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ORIGINAL PAPER

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A novel major gene on chromosome 6H for resistance of barley against the barley yellow dwarf virus

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Abstract In a mapping population derived from the Ethiopian barley line L94 × Vada, natural infection by barley yellow dwarf virus (BYDV) occurred. While line L94 hardly showed symptoms, Vada was severely affected. The 103 recombinant inbred lines segregated bimodally. The major gene responsible for this resistance mapped to chromosome 6H. We propose to name the locus *Ryd3*. A subset of recombinant inbred lines, L94, and Vada were planted in a subsequent field test which confirmed the previous field observations. Double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) indicated that the epidemic was due to a combination of the serotypes BYDV-PAV and BYDV-MAV. In the accessions with the least BYDV symptoms no virus was detected, justifying the consideration of the gene as conferring true resistance rather than tolerance to these viruses. In a laboratory/gauze house trial a near-isogenic line carrying the Vada chromosome 6H fragment in an L94 background was affected as much as Vada. The effect of *Ryd3* was quantified, and compared with that of the only other known major gene for resistance to BYDV, *Ryd2*, which is also of Ethiopian origin and is located on chromosome 3H. Both genes seemed to reduce the chance of the viral isolate used in this study to establish infection. In plants in which it became established, the virus concentration reached a similar level as in susceptible accessions, but with less dramatic symptom development. Inoculated plants in which the virus failed to multiply tended to show an increase in the number of ears per plant, resulting in higher grain yield per plant. *Ryd3* co-segregates with several PCR-based molecular markers that may serve for marker assisted selection.

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Introduction

Barley yellow dwarf (BYD) is the most prevalent and economically important virus disease of cereals in the world (Miller and Rasochova 1997). The disease is caused by barley yellow dwarf luteoviruses BYDV-PAV and BYDV-MAV, the BYDV-RMV, BYDV-SGV, BYDV-GPV which have not been classified to date, and the cereal yellow dwarf polerovirus CYDV-RPV, respectively (Regenmortel et al. 2000). These viruses cause a symptom complex consisting of stunted growth, late heading and discoloration of leaves. Several yield components can be affected (Suneson and Ramage 1957; Scheurer et al. 2001; McKirdy et al. 2002).

In barley, control of BYDV infection can be achieved by prophylactic sprayings of insecticides against the aphids transmitting the virus, and by growing resistant or tolerant cultivars. The latter option is more economical and environmentally desirable. However, only a few genes have been reported to protect barley cultivars sufficiently. One of these, *Ryd2*, may be considered a major gene. It was first reported in four Ethiopian barley accessions that shared this resistance or tolerance gene (Rasmusson and Schaller 1959). The gene has been commonly applied by barley breeders and occurs in many present-day cultivars (Burnett et al. 1995). It has been mapped to chromosome 3H (Schaller et al. 1964; Collins et al. 1996). The level of protection conferred by this gene varies according to the genetic background, environmental conditions, and the serotype and isolate of the virus. *Ryd2* has been reported to be effective against BYDV-PAV and BYDV-MAV, but may be ineffective against CYDV-RPV [compare Baltenberger et al. (1987) and Jefferies et al. (2003) with Banks et al. (1992)]. Some authors have suggested that *Ryd2* occurs in several allelic variants (Catherall et al. 1970; Chalhoub et al. 1995). At least five donors have been used

to incorporate a *Ryd2* allele for BYDV tolerance into cultivated barley (Burnett et al. 1995). Scheurer et al. (2001) mapped QTLs in mapping populations segregating quantitatively for tolerance to BYDV-PAV, and found that in the cultivar Vixen, which possesses *Ryd2*, this gene has a quantitative effect, suggesting that in some genetic backgrounds, or in some allelic forms, or against certain viral isolates, the gene may appear as a minor gene, requiring QTL-mapping software to establish the position.

In addition to *Ryd2*, several QTLs for tolerance to BYDV have been mapped in some cultivars (Scheurer et al. 2001; Toojinda et al. 2000). These QTLs can be accumulated to give substantial protection, but would be less convenient than a major gene giving almost full protection.

In 1999 we evaluated a mapping population consisting of 103 recombinant inbred lines (RILs) derived from a cross between the Ethiopian landrace line L94 [also known as Abyssinian 1102, HOR3036 and BBA1465 (see Jørgensen 1992) and as CIho11797 in the National Plant Germplasm System, USA, and at CGN Crop Collections, Wageningen, The Netherlands] and the Western European cultivar Vada for the level of partial resistance against the barley leaf rust fungus *Puccinia hordei* (Niks et al. 2000). The trial showed symptom expression typical of infection by a virus, presumably a BYDV strain. All three replicates of the trial were infected, with consistently great variation between the RILs. The L94 line hardly showed symptoms, while Vada showed strong symptom development.

