 28–30°C for 12 h in the light, 120 $\mu E\ m^{-2}\ s^{-1}$). Inoculation was performed mechanically on the last well-emerged leaf, generally the fourth, approximately 15 days after sowing. The virus isolate used for inoculation originated from Burkina Faso and has been previously used to detect QTL_{12} and QTL_7 (Albar et al. 1998; Pressoir et al. 1998). This isolate, belonging to the S2 strain (Pinel et al. 2000), 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 and a better discrimination between susceptible and resistant lines.

The date of appearance of the symptom was noted on individual plants up to 20 days after inoculation. Then, the blade of the last completely deployed leaf, generally the fifth, of each plant was assayed for virus content. The leaf virus content (LVC) of systemically infected leaves was measured using the Double Antibody Sandwich-ELISA method, as described by Albar et al. (1998). Leaf extracts were ground in phosphate buffer at the dilution of 1 g for 10,000 ml as this dilution gave the most-reliable results in previous studies. Absorbance at 405 nm was measured after different times of incubation at 37°C. The optical density measured after 20 min was retained for data analysis.

Results

Marker-assisted selection for QTL_{12} and QTL_7

The introgression process started with one of the doubled-haploid lines (DH P303) of the IR64/Azucena cross used for QTL detection. This line was selected because of: (1) its high level of resistance, (2) the presence of the resistant alleles coming from Azucena at the loci associated with QTL_{12} and QTL_7, (3) recombinations on each side of QTL12, near the RG869 locus, and (4) a predominant IR64 genetic background (60%) according to mapping data of more then 200 markers (Huang et al. 1997). In BC_1, a non-carrier chromosome selection was performed using convenient markers (Fig. 2a). The non-carrier chromosome selection took place on BC_1 plants because: (1) selection for the non-carrier chromosome is the most effective at early stages of backcross breeding, (2) too small a number of plants (only 28) was obtained in the first backcross generation, and (3) selection for recombination around the QTL to be introgressed had already been made in the choice of the P303 DH line.

In BC_2, selection was carried out only for the resistance allele on the two QTLs, as on non-carrier chromosomes, even without selection, the expected increase of the recipient genome proportion was 50%. The back-

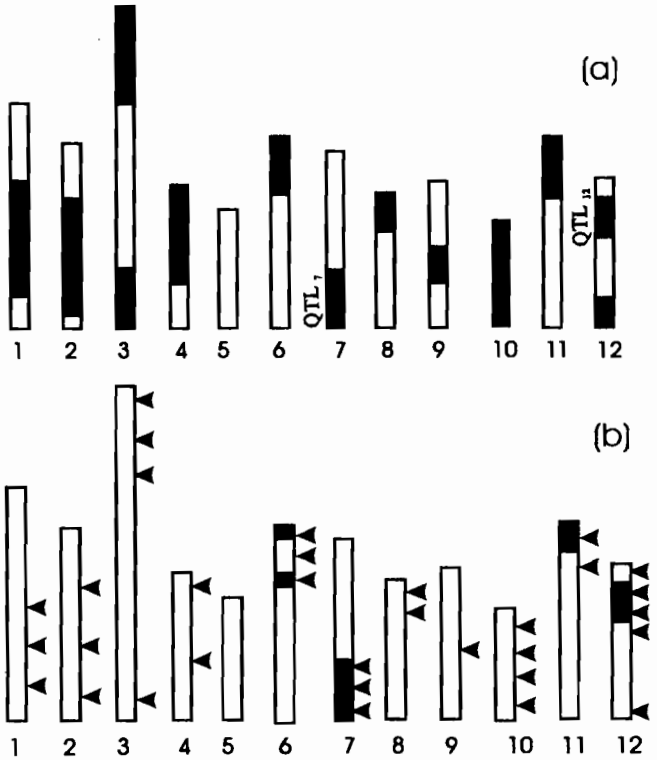

Fig. 2 Genetic conformation of the selected BC_1–F_1 (**a**) and BC_3–F_1 (**b**) plants. In *white* chromosomal segments with the recipient parent IR64 allele; in *black* chromosomal segments with the donor parent Azucena allele; *arrows* indicate the position of the microsatellite marker loci used for background selection in BC_3

