Theor Appl Genet (2011) 123:69–76
DOI 10.1007/s00122-011-1567-y

ORIGINAL PAPER

Pyramiding of *Ryd2* and *Ryd3* conferring tolerance to a German isolate of *Barley yellow dwarf virus*-PAV (BYDV-PAV-ASL-1) leads to quantitative resistance against this isolate

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Received: 3 December 2010 / Accepted: 26 February 2011 / Published online: 18 March 2011

Abstract *Barley yellow dwarf virus* (BYDV) is an economically important pathogen of barley, which may become even more important due to global warming. In barley, several loci conferring tolerance to BYDV-PAV-ASL-1 are known, e.g. *Ryd2*, *Ryd3* and a quantitative trait locus (QTL) on chromosome 2H. The aim of the present study was to get information whether the level of tolerance against this isolate of BYDV in barley can be improved by combining these loci. Therefore, a winter and a spring barley population of doubled haploid (DH) lines were genotyped by molecular markers for the presence of the susceptibility or the resistance encoding allele at respective loci (*Ryd2*, *Ryd3*, QTL on chromosome 2H) and were tested for their level of BYDV-tolerance after inoculation with viruliferous (BYDV-PAV-ASL-1) aphids in field trials. In DH-lines carrying the combination *Ryd2* and *Ryd3*, a significant reduction of the virus titre was detected as compared to lines carrying only one of these genes. Furthermore, spring barley DH-lines with this allele combination also showed a significantly higher relative grain yield as compared to lines carrying only *Ryd2* or *Ryd3*. The QTL on chromosome 2H had only a small effect on the level of tolerance in those lines carrying only *Ryd2*, or *Ryd3* or a combination of both, but the effect in comparison to lines carrying no tolerance allele was significant. Overall, these results show that the combination of *Ryd2* and *Ryd3* leads to quantitative resistance against BYDV-PAV instead of tolerance.

Communicated by P. Hayes.

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Introduction

Due to global warming, longer periods of higher temperature in autumn and winter are expected in many regions of the world, which may result in an expanded flight activity and overwintering of insects (Harrington et al. 2007; Jones 2009). This will have consequences for the transmission of insect-transmitted viruses like the aphid-transmitted viruses causing *Barley yellow dwarf* (BYD) (Habekuß et al. 2009; Harrington 2003; Roos et al. 2011).

The BYD viruses belong to the family of the *Luteoviridae* and were first grouped according to their vector specificity into five strains and named after their main vectors (Rochow 1969). On the basis of their serological relationships, cytopathology and nucleic acid sequences, they were later assigned to two subgroups, namely PAV, MAV and SGV to subgroup 1 and RPV and RMV to subgroup 2 (Gill and Chong 1979; Rochow and Duffus 1981). In the actual virus taxonomy, BYDV-MAV, -PAV and -PAS have been assigned to the genus *Luteovirus* and RPV and -RPS (Rhopalosiphum padi Severe) have been assigned as *Cereal yellow dwarf virus* (CYDV)-RPV and -RPS within the genus

Polerovirus. In addition, there are some up to now unassigned species (BYDV-GAV, -GPV, -RMV and -SGV) (Domier 2008).

BYDV-PAV with its vectors, *Rhopalosiphum padi* and *Sitobion avenae*, is the most prevalent BYDV serotype worldwide, followed by BYDV-MAV (Domier 2008). BYDV attacks all species of *Poaceae* (Domier and D'Arcy 2008) and causes severe economical losses in grain production especially in barley and wheat (Lister and Ranieri 1995). The main virus symptoms in barley are dwarfing of shoots, leaf yellowing, reduced number and sterility of ears, delay in heading and reduced winter hardiness.

