Theor Appl Genet (2013) 126:1201–1212
DOI 10.1007/s00122-013-2047-3

ORIGINAL PAPER

Genomics-based high-resolution mapping of the BaMMV/BaYMV resistance gene *rym11* in barley (*Hordeum vulgare* L.)

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Received: 5 September 2012 / Accepted: 13 January 2013 / Published online: 2 March 2013
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Abstract Soil-borne barley yellow mosaic virus disease, caused by different strains of Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV), is one of the most important diseases of winter barley (*Hordeum vulgare* L.) in Europe and East Asia. The recessive resistance gene *rym11* located in the centromeric region of chromosome 4HL is effective against all so far known strains of BaMMV and BaYMV in Germany. In order to isolate this gene, a high-resolution mapping population (10,204 meiotic events) has been constructed. F_2 plants were screened with co-dominant flanking markers and segmental recombinant inbred lines (RILs) were tested for resistance to BaMMV under growth chamber and field conditions. Tightly linked markers were developed by exploiting (1) publicly available barley EST sequences, (2) employing barley synteny to rice, *Brachypodium distachyon* and sorghum and (3) using next-generation sequencing data of barley. Using this approach, the genetic interval was efficiently narrowed down from the initial 10.72 % recombination to 0.074 % recombination. A marker co-segregating with *rym11* was developed providing the basis for gene isolation and efficient marker-assisted selection.

Introduction

Barley yellow mosaic virus disease caused by different strains of *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) (Huth 1991; Hariri et al. 2000, 2003; Kanyuka et al. 2004; Habekuss et al. 2008; Kühne 2009) has become one of the most important diseases of winter barley in East Asia and in Europe (Ordon et al. 2004; Kanyuka et al. 2003). The area infested is continuously growing and high yield losses of susceptible winter barley crops are frequently observed in Europe. Due to transmission of the viruses via the soil-borne plasmodiophorid *Polymyxa graminis*, breeding for resistant cultivars is the only way to ensure winter barley cultivation in the growing area of infested fields. Up to now, resistance in

Communicated by P. Hayes.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2047-3) contains supplementary material, which is available to authorized users.

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Europe mainly relies on the resistance gene *rym4*/*rym5*. This source of resistance has been overcome meanwhile by BaYMV-2 (*rym4*) and BaYMV-SIL/BaMMV-Teik (*rym5*), respectively (Habekuss et al. 2008). However, several resistance genes against BaMMV/BaYMV are known in the primary gene pool of barley (Friedt et al. 2008) suited to broaden the genetic basis of resistance. Among these, the recessive resistance gene *rym11* has been located in the centromeric region of chromosome 4HL (Bauer et al. 1997; Nissan-Azzouz et al. 2005). To date, this gene confers resistance to all known European strains of BaMMV and BaYMV under field conditions (Kanyuka et al. 2004; Habekuss et al. 2008). However, in a single report on studies conducted in pot trials using air-dried soil collected from different locations in France, *rym11* has been overcome by strains of BaYMV and BaMMV (Hariri et al. 2000). All bymovirus resistance genes derived from the primary gene pool of barley are inherited in a recessive manner (Friedt and Ordon 2007), with the exception of the newly identified dominant resistance gene *Rym17*, which confers resistance against Japanese strains of BaYMV (Kai et al. 2012). Furthermore, resistance genes transferred from *Hordeum bulbosum* are dominantly inherited, i.e., $Rym14^{Hb}$ (Ruge et al. 2003) and $Rym16^{Hb}$ (Ruge-Wehling et al. 2006).

Recessive inheritance is the common mode of resistance to potyviruses of which bymoviruses are a subgroup (López-Moya and Garcia 2008; Provvidenti and Hampton 1992; Maule et al. 2007). Recessive resistance is indicating a lack of host components vital for a particular step in the virus life cycle (Fraser 1990; Revers et al. 1999). In this respect, mutations in genes of the eukaryotic translation-initiation complex were identified as underlying mechanism of potyvirus resistance in plant species of different genera (Robaglia and Caranta 2006; Maule et al. 2007; Yeam et al. 2007; Hofinger et al. 2011). In barley, *rym4*/*rym5* mediated bymovirus resistance is conferred by mutations in the eukaryotic translation initiation factor 4E (*Hv-eIF4E).* Independent single nucleotide polymorphisms (SNPs) contribute to the differential genotypic response to different strains of BaMMV and BaYMV (Stein et al. 2005; Kanyuka et al. 2005).

In case no information on the putative function of a gene is available, map-based cloning has proven to be an efficient tool for gene isolation even in plants with complex genomes such as barley and wheat (Stein and Graner 2004). This approach requires the construction of a high-resolution mapping population followed by marker saturation of the target interval (Stein and Graner 2004; Pellio et al. 2005). While previously anonymous marker technologies like AFLPs had been used for marker saturation (e.g., Pellio et al. 2005), today, dense sequence-based marker maps either comprising non-genic (Ramsay et al. 2000; Hearnden et al. 2007; Varshney et al. 2007) or transcript-derived markers (e.g., Stein et al. 2007; Sato et al. 2009; Close et al. 2009) are available for targeted marker saturation. Moreover, synteny between barley, rice, sorghum and *Brachypodium* (Stein et al. 2007; Thiel et al. 2009; Mayer et al. 2011) can be efficiently employed (Smilde et al. 2001; Gottwald et al. 2004; Perovic et al. 2004; Shahinnia et al. 2009; Vu et al. 2010) especially if combined with sequencing information of flow-sorted barley chromosome arms (barley genome zipper, Mayer et al. 2011).