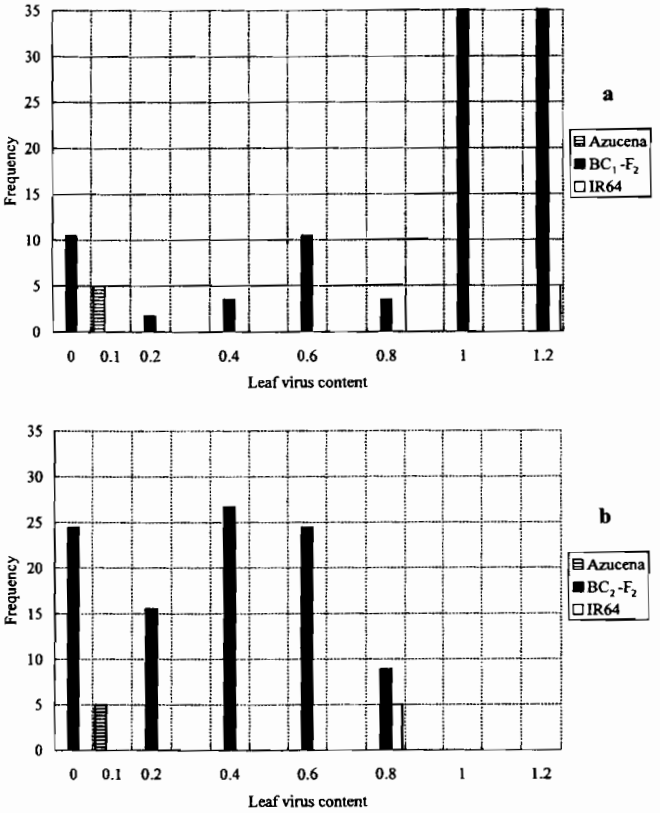

Fig. 3 Distribution of leaf virus content, estimated by absorbance (A 405 nm) in the ELISA test, in the systematically infected leaves of BC_1–F_2 (**a**) and BC_2–F_2 (**b**) populations. Resistant plants show low leaf virus content (*left*)

cross process was pursued with a BC_2–F_2 plant homozygous for the resistance allele of QTL_{12} and QTL_7. The use of such a BC_2–F_2 individual allowed us to obtain a BC_3 population in which the resistance allele of the two QTLs was present in a heterozygous condition in all plants. Therefore, it was possible to direct the whole selection effort toward the non-carrier chromosomes.

Background MAS in BC_3 was made with microsatellite markers. These microsatellites were chosen on the basis of the genetic conformation of the P303–17 BC_1 plant. In this plant, at least 13 chromosomal segments distributed on 11 chromosomes carried the alleles of the donor parent, Azucena (Fig. 2a). For each of these chromosomal segments, the return to the recipient parent was monitored with one to five microsatellites. For each segment, the choice of microsatellites was made in order to have a homogeneous coverage of the segment as far as possible. As shown in Fig. 2b, in the selected BC_3 plant, nine of the 13 target chromosomal segments were homozygous and carried the recipient parent allele of the corresponding microsatellite markers with a homozygous status. Among the four remaining heterozygous segments, two concerned the QTL carrier chromosomes, one bracketing QTL_7 and the other bracketing QTL_{12}. So, further background selection was needed only on the two non-carrier chromosomes, the sixth and the eleventh.

The closest BC_3 individual to the recipient parent IR64 (P303.17.18.8. 22) was selfed in order to produce a large BC_3 F_2 population serving for: (1) the finishing touches of background selection, (2) the selection on the carrier chromosome for recombination around the two QTL, (3) the creation of a NIL, and (4) the fine mapping of the QTL.