One way to avoid yield losses is to combat the aphids by chemical measures along with the elimination of alternate hosts like volunteer plants. However, the most environmentally sound alternative is breeding and growing of tolerant or resistant cultivars. In barley, different genes conferring tolerance have been identified. The first was *ryd1*, which was detected in the spring barley cultivar 'Rojo' (Suneson 1955), but was not used in barley breeding due to its low efficiency. Furthermore, *Ryd2* and *Ryd3*, with similar effects against BYDV-PAV and -MAV were identified in Ethiopian landraces. The effect of the semi-dominant *Ryd2* gene varies according to the genetic background, the environmental conditions and the virus isolate (Schaller et al. 1964; Schaller 1984). This gene is located on chromosome arm 3HL (Schaller et al. 1964; Collins et al. 1996). *Ryd2* has been reported to reduce the virus titre of BYDV-PAV and -MAV in young plants (Skaria et al. 1985; Baltenberger et al. 1987; Herrera and Plumb 1991; Ranieri et al. 1993; Chalhoub et al. 1994, 1995; Sip et al. 2006), but only to some isolates of CYDV-RPV (Banks et al. 1992). However, no differences in the virus titre of plants carrying *Ryd2* or *ryd2*, respectively concerning BYDV-PAV were observed in studies in which older plants were tested (Skaria et al. 1985; Scheurer et al. 2000). Besides this, different alleles may be present at this locus (Catherall et al. 1970; Chalhoub et al. 1995). *Ryd3* explaining about 75% of the phenotypic variance in the cross 'Vada' × 'L94' has been mapped on chromosome 6H (Niks et al. 2004). Furthermore, an association between SSR locus HVM054 on chromosome 2H and a large-effect gene for BYD resistance or tolerance was reported in a linkage disequilibrium study in European spring barley (Kraakman et al. 2006).

In addition to these loci, QTLs for tolerance have been mapped on chromosome 2HL (Scheurer et al. 2001) and on other chromosomes (Toojinda et al. 2000). *Ryd2* has been used in breeding for BYDV tolerant barley cultivars, e.g. cvs. 'CM 67', 'Atlas 68', 'Sutter', 'Coracle', 'Vixen', 'Wysor', 'Venus' and 'Naturel' (Brown et al. 1988; Catherall et al. 1977; Schaller and Chim 1969a, b; Schaller et al. 1973; Parry and Habgood 1986; Starling et al. 1987, 1994). *Ryd3* has been reported in for example cv. 'Granado' (Sayed et al. 2006), whereas cv. 'Laurel' was reported to contain both genes (Sayed et al. 2006). Besides this, the locus on 2H was detected in 15 out of 148 spring barley cultivars tested (Kraakman et al. 2006).

All the loci described above confer tolerance to the isolate BYDV-PAV-ASL-1 used in our study (Scheurer et al. 2000, 2001; Niks et al. 2004). It is important to differentiate between tolerance and resistance. The present paper uses the terms according to Cooper and Jones (1983), i.e. tolerance means that unrestricted virus infection and replication takes place but infected plants show only mild or no symptoms and no or minor decrease in grain yield, while resistance is characterised by a decreased virus replication in infected plants. In order to determine whether combining some of the above mentioned loci leads to a higher level of tolerance against a German isolate of BYDV-PAV, *Ryd2*, *Ryd3* and the QTL on chromosome 2HL were combined using DH-lines and molecular markers, a strategy which was efficient in pyramiding different resistance genes against the barley yellow mosaic virus complex (Werner et al. 2005, 2007).

Materials and methods

Plant material

For pyramiding, DH-lines of the cross 'RIL K4-56' (*Ryd3*, spring barley) × 'DH21-136' (*Ryd2* and positive allele at the QTL on chromosome 2HL of cv. 'Post', winter barley, population I) and DH-lines of 'RIL K4-56' (*Ryd3*) × 'Coracle' (*Ryd2*, spring barley, population II) were produced from F_1-seeds by the microspore or anther culture technique, respectively, by KWS-Lochow GmbH (Bergen) and the Saaten-Union Biotec GmbH (Leopoldshöhe). 'RIL K4-56' was one of the resistant *Ryd3* carrying lines from the cross 'Vada' × 'L94' (Niks et al. 2004). 'DH21-136' originates from the cross between 'Post' (2H QTL tolerance allele) and 'Vixen' (*Ryd2*, Scheurer et al. 2001).