The aim of the present study was to employ improved genomic tools of barley for (1) the rapid high-resolution mapping of *rym11* providing a starting point for the isolation of this resistance gene and (2) the identification of markers for efficient marker-based selection procedures.

Materials and methods

Plant material and construction of a high-resolution mapping population

For high-resolution mapping of *rym11*, a segregating population comprising 5,102 F_2 plants was constructed based on crosses between the *rym11*-carrying doubled haploid lines (DHs) 'W 757/112' and 'W 757/612', respectively, tracing back to an accession carrying a resistance gene allelic to the one of Russia 57 (*rym11*, Nissan-Azzouz et al. 2005) and the cultivar Naturel. Semi-automatic DNA-extraction according to Dorokhov and Klocke (1997) was carried out at the two-leaf stage using a 96-deep-well leaf crusher (Thieme et al. 2008). To identify F_2 plants recombinant for the target interval, screening with co-dominant SSR-markers HVM03 and HVM68 (Nissan-Azzouz et al. 2005) was performed as a duplex assay via fluorescence-detection on a CEQ 8000 (Beckman-Coulter; cf. Wang et al. 2009). Homozygous recombinant inbred lines (RILs) were selected among 12 F_3 plants of heterozygous recombinant F_2 plants by applying the same SSR-markers. Unintentionally, cultivar Naturel also exhibited recessive bymovirus resistance which was conferred by an unlinked recessive resistance at the locus *Rym4* (Stein et al. 2005). Therefore, plants carrying the recessive *rym4* allele were identified in F_2 by the closely linked marker Bmac29 (Graner et al. 1999) and were discarded from the further population development. For marker saturation, DNA of segmental RILs was extracted according to Stein et al. (2001) and respective seeds were harvested for repeated phenotyping.

Resistance tests

Resistance to BaMMV was tested in growth chamber experiments by mechanical inoculation of ten plants per each

RIL with the isolate BaMMV-ASL1 (Timpe and Kühne 1994; Habekuss et al. 2008). The presence of virus particles was assessed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) according to Clark and Adams (1977). Phenotyping for BaMMV resistance was also conducted in field trials under natural infection conditions at two infested fields (Schladen/Lower Saxony and Morgenrot/Saxony-Anhalt). Twenty plants per RIL were grown in double rows of 10 plants each in two replications. The experimental design contained negative (resistant cultivars Tokyo and Carola carrying resistance genes *rym5* and *rym4*, respectively) and positive controls (susceptible cvs. Uschi and Maris Otter) for monitoring infection.

Marker saturation

Marker saturation of the *rym11* target interval relied on publicly available marker resources. These included SSR (simple sequence repeat) and SNP (single nucleotide polymorphism) markers from published barley genetic maps (Ramsay et al. 2000; Hearnden et al. 2007; Varshney et al. 2007; Close et al. 2009; Stein et al. 2007; Sato et al. 2009; Table 1). Potential markers were amplified on DNA from the parental genotypes of the mapping population and subjected to amplicon sequencing on an ABI377XL sequencer using BigDye terminator sequencing chemistry (ABI Perkin Elmer, Weiterstadt, Germany). For the determination of polymorphisms, sequences were analysed by the help of the software Sequencher v4.9 (Gene Codes, Ann Arbor, MI). Insertion/Deletion (Indel) polymorphisms were directly assayed by PCR and gel electrophoresis. Sequences carrying SNPs were either converted to cleaved amplified polymorphic sequence (CAPS) markers using NEBcutter v.2.0 (http://tools.neb.com/NEBcutter2) and the TAIR (The Arabidopsis Information Resource) restriction tool (http://www.arabidopsis.org/cgi-bin/patmatch/RestrictionMapper.pl), or to pyrosequencing markers (PSQ™ Assay Design, Biotage AB, Uppsala, Sweden). For CAPS analysis, DNA amplicons were cleaved with the respective restriction endonuclease (12 µl reaction volume, 1.25 U, 3 h at appropriate temperature according to manufacturer's instructions, Fermentas or NEB) and subsequently resolved on ethidium bromide stained 1.5 % agarose gels. Pyrosequencing was performed on a PyroMark ID system (Biotage AB) using a biotinylated M13 universal primer according to Silvar et al. (2011).

In case of SNP markers previously mapped on the basis of multiplex SNP detection platforms like the Illumina Golden Gate assay (Close et al. 2009), the underlying EST sequence information (http://www.harvest-web.org/) and the corresponding sequences of orthologous rice genes (http://blast.ncbi.nlm.nih.gov/) were aligned (http://www.ncbi.nlm.nih.gov/spidey) and primers were designed (software: primer3; http://frodo.wi.mit.edu/primer3/; Rozen and Skaletsky 2000).

