Towards Positional Isolation of Three Quantitative Trait Loci Conferring Resistance to Powdery Mildew in Two Spanish Barley Landraces

Cristina Silvar1,2, Dragan Perovic2, Thomas Nussbaumer3, Manuel Spannagl3, Björn Usadel4, Ana Casas5, Ernesto Igartua5, Frank Ordon2

1 Department of Ecology, Plant and Animal Biology, University of Coruña, A Coruña, Spain, 2 Institute for Resistance Research and Stress Tolerance, Julius Kühn-Institute, Quedlinburg, Germany, 3 MIPS/IBS, Helmholtz Zentrum München, Neuherberg, Germany, 4 Institute for Biology I, RWTH Aachen University, Aachen, Germany, 5 Department of Genetics and Plant Production, Aula Dei Experimental Station (Consejo Superior de Investigaciones Científicas), Zaragoza, Spain

Abstract

Three quantitative trait loci (QTL) conferring broad spectrum resistance to powdery mildew, caused by the fungus *Blumeria graminis f. sp. hordei*, were previously identified on chromosomes 7HS, 7HL and 6HL in the Spanish barley landrace-derived lines SBCC097 and SBCC145. In the present work, a genome-wide putative linear gene index of barley (Genome Zipper) and the first draft of the physical, genetic and functional sequence of the barley genome were used to go one step further in the shortening and explicit demarcation on the barley genome of these regions conferring resistance to powdery mildew as well as in the identification of candidate genes. First, a comparative analysis of the target regions to the barley Genome Zippers of chromosomes 7H and 6H allowed the development of 25 new gene-based molecular markers, which slightly better delimit the QTL intervals. These new markers provided the framework for anchoring of genetic and physical maps, figuring out the outline of the barley genome at the target regions in SBCC097 and SBCC145. The outermost flanking markers of QTLs on 7HS, 7HL and 6HL defined a physical area of 4 Mb, 3.7 Mb and 3.2 Mb, respectively. In total, 21, 10 and 16 genes on 7HS, 7HL and 6HL, respectively, could be interpreted as potential candidates to explain the resistance to powdery mildew, as they encode proteins of related functions with respect to the known pathogen defense-related processes. The majority of these were annotated as belonging to the NBS-LRR class or protein kinase family.

Introduction

Barley (*Hordeum vulgare* L.) was domesticated in the Fertile Crescent about 10,000 years ago [1,2] and nowadays it ranks as the fourth cereal in worldwide production after wheat, rice and maize [3]. As for other crops, domestication and modern plant breeding have endangered the maintenance of the genetic diversity of barley [4,5]. Although, overall, barley genetic diversity has not decreased [6] local dominance of cultivars poses a serious threat to sustainable production, especially considering the risks associated to the appearance of new strains of pathogens that may be virulent on all cultivars grown in a region [7,8]. The limited genetic variation regarding disease tolerance is a great concern as it may result in widespread crop-yield and quality losses if new virulent pathogen populations appear [9]. Incorporation of new genes or alleles that confer pathogen resistance is needed to alleviate this genetic vulnerability. Wild relatives and landraces probably represent the most valuable reservoirs of unexploited variability within the primary gene pool of barley. For this reason, they have had, and still have, enormous relevance in breeding for disease resistance [10–13].

The Spanish Barley Core Collection (SBCC) [14] is constituted by a representative sample of the landraces cultivated in Spain before the advent of modern breeding. It consists of inbred lines derived from native landraces with an important history of adaptation and selection under Mediterranean conditions [15,16]. In order to evaluate its potential to contribute new genetic diversity to enhance the disease resistance of barley, the SBCC was screened with several fungal and viral pathogens. High levels of broad resistance to the fungus *Blumeria graminis f. sp. hordei*, responsible for powdery mildew, were detected in some lines tested with a large variety of isolates [17,18]. The resistances from the two most interesting lines were investigated further in mapping populations resulting in the identification of different sets of quantitative trait loci (QTL). Two QTL were identified on the chromosome 7H in the Spanish barley landrace-derived line SBCC097 [19]. In a recent work, the chromosomal intervals containing these resistances have been subjected to marker saturation following a comparative genomic approach based on the synteny of barley with the reference genomes of rice, sorghum and Brachypodium [20]. In a second line, i.e. SBCC145, a major QTL with a large effect was located to the long arm of...
chromosome 6H accounting for ca. 60% of the phenotypic variance [21]. The position and magnitude of effects of these QTLs, an exhaustive analysis based on a set of B. graminis pathotypes with broad spectra of virulences [18], and the characteristics of the defense reaction at the cellular level [22] suggested that they are newly identified loci or alleles for non-race specific resistance against powdery mildew in cultivated barley. The effective use of these resistance genes in barley breeding, avoiding linkage drag, requires a precise localization or, even better, the identification of candidate genes. Such identification of candidates is essential to ascertain the biochemical and physiological mechanisms underlying the resistances.

The progress towards map-based cloning of these QTLs and their exploitation in barley breeding programs follows a series of steps: first, positioning these resistance loci on the barley genome through the development of closely linked markers, then identifying putative candidate genes together with aid of high resolution mapping populations. In this regard, recent advances in barley genomics enable researchers to go one step further in the shortening and explicit demarcation on the barley genome of the regions conferring resistance to powdery mildew as well as in the identification of candidate genes. Mayer et al. [23,24] developed the first Genome Zipper of a Triticaceae genome, comprising a putative linear gene index of each chromosome in barley, embedded in a comparative grass genome organization model. The barley Genome Zipper led to the assignment of 86% of the estimated barley genes to individual chromosome arms and their organization in a virtual gene map. More recently, the International Barley Genome Sequencing Consortium (IBSC) published a cumulative physical map of 4.98 Gb and a draft of the barley genomic sequence holding 26,159 “high-confidence” genes [25].

In the present work, we took advantage of all these genomic resources to go ahead on our attempt to genetically dissect and physically circumscribe the barley regions specifically conferring resistance to powdery mildew in the Spanish lines SBCC097 and SBCC145.

Materials and Methods

Plant and Pathogen Materials

The SBCC097 × Plaisant F5 and F6 RIL population (262 lines) was used to select 13 lines as the most informative ones for their clear-cut phenotypic responses and the unequivocal presence of just one of the two QTL on 7H, based on marker information obtained previously [20]. The doubled haploid (DH) population SBCC145 × Beatrix, originally with over 400 lines, was employed for the selection of 13 DH lines showing recombination between flanking markers 11_1351 and 11_0509 on the chromosome 6HL [21].

Phenotypic disease scores of these lines against four B. graminis isolates (R79, R180, R126 and R178) were used to screen the SBCC097 × Plaisant RIL population and two isolates (R211 and R224) employed to inoculate the SBCC145 × Beatrix DH population were available from earlier works [17,18].