In the present paper, we report the identification and characterization of a major gene for resistance to BYDV that segregated in this RIL population.

Materials and methods

Mapping population and field trial 1999

The mapping population consisted of 103 RILs derived from the cross L94 × Vada, and was developed by single-seed descent to F_9. Each RIL was bulk-propagated thereafter (Qi et al. 1998a). They were planted on 28 April 1999 in Wageningen, in a randomized complete block design in three replicates. Each replicate consisted of eight strips. Each strip contained one plot each of the two parental barley lines as references and 13 RILs, alternating with plots of the oat cultivar Gigant. The oat plots served to reduce inter-plot interference, which would affect the reliability of the leaf rust resistance scores (Parlevliet and Ommeren 1984). Each barley and oat plot consisted of three plant rows of 1.25 m long, spaced at 25 cm, with about 50 seeds per row. Leaf rust was introduced into the field as reported in Qi et al. (1998b) and Niks et al. (2000).

Scoring of BYD symptoms and mapping of the responsible genes

In the course of the growing season, severe symptoms of BYD developed on Vada and most of the RILs. The severity of symptom development was scored on a 0–5 scale. The score took into account both the proportion of the plot that was affected and the severity of the symptom development per plant. Score 0 represented a whole plot without symptoms; score 1 represented a few plants showing some leaf discoloration; score 2 represented about 10% of the plot consisting of yellowish plants and mild stunting; score 3 represented 30% of plants showing stunting and yellowing; score 4 represented about 50% of the plot being affected with severe stunting; score 5 represented almost the whole plot being stunted with hardly any spikes emerging. This evaluation was done twice, on 24 June and 17 July (Zadoks scale about 54 and 60, respectively). The scores were averaged over the three replicates and processed as quantitative data by Map-QTL software. A molecular marker map for the population was already available for the purpose of leaf rust research (Qi et al. 1998a, b). Six SSR markers and two RFLP-derived SCAR and CAPS markers mapped on the RIL population in the relevant segment of chromosome 6H. Primer combinations of the six SSR markers were published by Ramsay et al. (2000). The sequences of the two RFLP markers were downloaded from the Graingene database (http://www.graingenes.org) and used to design primers. Before mapping, the two RFLP markers were converted into SCAR and CAPS markers.

Verification trial

In 2001 14 RILs were planted in a similar field layout, alternating with oat plots, and with L94 and Vada added as references. On the basis of the scores from the 1999 field trial, seven RILs were selected for particularly high symptom development and seven others for low symptom development scores. As for the initial trial, this trial consisted of three replicates. Again, BYD symptom development occurred from natural infection. Scores were taken on 10 July (Zadoks scale of development about 54), and three representative tillers were collected from each plot, of which all leaves were stripped and one sample per plot frozen at −80°C for future virus detection. The frozen leaves were ground, and serologically characterized by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using our in-house polyclonal antisera (BYDV-PAV) and reagents from Bioreba AG (Reinach, Switzerland) (BYDV-MAV, -RMV, CYDV-RPV). Preparation of the BYDV-PAV antisera was carried out on the purified BYDV-PAV 1 ASL isolate originating from a field near Aschersleben according to Proll et al. (1984). Prior to this, the isolate was characterized by aphid transmission tests and ELISA. Freeze dried reference samples of BYDV-PAV 1ASL-infected plants and healthy plants of the cultivar Rubina,

and positive controls for BYDV-MAV, -RMV, CYDV-RPV bought from Bioreba AG, were used to check the quality of the ELISA.

Comparison of the effect of the novel tolerance/resistance gene to known resistances

The Vada-derived segment containing the susceptibility allele for BYD has been introgressed into the L94 background by marker-assisted backcrossing (Berloo et al. 2001). The genetic composition of this line, L94-QTL3, will be described in a forthcoming paper (T. Marcel and R. E. Niks, unpublished data). The resulting near-isogenic line was evaluated by comparison with the recipient parent L94. This pair of lines was also compared to the spring barley cultivars Coracle (*Ryd2*), Vada and Femina as susceptible standards and to the winter barley cultivars Vixen (*Ryd2*) and Post (carrying several QTLs, Scheurer et al. 2001). In two replicates, each consisting of 12 seedlings (one per pot) of each line and cultivar, plants were inoculated with viruliferous aphids (BYDV-PAV 1 ASL, *Rhopalosiphum padi*, approximately ten adults per plant) in the laboratory 6 days after sowing. After an infestation time of 48 h the aphids were killed by insecticide. About 2 weeks after inoculation the plants were transferred to a gauze house, and transplanted into soil. For the estimation of the level of resistance or tolerance a healthy control variant with 24 plants of each genotype cultivated under the same conditions as those that had been inoculated was planted in the gauze house. To protect the plants from spontaneous BYDV infection they were treated regularly by insecticides. At 58 days after inoculation, pieces of three different leaves were combined into one sample for each plant in order to determine the virus extinction by DAS-ELISA. At the time of sampling the cultivars were on average in the flag leaf appearance stage (Zadoks 37–39). At ear emergence the severity of symptoms was rated on a 1–9 scale (score 1 represented a symptomless plant, score 9 represented a dead plant). At maturity, the plant height, number of ears per plant, kernel weight per plant and kernel weight were determined in the infected and in the control treatment groups.