On the basis of newly developed gene-based markers, the *rym11* target region could be related to the barley genome zipper (Mayer et al. 2011) representing a synteny-informed virtual gene-order map of barley genes detected by survey sequencing of sorted barley chromosomes. For further marker saturation at the *Rym11* locus, Roche/454-survey sequences of barley chromosome 4H (Supplemental data 1) underlying the barley chromosome 4H genome zipper were utilized for primer design using the software batchprimer3 (http://probes.pw.usda.gov/cgi-bin/batchprimer3/batchprimer3.cgi). Polymorphisms in amplicon sequences were exploited for marker development as outlined above.

As an additional resource, a preliminary and unpublished whole-genome-shotgun (WGS) sequence assembly of barley cultivar Morex (http://webblast.ipk-gatersleben.de/barley; assembly1_morex_rbca) was employed for selecting larger genomic segments for individual Roche/454 reads of the chromosome 4H survey sequences (Mayer et al. 2011) (Supplemental data 1).

PCR for all newly developed markers was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Darmstadt, Germany). PCR for CAPS and Indel-markers (Protocol A; cf. Supplemental data 1) was set up in a 15-µl reaction including 50 ng genomic DNA, 0.6 U Taq DNA polymerase, 1× PCR reaction buffer containing 1.5 mM $MgCl_2$ (Qiagen, Hilden, Germany), 0.2 mM dNTPs (Fermentas, St. Leon-Rot, Germany), 0.2 µM of each primer (Eurofins MWG Operon, Ebersberg, Germany). PCR amplification conditions were: 5 min at 94 °C, followed by 50 cycles of 30 s at 94 °C; 1 min annealing (annealing temperature was reduced by 0.5 °C/cycle from 62 to 56 °C), 1 min at 72 °C, respectively. PCR finished by 10 min at 72 °C (Silvar et al. 2012).

PCR for pyrosequencing markers (Protocol B; Supplemental data 1) was performed in a 30-µl reaction including 50 ng genomic DNA, 0.6 U AmpliTaq Gold DNA Polymerase, 1× buffer (Applied Biosystems, Darmstadt, Germany), 2.0 mM $MgCl_2$, 0.125 mM dNTPs (Fermentas, St. Leon-Rot, Germany), 0.02 µM of M13-tailed F-primer and 0.2 µM each of R-primer and biotin-labeled M13 universal primer (Eurofins MWG Operon, Ebersberg, Germany). PCR amplification conditions were: 15 min at 95 °C, followed by 62 cycles of 30 s at 94 °C; 30 s annealing (annealing temperature was reduced by 0.5 °C/cycle from 62 to 56 °C), 30 s at 72 °C, respectively. PCR finished by 10 min at 72 °C (Silvar et al. 2011).

Linkage analysis

Linkage analysis was performed by setting the number of recombinant gametes in relation to the number of gametes

Table 1 Polymorphism tests of the markers employed for marker saturation on the Naturel x W757-112/612 mapping population

Marker name	Marker type	Polymorphism (yes/no)	Endonuclease for CAPS assay; pyrosequencing primer	References
Bmac0030	SSR	No		1, 2
Bmac0181	SSR	Yes		1, 2, 3
Bmac0186	SSR	Yes		1, 2, 3
Bmac0298	SSR	Yes		1, 2, 3
Bmac0299	SSR	No		1, 2
Bmac0577	SSR	No		1, 2
Bmag0353	SSR	Yes		1, 2, 3
Bmag0375	SSR	No		1, 2, 3
Bmag0490	SSR	Yes		1, 2, 3
EBmac0540	SSR	No		1, 2
EBmac0669	SSR	No		1, 2
EBmac0701	SSR	Yes		1, 2, 3
EBmag0781	SSR	Yes		1, 2, 3
GBM1020	SSR	Yes		2, 3
GBMS049	SSR	Yes		2
GBMS081	SSR	Yes		2
GBS0001	SNP	No		4
GBS0023	SNP	No		4
GBS0026	SNP	No		8
GBS0081	SNP	Yes	*Bsr*G1	5
GBS0177	SNP	No		4
GBS0288	SNP	No		4
GBS0434	SNP	No		4
GBS0448	SNP	No		4
GBS0461	SNP	No		4
GBS0506	SNP	Yes	*Bsr*G1	4
GBS0547	SNP	No		4
GBS0589	SNP	No		4
GBS0692	SNP	Yes	InDel	4
GBS0751	SNP	No		4
GBS0887	SNP	Yes	*Eco*R1	4
HVM13	SSR	Yes		2, 3
HVM77	SSR	Yes		2, 3
POPA12_2977-1925	SNP	Yes	TGGCATTACCCTTATTT	6
POPA12_3127-273	SNP	No		6
POPA12_3699-1543	SNP	No		6
POPA12_3716-910	SNP	No		6
POPA12_4276-1082	SNP	No		6
POPA12_7942-948	SNP	No		6
POPA12_9208-919	SNP	No		6
k00391	SNP	No		7
k00813	SNP	No		7
k00824	SNP	No		7
k01212	SNP	Yes	*Psy*I	7
k01233	SNP	No		7
k01469	SNP	Yes	InDel	7
k02125	SNP	No		7
k02164	SNP	No		7
k02539	SNP	No		7