Genotyping

DNA of the DH-lines was extracted according to Stein et al. (2001). After RNAse treatment, nucleic acid concentration was measured using the NanoDrop ND-1000 spectrophotometer (peqLab, Biotechnology GmbH, Erlangen) and adjusted to a final concentration of 30 ng/µl for PCR. For the detection of *Ryd2*, the CAPS-Marker YlpPCRM was used according to Ford et al. (1998). Screening for the presence of *Ryd3* was conducted using the microsatellite marker, HVM74 (Niks et al. 2004), and the QTL on chromosome 2H derived from cv. 'Post' was detected by the microsatellite marker, HVCSG (Scheurer et al. 2001).

YlpPCRM was analysed on agarose gels and the microsatellite markers were analysed using a capillary sequencer (Beckman Coulter CEQ™ 8000, Brea, CA, USA).

Field trials

Phenotyping of the DH-populations was carried out at four locations in Germany [Gudow, Schleswig–Holstein (Nordsaat Saatzucht GmbH), Irlbach, Bavaria (Dr. J. Ackermann & Co. Saatzucht Irlbach), Bernburg (KWS-Lochow GmbH) and Quedlinburg, Saxony-Anhalt (JKI)] and in two growing periods (2007/2008, 2008/2009). For each trial, 40 seeds of each of 188 DH-lines per cross representing all possible allele combinations, as well as the parental lines and the susceptible standards 'Rubina' (winter barley) and 'Vada' (spring barley), were sown in the greenhouse (18–22°C, 16 h photoperiod, 10 klx). In the seedling stage (1 leaf stage) 10 plants per line and replication (20 plants) were inoculated in the greenhouse using BYDV-PAV (isolate BYDV-PAV-ASL-1) bearing *Rhopalosiphum padi* (10 aphids/plant, Habekuss and Lehmann 1991) and simultaneously the same number of healthy control plants—check—(20 plants) were grown in a separate greenhouse. After 5 days the aphids were killed by the insecticide Confidor® WG70 (Bayer CropScience AG, Germany).

Plants of population I were transferred to the field at the four locations in October 2007 and 2008 in a randomised complete block design in two replications (2 × 10 plants per infected variant and check) and the same was done for the spring barley population II in March 2008 and April 2009.

For each line the symptom expression was scored at the end of tillering by a scale from score 1 = plant without symptoms to score 9 = plant dead. The heading date was recorded and the plant height was measured at heading. Furthermore, the number of ears per plant, the number of kernels per ear, the thousand-kernel weight and the grain yield per plant were determined.

The level of tolerance for each DH-line was estimated as the relative performance of the infected variant in comparison to the check, i.e. (infected variant/non-infected variant) × 100.

Serological test for BYDV-PAV

At the end of tillering, the ELISA-extinction was determined for selected DH-lines of the different allele combinations as an indicator for the virus content. For this purpose, 0.1 g tissue of a mixed sample, of two leaves from half-way up the tiller, per plant was crushed and analysed by the double antibody sandwich (DAS) ELISA according to Clark and Adams (1977) with polyclonal antibodies against BYDV-PAV produced from isolate BYDV-PAV-ASL-1 (Serum bank of JKI) and used in many studies on BYDV-PAV, already (e.g. Scholz et al. 2009). Extinction was measured at a wavelength of 405 nm using a microtitre plate reader (Opsys MR, ThermoLabsystems, Franklin USA). Lyophilisated leaf samples of healthy barley plants were included as 'negative controls' in the test to calculate the threshold value of extinction between healthy and infected plants (estimated as mean extinction value of negative controls plus the threefold standard deviation). Based on these results, $E_{405} < 0.1$ was set as a threshold for infection. Furthermore, samples of infected plants were tested as 'positive controls'. In 2008, three DH-lines from population I and six from population II for each allele combination were tested with 10 plants per replication and from two (population I), respectively three (population II) different locations. In the same manner in November 2008, additional samples were taken from the test grown at Quedlinburg. In 2009, the number of samples tested was increased to 10 DH-lines in population I and to 20 DH-lines of population II for each allele combination, with five plants per replication and from all four locations. For each allele combination, 520 plants of population I and 1,160 of population II were tested by ELISA.

