Allele mining in barley genetic resources reveals genes of race-non-specific powdery mildew resistance

Annika Spies1†, Viktor Korzun2, Rosemary Bayles3, Jeyaraman Rajaraman1, Axel Himmelbach1, Pete E. Hedley4 and Patrick Schweizer1*

1 Leibniz-Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
2 KWS LOCHOW GMBH, Bergen-Wohld, Germany
3 National Institute of Agricultural Botany, Cambridge, UK
4 The James Hutton Institute, Dundee, UK

Race-non-specific, or quantitative, pathogen resistance is of high importance to plant breeders due to its expected durability. However, it is usually controlled by multiple quantitative trait loci (QTL) and therefore difficult to handle in practice. Knowing the genes that underlie race-non-specific resistance (NR) would allow its exploitation in a more targeted manner.

Here, we performed an association-genetic study in a customized worldwide collection of spring barley accessions for candidate genes of race-NR to the powdery mildew fungus Blumeria graminis f. sp. hordei (Bgh) and combined data with results from QTL mapping as well as functional-genomics approaches. This led to the identification of 11 associated genes with converging evidence for an important role in race-NR in the presence of the Mlo gene for basal susceptibility. Outstanding in this respect was the gene encoding the transcription factor WRKY2. The results suggest that unlocking plant genetic resources and integrating functional-genomic with genetic approaches can accelerate the discovery of genes underlying race-NR in barley and other crop plants.

Keywords: Hordeum vulgare, Blumeria graminis

INTRODUCTION

Since approximately 10,000 years crop domestication has laid the basis for food-, feed-, fiber-, energy-, and health promoting-compound production for an ever increasing world population. However, while important domestication traits such as seed non-shattering have been fixed early on during this process, other potentially valuable alleles from wild crop relatives were lost due to genetic bottlenecks during domestication and breeding (Pourkheirandish and Komatsuda, 2007). This problem was further accentuated by modern variety breeding starting up in the early decades of the twentieth century, often returning to relatively narrow breeding pools aiming at efficient combination of the best varieties with the best alleles (Hyten et al., 2006; Haudry et al., 2007; Li et al., 2009). Nevertheless, plant genebanks have been established around the globe to save and maintain plant genetic resources in ex situ collections that include wild crop relatives as well as landrace material etc. These resources will have to be accessed and mined systematically in order to meet the challenge of increasing and securing crop yields worldwide in the near future under increasingly volatile climatic conditions (Feuillet et al., 2008).

One important aspect of yield security is the prevention of losses due to diseases and pests, which will have to be achieved by resistance breeding or gene technology rather than by chemical pest control in order to fulfill the requirement of sustainability. As far as resistance to microbial pathogens is concerned and within this group of organisms, especially to fungal pathogens two strategies are being followed: firstly, introduction of major resistance (R) genes usually conferring strong protection against specific fungal races that carry the matching avirulence genes. However, it has become clear that the efficacy of major R gene-mediated monogenic resistance, though relatively easy to introgress, is often broken down by new pathogen races after few years of use in the field – a phenomenon that is further accelerated by the planting of monogenically resistant cultivars on large acreage (Brown et al., 1993). The second strategy includes the accumulation of useful quantitative trait loci (QTL) of resistance donors in susceptible genotypes of a target crop species, which confers more durable and race-non-specific resistance (NR) but is, due to its polygenic mode of inheritance, more difficult to handle in breeding practice (Kou and Wang, 2010). In recent years advances in the understanding of the molecular biology of plant–pathogen interactions has allowed replacement of the operational term “avirulence” gene or protein by the mechanistic term “effector” designating pathogen-encoded proteins that are delivered to host cells in order to interact with host factors and support pathogen growth (Koeck et al., 2011). The co-evolutionary model for the non-durability of race-specific resistance versus the durability of NR was proposed by Jones and Dangl (2006) and has gained strong experimental support since then. According to this model NR, also often referred to as quantitative resistance, reflects pathogen-associated molecular pattern (PAMP)-triggered innate immunity (PTI) minus effector-triggered susceptibility (ETS). Plants posses a range of pathogen-recognition receptors of the receptor-like kinase type that bind
to and are activated by PAMPs. R Gene-mediated, race-specific resistance on the other hand reflects effector-triggered immunity (ETI) in plant genotypes carrying matching NB-LRR-type R genes for direct or indirect effector recognition. Although the rice receptor-like kinase Xa21 conferring resistance to Xanthomonas oryzae was initially referred to as R gene it has recently been re-classified as PAMP receptor (Thomma et al., 2011). Thus, both resistance mechanisms are triggered by different types of pathogen-derived signals and involve different signal perception and transduction components (Pansstruga et al., 2009). They may, however, converge at some point resulting in overlapping sets of regulated genes and induced defense components such as reactive oxygen species or pathogenesis-related, antimicrobial proteins (Thomma et al., 2011).