Comparative Analysis to the barley Genome Zipper

The closest markers flanking the QTLs identified on chromosomes 6H and 7H in previous works, were employed to select the target region for comparison to the barley Genome Zipper developed by Mayer et al. [24]. Data available at the MIPS/IBIS (http://mips.helmholz-muenchen.de/plant/barley/gz/index.jsp) were used. The sequences of rice, sorghum and Brachypodium genes located at the promising regions on the barley Genome Zipper were downloaded from the Oryza sativa ssp. japonica IRGSP Build5 (http://www.rapdb.dna.affrc.go.jp), Sorghum bicolor release v1.0 (http://www.phytozome.net/sorghum.php) and Brachypodium distachyon ]GI| 8× (http://www.brachypodium.org). The sequences of those genes were used as queries for BlastN search at the ViroBlast tool implemented for barley at the Leibniz-Institute of Plant Genetics and Crop Plant Research (http://webblast.ipk-gatersleben.de/barley/viroblast.php). ViroBlast was performed with cut-off parameters of E-value $< e^{-10}$, identity, ≥80% and a minimum of 100 bp match length against the database “assembly_WGSMorex”, which holds contigs information on sequences from the cv. Morex at coverage of ~50×. The contigs with the best hit were employed in a second step of ViroBlast against the “sorted Chromosomes” database, harbouring 454 reads of flow sorted chromosomes of cv. Betzes. In those cases in which no reference gene (rice, sorghum or Brachypodium) was available, the “reads matching marker stringent” obtained from Mayer et al. [24] were directly employed for marker development.

Marker Development and Genotyping

With the aim of developing new molecular markers derived from Morex contigs at the target chromosomal regions, the 454 reads of Betzes, identified in the second step of BlastN, were aligned with their respective Morex contigs using the software Sequencher™ version 4.5 (Genes Codes Corporation, Ann Arbor, MI, USA). In silico SNPs were identified between cvs. Morex and Betzes and primers flanking those SNPs were designed using the software BatchPrimer3 v1.0 [26].

Routine PCR was done in 20 μl reaction volume including 25–50 ng genomic DNA, 0.5 U of Taq DNA Polymerase (Solis Biodyne, Tartu, Estonia) 1× PCR reaction buffer, 1.5 mM MgCl2, 0.2 mM dNTPs and 0.2 μM of each primer. All fragments were amplified using a previously published touchdown PCR profile [20]. Purified amplicons were subjected to cycle-sequencing from both ends on the ABI377XL sequencer using BigDye v3.1 terminator sequencing chemistry (ABI Perkin Elmer, Weiterstadt, Germany). Sequence analysis and identification of polymorphisms were conducted using the Sequencher™ software. The SNPs between the parental lines were transformed to CAPS (Cleaveed Amplified Polymorphism) markers. Restriction digestion was performed as described earlier [20]. Markers in which polymorphism was detected between the parental lines, either length or presence/absence, were genotyped directly. The markers developed were named after the corresponding Morex contig name (assembly_WGSMorex, ~55×, http://webblast.ipk-gatersleben.de/barley/docs/blast_databases.html) preceded by the prefix QB (Quedlinburg Barley).

Linkage and QTL Analysis

All informative lines for the two populations, i.e. lines that showed recombination across the target intervals, were used to map the new markers. The previously mapped BOPA or microsatellite markers on 6H and 7H [19,21] were employed as a framework to place the new markers. Genetic distances were calculated by minimizing the number of recombinants within the progeny. Linkage analyses were performed with JoinMap 4.0 [27], using Kosambi’s map function and a minimum logarithm of the odds ratio (LOD score) of 3. QTL analysis was performed using the Multiple QTL Model (MQM) [28] implemented in MapQTL 5.0 [29]. Several rounds of analysis with cofactors were conducted until a stable LOD profile was reached. The LOD threshold for QTL detection was calculated by permutation test with 1,000 iterations and a genome-wide significance level of 0.05.
Anchoring to the Barley Physical Map and Identification of Candidate Genes

The new markers derived from Morex contigs, which flanked the resistance region after the QTL analysis, were employed for anchoring of genetic and physical maps following the instructions available at the FTP download page hosted at MIPS/IBIS (ftp://ftpmpis.helmholz-muenchen.de/plants/barley/public_data/anchoring). Once the putative regions conferring resistance to powdery mildew were delimited on the barley genomic sequence, the “high-confidence” (HC) and “low-confidence” (LC) genes on those regions were extracted according to the information available at ftp://ftpmpis.gsf.de/plants/barley/public_data/ancho/genes_to_physMap_08062012.tab and the EnsemblPlants website for barley http://plantsensembl.org/Hordeum_vulgare. Annotation of HC genes was obtained from ftp://ftpmpis.gsf.de/plants/barley/public_data/genes/barley_HighConf_genes_MIPS_23Mar12_HumReadDesc.txt. The putative function of LC genes was defined using gene ontology (GO) and PFAM protein motifs computed with InterproScan (http://www.ebi.ac.uk/Tools/pfa/ipscan).

Orthologous sequences of rice, Brachypodium and sorghum corresponding to HC candidate barley genes were identified by BlastP search against the rice annotation project database (RAP-DB; http://rapdb.dna.affrc.go.jp/tools/blast), the Brachypodium distachyon project at MIPS/IBIS (http://mips.helmholz-muenchen.de/plant/brachypodium/) and the Sorghum bicolor database (http://www.phytozome.net/sorghum.php).

Results

Comparative Analysis to the Barley Genome Zipper

Markers QBS15 and GBM1060 on 7HS, markers QBS38 and QBS42 on 7HL and markers 11_1351 and 11_0509 on 6HL were designated as flanking markers for the QTL intervals according to previous works [20,21].

The data on the barley Genome Zipper held at the Barley Project hosted by the MIPS/IBIS was surveyed to find the barley unigenes, under the column “all non red, ESTs”, from which the flanking QBS markers were developed. This filtering of unigenes allowed the delimitation in the barley Genome Zipper of the regions corresponding to the two 7H intervals conferring resistance to powdery mildew in SBC0097. One region of 0.64 cM, holding 30 barley loci was delimited on the chromosome 7HS between markers QBS15 (unigene U35_32018) and GBM1060 (unigene U35_1176). The marker QBS15 only matched the Brachypodium gene Bradi1g50530.1, while GBM1060 matched genes Os06g016100, Sb10g001310 and Bradi1g50590, confirming our previous results with the comparative genomic approach [20] (Fig. 1a). Most of QBS markers at the 7HS interval showed the same counterpart in the rice, sorghum and Brachypodium genomes, as predicted previously, although substantial reshuffling was noticed. Namely, the region from QBS16 to QBS21, which corresponds to barley positions from 121 to 127, appeared proximal to marker GBM1060 in the Genome Zipper, whereas it was located distal to this marker in our previous map (Fig 1a). In preceding work, markers QBS23, QBS28 and QBS29 matched rice, sorghum and Brachypodium genes which are not represented in the Genome Zipper [20] (Fig. 1a). Surprisingly, the comparative analysis to barley predicted loci revealed the presence of two re-arrangements in rice, sorghum and Brachypodium genomes at the barley positions 99–104 (insertion) and 105–114 (inversion), which were not detected with the syntenic integration approach described earlier (Fig. 1a).