Statistical analysis

For the statistical analyses of the numeric phenotypic and ELISA data, the analysis of variance (ANOVA) was used. ANOVA and all other statistical tests were conducted using the software package SAS 9.1. The significance of differences between the allele combinations and their interactions with year and location and also the homogeneity of variance were tested using the general linear model procedure (proc GML). The Tukey test was used for the multiple comparison of means ($\alpha = 0.05$). The scores of symptom expression as ordinal data were tested for significance between the allele combinations by estimating the p values for contrasts (as t test) in a non-parametric permutation test (Neuhäuser and Jöckel 2006) using the MULTTEST procedure ($\alpha = 0.05$). The heritability was estimated according to Allard (1960) using the VARCOMP procedure of SAS for calculation of the variance components.

Results

Genotyping

The combination of three loci for tolerance in population I leads to eight possible allele combinations, and the two loci for tolerance in population II lead to four possible allele combinations in the DH-lines. All possible allele combinations were detected (Table 1), but while a good fit to the

expected segregation (χ^2) was observed in population II, in the other cross the observed segregation ratio deviated significantly from the expectation. This deviation was mainly due to an excess of *Ryd3* (310 DHs) over *ryd3* (160 DHs), and to a lesser extent by an excess of lines carrying the positive allele at the QTL on chromosome 2H (QTL+).

ELISA extinction

In both populations the combination of the alleles *Ryd2* and *Ryd3* resulted in a much decreased average ELISA-extinction (E_{405}), and hence a lower virus concentration, compared to those lines with only one of these genes and the lines carrying only the susceptibility encoding alleles (Figs. 1, 2). The positive allele of the QTL on chromosome 2H in population I leads to a slight, but significant reduction of the virus titre in each allele combination, except when lines carrying only the QTL were compared to lines without any tolerance allele (for explanation see "Discussion"). However, it has to be taken into account that the variance of the ELISA data as well as of the phenotypic data (see below) was not homogeneous ($p < 0.05$) leading to a possible underestimation of the significance level. The virus titre of the parental lines and the susceptible standard was similar to those of respective DH-lines, i.e. within the standard deviation of the respective allele combinations. In many plants of DH-lines carrying *Ryd2* and *Ryd3*, no virus was detected by DAS-ELISA, i.e. an E_{405} value lower than 0.1 was measured. This was the case in 65.3% of plants of population I carrying the allele combinations *Ryd2*/*Ryd3*/QTL+ and *Ryd2*/*Ryd3*/QTL- and in 23.2% of the *Ryd2* and *Ryd3* carrying spring barley lines (population II). An insufficient inoculation can be excluded as a major influencing factor due to the low proportion of virus-free plants in the other allele combinations (average population I: 5.0%, population II: 2.0%) and the susceptible standard. The average ELISA-extinction in plants of the combination *Ryd2*/*Ryd3* is still significantly lower when all samples with $E_{405} < 0.1$ are excluded, i.e. $E_{405} = 0.7$ in both populations in comparison to $E_{405} = 1.1$ for those DH-lines in both populations carrying only *Ryd2* or *Ryd3*, respectively. Although a significant influence of year and location and respective interactions was detected for the ELISA data and for the phenotypic data ($p < 0.001$), heritabilities for all traits analysed were estimated between 0.41 and 0.93. These high heritabilities give hint to the large influence of the genotype, e.g. herita-