Results

Field data

In the barley leaf rust field trial of 103 RILs and their parents, symptoms developed that suggested BYD disease. Plants showed yellowing of leaves, starting from the leaf tips and margins, and tillers did not elongate well, resulting in a stunted growth habit. Many spikes failed to emerge from the flag leaf sheath. In the alternating oat plots, strong red discoloration of leaves was observed. The symptom development occurred in patches within the plots, and never occurred in the whole plot. L94 remained almost free of symptoms (average score over the two dates 0.4), while Vada had an average score of 2.4.

RILs segregated bimodally, indicating majorgenic inheritance (Fig. 1). Heritability for the trait was 0.95.

Mapping of the gene(s)

The symptom scores at the two observation dates were averaged over the three replicatess, and used as two quantitative traits for which the responsible QTLs were mapped. The average score over the two dates was also mapped. The gene with the largest effect mapped to position 58 cM on chromosome 6H [the average score over the two dates, likelihood of odds (LOD) 33.1] at the position of AFLP marker E37M33-574. The gene explained 75% of the variation between the RILs. Such a high LOD score and this significant effect warrants calling the gene a major gene. We also found indications for minor QTLs for resistance near marker E37M38-340 at position 62.9 cM on chromosome 3H (only for the first observation date, LOD 2.7), and near marker E37M38-230 at position 54.2 cM on chromosome 1H (only for the second observation date, LOD 2.9). Their effects were marginal compared to that of the gene on chromosome 6H. All favourable alleles were contributed by the L94 parent.

In the case of one segregating major gene, one would expect a segregation ratio of 1:1 for the susceptible and resistant phenotypes in the RIL population. The bias towards susceptible phenotypes (Fig. 1) can be explained by skewed segregation towards the Vada alleles for that segment of chromosome 6H (Fig. 1 in Qi et al. 1998a). The L94 allele of the peak marker occurs in only 35 of the RILs, the Vada allele in the remaining 68 RILs, which is a significant deviation from 1:1 (χ^2 test, $P<0.05$).

Based on their marker identity, and using the Graphical Genotyping (GGT) software (Berloo 1999), we selected RILs that showed recombination in the area of the major gene on chromosome 6H. Their marker identities in this chromosome segment are graphically presented in Fig. 2. The diagram confirms that the marker at position 58 cM is associated with symptom development: the RILs with the

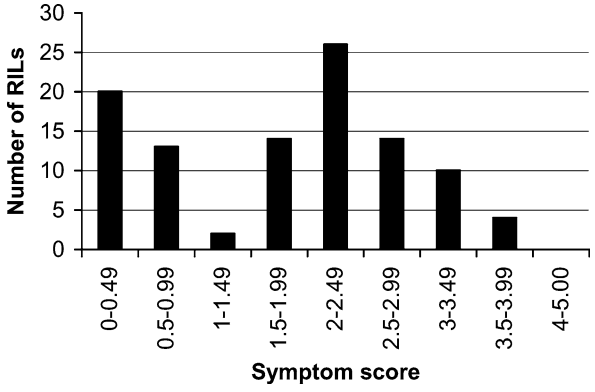

Fig. 1 Frequency distribution of average BYD symptom score of 103 RILs from the cross L94 × Vada, in a spontaneously infected field trial in 1999

Vada allele had field symptom scores between 1.0 and 3.0, the RILs with the L94 allele had symptom scores 0–1.0. This indicates that the gene should be located between position 56.6 and 58.9 cM on that chromosome. To relate this segment to chromosome positions in other barley crosses, we mapped SSR markers and RFLP-derived CAPS markers in the L94 × Vada mapping population. One CAPS marker (ABG458) and six SSR markers mapped close to the position of the E37M33-574 AFLP marker (Fig. 2). The resistance against BYDV is closely associated with a cluster of SSR markers, (HVM22, HVM14, HVM65, HVM74, Bmac0018 and Bmac0009) that fully cosegregate with E37M33-574 in the L94 × Vada mapping population .