Table 1 continued

Marker name	Marker type	Polymorphism (yes/no)	Endonuclease for CAPS assay; pyrosequencing primer	References
k02715	SNP	No		7
k02829	SNP	No		7
k03071	SNP	No		7
k03193	SNP	Yes	(not mapped)	7
k03198	SNP	Yes	(not mapped)	7
k03209	SNP	No		7
k03423	SNP	No		7
k03979	SNP	No		7
k04127	SNP	No		7
k04165	SNP	No		7
k04266	SNP	No		7
k04427	SNP	No		7
k04725	SNP	Yes	InDel	7
k04748	SNP	No		7
k04779	SNP	No		7
k06631	SNP	No		7
k06701	SNP	No		7
k06721	SNP	No		7
k06766	SNP	No		7
k07237	SNP	No		7
k07728	SNP	No		7
k07964	SNP	No		7
k08238	SNP	No		7
k08908	SNP	No		7
k09244	SNP	No		7
k09860	SNP	No		7
k09907	SNP	No		7
k09982	SNP	No		7
k10036	SNP	No		7
k10188	SNP	No		7
k10515	SNP	Yes	(not mapped)	7
k10659	SNP	Yes	*Bfa*I	7
k10901	SNP	Yes	*Dra*I	7

Markers originating from: Ramsay et al. (2000) (1), Varshney et al. (2007) (2), Hearnden et al. (2007) (3), Stein et al. (2007) (4), Kota et al. (2008) (5), Close et al. (2009) (6), Sato et al. (2009) (7), Perovic et al. (2007) (8)

analyzed (Pellio et al. 2005). To correct for those plants carrying *rym4* and those dying during the cultivation, a 'Corrected genetic resolution' for the remaining RILs was applied by dividing the % recombination identified for the F_2-generation by the number of those remaining RILs.

Testing the diagnostic value of identified markers

Closely linked markers were tested for their diagnostic value on a panel of 93 susceptible and resistant cultivars and on accessions carrying different *rym*-genes (Supplemental data 2).

Results

Construction of the high-resolution mapping population and marker saturation

Development of the high-resolution mapping population involved three subsequent steps. Initially, 1,270 F_2-plants providing a genetic resolution of 0.0394 % recombination were screened for the recombination between the flanking markers HVM03 and HVM68 and a genetic distance of 8.94 % recombination was determined. Due to the loss of plants (i.e., homozygous *rym4* or non-survival of recombinant plants) the corrected genetic resolution provided by

83 remaining RILs equalled 0.1077 % recombination (Fig. 1a).

In the aim of marker saturation at the *rym11* target region, 82 markers (Table 1) were selected from seven published genetic maps of barley (Ramsay et al. 2000; Hearnden et al. 2007; Varshney et al. 2007; Kota et al. 2008; Stein et al. 2007; Close et al. 2009; Sato et al. 2009). Twenty-five markers were polymorphic of which, 4 markers (EBmac0701, EBmag0781, GBS0081, GBS0692) mapped outside the target interval and two marker polymorphisms (k03193 and k03198) could not be reproduced in the population. The polymorphic marker k10515 has not been mapped since it co-segregated with marker k04725 in the map of the original publication and marker k01212 was later identified and only mapped at higher resolution (Fig. 1b). So, 17 markers were mapped to the target region (Table 1; Fig. 1a). Sequences of all newly mapped markers were compared to the rice genome. This revealed for the *rym11* region, a highly preserved macro-collinearity to rice chromosome 3 between genes Os03g0223400 and Os03g0327800 (Fig. 1a). On the basis of this information, an additional barley EST unigene marker (U35_15555; Table 2) with sequence similarity to Os03g0273800 was developed and mapped confirming the observed macro-collinear context between barley and rice at the *Rym11* locus. Overall, at this stage, 18 new markers were mapped reducing the *rym11* target interval to 1.72 % recombination (Fig. 1a).

In a second step, the (corrected) genetic resolution of the mapping population could be increased to 0.0481 % recombination by screening additional 1,105 F_2-plants. Based on the cumulative 2,375 F_2-plants, a genetic distance of 10.11 % recombination was determined between markers HVM03 and HVM068; the newly established markers GBS0887, GBS0506 and k10659 could be resolved at higher genetic resolution flanking the gene *rym11* (Fig. 1b). Based on this result, further marker saturation was restricted to the region flanked by markers GBS0506 and GBS0887 (Fig. 1b). Markers from published maps and barley EST unigenes putatively orthologous to genes of the collinear region in rice (Sato et al. 2009; Table 1; Close et al. 2009; Table 2) were tested. Unigene U32_3699A (similar to Os03g0291500) was mapped proximal to *rym11* whereas k01212 (similar to Os03g0284100) mapped distal to *rym11* delimiting the flanking interval to 0.336 % recombination (Fig. 1b).