One region of ca. 4.30 cM, harboring 34 loci, was identified on the long arm of chromosome 7H, between flanking markers QBS58 (unigene U33_10765) and QBS42 (unigene U35_11617) (Fig. 1b). An unexpected good colinearity was observed between our genetic map and the linear order of barley genes predicted by the Genome Zipper, contradicting our previous results, which showed an inversion in the rice, sorghum and Brachypodium physical maps compared to the genetic region of barley flanked by QBS58 and 11_0115 [20]. Markers QBS60 and QBS61 detected rice, sorghum and Brachypodium genes which are not represented in the Genome Zipper. Once more, some re-organizations on the reference genomes, among predicted barley loci 2937–2948 were observed for the first time (Fig. 1b).

The search for homology on chromosome 6H was directly based on BOPA markers. Flanking markers 11_1351 and 11_0509 were identified as markers 1_1111 and 2_0537, respectively, on the Genome Zipper, and one region of 0.9 cM, comprising 36 barley loci was defined (Fig. 2). This interval corresponded to a syntenic region on chromosome 2 (Os02) of rice (comprising 18 genes), chromosome 4 (Sb04) of sorghum (22 genes) and chromosome 3 (Bd03) of Brachypodium (28 genes). Due to the tricky position of the QTL at 6HL, which is located at the telomeric end of the chromosome, an additional region of 11 loci, proximal to marker 11_0509, was also selected for subsequent analysis (Fig. 2).

Fifteen, nine and twelve genes putatively located within the target intervals at 7HS, 7HL and 6HL, respectively, in the barley Genome Zipper were selected for further work (Table 1). In the case of the intriguing zipper loci 2953 (7HL), at which no reference genes were available, the 454 read CUST_39488_-PI390587928_13937_7HL was directly employed for marker development. The sequences of rice, Brachypodium and sorghum showing synteny with those barley loci were employed for ViroBlast search against the “assembly_WGSMorex” database. The Morex contig with the best hit at each locus was selected for further work (Table 1). E-values for the selected contigs after ViroBlast search ranged from 0.0 to 2E-42, with a length between 1661 to 19133 bp (Table 1). All contigs, except three on 7HS (contig_43731, contig_53679, contig_2552675) were assigned to the expected barley chromosome according to the information available at the “assembly_WGSMorex” database. Those contigs without assignation were blasted against the rice, sorghum and Brachypodium genomes, showing a great homology to the chromosomes Os06, Sb10 and Bd1 (data not shown), and therefore, they were also considered for further work on marker development. The 36 Morex sequences were employed in a second step of ViroBlast against the “Sorted Chromosomes” database in order to identify their homologous Betzes 454 reads. The number of reads identified per each contig at E-values smaller than e^-30 ranged from 3 to 20, with an average of ~14 reads per contig (Table 1). The alignment of the sequences from Morex contigs and Betzes reads allowed the identification of those regions with higher number of in silico SNPs, and permitted to focus the primer design on those intervals presumably containing the highest polymorphism rates.

Primers were designed for amplification and sequencing of promising regions - in the range of 1000 bp - on the selected barley contigs (Table S1). The contigs_69947 and 39067 on 7HS, contig_168471 on 7HL and contig_160010 on 6HL did not amplify any fragment with none of two primers pairs tested at different positions on the contig (Table S1). The marker QB_1600008 on 7HL was monomorphic. The rest of markers developed from contigs resulted highly polymorphic between the parental lines, with an average number of 2 SNPs per 1 kb.
fragment (data not shown). The read CUST_39488_-_PI390587928_13937_7HL also discerned between SBCC097 and Plaisant. Thirty-one new markers (twelve at 7HS, eight at 7HL and eleven at 6HL) were genetically mapped in the lines selected from the two populations yielding thirty-one new loci. Five markers were genotyped based on the presence/absence of

Table 1. Genetic and physical distances of Brachypodium, Rice and Sorghum loci at 7HS, 7HL and 6HL.

| Loci | Marker | cM | Brachypodium | Rice | Sorghum |
|------|--------|----|--------------|------|---------|
| 2258 | _1_111 | 128.48 | Brachypodium56080.1 | Os02g0822400 | Sb04g037720.1 |
| 2259 | - - | Brachypodium56087.1 | Os02g0793500 | - | - |
| 2260 | - - | - | Os02g0828566 | Sb04g038140.1 | |
| 2261 | - - | Brachypodium60420.1 | Os02g0829100 | Sb04g038190.1 | |
| 2262 | - - | - | - | Sb04g038210.1 | |
| 2263 | - - | Brachypodium60430.1 | Os02g0829200 | Sb04g038220.1 | |
| 2264 | - - | Brachypodium60446.1 | - | - | |
| 2265 | - - | Brachypodium60452.1 | - | - | |
| 2266 | - - | - | - | Sb04g038230.1 | |
| 2267 | - - | Brachypodium60460.1 | Os02g0829400 | Sb04g038250.1 | |
| 2268 | - - | Brachypodium60470.1 | Os02g0829500 | Sb04g038260.1 | |
| 2269 | - - | Brachypodium60477.1 | - | Sb04g038270.1 | |
| 2270 | - - | Brachypodium60510.1 | Os02g0829800 | Sb04g038280.1 | |
| 2271 | - - | Brachypodium60517.1 | - | Sb04g038290.1 | |
| 2272 | - - | Brachypodium60550.1 | Os02g0830100 | Sb04g038310.1 | |
| 2273 | - - | Brachypodium60557.1 | Os02g0830200 | Sb04g038320.1 | |
| 2274 | - - | Brachypodium60570.1 | - | - | |
| 2275 | - - | Brachypodium60580.1 | - | - | |
| 2276 | - - | Brachypodium60590.2 | - | Sb04g038330.1 | |
| 2277 | - - | Brachypodium60593.1 | - | - | |
| 2278 | - - | Brachypodium60600.1 | - | Sb04g038340.1 | |
| 2279 | - - | - | - | Sb04g038350.1 | |
| 2280 | - - | Brachypodium60610.1 | Os02g0830900 | Sb04g038360.1 | |
| 2281 3_0627 | 129.38 | Brachypodium60617.1 | Os02g0831100 | Sb04g038370.1 | |
| 2282 | - - | - | - | Sb04g038410.1 | |
| 2283 | - - | Brachypodium60630.1 | - | - | |
| 2284 | - - | Brachypodium60640.1 | - | - | |
| 2285 | - - | Brachypodium60687.1 | Os02g0831500 | - | |
| 2286 | - - | - | Os02g0831600 | - | |
| 2287 | - - | Brachypodium60750.1 | Os02g0832000 | Sb04g038450.1 | |
| 2288 | - - | - | - | Sb04g038460.1 | |
| 2289 | - - | Brachypodium60756.1 | - | - | |
| 2290 | - - | Brachypodium60770.1 | Os02g0832150 | - | |
| 2291 | - - | Brachypodium60780.1 | Os02g0832200 | - | |
| 2292 | - - | Os02g0832400 | - | - | |
| 2293 2_0537 | 129.38 | Brachypodium60790.1 | - | Sb04g038510.1 | |
| 2294 | - - | - | Os02g0832500 | - | |
| 2295 | - - | Brachypodium60802.1 | Os02g0832700 | - | |
| 2296 | - - | Brachypodium60810.1 | - | - | |
| 2297 | - - | Brachypodium60820.1 | Os02g0832800 | - | |
| 2298 | - - | Brachypodium60827.2 | - | - | |
| 2299 | - - | Brachypodium60850.1 | Os02g0833400 | - | |
| 2300 | - - | Brachypodium60900.1 | - | - | |
| 2301 3_0956 | 129.38 | Brachypodium60920.1 | - | - | |
| 2302 | - - | Brachypodium60950.1 | - | - | |
| 2303 | - - | Brachypodium60980.1 | - | - | |
| 2304 | - - | Brachypodium61020.1 | - | - | |