In order to exploit NR in a more efficient manner for crop breeding knowledge about loci and – more importantly – underlying host genes are required. Prerequisites for such an approach will be the availability of extensive genetic and genomic resources and tools for high-resolution mapping, efficient back-crossing, and highly informed candidate-gene approaches. barley (Hordeum vulgare ssp. vulgare) belonging to the Triticeae tribe of cereals, which includes wheat and rye, fulfills these requirements because well-characterized experimental populations and large collections of natural genetic variability (Rostoks et al., 2006; Waugh et al., 2009), high-density genetic maps (Close et al., 2009; Sato et al., 2009; Aghnoum et al., 2010), a physical genome map (Schulte et al., 2009), and extensive gene-expression data (Wise et al., 2007; Druka et al., 2010) have been made publicly available.

One of the major diseases of barley and wheat is powdery mildew caused by the obligate biotrophic ascomycte fungus Blumeria graminis f. sp. hordei (Bgh). The interaction of barley with Bgh can be regarded as a model plant–pathogen interaction due to a large body of physiological, cellular, biochemical, and molecular information about changes in the host during compatible or resistant interactions (Collins et al., 2002; Huckelhoven, 2007; Wise et al., 2009) also taking advantage of high-throughput functional tools (Douchkov et al., 2005; Ihlow et al., 2008). Moreover, progress in identifying genes as well as effector proteins of Bgh has also been made recently (Bindschedler et al., 2009; Godfrey et al., 2010) and will be strongly supported by the availability of the genome sequence of the pathogen (Spanu et al., 2010). In summary, a better understanding of the barley–Bgh interaction – together with the Arabidopsis thaliana–Golovinomyces orontii interaction – will serve as important model for hundreds of powdery mildew–crop interactions, the clade of Erysiphales being extremely widespread and economically most important phylogenetic group of pathogens worldwide.

Here, we describe a candidate gene (CG) based approach in barley attacked by Bgh in order to identify potentially important host factors of NR. For this purpose, a trait-customized collection of barley ex situ genebank accessions was established that is enriched in lines exhibiting strong interaction phenotypes (strong NR versus high susceptibility). The collection was used for the re-sequencing of CGs derived either from previous functional-genomics approaches in barley or from a priori knowledge of genes relevant for plant innate immunity. As a result we generated combined genetic and functional-genomic evidence (“convergent evidence” CE throughout) for 11 barley genes to be involved either in host ETS or NR.