By screening additional 2,727 F_2-plants in a third step, the mapping population was further increased to altogether 5,102 F_2-plants and a genetic distance of 10.72 % recombination was determined between markers HVM03 and HVM068. Fifty-seven RILs were selected between flanking markers GBS0506 and GBS0887. Altogether, 100 RILs were detected for this respective marker interval providing a final (corrected) genetic resolution of 0.0187 % recombination. Phenotypic analysis of resistance to BaMMV both under greenhouse and field conditions showing the same results revealed the expected 1:1 segregation ratio among these RILs (52r : 48 s; $\text{Chi}^2_{(1r:1s)} = 0.163$) and allowed for precise allocation of the resistance gene *rym11* to the final map (Fig. 2).

At this stage, further marker saturation was performed on the basis of information provided by the virtual gene

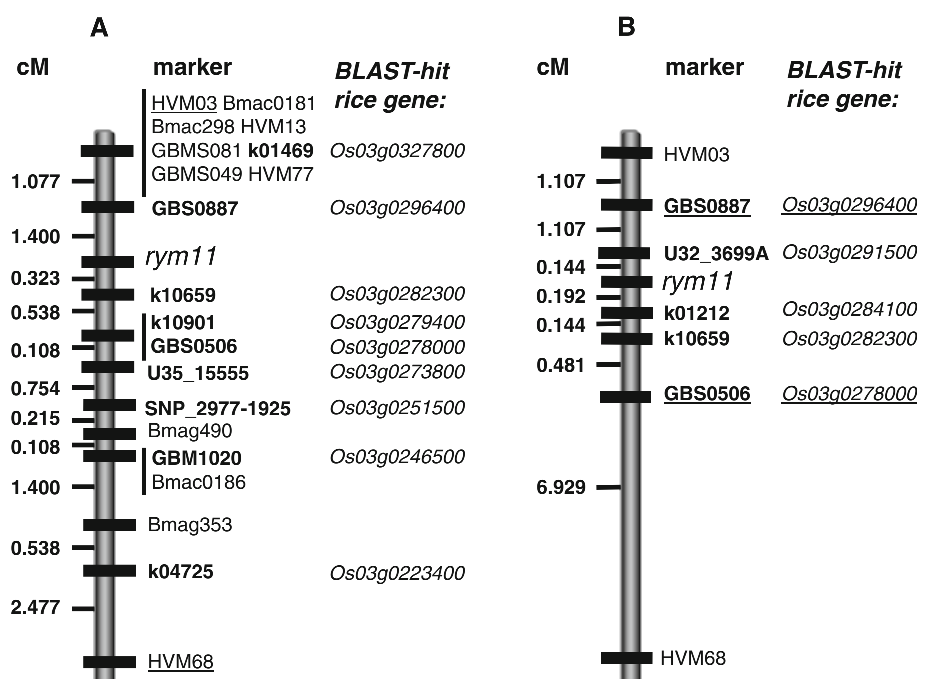

Fig. 1 High-resolution mapping of the gene *rym11*. **a** In phase I, genetic mapping of *rym11* was based on the analysis of 83 RILs originating from 1,270 F_2-plants. **b** In subsequent steps, the genetic resolution was further increased by extending the population to 210 RILs derived from 2,375 F_2-plants. Gene-based markers are in **bold** and their similarity to rice genes is depicted; *underlined markers and rice genes* indicate the genetic interval analysed and the syntenic region of interest. The similarity of k10659 to Os03g0282300 was identified via *Triticum aestivum* clone AK336063.1

Table 2 Barley unigenes used for marker development and their homology to rice genes

Barley unigene	Chromosome	Best rice hit	*e* value
U35_13941	4H	Os03g0255200	0
U35_15555	4H	Os03g0273800	0
U35_18478	4H	Os03g0292100	0
U32_7347	4H	Os03g0286900	4,00E-45
U32_2274	4H	Os03g0283100	0
U32_3699	4H	Os03g0291500	4,00E-146
U32_2386	4H	Os03g0294200, Os05g0164100	0
U32_7942	4H	Os03g0294900, Os07g0692900, Os11g0106400	0
U32_1169	4H	Os03g0285700	0
U32_3917	4H	Os03g0285800	0
U32_7539	4H	Os03g0288300	0
U35_6623*	4H	Os03g0280400	1,00E-77
U35_13812*	4H	Os03g0177500, Os03g0177400, Os03g0178000, Os03g0177900	0
U35_5418*	4H	Os03g0288400	2,00E-128
U35_47644*	4H	Os03g0278000	0
U35_5294*	4H	Os03g0271600	0
U35_158*	4H	Os03g0276500, Os01g0840100, Os11g0703900, Os05g0460000	0