Phenotypic evaluation of F_2 progenies

The transfer of resistance was verified in F_2 progeny of the selected BC_1 and BC_2 plants. The BC_1–F_2 and BC_2–F_2 populations still segregated for resistance to RYMV (Fig. 3). By comparison with a dilution curve of purified virus, the virus concentration ratio between the most-resistant and the most-susceptible individual was approximately 1/1,000 (data not shown). The two F_2 populations did not show similar resistance distributions. The proportion of plants with a low virus content is much higher in the BC_2–F_2 population. This difference suggests the fixation of other non-controlled favourable factors, but it could also reflect uncontrolled differences in the experimental conditions.

Among the F_2 plants checked for LVC, 28 BC_1–F_2 and 45 BC_2–F_2 were genotyped with the QTL_{12} marker

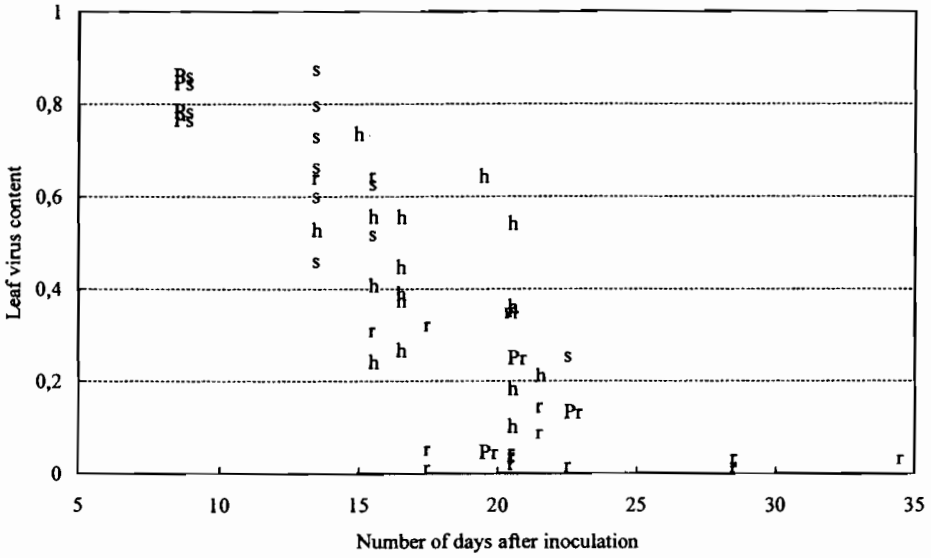

Fig. 4 Relationship between leaf virus content and symptom appearance time in the BC_2–F_2 population. *Letters* give the allelic shape of each plant at the QTL12 marker locus: *r* resistant homozygous, *s* susceptible homozygous, and *h* heterozygous, *Pr* resistant parent, *Ps* susceptible parent

and with the QTL_{12} and QTL_7 markers respectively. In the BC_1–F_2 population, the relationship between LVC and the allelic status of the QTL_{12} marker was not significant, most likely due to the small number of genotyped individuals. In the BC_2–F_2 population, the hypothesis of the independence between LVC and the allelic status of the QTL_{12} marker was rejected ($P<0.001$) for the 11 most-resistant plants. Among these 11 plants, ten were homozygous for the allele coming from the resistant parent Azucena at the QTL_{12} marker and none of them were homozygous for the allele coming from the susceptible parent IR64 at the locus of the QTL_7 marker.

These results confirmed that the resistance is actually quantitative and that its major component has not been lost after two generations of marker-assisted backcross selection. They also suggest that the presence of the Azucena allele at the locus of the QTL_{12} marker cannot on its own explain the resistance phenotype.