Verification trial

In 2001, seven RILs that showed low symptom development and seven with high symptom development in the 1999 field trial were planted in a verification trial in Wageningen under high natural BYDV infection. There was very good agreement in the symptom scores between the 1999 trial and the 2001 verification trial (Table 1). Again Vada showed strong symptom development, while L94 remained almost free of symptoms. The RILs that showed low symptom development in 1999 also developed few symptoms in 2001, and the RILs with high symptom level in 1999 also were severely affected in 2001. The samples reacted positively in DAS-ELISA against BYDV-PAV and also against BYDV-MAV. All accessions that had been virtually free of symptoms in 1999 and 2001 tested negatively in the ELISA (extinction values for BYDV-PAV lower than 0.1), whereas the accessions with clear symptom development tested positively (Table 1). The data indicate that the epidemic in 2001 was due to a combination of BYDV-PAV and BYDV-MAV. Due to lack of sufficient samples, not all accessions could be tested for presence of BYDV-MAV. However, the data suggested that resistance to both viral serotypes is correlated. We cannot rule out the possibility that the antiserum of BYDV-MAV had some affinity to PAV virions. BYDV-RMV and CYDV-RPV could not be detected in the samples (data not presented).

Comparison of the effect of the novel tolerance/resistance gene to known resistances

The experiment on individual plants that were artificially infected with BYDV-PAV was performed to determine the effect of the gene that was found in L94 and compare it with that of *Ryd2*. In all accessions at least some plants showed BYD symptoms (Table 2). In all accessions except Vixen, the plants not showing symptoms tested negatively by ELISA, i.e. gave extinction values lower than 0.1. In Vixen, six out of 16 plants without symptoms contained virus, the others did not.

The lines carrying the L94 allele (L94 and RIL K4-56) and the cultivars containing *Ryd2* had a lower frequency of plants showing symptoms than the susceptible accessions and the tolerant cultivar Post (Table 2). The symptoms were less severe (maximum score 4 on the scale of 1–9), but the virus concentrations were similar or only slightly lower than in the susceptible accessions.

The major gene for resistance is confirmed by the performance of the line L94-QTL3 that is near-isogenic with L94, with the relevant segment replaced by an introgression from Vada, carrying the susceptibility allele. L94-QTL3 was as susceptible as Vada, and therefore very different from L94 (Table 2). It is further confirmed by the performance of the two RILs. RIL K4-56 was the most resistant of the two in terms of disease incidence (41% versus 65%) and the most tolerant in terms of maximum symptom score (3 versus 7) (Table 2). This is the RIL with the L94 allele of the relevant chromosome segment on 6H,

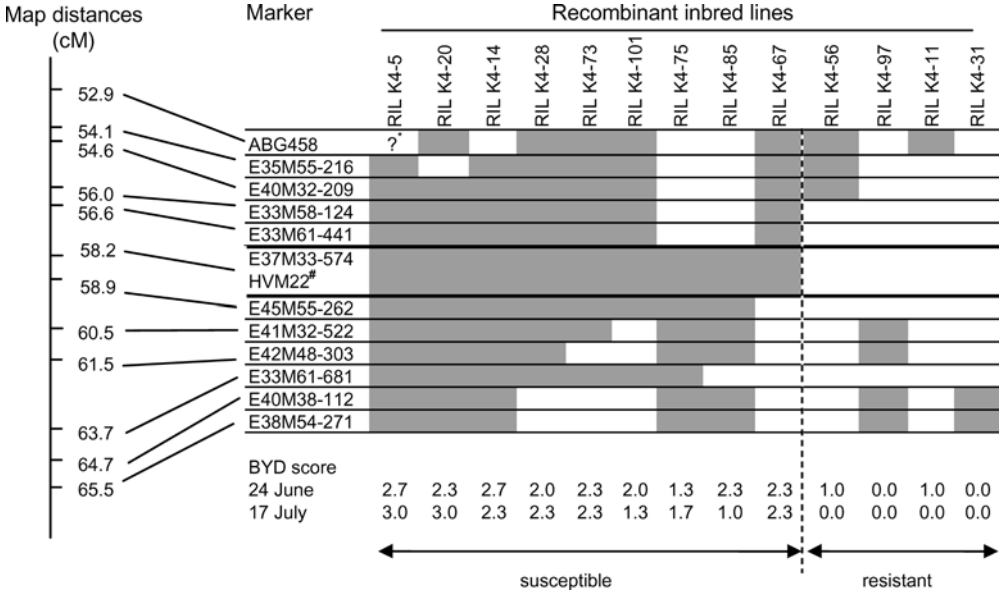

Fig. 2 Diagram of central part of chromosome 6H of barley, presenting the allelic identity of 13 RILs from the mapping population L94 × Vada for 11 AFLP markers, CAPs marker ABG458 and SSR marker HVM22. *Grey cells* indicate that the inbred line possesses the Vada-derived allele, *white* the L94-derived allele. For each inbred line the score for BYD symptom expression (ranging from 0 to 5) is also presented for two observation dates in the 1999 field test. * marker identity unknown; # SSR marker HVM22 maps at same position of the SSR markers HVM14, HVM65, HVM74, Bmac0018 and Bmac0009