Unigenes with an asterisk were selected from the Genome zipper (Mayer et al. 2011)

order map of chromosome 4H (genome zipper; Mayer et al. 2011). Markers k01212 (similar to Os03g0284100; Bradi1g65910.1; Sb01g038820.1) and U32_3699A (similar to Bradi1g65540.1; Sb01g038460.1) delimited in the chromosome 4H genome zipper a 1.38-cM interval comprising 32 barley genes and the orthologs from rice, *Brachypodium* and/or sorghum as well as five additional barley EST-based unigenes without any identified orthologs from the above-mentioned model grass species (Supplemental data 1). Out of this information, 70 sequence-tagged site (STS) markers were amplified (data not shown). Mapping of marker sel.1167 (similar to Os03g0285100; Bradi1g65850.1) shortened further the target interval (Fig. 2, Supplemental data 1). Marker 4HL_1159A mapped outside the initial target interval and marker U35_158_255-414 was co-segregating with GBS0506 (Fig. 2). For marker U35_158, no orthologous gene was indicated in the 4H genome zipper, however, individual sequence similarity analysis identified similarity to rice gene Os03g0276500 (Table 2). All predicted open reading frames between rice and *Brachypodium* genes Os03g0288800-Os03g0287100, and Bradi1g65650.1-Bradi1g65767.1, respectively (Supplemental data 1), were compared to a draft assembly of whole genome shotgun sequence of barley cultivar 'Morex' (http://webblast.ipk-gatersleben.de/barley; assembly1_morex_rbca) and eight contig sequences could be identified which were employed for additional marker development. Three polymorphic markers out of 24 amplified STS could be generated with C_1030750_B mapping proximal, C_1012894_B mapping distal to *rym11*, and C_205243_B

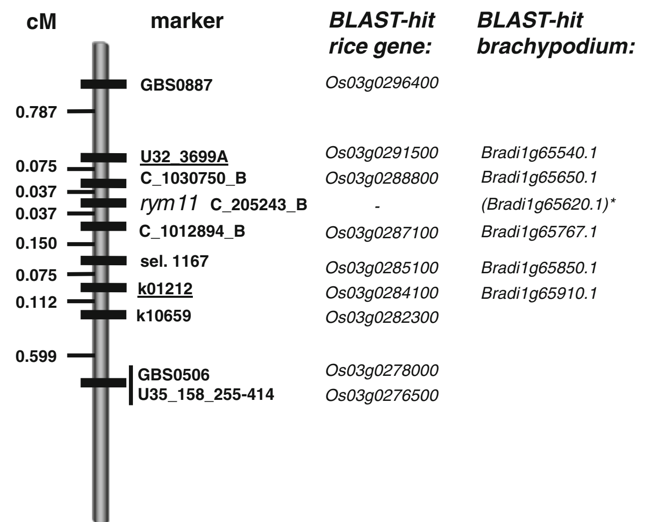

Fig. 2 High-resolution mapping of the gene *rym11*. In the final-phase genetic mapping of *rym11* was based on the analysis of RILs recombinant between GBS0887 and GBS0506. Genetic mapping of *rym11* on a set of 100 RILs providing all informative crossover events in the mapping population of 5,102 F_2-plants. Gene-based markers are in *bold* and their similarity to rice genes is depicted; underlined are markers used for confining the target interval of *rym11* in the genome zipper by Mayer et al. (2011)

co-segregating with *rym11* (Fig. 2). Mapping of C_205243_B was based on a direct size polymorphism due to a deletion in a non-coding region in close proximity to the barley unigene U35_13812 (http://www.harvest-web.org/hweb/bin/viewsetno.wc?assembly=35&setno=13812). This gene, according to the genome zipper, is orthologous

to Bradi1g65720.1 (Supplemental data 1) which is most likely coding for an elongation factor 1-alpha activity (cf. http://www.ebi.ac.uk/interpro/, http://pfam.sanger.ac.uk/) and was, therefore, considered as a putative candidate gene. However, sequencing of this gene revealed no polymorphism in the coding region of the gene between parental lines. In addition, a test of the gene expression showed that the elongation factor 1-alpha is expressed in both parents and that there are no significant differences in the expression level (Ping Yang, pers. comm.). Nevertheless, this marker provides a starting point for positional cloning of the gene *rym11*.