Symptomatology data confirmed the results obtained through LVC assessment. Actually, a close relationship between LVC and the number of post-inoculation days needed for visible leaf yellow discoloration was observed. Early symptoms were associated with a high virus titre and late symptoms with low virus titres (Fig. 4). This pattern can be fitted to a sigmoid ('S' shaped) curve. Such a relationship between LVC and the date of appearance of the symptom has been observed with many plant virus diseases (Bos 1999). In the same way, early symptoms were associated with the susceptible parent allele at the locus of the QTL12 marker and late symptoms were associated with the resistant parent allele at the locus of the QTL12 marker.

Complementary epistasis between QTL_{12} and QTL_7

The relationship between the allelic status of the QTL_{12} and QTL_7 markers and LVC was analysed in two groups of F_3 lines derived from the BC_1 and BC_2 plants selected for the introgression process and in one BC_1–F_3 population.

Table 1 Effects of QTL_{12}, QTL_7, and their interaction on the leaf virus content of BC_1–F_3 and BC_2–F_3 lines

Factor	BC_1–F_3			BC_2–F_3		
	MS[a]	*df*[b]	Significance limit	MS	*df*	Significance limit
QTL_{12}	0.460	1	0.05	0.791	1	0.0005
QTL_7	0.010	1	Ns	0.091	1	0.001
QTL_{12}×QTL_7 interaction	0.459	1	0.05	0.039	1	0.05
Residual	0.115	16	–	0.007	32	–

[a] MS: mean square
[b] *df*: degree of freedom

The BC_1–F_3 and BC_2–F_3 groups of 12 lines were all homozygous for the two QTL markers. Within these lines, the allelic status of QTL_{12} had a significant effect on plant LVC (Table 1). The statistical significance of the allelic status on LVC is higher in BC_2–F_3 lines compared to BC_1–F_3 lines, due to the higher number of replicates. The resistant-parent allele R_{12} R_{12} was clearly associated with the low values of LVC while the susceptible-parent allele S_{12} S_{12} was associated with its high values (Table 2). The effect of the allelic status of QTL_7 on LVC was significant only in the BC_2–F_3 progenies. The susceptible parent allele was associated with high LVC values. The effect of interaction between the allelic status of the two QTLs was significant in both BC_1–F_3 and BC_2–F_3 lines (Table 1). The lowest values of LVC were observed in F3 progenies with the resistant parent allele in both QTL_{12} and QTL_7 marker loci. This significant interaction between the two QTLs confirmed the existence of a complementary epistasis between QTL_{12} and QTL_7.

The BC_1–F_3 population was derived from a BC_1–F_2 plant with $R_{12}S_{12}$–R_7R_7 genotype. Within this popula-

Table 2 Allelic status of QTL_{12} and QTL_7 markers and the leaf virus content of BC_1–F_3 and BC_2–F_3 progenies

Genotype		Leaf virus content[a]		
QTL_{12} marker	QTL_7 marker	BC_1–F_3 population	BC_1–F_3 lines[b]	BC_2–F_3 lines[b]
$R_{12}\,R_{12}$	$R_7\,R_7$	0.229 a	0.443 a	0.305 a
$R_{12}\,R_{12}$	$S_7\,S_7$	–	0.553 ab	0.450 b
$S_{12}\,S_{12}$	$R_7\,R_7$	0.710 b	0.597 ab	0.619 c
$S_{12}\,S_{12}$	$S_7\,S_7$	–	0.815 b	0.650 c
$R_{12}\,S_{12}$	$R_7\,R_7$	0.627 b	–	–
Azucena		0.101 a	0.184	0.238
IR64		0.808 b	0.968	0.661

[a] Leaf virus content (LVC) is expressed in absorbance (405 nm) measured by the ELISA test
[b] Parents LVC data were not submitted to statistical analysis; LVC figures marked with the same letter (a, b, c) in each column are not significantly different according to the Newman-Keuls test, P=0.05 for BC_1–F_3 and P=0.01 for BC_2–F_3; –: not tested

tion, the LVC of plants with $R_{12}R_{12}$–R_7R_7 genotype is not different from the LVC of the resistant parent Azucena (Table 2). By contrast, the LVC of plants with $R_{12}S_{12}$–R_7R_7 and $S_{12}S_{12}$–R_7R_7 genotypes were not different from the susceptible parent IR64. These data confirmed: (1) the recessive nature of resistance associated with QTL_{12}, and (2) the indirect (QTL_{12}-dependent) effect of the Azucena allele in QTL_7 which has a reducing effect on LVC only when associated with the homozygous allele of Azucena in QTL_{12}.