Figure 1. Anchoring of the QTL target intervals to the Genome Zipper of chromosome 7H. Comparison of chromosomes 7HS (A) and 7HL (B) genetic maps developed earlier [20] to the 7H Genome Zipper described by Mayer et al. [24]. For the sake of clarity, marker GBM1060 is only anchored to barley loci 118.
doi:10.1371/journal.pone.0067336.g001

Figure 2. Anchoring of the QTL target intervals to the Genome Zipper of chromosome 6H. Comparison of chromosome 6HL genetic map developed earlier [20] to the 7H Genome Zipper described by Mayer et al. [24]. For the sake of clarity, only the telomeric part of chromosome 6H is represented.
doi:10.1371/journal.pone.0067336.g002
and the rest were genotyped as CAPS by using a restriction 

the PCR product, three were detected as a length polymorphism 

the 7H interval (Fig. 3b), one (QB_102319) was genetically mapped 0.4 cM distal to QBS58 and the other two (QB_153867 and QB_43456), which correspond to the predicted rearrangement at loci 2937–2948, were located out of the interval. The other markers mapped in good colinearity with their predicted positions in the Genome Zipper (Fig. 3b). All fourteen new markers positioned on chromosome 7H in the SBCC097 × Plaisant population, co-segregated with other previously developed QBS markers. The constructed linkage map of 7HS and 7HL resulted in 7 and 5 groups, respectively, of non-

Anchoring to the Barley Physical Map and Identification of Candidate Genes

The information available for those Morex contigs bearing the new flanking markers on each QTL was used for anchoring the SBCC097 × Plaisant and SBCC145 × Beatrix genetic maps to the physical map of barley. A genomic region of 4 Mb was identified for the 7HS interval between markers QB_273608 and QB_45091 (Table 2). Morex contigs underlying those markers were anchored to FP contigs (FPC) and they were positioned according to the AC1 anchoring strategy described by IBSC [25]. Two regions of 3.7 Mb and 3.2 Mb covered the QTL intervals on 7HL and 6HL, respectively (Fig. 3a, b). All eleven markers developed from contigs on 6HL chromosome mapped as expected at the region spanning the QTL on SBCC145 (Fig. 3c). These new markers permitted to narrow down the chromosomal sections containing the QTLs. An examination of the three most informative lines (DH-122, DH-268 and DH-320) suggested the presence of the QTL co-segregating with the marker derived from contig_135962 (Fig. 3c).

| Chrom. | Barley Loci | Morex Contig | E-value | Contig size (bp) | Betzes reads |
|--------|-------------|--------------|---------|-----------------|--------------|
| 7HS    | 99          | 86947        | 5.0E-67 | 5180            | 5            |
|        | 100         | 39067        | 5.0E-149| 19133           | 20           |
|        | 101         | 39067        | 1.0E-176| 19133           | 20           |
|        | 103         | 56519        | 0.0     | 3100            | 14           |
|        | 104         | 43731        | 0.0     | 6468            | 3            |
|        | 105         | 45091        | 1.0E-122| 9419            | 17           |
|        | 106         | 56996        | 0.0     | 13259           | 19           |
|        | 108         | 62161        | 1.0E-149| 5735            | 16           |
|        | 110         | 335030       | 0.0     | 8139            | 20           |
|        | 111         | 53679        | 4.0E-62 | 7130            | 8            |
|        | 112         | 275008       | 8.0E-85 | 2978            | 7            |
|        | 113         | 2552675      | 4.0E-54 | 2651            | 4            |
|        | 114         | 335030       | 0.0     | 8139            | 20           |
|        | 120         | 6245         | 1.0E-128| 7446            | 18           |
|        | 126         | 45274        | 0.0     | 4892            | 16           |
| 7HL    | 2927        | 36988        | 2.0E-164| 3995            | 20           |
|        | 2933        | 102319       | 3.0E-136| 6141            | 10           |
|        | 2937        | 43456        | 0.0     | 18642           | 20           |
|        | 2942        | 160008       | 9.0E-90 | 3156            | 16           |
|        | 2946        | 135867       | 0.0     | 4214            | 16           |
|        | 2948        | 1562518      | 0.0     | 5683            | 17           |
|        | 2949        | 168471       | 4.0E-90 | 1661            | 5            |
|        | 2950        | 7066         | 0.0     | 8199            | 20           |
|        | 2953        | CUST_39488   | –       | 689             | –            |
|        | 2956        | 1561792      | 3.0E-32 | 2569            | 10           |
|        | 2261        | 98708        | 7.0E-177| 11121           | 19           |
|        | 2264        | 165059       | 0.0     | 3889            | 17           |
|        | 2267        | 2549444      | 0.0     | 6855            | 16           |
|        | 2270        | 138749       | 0.0     | 6971            | 13           |
|        | 2273        | 160010       | 2.0E-101| 5220            | 11           |
|        | 2276        | 38804        | 0.0     | 6643            | 13           |
|        | 2278        | 159682       | 3.0E-114| 4182            | 7            |
|        | 2281        | 50047        | 0.0     | 10166           | 5            |
|        | 2285        | 66958        | 0.0     | 14049           | 14           |
|        | 2287        | 1568412      | 3.0E-161| 4360            | 12           |
|        | 2291        | 57887        | 2.0E-42 | 6909            | 12           |
|        | 2299        | 46523        | 4.0E-131| 4512            | 13           |