Table 1 BYD symptom scores and results of DAS-ELISA of 14 RILs from the mapping population L94 × Vada in naturally infected field tests in 1999 and 2001. *NT* not tested (due to lack of sample material). The BYD score is given on a 0–5 scale; 0 being most resistant. For the 1999 trial, the score is an average of the two evaluation dates. The virus extinction data is for leaf samples collected in 2001 measured at a wavelength of 405 nm. For BYDV-MAV, the numbers in *parentheses* indicated the number of samples tested

Accession	BYD score		Virus extinction	
	1999	2001	BYDV-PAV	BYDV-MAV
Vada	2.4	2.0	1.13	0.64 (2)
RIL K4-2	3.7	3.2	0.86	NT
RIL K4-21	2.7	3.0	0.81	NT
RIL K4-34	3.5	2.8	1.10	0.93 (1)
RIL K4-45	3.2	2.7	1.07	0.23 (1)
RIL K4-59	3.2	3.7	1.58	1.14 (1)
RIL K4-61	3.5	4.0	1.47	1.11 (1)
RIL K4-89	3.7	3.7	0.58	0.76 (1)
RIL K4-3	0.0	0.3	0.05	0.12 (2)
RIL K4-12	0.0	0.3	0.02	NT
RIL K4-31	0.0	0.7	0.08	NT
RIL K4-35	0.0	0.7	0.05	0.09 (2)
RIL K4-38	0.0	0.3	0.09	0.13 (3)
RIL K4-40	0.0	1.3	0.07	0.07 (3)
RIL K4-46	0.0	0.3	0.05	0.09 (2)
L94	0.4	0.3	0.08	NT
Reference samples				
Negative			0.00[a]	0.02[a]
Positive			2.02[b]	0.29[c]

[a]Healthy leaves of cultivar Rubina
[b]Infected leaves of cultivar Rubina
[c]Leaf sample purchased from Bioreba AG

whereas the more susceptible RIL K4-67 carries the Vada allele of the segment (Fig. 2).

In the plants that tested positively by ELISA ("virus present" in Table 3) the number of ears per plant, and to lesser extent the 1,000 kernel weight were reduced (Table 3) compared to the non-inoculated control plants. Interestingly, for L94 and Vixen the plants that tested negatively by ELISA developed significantly more ears per plant than the non-inoculated plants, resulting in a higher total kernel weight per plant ("no virus detected" in Table 3).

Discussion

In this paper we report a major gene in the barley line L94 that protects against two serotypes of BYDV: BYDV-PAV and BYDV-MAV. The effect of this gene appears to be very similar to that of the only other known major gene for resistance to BYD, *Ryd2* (Tables 2 and 3), located on chromosome 3H (Schaller et al. 1964; Collins et al. 1996). We propose to name the locus of the L94 gene *Ryd3*, and show in this paper that it is located on chromosome 6H. We have not tested heterozygous populations, and therefore the degree of dominance of the resistance allele remains unknown.

The *Ryd3* gene maps to the same position as the SSR markers HVM22, HVM14, HVM65, HVM74, Bmac0018 and Bmac0009 (Fig. 2). These markers have been placed in barley-BIN 6H-006 by Spaner et al. (1998). Their relative position is in agreement with Ramsay et al. (2000), near the centromere of chromosome 6H. These markers can serve to efficiently transfer the *Ryd3* gene from line L94 to modern, adapted barley germplasm by

Table 2 Infection rate and virus concentration in seven barley lines in comparison to the resistant line L94 and the susceptible line Vada, after artificial BYDV-PAV inoculation. The percentage of plants showing symptoms of infection is given in *parentheses*. The virus concentration was measured as virus extinction at a wavelength of 405 nm