Discussion

Having identified the QTLs for resistance to RYMV, and the markers associated with them, in an upland *japonica* rice variety not adapted to lowland cultivation (Albar et al 1998; Pressoir et al. 1998), the aim of our marker-assisted introgression program was to fix the favourable alleles of these QTLs in a lowland *indica* variety with as little as possible of the remainder of genome from the *japonica* donor.

The recourse to MAS versus phenotypic selection is justified by: (1) the concern for the elimination of the donor parent genome as it can be detrimental for lowland adaptive traits and yield, and (2) the fact that a significant part of the effect of the QTL came from a complementary epistasis which cannot be followed in phenotypic selection. Indeed, the percentage of LVC variance explained by the interaction between QTL_{12} and QTL_7 is 36.5% while that explained by QTL_{12} alone is only 21% (Albar et al. 1998; Pressoir et al. 1998).

MAS for monogenic traits in which the position of the gene of interest on the chromosome is known with certainty, especially the complete resistance to insects and pathogens, have been reported by many authors (see Mohan et al. 1997 for a review). There are fewer MAS reports dealing with QTLs whose chromosomal position is not known with certainty but only estimated (Han et al. 1997; Bernacchi et al. 1998; Toojinda et al. 1998; Van Berloo and Stam 1999). The efficiency of MAI for genes and for QTLs was investigated using analytic and simulation results, notably by Hospital et al. (1992), Visscher et al. (1996), Hospital and Charcosset (1997) and Van Berloo and Stam (1998). We adapted the recommendations of these theoretical approaches to the practical case of two QTLs of resistance to RYMV.

In "foreground" selection, optimal marker spacing, in relation to the length of the confidence interval (S) of the QTL position, provides a high probability for having the desired genotype at the QTL, given the desired genotype at the markers. For short confidence intervals, the number of markers (m) has an almost non-visible effect and, when m is equal to one, the optimal position of the marker is the centre of confidence interval of the QTL. For two unlinked QTLs, with S=40 cM, and one marker per QTL situated at the middle of S, $P_{Q/M}$ values of 0.890 and 0.713 were obtained with a minimum population size of 17 and 20 individuals respectively in first and third backcross generations (Hospital and Charcosset 1997). In our case, the estimated value of S was approximately 20 cM for QTL_{12} and 15 cM for QTL_7. The marker of QTL_{12} is actually situated in the centre of its confidence interval (Ghesquière et al. 1997) whereas the marker of QTL_7 was situated in the upper extremity of its confidence interval. The population size was 28 in BC_1 and close to 60 in BC_2 and BC_3. Moreover, during the three BC generations, priority was given to foreground versus background selection. This explains why we did not lose the QTLs during the backcross process even if we used only one marker per QTL.

In background selection two cases have to be distinguished: chromosomes that do not carry the introgressed QTL and carrier chromosomes. In non-carrier chromosomes, two markers per 100 cM are sufficient to get the highest possible response to selection in the early generations. However, even without selection, in the third generation the expected proportion of the recipient genome reach values very close to unity (Hospital et al. 1992). In our case, the background selection on non-carrier chromosomes began with the introgression process and was pursued as far as BC_3 with an interruption in BC_2. First, it is important to emphasise the fact that in our MAI procedure the donor parent was not the original *japonica* variety, Azucena, but a doubled-haploid line of the IR64/Azucena cross. Therefore, there is a negative shift