The information available for those Morex contigs bearing the new flanking markers on each QTL was used for anchoring the SBCC097 × Plaisant and SBCC145 × Beatrix genetic maps to the physical map of barley. A genomic region of 4 Mb was identified for the 7HS interval between markers QB_273608 and QB_45091 (Table 2). Morex contigs underlying those markers were anchored to FP contigs (FPC) and they were positioned according to the AC1 anchoring strategy described by IBSC [25]. Two regions of 3.7 Mb and 3.2 Mb covered the QTL intervals on 7HL and 6HL, between markers QB_1562518 and QB_1561792 and QB_138749 and QB_46523, respectively (Table 2). The Morex contigs anchored to FPC were positioned as described above, while those Morex contigs (morex_contig_1561792 for 7HL and morex_contig_46523 for 6HL) without sequence homology to any FP contigs (FPC) and they were positioned according to the AC1 anchoring strategy described by IBSC [25]. Two regions of 3.7 Mb and 3.2 Mb covered the QTL intervals on 7HL and 6HL, between markers QB_1562518 and QB_1561792 and QB_138749 and QB_46523, respectively (Table 2). The Morex contigs anchored to FPC were positioned as described above, while those Morex contigs (morex_contig_1561792 for 7HL and morex_contig_46523 for 6HL) without sequence homology to any FPC were anchored according to the AC2 strategy [25]. All newly developed markers within the target intervals matched a FPC contig or a Morex contig in the expected order, except for markers QB_7066 and QB_135962, whose Morex contigs were physically positioned elsewhere in the chromosome 7HL and 6HL, respectively.

Only those Morex contigs bearing “high-confidence” or “low-confidence” genes, according to the definition established by IBSC [25], were considered for drawing a minimum tiling path at the three target genomic regions. The region putatively carrying the powdery mildew resistance on 7HS comprises 10 FPC and 98 Morex contigs (Table S2). Three FPC do not contain any Morex contig with an assigned gene. Fifty-nine morex contigs were anchored to the other seven FPCs (Table S2). This genomic region contains 99 LC and 53 HC genes. The region on 7HL displayed 10 FPC, 84 morex contigs and 122 genes (81 LC and 41 HC) (Table S2). Thirty-six Morex contigs were anchored to seven FPC and the additional three FPC did not have any gene assigned (Table S2). Regarding the genomic interval on 6HL, the physical region covered 6 FPC and 66 Morex contigs (Table S2). Forty-one of these contigs were anchored to FPC. In total, 87 genes (47 LC and 40 HC) were assigned to this region (Table S2).
Among the genes annotated from the Morex contigs on the 7HS, 7HL and 6HL regions, 10, 7 and 10 HC genes, respectively, showed a functional annotation that hints to an involvement in the disease resistance mechanisms. They might be considered as candidates for the resistances described at these genomic regions (Table 3, Table S2). In total, five genes were annotated as Nucleotide Binding (NB)-Leucine Rich Repeats (LRR) proteins (PFAM: PF00931), four were identified as belonging to the serine/threonine protein kinase (S/TPK) family (PF00069), and two genes contained domains that could be involved in the recognition of the pathogen (Table 3). Additional candidate genes encoded proteins that could be involved in other mechanisms of plant defense or signal transduction more than in the perception of pathogen effectors (Table 3).

BlastP search for orthologous genes in the three grasses reference genomes showed low levels of microsynteny for the target regions. In total, six (22.2%), eight (29.6%) and eleven (40.7%) proteins predicted from the most promising HC genes matched their corresponding counterpart on the expected chromosome of rice, sorghum and Brachypodium, respectively (Table 3).

Analysis of GO/PFAM terms on the protein sequences derived from LC genes revealed that 11 (7HS), 3 (7HL) and 6 (6HL) genes might be involved in the disease resistance, exhibiting mainly the terms GO:0005524 (ATP-binding), GO:0005515 (protein-binding), GO:0004672 (protein kinase activity), PF00069 (protein kinase domain), PF08263 (Leucine rich repeat N-terminal domain) and PF00931 (NB-ARC domain) (Table S2).

Discussion

Positioning of disease resistance QTL on the physical map of barley constitutes an essential step towards the map-based gene cloning, but it also paves the way towards the suitable exploitation of these resources in breeding programs, through the development of tightly linked molecular markers. In barley, such steps were typically hampered by the large genome size of the crop and its highly repetitive nature [30]. Advances in barley genomics have abounded over the past decade greatly increasing the opportunities for interrogating the molecular mechanisms underlying the formation of interesting traits [31–36]. Among these, two recent milestones stand out as the main contributions to facilitate the access and full exploitation of the barley genome sequence. First, the construction of a genome-wide putative linear gene index of barley (Genome Zipper) based on flow sorted chromosomes and shotgun sequencing [24] and, more recently, the publication of the first draft of the physical, genetic and functional sequence of the barley genome [25]. The combined use of these resources allows reaching enhanced resolution of an extremely complex genome, making full use of the resolution available in classical biparental populations used for QTL search. We report here an example of this use to dissect to new depths the chromosomal regions conferring resistance to B. graminis in two Spanish barleys and as a conduit to identify candidate genes at the target intervals.