Accession	Number of plants		Symptom severity score (scale 1–9)		Virus concentration	
	Without symptoms	With symptoms	Mean score all plants	Maximum score	Plants without symptoms	Plants with symptoms
L 94	16	4 (20)	1.6	4	0.02	1.67
RIL K4-56	7	5 (41)	2.0	3	0.02	1.53
Coracle (*Ryd2*)	16	3 (16)	1.3	3	0.03	1.92
Vixen (*Ryd2*)	16[a]	4 (20)	1.5	4	0.02 (*n*=10)[a] 1.93 (*n*=6)[a]	1.88
Vada	0	20 (100)	4.0	7	–	2.05
RIL K4-67	6	11 (65)	4.4	7	0.02	1.87
L94-QTL3	0	18 (100)	3.4	7	–	2.02
Femina	0	20 (100)	3.0	6	–	1.79
Post	2	18 (90)	3.3	5	0.04	1.80
Reference samples						
Negative					0.01	
Positive						1.92

[a]Out of the 16 plants, six contained virus, the other ten did not

Table 3 Ratio of plant height and yield components (relative to non-inoculated control plants) of six barley accessions in comparison to the resistant line L94 and the susceptible cultivar Vada after artificial BYDV-PAV inoculation. *NS* not significant (different from the non-inoculated plants). No virus detected indicates an extinction value on ELISA of lower than 0.1. Due to combined harvesting, no statistical test was performed for the relative kernel yield per plant, or the relative 1,000 kernel weight (*TKW*)

Accession	Number of plants		Relative plant height (%)		Relative number of ears/plant (%)		Relative kernel yield per plant (%)		Relative TKW (%)	
	No virus detected	Virus detected	No virus detected	Virus detected	No virus detected	Virus detected	No virus detected	Virus detected	No virus detected	Virus detected
L94	16	4	101 NS	86[b]	150[b]	34[b]	158	31	100	87
RIL K4-56	7	5	101 NS	103 NS	100 NS	59[b]	93	48	89	87
Coracle (*Ryd2*)	16	3	102[c]	106 NS	108 NS	99 NS	105	87	97	83
Vixen (*Ryd2*)	9[a]	7	104 NS	88[b]	153[b]	107 NS	160	73	103	85
Vada	0	20	–	75[b]	–	43[b]	–	28	–	93
RIL K4-67	6	11	114 NS	86 NS	154 NS	25[b]	161	22	107	90
L94-QTL3	0	18	–	87[b]	–	46[b]	–	45	–	90
Femina	0	20	–	93 NS	–	69 NS	–	57	–	96

[a]Four plants without symptoms (one of them containing virus, see Table 2) had winter habit, so no yield components could be determined
[b]Significantly different from the non-inoculated control (α=0.05). Tested by Wilcoxon test
[c]Due to combined harvesting no statistical test was performed

marker-assisted selection. The six markers co-segregating with *Ryd3* are flanked by markers ABG458 (about 5 cM distally) and ABG388 (at 14 cM proximally) (T. Marcel and R.E. Niks, unpublished data). These two markers delimit a region of suppressed recombination where physical distances are more than 14 Mb/cM (Künzel et al. 2000). Because of its location on a different chromosome than *Ryd2* it would be easy to combine both genes in one cultivar, and possibly attain an even higher level of BYD resistance.

The locus that contains *Ryd3* co-segregates with the peak marker for a quantitative resistance locus to *Puccinia hordei*, for which L94 carries the susceptibility allele, and Vada the resistance allele (Berloo et al. 2001; Qi et al. 1998b). Since the area shows suppressed recombination (Künzel et al. 2000) each centimorgan on the map corresponds to large stretches of DNA (at least 4.4 Mb) that may contain several to many genes. The markers near *Ryd3* also are in the confidence interval of QTLs for several yield components (Stam et al. 1997). A locus for BaMMV resistance, the gene *rhym15*, has been reported recently (Le Gouis et al. 2004) and is close to the *Ryd3* locus. These associations are probably coincidental.

It is interesting, but not surprising, that *Ryd3* occurs in a barley from Ethiopia. This was also the case for *Ryd2* (Rasmusson and Schaller 1959). Schaller et al. (1963) tested 6,689 barley accessions of worldwide germplasm, and found 117 accessions to be quantitatively resistant or tolerant to BYDV. All except one were of Ethiopian origin. Genetic analyses on 20 BYD resistant Ethiopian accessions suggested that the resistance was due to *Ryd2*, the only major gene reported so far, in all cases (Schaller 1984). Ethiopian L94 is among the Ethiopian lines in the European Barley Disease Nursery of 1970, and has been reported to contain the *mlo* gene for powdery mildew resistance (Jørgensen 1992). Therefore, it should have circulated in germplasm collections for over 30 years. It is very surprising that this line contains a BYD resistance gene of as strong effect as *Ryd2*, though located on another chromosome, without having been detected before.