Improved tool for marker-assisted selection (MAS) of rym11-based resistance

The exploitation of genetic resources and the efficient breeding for virus resistance requires access to closely linked or diagnostic markers. Therefore, it was analysed whether any of the newly developed markers could serve this purpose. The newly developed closely flanking markers C_1030750_B, C_1012894_B as well as the co-segregating marker C_205 243_B were tested for their diagnostic value for *rym11*-based resistance in a panel of 93 recent cultivars and accessions (Supplemental data 2). Among these, only accession Russia 57 carries resistance conferred by the *Rym11* locus (Götz and Friedt 1993; Ordon et al. 1993; Bauer et al. 1997). The SNP-marker C_1012894_B revealed the same SNP as Naturel (T) on 56 barley cultivars and accessions, and on 33, the same SNP as on W757-112/612 (C) which is the donor of *rym11*. Therefore, this marker is of limited value for marker-assisted selection for *rym11*. The SNP-marker C_1030750_B is better suited as it revealed the SNP of the susceptible parent Naturel (G) in 86 accessions and only in 5 genotypes the SNP of W757-112/612 (A) indicative for *rym11*, i.e., Morex, Uschi, Bulgarien347, BCC No. 2 and No. 4. While nothing is known about the resistance genes present in BCC No. 2 and No. 4, Bulgarian 347 carries *rym9*, and Uschi and Morex are susceptible. For C_205243_B, altogether, 69 accessions and cultivars revealed the fragment of Naturel (614 bp), 21 produced a fragment of about 650 bp (including Russia 57) and 2 genotypes (susceptible cultivar Uschi, and *rym8*-donor 10,247) showed the same fragment as W757-112/612 (513 bp), leading to the conclusion that none of the markers is of absolute diagnostic value for *rym11*. However, MAS C_205243_B performed superior compared to previously available markers.

Discussion

In this paper, we describe high-resolution mapping of the recessive bymovirus resistance gene *rym11* taking advantage of recently developed genomics resources of barley. Compared with anonymous marker technologies like AFLPs, the exploitation of dense transcript-derived marker maps (e.g., Stein et al. 2007; Sato et al. 2009; Close et al. 2009) in combination with newly acquired sequencing information (Mayer et al. 2011) led to a more straightforward and faster delimitation of the target region and the rapid development of a marker co-segregating with the resistance gene.

After the first linkage map around *rym11* had been constructed (Bauer et al. 1997), initial fine mapping of the resistance gene using RAPD, AFLP and SSR-markers was performed by Nissan-Azzouz et al. (2005) on 352 DH-lines resulting in the identification of two co-segregating AFLP and several closely linked SSR markers. These studies included markers HVM03 and HVM68 providing the basis for the construction of a high-resolution mapping population in this study. Whereas the mapping of HVM03 at 2.36 % recombination proximal to *rym11* is in good accordance with the genetic distance of HVM03 presented by Nissan-Azzouz et al. (2005), the genetic distance of HVM68 to *rym11* (7.75 % recombination) estimated in our study is almost two times larger than that in the DH mapping population of Nissan-Azzouz et al. (2005). Such variation in recombination rates between crosses is well known (Stein and Graner 2004; Langridge 2005). Even in crosses with a comparable genetic background, the recombination rate within a defined interval could vary tremendously such as shown for the calculated distances (0 vs. 13.8 cM) between the markers HVM03 and HVM68 in the map of Ramsay et al. (2000) and Hearnden et al. (2007), respectively. However, Perovic et al. (2009), in their study, came to the conclusion that such differences are not significant. Since stress factors like heat could enhance the somatic recombination rate (Lebel et al. 1993; Pecinka et al. 2009), the cultivation of a mapping population may also have an influence on the genetic map distance, bearing in mind that the temperature in a greenhouse could rise enormously during summer. In addition, a variation in recombination rates between DH and RIL mapping populations derived from the same cross was observed (He et al. 2001).

The screening of parental lines with SSR-markers from the already-published genetic maps of barley (Ramsay et al. 2000; Hearnden et al. 2007; Varshney et al. 2007) identified a fairly high degree of polymorphic markers (12 polymorphic vs. 6 monomorphic) compared to the polymorphism rate described by Nissan-Azzouz et al. (2005). One reason for this is maybe the fact that the DH lines analyzed by Nissan-Azzouz et al. (2005) derived from a cross of susceptible and resistant DH-lines derived from the same F_1, while in our case, one of these lines was crossed to an adapted European barley cultivar. While mapping of

the polymorphic SSRs was in good accordance with the map of Hearnden et al. (2007), different map positions in relation to the maps of Ramsay et al. (2000) and Varshney et al. (2007) were detected. This may be attributed to the clustering of SSRs around the centromere in the individual map of Ramsay et al. (2000), whereas the merging of individual maps into one consensus map is generally problematic (Wu et al. 2008).

The level of polymorphism identified for the already-known gene-based markers (Stein et al. 2007; Close et al. 2009; Sato et al. 2009) as well as those developed in this study is rather low compared with the polymorphism rate of the tested SSR-markers and is in contrast to the results of Stein et al. (2007) who had identified SNP-markers being more polymorphic than the SSRs. Furthermore, the frequency of <1 SNP per 1,000 bp detected for the parental lines is rather low in comparison to the SNP frequency of 1 SNP per 130 bp detected by Kota et al. (2008) or 1 SNP per 240 bp assessed by Duran et al. (2009). In addition, Silvar et al. (2012) detected a higher degree of polymorphic SNP-markers (64 polymorphic vs. 96 monomorphic) derived from Close et al. (2009) in their studies. Besides the different relatedness between the parental lines, this is maybe due to the fact that Silvar et al. (2012) tested SNPs assigned to the whole chromosome 7H for polymorphism while in the present study, only SNPs located in a short centromeric region were of interest. In centromeric regions, loci on average are less polymorphic than loci located on the rest of the chromosome (Dvorák et al. 1998) as rarely recombining haplotype blocks extend across the genetic centromeres (Comadran et al. 2010).