Table 1 Observed segregation ratio for the possible allele combinations in population I ('RIL K4-56' × 'DH21-136', upper part) and in population II ('RIL K4-56' × 'Coracle', lower part)

Alleles	*Ryd2 Ryd3* QTL+	*Ryd2 Ryd3* QTL−	*Ryd2 ryd3* QTL+	*Ryd2 ryd3* QTL−	*ryd2 Ryd3* QTL+	*ryd2 Ryd3* QTL−	*ryd2 ryd3* QTL+	*ryd2 ryd3* QTL−
Number of DH-lines	93	49	43	37	92	76	52	28
χ^2 (1:1:1:1:1:1:1:1) = 74.612								
Alleles		*Ryd2 Ryd3*		*Ryd2 ryd3*		*ryd2 Ryd3*		*ryd2 ryd3*
Number of DH-lines		68		66		76		85
χ^2 (1:1:1:1) = 3.04								

+, tolerance encoding allele; −, susceptibility encoding allele

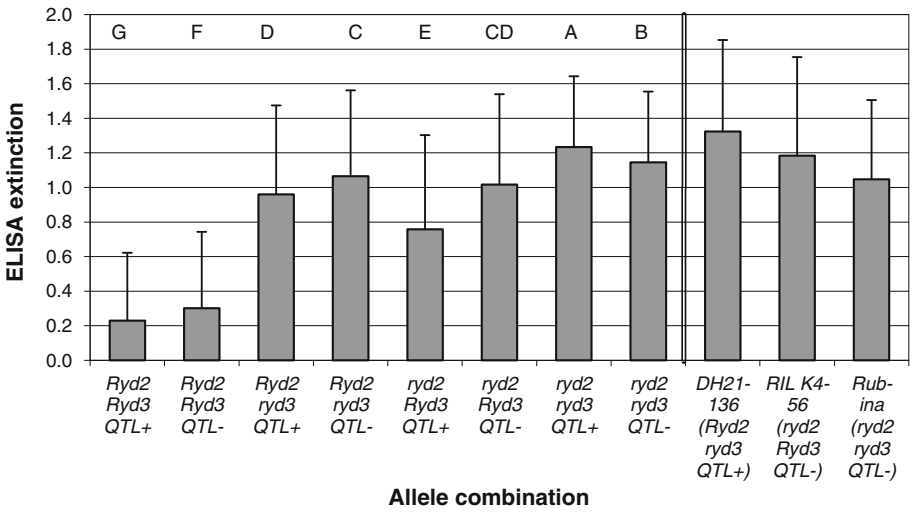

Fig. 1 Average ELISA-extinction (405 nm) and standard deviation in DH-lines of population I ('RIL K4-56' × 'DH21-136') carrying different allele combinations at the *Ryd2*, *Ryd3* locus and the QTL on chromosome 2H determined after experimental BYDV-PAV inoculation on four locations and in 2 years in field trials. *Different letters* indicate significant differences (Tukey test, $\alpha = 0.05$). Data of parental lines and the susceptible standard are shown for comparison