In addition to the *Ryd2* and *Ryd3* genes, several genes conferring minor effects have been reported, one of them named *ryd1* (Suneson 1955) and considered to be of low efficiency (Rasmusson and Schaller 1959; Schaller et al. 1963; Scheurer et al. 2001), others have been reported as minor genes that required QTL-mapping approaches to be detected (Toojinda et al. 2000; Scheurer et al. 2001). The location of *ryd1* is unknown, and none of the reported QTLs maps to chromosome 6H. This implies that the locus has never been previously implicated in controlling BYD in barley. This is in contrast to experience with *Ryd2*, which has been reported both as a major effect gene, showing discrete segregation like *Ryd3* in Fig. 1, but also as a minor gene, with only a small quantitative effect, and requiring a QTL-mapping approach (Scheurer et al. 2001). This may either be due to allelic variation of the *Ryd2* gene, to modifying effects of the genetic background, to environmental factors or to the virus isolate used. It remains to be determined whether the effect of *Ryd3* is similarly dependent on the above-mentioned factors.

Both presently known major genes for resistance can be handled conveniently in barley breeding programmes by marker-assisted selection. For *Ryd2* the tightly linked markers YLM (Jefferies et al. 2003) and YLP (Ford et al. 1998), while for *Ryd3* several SSR markers, like HVM22, can be used.

Virus concentration and levels of BYD symptom expression, often measured as percentage biomass or yield reduction, are not consistently correlated (Banks et al. 1992; Scheurer et al. 2000; Skaria et al. 1985). Our experiments add some new aspects to the complicated relationship between these two features. The test carried out in the gauze house indicates that the resistance may reduce the chance for the virus to become established.

Such low BYD incidence was specifically observed for lines carrying *Ryd2* or *Ryd3*, and therefore seems to be the effect of these genes, rather than of fortuitous poor aphid transmission rates. Low incidence due to *Ryd2* has not been reported before. Another interesting observation is that in the gauze house tests, *Ryd2* and *Ryd3* tended to increase yields in those inoculated plants that did not develop symptoms, and in which the virus failed to build up high concentrations (Table 3). Such tillering-promoting effects have also been reported by Friedt et al. (2003) and Scheurer et al. (2000). Both the apparently low incidence and the increased tillering on those apparently virus-free plants warrant studies that monitor virus concentration during the life span of the plant, in shoots and roots, similar to the study by Ranieri et al. (1993).

The gauze house tests suggest that the effect of *Ryd3* is very similar to that of *Ryd2*. Further studies are required to test the effectiveness of *Ryd3* in different genetic backgrounds and against different isolates and serotypes of BYDV and against cereal yellow dwarf virus, and the possible association with yield and malting quality aspects in absence of BYD infection.

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References

Baltenberger DE, Ohm HW, Foster JE (1987) Reactions of oat, barley and wheat to infection with barley yellow dwarf isolates. Crop Sci 27:195–198

Banks PM, Waterhouse PM, Larkin PJ (1992) Pathogenicity of three RPV isolates of barley yellow dwarf virus on barley, wheat and wheat alien addition lines. Ann Appl Biol 121:305–314

Berloo R van (1999) GGT: software for the display of graphical genotypes. J Hered 90:328–329

Berloo R van, Aalbers H, Werkman A, Niks RE (2001) Resistance QTL confirmed through development of QTL-NILs for barley leaf rust resistance. Mol Breed 8:187–195

Burnett PA, Comeau A, Qualset CO (1995) Host plant tolerance or resistance for control of barley yellow dwarf. In: D'Arcy CJ, Burnett PA (eds) Barley yellow dwarf. Forty years of progress. APS, St Paul, pp 321–343

Catherall PL, Jones AT, Hayes JD (1970) Inheritance and effectiveness of genes in barley that condition tolerance to barley yellow dwarf virus. Ann Appl Biol 65:153–161

Chalhoub BA, Sarrafi A, Lapierre HD (1995) Partial resistance in the barley (*Hordeum vulgare* L.) cultivar Chikurin Ibaraki 1 to two PAV-like isolates of barley yellow-dwarf virus: allelic variability at the *Yd2* gene locus. Plant Breed 114:303–307

Collins NC, Paltridge NG, Ford CM, Symons RH (1996) The *Yd2* gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3. Theor Appl Genet 92:858–864

Ford CM, Paltridge NG, Rathjen JP, Moritz RI, Simpson RJ, Symons RH (1998) Rapid and informative assays for *Yd2*, the barley yellow dwarf virus resistance gene, based on the nucleotide sequence of a closely linked gene. Mol Breed 4:23–31

Friedt W, Scheurer KS, Huth W, Habekuß A, Ordon F (2003) Genetic analysis of BYDV-tolerance in barley (*Hordeum vulgare* L.). J Plant Dis Prot 110:278–286

Jefferies SP, King BJ, Barr AR, Warner P, Logue SJ, Langridge P (2003) Marker-assisted backcross introgression of the *Yd2* gene conferring resistance to barley yellow dwarf virus in barley. Plant Breed 122:52–56