Comparative Analysis to the Barley Genome Zipper

First, we took advantage of the previously established barley Genome Zipper of chromosomes 7H and 6H, constructed by integrating next generation sequencing information of barley with
Table 2. Anchoring of the genetic markers developed from Morex contigs to the physical map of barley via FP contigs (AC1 strategy) or Morex contigs (AC2 strategy) [25].

| Chrom | Marker name | Morex_contig | FP_contig | cM<sup>1</sup> | Bp<sup>2</sup> |
|-------|-------------|--------------|-----------|---------------|-------------|
| 7HS   | QB_275608   | morex_contig_275608 | 45784 | 8.286119 | 9055720 |
|       | QB_2552675  | morex_contig_2552675 | – | – | – |
|       | QB_6245     | morex_contig_6245 | – | – | – |
|       | QB_335030   | morex_contig_335030 | 44369 | 12.747875 | 11229440 |
|       | QB_45091    | morex_contig_45091 | 44313 | 12.747875 | 13155160 |
| 6HL   | QB_156215    | morex_contig_156215 | 1622 | 120.82153 | 574959480 |
|       | QB_36988    | morex_contig_36988 | – | – | – |
|       | QB_7066     | morex_contig_7066 | – | – | – |
|       | QB_1561792  | morex_contig_1561792 | – | 124.57507 | 578735280 |
|       | QB_138749   | morex_contig_138749 | 8992 | 119.33428 | 534422080 |
|       | QB_2549444  | morex_contig_2549444 | 48820 | 119.33428 | 537882240 |
|       | QB_159682   | morex_contig_159682 | – | – | – |
|       | QB_57887    | morex_contig_57887 | – | 123.79603 | 537882240 |
|       | QB_50047    | morex_contig_50047 | – | 126.48725 | 537882240 |
|       | QB_66958    | morex_contig_66958 | 7137 | 126.48725 | 538665920 |
|       | QB_1568412  | morex_contig_1568412 | – | – | – |
|       | QB_46523    | morex_contig_46523 | – | 126.6289 | 538665920 |

<sup>1</sup>cM position according to IBSC [25]. <sup>2</sup>Bp position according to IBSC [25].

doi:10.1371/journal.pone.0067336.t002

virtually ordered gene inventory, between barley loci 99–104 (7HS) and loci 2937–2948 (7HL), were not confirmed in our results, which on the contrary, suggest an upstream location for the homologous genes in rice, sorghum and Brachypodium. Such absence of syntenic relationships was also observed for the inverted regions on chromosomes 7HS and 7HL, in which new markers were not genetically mapped according to the gene order expected from the Genome Zipper. Changes in marker order could be attributed to the fact that some of the barley loci anchored at those positions along the Genome Zipper of chromosome 7H are supported only by the order of their counterpart in one or two reference genomes [24]. This could explain some misinterpretation in the gene order when constructing the virtual barley model. At this medium resolution level of synteny, it is expected that the accuracy of the one-to-one relationship between orthologous will vary depending on the density of reference genomes and the ancestral rearrangements affecting few linked or unlinked genes under selection pressure [41,42]. Several reports also demonstrated that colinearity is commonly less conserved at the telomeric regions of the chromosomes [43,44], which is the case for 7HS and 6HL. Beyond this, a good performance was observed for the Genome Zipper, allowing the positioning of eight and five new markers at the 7HS and 7HL intervals, respectively. Regarding the 6HL region, a high level of colinearity was found between our new genetic map and the barley Genome Zipper, whereas new markers developed from contigs ordered according to the position defined by the barley virtual map. Similar results have been found for chromosomes 1H, 2H and 4H (D. Perovic, unpublished data). These data suggest that the Genome Zipper should be retained as an extremely powerful resource for fine mapping, chromosome dissection and physical map anchoring, provided that such approach will also meet some limitations depending on the features of the target region.
| Chrom | Barley gene | FP contig | Morex_contig | Annotation | Protein length | PFAM | Rice | Brachypodium | Sorghum |
|-------|-------------|-----------|--------------|------------|----------------|------|------|--------------|---------|
| 7HS   | AK2S1676.1  | –         | morex_contig_6245 | Protein kinase superfamily protein LENGTH = 464 | 483  | PF00669 | Os02|07200600-01 | Bradi1g50590.1 | Sb10g001310.1 |
|       | MLOC_5217.3 | –         | morex_contig_136027 | Disease resistance protein (CC-NBS-LRR) | 1035 | PF00931 | Os11|0707014100-00 | Bradi4g10987.1 | Sb05g004310.1 |
|       | MLOC_70200.1 | contig_41890 | morex_contig_57570 | Leucine-rich repeat receptor kinase-like protein | 884  | PF00560 | Os11|0202089900-01 | Bradi4g10987.1 | Sb05g0027140.1 |
|       | MLOC_63994.1 | contig_44369 | morex_contig_48252 | F-box domain containing protein | 251  | –     | Os06|01136000-01 | Bradi1g50426.1 | Sb05g001110.1 |
|       | MLOC_72805.2 | contig_44369 | morex_contig_6241 | NBS-LRR disease resistance protein, putative, expressed | 914  | PF00931 | Os10|03358200-00 | Bradi5g01167.1 | Sb02g027790.1 |
|       | MLOC_7548.1 | contig_44369 | morex_contig_139505 | Protein kinase-3 | 427  | PF00669 | Os11|06068300-01 | Bradi4g09457.1 | Sb09g000630.1 |
|       | MLOC_69817.1 | contig_44369 | morex_contig_64333 | Superoxide dismutase | 393  | PF02777 | Os06|01154000-01 | Bradi1g50550.1 | Sb10g001280.1 |
|       | MLOC_16184.1 | –         | morex_contig_157265 | Peroxidase 1 | 324  | PF00141 | Os07|01570000-00 | Bradi2g11300.1 | Sb09g002760.1 |
|       | MLOC_2395.1 | –         | morex_contig_121333 | Leaf rust resistance protein Lr10 | 464  | –     | Os11|02490000-01 | Bradi2g39537.1 | Sb07g002160.1 |
|       | MLOC_5842.1 | contig_3887 | morex_contig_136766 | GDSL esterase/lipase | 370  | PF00657 | Os01|06651000-01 | Bradi2g45230.1 | Sb03g002690.1 |
|       | MLOC_39511.4 | –         | morex_contig_255327 | Phospholipase D4 | 413  | PF08371 | Os06|06499000-01 | Bradi1g39302.1 | Sb10g025690.1 |
|       | MLOC_11419.3 | contig_6970 | morex_contig_1561039 | Protein kinase superfamily protein LENGTH = 579 | 606  | PF00669 | Os08|02491000-01 | Bradi3g19020.1 | Sb07g008540.1 |
|       | MLOC_62970.1 | –         | morex_contig_47144 | F-box domain containing protein, expressed | 410  | PF00646 | Os12|01280000-01 | Bradi1g31010.1 | Sb01g037880.1 |
|       | MLOC_39346.1 | –         | morex_contig_2553094 | GDSL esterase/lipase | 359  | PF00657 | Os01|06651000-01 | Bradi2g45230.1 | Sb03g029600.1 |
| 6HL   | MLOC_60881.1 | contig_591 | morex_contig_44875 | WD-repeat protein 57 | 346  | PF00400 | Os06|06538000-01 | Bradi1g31170.1 | Sb10g025990.1 |
|       | MLOC_2395.1 | –         | morex_contig_121333 | Leaf rust resistance protein Lr10 | 464  | –     | Os11|02490000-01 | Bradi2g39537.1 | Sb07g002160.1 |
|       | MLOC_5842.1 | contig_3887 | morex_contig_136766 | GDSL esterase/lipase | 370  | PF00657 | Os01|06651000-01 | Bradi2g45230.1 | Sb03g002690.1 |
|       | MLOC_39511.4 | –         | morex_contig_255327 | Phospholipase D4 | 413  | PF08371 | Os06|06499000-01 | Bradi1g39302.1 | Sb10g025690.1 |
|       | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
|       | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
|       | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
| 6HL   | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
|       | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
|       | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
|       | MLOC_68014.1 | –         | morex_contig_61285 | Leucine-rich repeat-containing protein 40 | 586  | PF00560 | Os02|08266000-01 | Bradi3g56757.1 | Sb04g038010.1 |
Anchoring to the Barley Physical Map and Identification of Candidate Genes