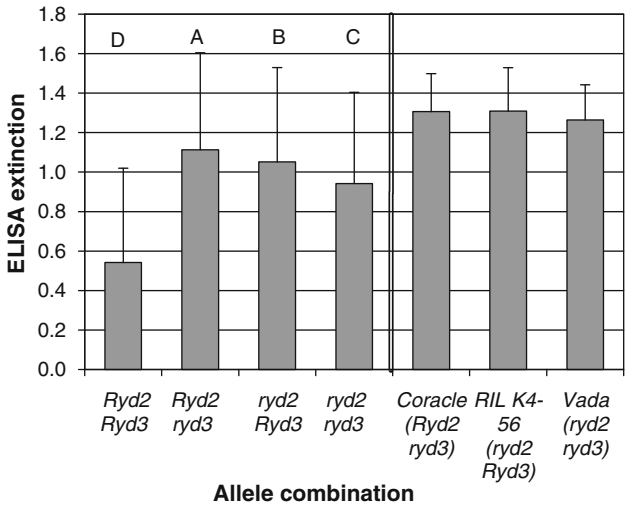

Fig. 2 Average ELISA-extinction (405 nm) and standard deviation in population II ('RIL K4-56' × 'Coracle'), carrying different allele combinations at the *Ryd2* and *Ryd3* locus, determined after experimental BYDV-PAV inoculation on four locations and in 2 years in the field. *Different letters* mean significant differences (Tukey test, $\alpha = 0.05$). Data of parental lines and the susceptible standard are shown for comparison

bility (h^2) of the ELISA-extinction was estimated at $h^2 = 0.85$ for population I and $h^2 = 0.74$ for population II.

Growth and yield parameters

In the DH-lines of population II, significant differences between the four allele combinations were detected for nearly all traits measured (Tables 2, 3). DH-lines of the allele combination *Ryd2*/*Ryd3* showed the highest relative grain yield, number of ears, number of kernels per ear and plant height. In addition, these lines had on average a lower symptom expression and a reduced delay in heading after virus inoculation. The DH-lines of population I reacted similarly to those of population II, but the differences were not so clear between lines carrying only *Ryd2* or *Ryd3* and those carrying a combination of both. DH-lines of both populations carrying no tolerance alleles showed a decrease in all plant growth parameters, especially in grain yield, in which mean losses up to 86% were detected. In general, the QTL on chromosome 2H had only a small effect on the level of tolerance in those lines carrying *Ryd2*, *Ryd3* or a combination of both, but the effect in comparison to lines carrying no tolerance allele was significant. The mean parental value for grain yield relative to un-inoculated plants of the same accession was 94% for 'Coracle' and 116% for 'RIL K4-56' in population II, whereas the susceptible standard 'Vada' reached 9%. In population I 'DH21-136' gave a mean relative grain yield of 108%, 'RIL K4-56' of 103% and the susceptible standard 'Rubina' of 2%. Heritability of the relative grain yield was estimated at $h^2 = 0.90$ in the population II and $h^2 = 0.89$ in population I.

Discussion

In both DH-populations, besides the detection of a higher level of tolerance expressed by a higher relative grain yield and higher relative values for the other traits estimated (Tables 2, 3), it turned out that the combination of *Ryd2* and *Ryd3* leads to a significant reduction in the virus titre, i.e. to quantitative resistance (Figs. 1, 2). Similar observations were made by Jahier et al. (2009) in wheat by combining *Bdv2* and the group-2 chromosome arm carrying BYDV resistance derived from *Thinopyrum intermedium*. In their studies, they also found a very low virus titre and a significantly lower number of infected plants in those lines carrying both genes compared to the single loci. While in several virus host pathosystems like *Cucumber mosaic virus*—cucumber (Weber et al. 1990) or *Potato leafroll virus*—potato (Barker and Harrison 1986) the virus titre is correlated to the resistance level, no consistent results are available for BYDV. Concerning the effectiveness of *Ryd2*, contradictory results are known. Sip et al. (2006) found a positive correlation between ELISA values estimated in barley seedlings of the cross 'Igri' (*ryd2*) × 'Atlas 68' (*Ryd2*) and the symptom level observed in field experiments, indicating that *Ryd2* decreases viral reproduction in the plant at least in the seedling stage, and hence is a gene for resistance rather than tolerance. Furthermore, Larkin et al. (1991) and Ranieri et al. (1993) found a positive correlation between symptom expression and ELISA values, which was also more pronounced in younger plants, whereas Scheurer et al. (2000) and Skaria et al. (1985) found no relation between the virus titre and the level of tolerance. However, in these experiments the virus titre was measured in older plants. The latter two studies therefore indicate that *Ryd2* confers tolerance. Niks et al. (2004) detected a lower infection rate determined by DAS-ELISA of plants carrying *Ryd3*. However, such an effect was not corroborated in the present studies, in which it turned out that both *Ryd2* and *Ryd3* confer tolerance (Figs. 1, 2). The reduced virus titre in plants carrying *Ryd2*/*Ryd3* seems to have no epidemiological advantages as could be demonstrated in preliminary greenhouse tests (unpublished data). In these studies plants having a low ($E_{405} = 0.26$; *Ryd2*/*Ryd3*) and a high ($E_{405} = 1.66$; *ryd2*/*ryd3*) virus titre were used as the source for virus acquisition, resulting in extinction values of $E_{405} = 0.48$ and $E_{405} = 0.25$ on lines carrying *Ryd2*/*Ryd3* and in values of $E_{405} = 1.38$ and $E_{405} = 1.19$ on carriers of *ryd2*/*ryd3*, measured in seedlings 4 weeks after infection. Apparently, the plants with *Ryd2*/*Ryd3* can still serve as sources of infection, and it may be concluded that