Jørgensen JH (1992) Discovery, characterization and exploitation of *Mlo* powdery mildew resistance genes in barley. Euphytica 63:141–152

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154:397–412

Le Gouis J, Devaux P, Werner K, Hariri D, Behrman N, Béghin D, Ordon F (2004) *Rym15* from the Japanese cultivar Chikurin Ibaraki1 is a new barley mild mosaic virus (BaMMV) resistance gene mapped on chromosome 6H. Theor Appl Genet 108:1521–1525

McKirdy SJ, Jones RAC, Nutter FW Jr (2002) Quantification of yield losses caused by Barley yellow dwarf virus in wheats and oats. Plant Dis 86:769–773

Miller WA, Rasochova L (1997) Barley yellow dwarf viruses. Annu Rev Phytopathol 35:167–190

Niks RE, Fernández E, Haperen B van, Bekele-Aleye B, Martínez F (2000) Specificity of QTLs for partial and non-host resistance of barley to leaf rust fungi. Acta Phytopathol Hung 35:13–21

Parlevliet JE, Ommeren A van (1984) Interplot interference and the assessment of barley cultivars for partial resistance to leaf rust, *Puccinia hordei*. Euphytica 33:685–697

Proll E, Richter J, Hamann U, Stanarius A, Eisenbrandt K, Spaar D (1984) Kartoffelblattrollvirus (potato leaf roll virus): Reinigung und Herstellung von Antiseren. Arch Phytopathol Plant Prot 20:1–13

Qi X, Stam P, Lindhout P (1998a) Use of locus specific AFLP markers to construct a high density molecular map in barley. Theor Appl Genet 96:376–384

Qi X, Niks RE, Stam P, Lindhout P (1998b) Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theor Appl Genet 96:1205–1215

Ramsay L, Macaulay M, Ivanissevich S degli, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Ranieri R, Lister RM, Burnett PA (1993) Relationship between barley yellow dwarf virus titer and symptom expression in barley. Crop Sci 33:968–973

Rasmusson DC, Schaller CW (1959) The inheritance of resistance in barley to the yellow-dwarf virus. Agron J 51:661–664

Regenmortel MHV van, Fauquet CM, Bishop DHL (2000) Virus taxonomy. Classification and nomenclature of viruses. In: Seventh report of the international committee on taxonomy of viruses. Academic, San Diego, pp 775–784

Schaller CW (1984) The genetics of resistance to barley yellow dwarf virus in barley. In: Burnett PA (ed) Barley yellow dwarf. A proceedings of the workshop. CIMMYT, Mexico, pp 93–99

Schaller CW, Rasmusson DC, Qualset CO (1963) Sources of resistance to the yellow-dwarf virus in barley. Crop Sci 3:342–344

Schaller CW, Qualset CO, Rutger JN (1964) Inheritance and linkage of the *Yd2* gene conditioning resistance to the barley yellow dwarf disease in barley. Crop Sci 4:544–548

Scheurer KS, Huth W, Friedt W, Ordon F (2000) First results on BYDV-tolerance in barley estimated in pot experiments. J Plant Dis Prot 107:427–432

Scheurer KS, Friedt W, Huth W, Waugh R, Ordon F (2001) QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.). Theor Appl Genet 103:1074–1083

Skaria M, Lister RM, Foster JE, Shaner G (1985) Virus content as an index of symptomatic resistance to barley yellow dwarf virus in cereals. Phytopathology 75:212–216

Spaner D, Shugar LP, Choo TM, Falak I, Briggs KG, Legge WG, Falk DE, Ullrich SE, Tinker NA, Steffenson BJ, Mather DE (1998) Mapping of disease resistance loci in barley on the basis of visual assessment of naturally occurring symptoms. Crop Sci 38:843–850

Stam P, Bos I, Qi X, Niks RE (1997) QTL mapping of yield and yield-related traits in a wide cross of spring barley (*Hordeum vulgare* L.). Advances in biometrical genetics. In: Krajewski P, Kaczmarek Z (eds) Proceedings of 10th meeting of the EUCARPIA section biometrics in plant breeding. Poznan, pp 277–280

Suneson CA (1955) Breeding for resistance to barley yellow dwarf virus in barley. Agron J 47:283

Suneson CA, Ramage RT (1957) Studies on the importance of the yellow-dwarf virus. Agron J 49:365–367

Toojinda T, Broers LH, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Ramsay L, Vivar H, Waugh R (2000) Mapping quantitative and qualitatitve resistance genes in a doubled haploid population of barley (*Hordeum vulgare*) Theor Appl Genet 101:580–589