RESULTS
A TRAIT-CUSTOMIZED BARLEY COLLECTION
By referring to primary evaluation data of accessions from the IPK genebank over more than five decades, a list of 212 spring barley accessions was assembled that had been described as either resistant in a race-non-specific manner or as highly susceptible (Nover and Mansfeld, 1955; Nover and Lehmann, 1972, 1973). Re-evaluation of these accessions by detached leaf assays using two polyviral German Bgh isolates that together have overcome 44 major R genes representing almost all resistance specificities ever introduced into European barley germplasm (Figure A1 in Appendix) resulted in the identification of 30 susceptible and 35 resistant accessions, most of them exhibiting strong phenotypes (Figures 1A,B). Because resistance segregated in some of the accessions, two single-seed descendant lines (referred to as “genotypes” throughout) were established from each of the 65 accessions and re-scored in the detached leaf assay. Field trials for Bgh resistance in Germany in 2007 (data not shown) and 2008 (Figure 1C) under high infection pressure derived from spreader rows revealed a strong correlation with resistance data from the detached leaf assay. NR in spring barley accessions could have been caused by known or unknown recessive loss-of-function alleles of the Mlo gene, a well-known susceptibility factor for powdery mildew in barley and other plant species (Buschges et al., 1997). We addressed this possibility in a transient complementation assay by bombarding a BAC clone carrying wildtype Mlo into epidermal cells of the resistant genotypes. As shown in Figure 1D, three lines (HOR 1457, HOR 1506, and HOR 3537) plus the positive control line Ingrid BC mlo5 responded to BAC complementation with an increase in penetration efficiency of Bgh by a factor of at least five. Therefore, these genotypes were assumed to carry mlo-resistance alleles and excluded from the panel. Four out of five lines showing partial complementation by a factor of less than fivefold were not excluded because they might carry partially functional alleles of Mlo (highlighted by “?” below the bar chart) not completely masking the effect of other resistance QTL. Because Mlo was transiently expressed under the control of its own promoter, we expected not to observe hypersusceptibility in Mlo-containing genotypes as reported upon over-expression driven by a strong constitutive promoter (Elliott et al., 2002). Interestingly, the four partially Mlo-complementing lines exhibited HR-associated resistance, which is atypical for the mlo-resistance gene therefore suggesting indeed the effect of additional resistance loci. We also searched for the presence of the only known natural mlo-11 allele, which was found in Ethiopian landraces and subsequently introgressed into European germplasm, by using a PCR-based DNA marker (Figure A2 in Appendix; Piffanelli et al., 2004). Five lines including the three lines already excluded due to mlo-complementation were found to contain mlo-11 and subsequently excluded from the collection. The two remaining mlo-11 containing lines were partially (HOR 2543) or not (HOR 4408) complementing, which suggests presence of additional resistance loci effective against the Bgh isolate CH4.8 used here. The final trait-customized collection for race-non-specific, mlo-independent seedling resistance to
**FIGURE 1** | A trait-customized spring barley collection differing in seedling and adult-plant resistance to *Bgh*. (A) Examples of strong (HOR 3075) and intermediate seedling resistance (HOR 200) as well as full susceptibility (HOR 3941) to *Bgh*. The pictures were taken 7 days after inoculation. (B) Phenotypic distribution of the barley collection with respect to seedling resistance against the polyvirulent German isolates P78 and D12–12 of *Bgh*. Mean values from inoculations with both isolates are shown. (C) Correlation of seedling and adult-plant resistance determined by a detached leaf assay of greenhouse-grown plants and in a field experiment performed in 2008 in Germany, respectively. (D) Functional assessment of *mlo*-allelic status of resistant accessions by Mlo BAC complementation. Red dots indicate genotypes carrying *mlo-1*.

*Bgh* consisted of 30 highly susceptible and 31 strongly to moderately resistant genotypes against two polyvirulent German *Bgh* isolates (Table 1). Fourteen of the resistant genotypes were also completely or partially resistant to four *Bgh* isolates from Israel that contained virulences for different *Mla* alleles present in wild barley (*H. vulgare* ssp. *spontaneum*; Dreiseitl and Dinoor, 2004). With the exception of HOR 3988, a landrace from India, no racespecific resistance pattern was observed with the Israeli isolates either. The customized collection represents only cultivated spring barley except for two genotypes of wild barley from Turkmenistan (HOR 2826) and from an unknown origin (HOR 1647).