The addition of new markers to the 7H linkage map did not shed much more light to resolve the resistance regions, due to the resolution of the current mapping population. However, they will be very useful for a more precise screening for recombinants of large F2 populations and further positional isolation, allowing increase in marker density at the high-resolution mapping population from 1 marker/0.34 cM to 1 marker/0.27 cM in the case of 7HL [unpublished data]. Regarding the QTL on 6HL, the addition of new markers narrowed down the resistance regions to smaller intervals, i.e. from 3.3 cM to 1.1 cM (1 marker/0.1 cM), and pointed out to QB_159682 as the most likely marker co-segregating with the QTL (based on the recombination events displayed by three DH lines). The generation of these saturated genetic maps is useful to exploit fully the potential of physical maps for map-based cloning strategies, since the sequenced contigs should be anchored at a density as high as possible with molecular markers [45]. Dense genetic maps are also valuable for the breeding community, to perform precise introgression of the novel resistances in elite cultivars via marker-assisted selection approaches.

These new markers developed from Morex contigs provided the framework for anchoring of genetic and physical maps, figuring out the outline of the barley genome at these regions conferring resistance in SBCCC97 and SBCCC145. The outermost flanking markers of QTLs on 7HS, 7HL and 6HL defined a physical area of 4 Mb, 3.7 Mb and 3.2 Mb, respectively. The accurate relationship of physical to genetic distance was hard to predict for the target loci due to co-segregation of markers. According to the map of Kunzel et al. [46], the recombination rate at the distal end of chromosome 7HS corresponded to 1.3 Mb/cM. Our estimation, considering the block of co-segregating markers, was the ~4 Mb/cM, which is three times larger than expected. This may be partially explained by the presence of two important gaps of 1.4 (between 9824520 and 11292440 bp) and 1.2 Mb (from 11938760 to 13155160 bp) in the barley genomic sequence predicted for this region. Correcting for this fact, the obtained ratio would be ~1.4 Mb/cM, which is closer to that reported by Kunzel et al. [46]. The recombination rate of the regions flanking the 7HL co-segregating area was of ~1.5 Mb/cM, which is in accordance with Kunzel et al. [46], who proposed rates between 1.3 and 3.4 Mb/cM. The estimated physical to genetic ratio for the 6HL was of 2.9 Mb/cM, which is within the predictions of 1.8 and 3.4 Mb/cM. The estimated physical to genetic ratio for 6HL, which is also exhibited the structure of disease resistance proteins. Both results are in agreement with data from the IBSC [23] who reported up to 191 NBS-LRR type genes, which tended to cluster in gene families towards the distal ends of barley chromosomes.

The largest class of plant resistance genes encodes a NBS-LRR class of proteins [47]. The carboxy-terminal LRR domains are found in diverse proteins and function in the recognition of pathogen effectors as sites of protein–protein interaction [52]. The nucleotide-binding site (also termed as NB-ARC) is part of a larger domain with homology to some eukaryotic cell death effectors and it seems to play a role in the subsequent signaling events that trigger the resistance, through the hydrolysis of ATP [53,54]. S/TPKs are another important group of resistance genes which may act directly conferring resistance to the pathogen or indirectly through its cooperation with a NBS-LRR gene, as happens with the tomato Pto gene [55,56]. This collaboration of different protein domains to provide resistance to plant pathogenic organisms could explain their grouped positions at the distal regions of barley chromosomes, as they may sometimes work together for the resistance response to occur. One additional gene was annotated as a “Leucine-rich repeat receptor kinase-like protein”, which constitutes a third class of relatively few members that possesses the LRR and PK domains within the same transcript [57]. Another HC gene was annotated as a “WD-repeat protein 57”, which does not display the typical structure of a resistance protein but it maintains a domain (WD40) that could be involved in protein-protein interactions, in a similar way as the LRR domains [58]. Apart from these, other annotated genes were also judged as putative candidates based on the predicted function of the translated protein. Thus, CCC1-type zinc finger proteins, glucan synthase-like proteins, superoxide dismutase, peroxidase, GDSSL esterase, etc. seem to play important roles in imparting host resistance through some function in the signaling networks triggering the multilayered mechanisms involved in the defense response [59–62].

Orthologous rice, Brachypodium and sorghum genes were identified for the most promising barley candidate genes at the protein level. Only ten of them (40%) lay in at least one of the syntenous regions described previously [37]. The number of conserved syntenic loci was similar in comparison with rice and sorghum (22.2 and 29.6%, respectively) but was higher with Brachypodium (40.7%), confirming a closer relationship and a better conservation of genetic material between this grass and the Triticeae [40,63]. The lack of large microsynteny suggests that the regions conferring resistance to powdery mildew in Spanish barleys likely underwent some rearrangements compared to the three reference genomes. These results provide additional clues that explain the frequent lack of success of comparative genomics approaches for gene isolation in the Triticeae and support previous reports that suggested unique features in the barley genome [64]. Thus, the barley genes ROR2, ryn4/f5 and Phl-H1 are all present within the syntenic positions of the rice genome [65–67]. However, the orthologs of the barley genes Vir1, Rpg1 or Rdp2a are either within non-syntenic positions or absent in the rice genome [41,49,68]. Such limited success of synteny-based strategies, even when integrating more than one reference genome, is frequently observed at disease resistance loci, which are particularly unstable and frequently subjugated to tandem or
segmental duplications of the entire chromosomal regions where they are allocated [69,70].

One marker on 7HS and two markers on 6HL were directly associated with candidate genes. Additionally, all the Morex contigs anchored to FP contigs could be used to identify BAC clones from the Morex barley libraries, according to the algorithms available at IBSC [25]. This information together with further analysis of high-resolution mapping populations, which were separately constructed for the analysis of each QTL independently, will serve to construct a more accurate and reliable minimum tiling path containing the regions that confer resistances to powdery mildew in Spanish barley landraces. As far as we know, the current report places among the earliest efforts to put into practice the recently developed barley genomic resources to deal with old breeding dilemmas, such as accurate identification and exploitation of novel disease resistances.