of one generation between the formal count of the BC generations that we use, and the real one. The introgression process started with a doubled-haploid line of the IR64/Azucena cross which carried approximately 60% of the recipient parent genome. Then, in BC_1, the donor parent alleles were counter-selected at five chromosomal segments using one marker per segment. And finally, in BC_3, background selection was targeted at the remaining 11 chromosomal segments of the donor parent. For this last selection step, as recommended by Hospital et al. (1992), markers were chosen in order to be situated near the extremities of each non-desired chromosomal segment. According to Hospital et al. (1992) the optimal marker density for background selection is two to three per 100 cM. We used a density of approximately one marker per 25 cM in order to take into account the accumulation of recombination events through generations. This accumulation induces an increase in the number of donor chromosomal segments and a decrease in their length. According to the results of this genome survey, the proportion of recipient genomes in the selected BC_3 plant was approximately 95% for the ten non-carrier chromosomes, but some risk of non-detected residual segments of the donor genome subsists. We will test this possibility in the next generation with a new set of markers.

In QTL carrier-chromosomes, selection is mostly devoted to the reduction of the donor chromosome segments surrounding the QTL. Therefore, the position of the markers against the QTL becomes critical. Markers distant from the introgressed QTL should be used in the early generations whereas markers close to the QTL are more useful in later generations (Hospital et al. 1992; Hospital and Charcosset 1997). For our part, we carried out background selection on carrier chromosomes as early as the starting point of the introgression process. Indeed, the doubled-haploid lines of the IR64/Azucena cross selected for the backcross process was endowed with a favourable recombination on each side of QTL_{12}. In BC_1 and BC_2, as the size of the population was rather small, the donor chromosome segments surrounding the two QTLs were not very long – less then 20 cM for QTL_{12} and approximately 30 cM for QTL_7 –, and, as PCR-like markers were not available, we did not attempt any selection on carrier chromosomes. In BC_3, the microsatellite markers used did not allow the detection of any favourable recombinant, most likely because of the rather small size of the population. Reduction of the donor chromosome segment surrounding the QTL will be attempted again in a larger population of BC_3–F_2 plants. The availability of a new set of microsatellite markers (Temnykh et al. 2000) bracketing the two QTLs more closely, should be helpful in such an experiment.

Finally, our results pointed out the methodological flexibility of MAI and the fact that it was possible to conduct a successful MAI without strict conformation to recommendations of analytical and simulation studies.

The assumption that QTL-alleles detected in segregating populations could be treated as units of Mendelian inheritance, and that the incorporation of these alleles into elite lines would result in an enhanced performance, have rarely been verified by experiments (Mohan et al. 1997). Our results on the evaluation of the level of resistance to RYMV in F_2 and F_3 offspring of different backcross generations confirmed these assumptions experimentally. In the same way, these results confirmed experimentally the existence of the complementary epistasis between QTL_{12} and QTL_7 detected by a systematic research of interactions between QTL_{12} and the rest of the genome in the DH population (Pressoir et al. 1998). Moreover, these results allowed the resolution, at least partly, of the complex trait for quantitative resistance to RYMV into its single Mendelian components, which is indispensable for the analysis of the resistance mechanisms involved. The LVC value of all F_3 progenies carrying the resistance allele of both QTL_{12} and QTL_7 was higher than that of the resistant donor Azucena. This confirms the fact that these two QTLs are the major genetic factor controlling the LVC but not the whole of it. In all F_3 progenies, QTL_{12} has a significant effect on LVC even in the absence of QTL_7. This demonstrates the fact that QTL_{12} has a significant additive component, and this component seems to be much more important then the complementary epistasis component.