In the synteny-based marker saturation approach, the perfectly conserved inverted collinearity between barley chromosome 4H and rice chromosome 3 (Stein et al. 2007; Thiel et al. 2009; Shahinnia et al. 2009; Mayer et al. 2011) was successfully exploited and, furthermore, verified by mapping of k01212 (similar to Os03g0284100) proximal to k10659 (similar to Os03g0282300). This could be expected but is, however, in contrast to the map of Sato et al. (2009) which was suggesting a disturbed collinearity.

The rice genes depicted in the map-interval of Close et al. (2009) displayed also a perfect collinearity in this region as the orthologs in the much denser genome model of Mayer et al. (2011) for which the map of Close et al. (2009) provided the genetic framework. The deviating assignment of one barley 454-read and one unigene revealed by the mapping of 4HL_1159A and U35_158_255-414 outside the interval in our study does not afflict the damage to the overall value of the genome zipper for synteny-based marker saturation and is much lower than the frequency detected by Shahinnia et al. (2012). The accordance between the genome zipper gene order and the map of *rym11* elucidates that the genome zipper is a very efficient tool for marker saturation also at high resolution, as has been already proven at lower resolution (Silvar et al. 2012). The usefulness of the genome zipper for map-based cloning will even increase in the future by integrating WGS sequencing data as shown by the contig sequence C_205243 from the Morex-assembly of the IPK Gatersleben (http://webblast.ipk-gatersleben.de/barley; assembly1_morex_rbca) which finally led to the development of marker C_205243_B which is co-segregating with *rym11* for 5,102 analysed F_2-plants.

Despite C_205243_B not being a functional marker (Andersen and Lübberstedt 2003), it was shown that the application of next-generation sequencing data can meet the expectations concerning a straightforward delimitation of the gene containing interval and the identification of a marker co-segregating with *rym11*. Like in previous studies (Perovic et al. 2004; Vu et al. 2010), the existence of extensive collinearity between barley and rice or *Brachypodium* was the basis for this. In addition, it was important that *rym11* is not localized in a region of suppressed recombination (Petes 2001), like it holds true for the *Rrs2* scald resistance gene of barley, for which even a mapping population of 9,179 F_2-plants was not sufficient to resolve a co-segregating area of suppressed recombination (Hanemann et al. 2009). In this respect, it has to be mentioned that according to the genetic maps (Bauer et al. 1997; Nissan-Azzouz et al. 2005) and the updated map of Künzel et al. (2000) (http://pgrc.ipk-gatersleben.de/kuenzel/image6h.gif) *rym11* is located in a region of moderately suppressed recombination rates of 5.9–8.5 Mb/cM. Nevertheless, marker saturation using the strategy described above could be successfully conducted. During this approach, the barley ortholog of Bradi1g65720.1 was identified. This elongation factor 1-alpha was regarded as the most promising candidate gene for *rym11*, as the already-isolated resistance gene *rym4/rym5* has also a translation-initiation factor activity. But the monomorphic outcome of the sequencing of the exons together with same level of gene expression identified for the parents in an expression study (Ping Yang, pers. comm.) has shown that the resistance encoded by *rym11* is not due to a loss-of-function of this gene.

Anyway, the identification of two flanking co-dominant markers comprising an interval of 0.074 % recombination, in addition to the co-segregation of C_205243_B, are providing an excellent starting point for a rapid identification of further candidate genes. Furthermore, these markers provide useful tools in marker-based breeding strategies for virus resistance (cf. Palloix and Ordon 2011), due to their close linkage to *rym11*. However, they are of limited diagnostic value (Supplemental data 2). Therefore, screening of parental lines for polymorphisms is needed for each combination.

The newly acquired genomic information of barley (Mayer et al. 2011) used in the present study was of great value for marker saturation. The presence of two different resistance genes (*rym11* and *rym4*) harbored by the two parents was entailed with the peril of false-negative outcomes regarding the virus-testing for a *rym11*-conferred resistance. But by an additional selection step, individuals with a *rym4*-conferred resistance were easily separated from the mapping population. Such an approach was beforehand successfully done in the study of Werner et al. (2003) and despite this additional selection step, the high-resolution mapping of *rym11* was much faster and more efficient than the one for the *rym4/rym5* locus (Pellio et al. 2005). Therefore, it may be expected that, based on this resource and the further growing sequence information becoming available for barley, many major genes and QTL for traits of major agronomic importance will be isolated, helping to move breeding and trait analysis in barley to an unprecedented level of precision and productivity (Bevan and Waugh 2007). With regard to *rym11*, a BAC contig harboring this gene has been identified meanwhile and gene isolation and functional analyses is in progress.

Acknowledgments The authors thank Karin Ernst and Dörte Grau (JKI) for excellent technical assistance and Susanne König (IPK) for sequencing service. Furthermore, we thank the Federal Ministry of Education and Research (BMBF) for funding this project (FKZ 0314000D) within the BARLEX project.

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