**INHERITANCE OF RESISTANCE TO *Bgh***

In order to characterize the mode of inheritance of *Bgh* resistance in the customized collection, three resistant lines were selected and crossed with the highly susceptible barley cv. Morex. The resistance donors were HOR 3271 (German cv “Steffi” possessing HR-associated resistance), HOR 3726 (North American cv “Nigrate” possessing papilla-associated resistance), and HOR 2932 (an Ethiopian landrace possessing HR-associated resistance). Phenotyping of F2 populations revealed quantitative trait segregation (Figure 2). Because major *R* genes can be inherited in a semi-dominant manner, a chi-square test for 1:2:1 segregation of strong (same phenotype as resistance donor) to intermediate resistance (all degrees of resistance between the parents) to susceptibility (same degree of susceptibility as cv. Morex) was performed. All three populations differed from the hypothetical 1:2:1 segregation pattern with *p* = 0.002 or lower suggesting that NR was based on several unlinked loci. QTL mapping was performed by using a panel of 384 highly polymorphic SNP markers. In each population 3–4 QTL were detected with profile LOD scores of composite interval mapping ranging from 3.0 to 18.8 (Table 2). Map positions of these QTL in the two populations showing HR-associated resistance were found to be in proximity of the race-specific resistance genes *Mlra*, *Mla*, *Mlk*, *MLa*, and *mlt*, which opens up the question if resistance of the corresponding resistant parent was due to any of these *R* genes, especially because major *R* genes are known to mediate HR-associated resistance. This however appears unlikely for the following reasons: first, LOD scores of major *R* genes in populations of the size used here are usually higher (≥ 20) than the scores of the detected QTL. Second, each population possessed several additional weaker QTL with LOD scores between 2 and 3.
### Table 1 | Characterization of the customized barley collection for race-non-specific resistance to Bgh.