Table 2 Average tolerance level relative to the check of the same DH-line (except for symptom expression) of the different allele combinations in DH-lines of population II ('RIL K4-56' × 'DH21-136')

Trait/alleles	*Ryd2 Ryd3* QTL+	*Ryd2 Ryd3* QTL−	*Ryd2 ryd3* QTL+	*Ryd2 ryd3* QTL−	*ryd2 Ryd3* QTL+	*ryd2 Ryd3* QTL−	*ryd2 ryd3* QTL+	*ryd2 ryd3* QTL−
Grain yield (%)	106a	105a	94b	94b	100ab	101ab	43c	20d
Number of ears (%)	109a	109a	100b	101ab	106ab	105ab	54c	32d
Thousand-kernel weight (%)	100a	100a	99a	99a	100a	99a	87b	81c
Kernels per ear (%)	101a	100a	97ab	94b	98ab	101a	79c	62d
Plant height (%)	99a	99a	98a	98a	98ab	98a	87b	72c
Heading date*	0.48d	0.55cd	1.02cd	1.19c	0.99cd	1.04cd	2.57b	3.59a
Symptom expression**	2.46a	2.60ab	2.91c	3.16c	2.96c	2.88bc	4.39d	5.51e

Results were obtained after experimental BYDV-PAV inoculation with two replications at four locations and in 2 years (2007/2008, 2008/2009) in field trials. Different letters within each row mean significant differences (Tukey test, $\alpha = 0.05$; except for symptom expression tested by a permutation test according to Neuhäuser and Jöckel 2006)

* number of days that infected plants were delayed, ** average symptom scoring in infected plants

Table 3 Average tolerance level relative to the check of the same DH-line (except for symptom expression) of the different allele combinations in DH-lines of population II ('RIL K4-56' × 'Coracle')

Trait/alleles	*Ryd2 Ryd3*	*Ryd2 ryd3*	*ryd2 Ryd3*	*ryd2 ryd3*
Grain yield (%)	116a	88c	101b	14d
Number of ears (%)	114a	93c	103b	25d
Thousand-kernel weight (%)	99a	96c	97b	70d
Kernels per ear (%)	101ab	99b	102a	79c
Plant height (%)	100a	97b	100a	70c
Heading date*	1.11c	2.14b	2.39b	6.19a
Symptom expression**	2.43a	2.39c	3.20b	6.06d

Results were obtained after experimental BYDV-PAV inoculation with two replications at four locations and in 2 years (2008, 2009) in field trials. Different letters within each row mean significant differences (Tukey test, α=0.05; except for symptom expression tested by a permutation test according to Neuhäuser and Jöckel 2006)