QTL_{12} is also involved in field resistance (Ghesquière et al. 1997) and in other epistatical interactions with other resistance and morphology QTLs (Pressoir et al. 1998). In particular, the QTL_{12} interaction with tillering QTLs, mapped on chromosomes 1 and 7, is a major explanatory variable of field resistance. For all of these QTLs, except QTL_7, the backcross process has fixed the allele of the susceptible parent IR64. Therefore, it is already possible to affirm that the backcross process has broken the interaction between QTL_{12} and the tillering QTL mapped in chromosome 1 (QTL_1). Indeed, despite the fact that all BC_2F_3 lines have reached the tillering capacity of IR64 (data not shown), and despite the presence of the IR64 allele on QTL_1, all BC_2F_3 lines with the Azucena allele on QTL_{12} have a low LVC. In the same way, it is now possible to evaluate precisely the degree and the genetic nature, additive or epistatic, of the involvement of QTL_{12} in other resistance criteria. We have not developed a complete set of NILs taking into account the 15 resistance QTLs identified. It would be too time-consuming, especially for QTLs involved in field resistance, i.e. on the disease impact for growth and the yield under field conditions. Nevertheless, the comparison of the field resistance of NILs for QTL_{12} and QTL_7 derived from the backcross process, with DH lines of the IR64×Azucena cross holding a specific QTL, would determine whether the different QTLs are involved in different resistance mechanisms or if a single factor of resistance is underlying the detection of several co-located resistance factors.

As the introgression process is close to achievement, at least four research directions have to be explored: (1) designing the tools necessary for the routine use of QTL_{12} in breeding for partial resistance to RYMV, (2)

pyramiding different types of resistance to RYMV, (3) developing material for the analysis of resistance mechanisms, and (4) mapping QTL_{12} more finely to advance toward the analysis of the structure of a QTL conferring resistance to a virus.

The design of tools for the routine use of QTL_{12} has already started with the ongoing development of a sequence-characterised amplified region (SCAR) from the QTL_{12} marker, RG869. As RG869 is situated in the centre of the confidence interval of QTL_{12} (Ghesquière et al. 1997), the development of a SCAR from this marker will eliminate the risk of the loss of the QTL during the new introgression processes. Indeed, even with two closely bracketing markers the risk of losing the QTL through double cross-overs between flanking markers remains. Moreover, a SCAR from the centre of the confidence interval of the QTL will be more powerful in crosses which do not involve the Azucena variety as a resistance donor.

Pyramiding the partial resistance and the complete resistance in the same variety may provide a more durable resistance to RYMV, less likely to be overcome by aggressive isolates. As the effect of QTLs for partial resistance will be hidden by the effect of the complete resistance gene, the recourse to MAS seems necessary. We already have at our disposal the tools for the pyramiding exercise. A better knowledge of the resistance mechanism controlled by each genetic factor would help the efficient deployment of such a combination of resistances. The ongoing creation of NILs carrying different combinations of QTL_{12} and QTL_7 will be helpful in this way. Relieved of the effects of interactions with the *japonica* genetic background, the resistance mechanisms associated with QTL_{12} could also be analysed at the cytological level.

The fine-mapping and the isolation of QTL_{12} is all the more important in that: (1) the QTL_{12} seems to be part of a chromosomal cluster dedicated to resistance to blast (McCouch et al. 1994; Yu et al. 1996), (2) a gene for resistance to RHBV has been tentatively mapped on chromosome 12, and an *indica* variety resistant to RHBV has also shown partial resistance to RYMV (data not shown), (3) in different mapping populations, several QTLs contributing to tolerance to drought related to root development and provided by the upland parent have been mapped in the vicinity of the QTL_{12} marker locus RG869 (Champoux et al. 1995; Yadav et al. 1997), and (4) the allelic status of RG869 discriminates very well between the *indica* and *japonica* varieties (Qian et al. 1995). The production of NILs carrying a small part of the present chromosomal segment of the *japonica* parent surrounding the QTL will help to clarify the relationship between the partial resistance to RYMV and the disease resistance cluster of chromosome 12 on the one hand, and between the partial resistance to RYMV and morphological traits related to the *indica/japonica* subdivision of *O. sativa*, on the other hand. The fine mapping of QTL_{12}, especially with resistance gene analogues, and its isolation, will provide a good model for the analysis of the interaction between the virus pathogenicity factors and the product of the host plant resistance gene.

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