| Accession | Origin | Row number | Status of accession | % Inf. EU isol. | % Inf. Israel isol. | Resistance response | mlo-11 | Mio compl. |
|-----------|--------|------------|---------------------|-----------------|---------------------|---------------------|--------|-----------|
| Ingrid BC mlo5 | Denmark | 2 | Breeding line | 2.5 | n.a. | Papillae | NO | Full |
| HOR 2573 | Ethiopia | 6 | Landrace | 2.5 | 32.4 | HR | NO | NO |
| HOR 3270 | Turkey | 6 | Breeding line | 2.5 | 5.8 | HR | NO | NO |
| HOR 3726 | USA | 6 | Cultivar | 2.5 | 11.4 | Papillae | NO | NO |
| HOR 1647 | Unknown | 2 | Wild | 2.9 | n.a. | HR | NO | NO |
| HOR 1036 | Greece | 6 | Landrace | 3.3 | 40.8 | HR | NO | NO |
| HOR 2591 | New Zealand | 6 | Cultivar | 3.3 | 49.7 | HR | NO | NO |
| HOR 4021 | USA | 6 | Cultivar | 3.3 | 7.4 | HR | NO | NO |
| HOR 842 | China | 6 | landrace | 3.6 | 14.5 | HR | NO | Partial |
| HOR 3075 | Ethiopia | 6 | Landrace | 3.8 | 29.6 | HR | NO | NO |
| HOR 2932 | Ethiopia | 6 | Landrace | 4.5 | 32.4 | HR | NO | NO |
| HOR 3983 | USA | 6 | Breeding line | 7.8 | 42.7 | HR | NO | NO |
| HOR 795 | Ethiopia | 2 | Landrace | 8.1 | 26.8 | HR | NO | NO |
| HOR 728 | Greece | 6 | Landrace | 9.7 | 43.6 | HR | NO | NO |
| HOR 1379 | Greece | 6 | Landrace | 9.9 | 52.1 | HR | NO | NO |
| HOR 736 | Albany | 6 | Landrace | 11.2 | 54.4 | HR | NO | NO |
| HOR 804 | USA | 6 | Cultivar | 11.3 | 46.0 | HR | NO | NO |
| HOR 3984 | USA | 6 | Breeding line | 12.0 | 42.7 | HR | NO | NO |
| HOR 261 | USA | 6 | Cultivar | 13.3 | 42.7 | HR | NO | NO |
| HOR 683 | Greece | 6 | Landrace | 13.4 | 42.7 | HR | NO | NO |
| HOR 3988 | India | 6 | Landrace | 13.9 | 10.0 | HR | NO | Partial |
| HOR 1159 | Greece | 6 | Landrace | 16.5 | 34.7 | HR | NO | NO |
| HOR 262 | Unknown | 6 | Breeding line | 16.6 | 41.3 | HR | NO | NO |
| HOR 3041 | India | 2 | Breeding line | 17.4 | 5.8 | HR | NO | NO |
| HOR 3271 | Germany | 2 | Cultivar | 19.1 | 2.5 | HR | NO | Partial |
| HOR 214 | Japan | 6 | Landrace | 20.1 | 45.0 | HR | NO | NO |
| HOR 800 | Yugoslavia | 6 | Breeding line | 20.5 | 41.3 | HR | NO | NO |
| HOR 1488 | India | 2 | Breeding line | 21.3 | 4.1 | HR | NO | NO |
| HOR 4400 | Germany | 2 | Cultivar | 22.4 | 2.5 | HR | NO | NO |
| HOR 1873 | Greece | 6 | Landrace | 23.8 | 48.3 | HR | NO | NO |
| HOR 303 | USA | 2 | Cultivar | 28.4 | 38.9 | HR | NO | NO |
| HOR 831 | Unknown | 2 | Landrace | 33.8 | 45.0 | HR | NO | Partial |
| HOR 2826 | Turkmenistan | 2 | Wild | 38.0 | n.a. | n.a. | n.a. | n.a. |
| HOR 844 | Unknown | 2 | Landrace | 38.0 | n.a. | n.a. | n.a. | n.a. |
| HOR 3997 | USA | 2 | Cultivar | 42.7 | n.a. | n.a. | n.a. | n.a. |
| HOR 4031 | India | 6 | Landrace | 54.4 | n.a. | n.a. | n.a. | n.a. |
| Ingrid | Sweden | 2 | Cultivar | 55.4 | 53 | NO | NO | |
| HOR 3275 | China | 6 | Cultivar | 59.1 | n.a. | n.a. | n.a. | n.a. |
| BCC 3 | Afghanistan | 2 | Landrace | 61.4 | n.a. | n.a. | n.a. | n.a. |
| BCC 1389 | Ireland | 2 | Cultivar | 61.4 | n.a. | n.a. | n.a. | n.a. |
| BCC 190 | Syria | 2 | Landrace | 67.4 | n.a. | n.a. | n.a. | n.a. |
| BCC 1376 | Denmark | 2 | Cultivar | 68.5 | n.a. | n.a. | n.a. | n.a. |
| BCC 1408 | UK | 2 | Cultivar | 68.5 | n.a. | n.a. | n.a. | n.a. |
| BCC 1405 | UK | 2 | Cultivar | 70.9 | n.a. | n.a. | n.a. | n.a. |
| BCC 852 | Canada | 6 | Cultivar | 73.2 | n.a. | n.a. | n.a. | n.a. |
| HOR 3941 | Israel | 2 | Breeding line | 73.2 | n.a. | n.a. | n.a. | n.a. |
| HOR 4060 | Israel | 2 | Cultivar | 73.2 | n.a. | n.a. | n.a. | n.a. |
| BCC 1431 | Austria | 2 | Cultivar | 75.5 | n.a. | n.a. | n.a. | n.a. |
| BCC 888 | Canada | 6 | Cultivar | 75.5 | n.a. | n.a. | n.a. | n.a. |
| BCC 903 | Canada | 2 | Cultivar | 75.5 | n.a. | n.a. | n.a. | n.a. |
| BCC 423 | China | 6 | Cultivar | 75.5 | n.a. | n.a. | n.a. | n.a. |

(Continued)
A total of 73 CGs were nominated based on previous transcript-association-genetic studies in barley and probably reflects the high two-rowed and six-rowed barley genotypes differs from other (Figure A3 in Appendix). The non-structuring of the data into with respect to observed resistance or row number (Figure 3A; Figure 3B). Genotypes from USA, which all represent breeding lines or cultivars, were grouped together with genotypes from East Asia and the Balkans, suggesting that US breeders used such material rather frequently in their pedigree. The grouping of European cultivars with Ethiopian landraces suggests some preferred use of this exotic germplasm by European breeders, possibly for the introgression of major R genes.