* number of days that infected plants were delayed; ** average symptom scoring in infected plants

the virus titre of newly infected plants is independent from the virus titre of plants being the source for virus acquisition. In contrast, Barker and Harrison (1986) noticed that the aphid *Myzus persicae*, the vector of *Potato leafroll virus*, acquired less virus from resistant plants with a low virus concentration, resulting in strongly reduced infection efficiency (3%), compared to 58% when aphids acquired virus from susceptible plants. Nevertheless, the reduced virus titre obtained by the combination of *Ryd2* and *Ryd3* and the higher number of plants expressing extinction values of $E_{405} < 0.1$ reduces the chance of aphids acquiring virus thereby potentially influencing the epidemics of BYDV.

The effect of the QTL on chromosome 2H on tolerance against BYDV-PAV (Scheurer et al. 2001) was confirmed in this study but the level of tolerance turned out to be much smaller than that conferred by *Ryd2* or *Ryd3*. The reduced ELISA-extinction in lines without tolerance encoding alleles compared to lines carrying only the QTL on chromosome 2H (Fig. 1) can be explained by the fact that at sampling time of ELISA probes in spring those lines without any tolerance encoding allele were already severely damaged by BYDV leading to a reduced virus replication at that time. This assumption is confirmed by the fact that in autumn 2008 (data not shown) no significant differences in ELISA values were detected between lines carrying the positive allele at the QTL on chromosome 2HL and the ones without any positive allele.

In barley breeding, marker assisted combining of both genes, *Ryd2* and *Ryd3*, can easily be conducted because of the localisation of these genes on different chromosomes and due to the availability of the closely linked PCR-based markers, YlpPCRM and HVM74 (amongst others like HVM22 and HVM14, see Niks et al. 2004). Up to now, *Ryd2* was mainly used in breeding BYDV tolerant barley cultivars. Several cultivars like 'Sutter' (Schaller et al. 1973), 'Coracle' (Catherall and Wilkins 1977) 'Vixen' (Parry and Habgood 1986) and 'Franklin' (Vertigan 1991)

were developed, however, they have no economical relevance any more due to agronomical shortcomings in comparison to recently released cultivars. In the present study, for the first time the *Ryd3* tolerance allele was transferred from spring barley to winter barley in which yield losses due to BYDV are higher than in spring barley. Therefore, winter barley as well as spring barley lines with both BYDV-tolerance genes, i.e. *Ryd2* and *Ryd3*, are available for breeding of barley cultivars with quantitative BYDV resistance.

In 2009, a gene for qualitative BYDV resistance in barley, named $Ryd4^{Hb}$, was identified and localised on chromosome 3HL (Scholz et al. 2009). This resistance was introgressed from *Hordeum bulbosum*, the secondary gene pool of barley. According to Scholz et al. (2009), this gene cannot yet be efficiently used in barley breeding because it is linked to undesirable traits derived from *H. bulbosum*. Therefore, actually using *Ryd2* and *Ryd3* in combination is the most promising way to breed barley cultivars expressing quantitative resistance against *Barley yellow dwarf virus*. Further studies are required to test the present plant material in detail for its reaction to BYDV-MAV and the *Cereal yellow dwarf virus*-RPV. Furthermore, the effect of the QTL on chromosome 2 detected by Kraakman et al. (2006) should be analysed in combination with the *Ryd2* and *Ryd3* genes.

Acknowledgment The authors thank the Federal Ministry for Nutrition, Agriculture and Consumer Protection (BMELV) and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V (GFP) for financial support (PGI-06.01-28-1-41.002-06). For carrying out field trials we thank Dr. Claus Einfeldt from Saatzucht Dr. J. Ackermann & Co, Irlbach, Dr. Viktor Korzun from KWS-Lochow GmbH, Dr. Eberhard Laubach from Nordsaat Saatzuchtgesellschaft mbH and Dr. Jens Weyen from Saaten-Union Biotec GmbH for producing the DH-lines.

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