Supporting Information

Table S1 Contig-based markers developed and evaluated for the marker enrichment of the chromosome 7HS, 7HL and 6HL regions harboring the QTLs for resistance to powdery mildew.

References

1. Komatsuda T, Pourkheirandish M, He C, Ashagulov P, Kamamori H, et al. (2007) Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. Proc Natl Acad Sci USA 104: 1424–1429.
2. Morrell PL, Cling MT (2007) Genetic evidence for a second domestication of barley (Hordeum vulgare) east of the Fertile Crescent. Proc Natl Acad Sci USA 104: 3289–3294.
3. FAOSTAT Website. Available: http://faostat.fao.org. Accessed 2012 November 12.
4. Kilian B, Oitkin H, Kolj J, von Haeseler A, Barale F, et al. (2006) Haplotype structure at seven barley genes: relevance to gene pool bottlenecks, phylogeny of ear type and site of barley domestication. Mol Genet Genomics 276: 230–241.
5. Condon F, Gustav C, Rasmussen DC, Smith KP (2008) Effect of advanced cycle breeding on genetic diversity in barley breeding germplasm. Crop Sci 48: 1027–1036.
6. Malyheva-Otto I, Ganal MW, Law JR, Reeves JC, Roder MS (2007) Temporal trends of genetic diversity in European barley cultivars (Hordeum vulgare L.). Mol Breed 20: 309–322.
7. Lopez PR (1994) A new plant disease: uniformity. CERES 26: 41–47.
8. Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kiniya MG, et al. (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Up99 (TTKS) of stem rust pathogen. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 1: 1–13.
9. van de Wouw M, van Hintum T, Kik C, van Treuren R, Visser B (2010) Genetic diversity trends in twentieth century crop cultivars: a meta-analysis. Theor Appl Genet 120: 1241–1252.
10. Fischbeck G, Jahoor A (1993) The transfer of genes for mildew resistance from Hordeum spontaneum. In: Jorgensen JH, editor. Integrated control of cereal mildews: virulence patterns and their change. 247–255.
11. Jorgensen JH, Jensen HP (1997) Powdery mildew resistance in barley landrace material. I. Screening for resistance. Euphytica 97: 227–233.
12. Dreswiat A, Bockelman HE (2003) Sources of powdery mildew resistance in a wild barley collection. Genet Resour Crop Ev 50: 345–350.
13. Konig J, Kopahnke D, Steffenson BJ, Przulj N, Romero T, et al. (2012) Genetic mapping of a leaf rust gene resistance in the former Yugoslavian barley landrace MBR1012. Mol Breed 30: 1253–1264.
14. Igartua E, Gracia MP, Lasa JM, Medina B, Molina-Cano JL, et al. (1998) The Spanish barley Core Collection. Genet Resour Crop Ev 45: 475–481.
15. Lasa JM (2008) Spanish Barley Core Collection. Monografias INIA n 25, Madrid, 222 pp.
16. Yoneshiro S, Igartua E, Moralez M, Ramos L, Molina-Cano JL, et al. (2008) Patterns of genetic and eco-geographical diversity in Spanish barley. Theor Appl Genet 116: 271–282.
17. Silvá C, Casas AM, Kopahnke D, Habecker A, Schweizer G, et al. (2010) Screening the Spanish barley Core Collection for disease resistance. Plant Breed 129: 45–52.
18. Silvá C, Flath K, Kopahnke D, Gracia MP, Lasa JM, et al. (2011) Analysis of powdery mildew resistance in the Spanish barley core collection. Plant Breed 130: 195–202.
19. Silvá C, Dhif H, Igartua E, Kopahnke D, Gracia MP, et al. (2010) Identification of quantitative trait loci for resistance to powdery mildew in a Spanish barley landrace. Mol Breed 25: 361–392.
20. Silvá C, Porovic D, Scholz U, Casas AM, Igartua E, et al. (2012) Fine mapping and comparative genomics integration of two quantitative trait loci controlling resistance to powdery mildew in a Spanish barley landrace. Theor Appl Genet 124: 49–62.
21. Silvá C, Casas AM, Igartua E, Perez-Molina JL, Gracia MP, et al. (2011) Resistance to powdery mildew in Spanish barley landraces is controlled by different sets of quantitative trait loci. Theor Appl Genet 123: 1019–1028.
22. Silvá C, Kopahnke D, Flath K, Serfling A, Porovic D, et al. (2013) Resistance to powdery mildew in one Spanish barley landrace hardly resembles other previously identified wild barley resistances. Eur J Plant Pathol DOI 10.1007/s10658-013-0178-7.
23. Mayer KFX, Taudienn S, Martin M, Imkova H, Suchanckova P, et al. (2009) Gene content and virtual gene order of barley chromosome 1H. Plant Physiol 151: 496–505.
24. Mayer KFX, Martin M, Hedley PF, Kimkova H, Liu H, et al. (2011) Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23: 1249–1263.
25. The International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491: 711–716.
26. You FM, Hua N, Qiang Gu Y, Lao MC, Ma Y, et al. (2008) BatchPrimer3: a high throughput web application for PCR and sequencing primer design. BMC Bioinformatics 9: 253.
27. van Ooijen JW (2006) JoinMap 4, software for the calculation of genetics linkage maps in experimental populations. Kyazma, BV, Wageningen.
28. Jansen R, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetica 130: 1447–1453.
29. van Ooijen JW (2009) MapQTL 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen.
30. Varshney RK, Langridge P, Graner A (2007) Application of genomics to molecular breeding of wheat and barley. Adv Genet 50: 121–153.
31. Sato K, Shiu T, Seki M, Shinzaki K, Yoshida H, et al. (2009) Development of 5006 Full-Length CDNAs in barley: a tool for accessing cereal genomics resources. DNA Res 16: 81–89.
32. Sato K, Nankaku N, Takeki K (2009) A high-density transcript linkage map of barley derived from a single population. Heredity 103: 110–117.
33. Wicker T, Taudien S, Houben A, Keller B, Graner A, et al. (2009) A whole-genome snapshot of 454 sequences exposes the composition of the barley genome and provides evidence for parallel evolution of genome size in wheat and barley. Plant J 59: 712–722.
34. Muttoo-Amatriain M, Moscou MJ, Bhat PR, Svensson JT, Bartol J, et al. (2011) An improved consensus linkage map of barley based on flow-sorted chromosomes and single nucleotide polymorphism markers. Plant Genome 4: 239–249.
52. Jones DA, Jones JDG (1996) The roles of leucine rich repeats in plant defences. Adv Bot Res Adv Plant Pathol 24: 90–167.