### Table 3 | Continued

| Accession | Origin   | Row number | Status of accession | % Inf. EU isol. | % Inf. Israel isol. | Resistance response | mlo-11 | Mlo compl. |
|-----------|----------|------------|---------------------|-----------------|---------------------|---------------------|--------|-----------|
| BCC 1450  | Finland  | 6          | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1430  | France   | 2          | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| HOR 2800  | Iran     | 6          | Landrace            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1468  | Kazakhstan | 2      | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 745   | Nepal    | 6          | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1420  | Netherlands | 2     | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1452  | Netherlands | 6     | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1488  | Russia   | 6          | Landrace            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1412  | Sweden   | 2          | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1404  | UK       | 2          | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 893   | USA      | 6          | Cultivar            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |
| BCC 1498  | Uzbekistan | 6     | Landrace            | 75.5            | n.a.                | n.a.                | n.a.   | n.a.      |

Scoring results below the median of class II (Altpeter et al., 2005) reflecting moderate to strong resistance are highlighted in bold.

1. Accession Nr. from IPK genebank.
2. Mean resistance to two polyvirulent German isolates (total 44 virulences).
3. Mean resistance to four Israeli isolates (total 18 virulences).
4. Microscopic analysis of attacked epidermal cells.
5. Complementation by bombarding epidermal cells with a BAC clone containing Mlo.
6. Used as resistance donor for crosses with susceptible cv Morex to establish F2-mapping populations.
7. H. vulgare ssp. Spontaneum.
8. Not analyzed.

(data not shown), in agreement with previous studies on quantitative resistance to Bgh that revealed a complex genetic setup of NR (e.g., Aghnoum et al., 2010). In summary, the QTL mapping of three selected barley genotypes exhibiting strong, race-non-specific seedling and adult-plant resistance to Bgh confirmed a polygenic mode of inheritance, as suggested by the phenotypic distribution of F2 progenies.

### ASSOCIATION OF ALLELIC VARIANTS WITH RESPONSE TO Bgh

A total of 73 CGs were nominated based on previous transcript-profiling and transient-induced gene silencing (TIGS) data, a literature survey for genes that may be relevant for NR in barley, and genetic map information. Five additional genes were selected as negative controls because they were described to be involved in other traits such as the number of seed rows per spike (“row number” throughout) or seed development (Table S1 in Supplementary Material). Out of a total of 78 re-sequenced genes, 9 did not yield high-quality sequence data and 8 were monomorphic. The remaining 61 polymorphic genes were analyzed for SNP and deduced haplotypes (Table 3). Sequence diversity with 1 SNP per 48 bp was similar to what has been reported before in a worldwide collection of cultivated barley (Haseneyer et al., 2010b).

Population structure and kinship were determined by using 42 SSR markers evenly distributed over the barley genome (Tables S2 and S3 in Supplementary Material). Principal-component analysis (PCA) of kinship revealed no clear structuring of the population with respect to observed resistance or row number (Figure 3A, Figure A3 in Appendix). The grouping of European cultivars with Ethiopian landraces suggested a high degree of genetic unrelatedness of members of the customized worldwide population (Haseneyer et al., 2010a). On the other hand, strong population structure was observed with respect to geographic origin (Figure 3B). Genotypes from USA, which all represent breeding lines or cultivars, were grouped together with genotypes from East Asia and the Balkans, suggesting that US breeders used such material rather frequently in their pedigree. The grouping of European cultivars with Ethiopian landraces suggests some preferred use of this exotic germplasm by European breeders, possibly for the introgression of major R genes.

Structured SNP-trait as well as haplotype–trait associations were calculated by using: a general-linear model; a general-linear model including row number as covariate; and a mixed-linear model (see Materials and Methods). Row number was included as a covariate into one model because of significant correlation between this trait and mean resistance to both Bgh isolates (Table S4 in Supplementary Material). The results of marker–trait associations from the general-linear model including population structure were highly similar to those from the mixed-linear model including both population structure and kinship, which indicates again a high degree of phylogenetic non-relatedness of members of the population (Johrde, 2010). Therefore, only results from the two general-linear models with or without row number as covariate are discussed here. Genes were assumed to be associated with a trait if Bonferroni-corrected $p$-values were smaller than 0.05. $p$-Values smaller than 0.01 were taken as sign of strong association with the trait. Bonferroni correction was based on the assumption that each polymorphic CG ($n = 87$ including some genes to be discussed elsewhere) represents 0 linkage block. For any set of randomly chosen markers and for any statistical model used in
 association genetics, the over-representation of spurious associations would result in a skewed distribution of p-values toward zero. In the perfect model for a given population, on the other hand, p-values would be expected to be randomly distributed between zero and one and lie on a diagonal when sorted. In order to test the accuracy of the selected models, we plotted sorted p-values of all SNP for traits “row number” and “Bgh interaction” (Figure 4). The distribution of p-values from both traits using the naïve model was clearly skewed toward zero, as expected in the presence of population structure producing spurious associations. Using either the general- or mixed-linear models that take into account population structure resulted in an approximated random p-value distribution for “row number” but not for “Bgh interaction.” This exactly meets the expectation because the CGs (and their SNP markers) were not selected at random but due to a priori knowledge about a function in NR or disease resistance in general. The same skewness toward significant p-values has also been observed in sets of CGs of A. thaliana for 107 observed traits (Atwell et al., 2010).

Figure 5 shows the result from analysis of structured association of SNPs and haplotypes with the traits “Bgh interaction” and “row number.” The fact that control gene Vrs1, a known factor determining row number in barley (Komatsuda et al., 2007) exhibited the strongest, significant association with “row number” indicates that the customized population, although relatively small, was suitable to detect marker–trait associations. A second CG (Contig5974_s_at) encoding HvWIR1a also showed highly significant association with row number. This may reflect genetic linkage with the Vrs2 gene for row number (Pourkheirandish and Komatsuda, 2007), which was discovered in a mutant population of barley and mapped ca. three centimorgans away from HvWIR1a in the consensus map “Barley, Integrated, Marcel 2009”1. A total of 11 CGs were found to be associated with the Bgh-interaction phenotype at SNP or haplotype level (see also Figure A4 in Appendix for graphical haplotypes). In most of the cases, association was also detected by using the general-linear model including row number as covariate. This indicates that the marker–trait associations were robust despite the fact that row number was correlated with resistance to Bgh.

The previously identified Mla gene for race-specific and mlo for race-NR of barley to Bgh were also included in the re-sequencing approach. In the case of Mla, a number of SNPs produced apparently heterozygotic calls, which most likely reflects PCR amplification of closely related Mla- and RGH1 genes in several genotypes, as previously described (Wei et al., 2002). Therefore, only SNPs with homozygous calls derived from either gene were used for the calculation of association with the response to Bgh. Neither Mla nor mlo was associated with the response to Bgh, irrespective of the statistical model used for the analysis (Table S1 in Supplementary Material).

CO-LOCALIZATION OF GENES WITH RESISTANCE QTL

The 11 CGs that were significantly associated with the Bgh-interaction phenotype were genetically mapped, and map positions projected onto the “Barley, Integrated, Marcel 2009” map containing 6990 markers (see text footnote 1). This integrated map includes five biparental populations that have also been used for mapping of resistance QTL to Bgh (Aghnoun et al., 2010). Eight out of 11 CGs co-localized with resistance QTL to Bgh that have been described over the last 20 years (Schweizer and Stein, 2011) indicating a significant ($\chi^2$, one-tailed $p = 0.0431$) over-representation (Figure 6). Thus, the associated CGs might be causally related to the QTL previously mapped to corresponding genome positions, or they reveal linkage to nearby causative genes within the QTL confidence intervals.

1http://wheat.pw.usda.gov/ggpages/map_shortlist.html
Table 2 | Mapping of resistance QTL against Bgh in three populations of resistant accessions x Morex.

| F2-Population        | Chr | cM   | Left Marker       | Right Marker       | LODa | Typeb | Effectc | FreqCVd |
|----------------------|-----|------|------------------|-------------------|------|-------|---------|---------|
| Morex-1xHOR3726-1    | 4H  | 8    | ge00201s01       | ge00290s01        | 3.0  | add   | ~6.5    | 0.03    |
| Morex-1xHOR3726-1    | 4H  | 113  | ge00196s01       | ge00212s01        | 4.5  | add   | ~16.4   | 0.7     |
| Morex-1xHOR3726-1    | 4H  | 113  | ge00196s01       | ge00212s01        | 4.5  | dom   | 10.1    | 0.7     |
| Morex-1xHOR3726-1    | 5H  | 96   | ge00129s01       | ge00374s01        | 4.9  | add   | ~13.0   | 0.46    |
| Morex-1xHOR3726-1    | 5H  | 96   | ge00129s01       | ge00374s01        | 4.9  | dom   | 0.0     | 0.46    |
| Morex-1xHOR3726-1    | 7H  | 17   | ge00317s01       | ge00180s01        | 7.7  | add   | ~28.2   | 0.69    |
| Morex-1xHOR3726-1    | 7H  | 17   | ge00317s01       | ge00180s01        | 7.7  | dom   | ~13.4   | 0.69    |
| Morex-2xHOR2932-2    | 1H  | 16   | ge00346s01       | ge00275s01        | 8.8  | add   | ~19.7   | 0.94    |
| Morex-2xHOR2932-2    | 1H  | 16   | ge00346s01       | ge00275s01        | 8.8  | dom   | 0.0     | 0.94    |
| Morex-2xHOR2932-2    | 3H  | 108  | ge00033          | ge00365s01        | 3.5  | add   | 6.6     | 0.02    |
| Morex-2xHOR2932-2    | 3H  | 155  | ge00146          | ge00227s01        | 3.1  | add   | 6.2     | 0.05    |
| Morex-2xHOR3271-2    | 2H  | 156  | ge00372s01       | ge00260s01        | 18.9 | add   | ~21.7   | 0.91    |
| Morex-2xHOR3271-2    | 2H  | 156  | ge00372s01       | ge00260s01        | 18.9 | dom   | ~15.5   | 0.91    |
| Morex-2xHOR3271-2    | 4H  | 111  | ge00023          | ge00212s01        | 5.3  | add   | ~5.2    | 0.08    |
| Morex-2xHOR3271-2    | 7H  | 22   | ge00180s01       | ge00384s01        | 4.0  | add   | ~9.3    | 0.25    |
| Morex-2xHOR3271-2    | 7H  | 22   | ge00180s01       | ge00384s01        | 4.0  | dom   | 0.0     | 0.25    |

| LOD, logarithm of odds, obtained by composite interval mapping. |
| Additive QTL effect; dom, dominant QTL effect. |
| QTL effect in percent of phenotypic variation. |
| Frequency in cross validation. |

Table 3 | Summary of polymorphic candidate genes used for association of allelic variants with traits by re-sequencing.

| Selection criterion        | Number of CG | Number of SNP (bp/SNP) | Number of haplotypes |
|----------------------------|--------------|------------------------|----------------------|
| TIGS, OEX, Transcr. profiling | 33           | 515 (42.2)             | 274                  |
| Evidence from literature   | 27           | 343 (60.3)             | 231                  |
| Negative control genes     | 1            | 7 (150.7)              | 8                    |
| Total                      | 61           | 866                    | 512                  |
| Average/gene               | 14.2 (47.8)  | 8.4                   |                      |

| CG, candidate gene (only polymorphic sequences taken into account). |

TRANSSCRIPT LEVELS OF ASSOCIATED CGs

The CGs significantly associated with Bgh-interaction phenotype are represented as oligonucleotides on a custom 44K barley transcript-profiling microarray (Chen et al., 2011) and were assessed for regulation of the corresponding transcripts in peeled barley epidermis attacked by either Bgh or the non-adapted wheat powdery mildew B. graminis f. sp. tritici (Bgt; Figure 7; Table S5 in Supplementary Material). Seven out of the 11 transcripts were either significantly up- or down-regulated upon pathogen attack, with no clear difference between host and non-host interaction. The strongest up-regulation was observed at 24 h.a.i., except for transcription factor HvWRKY2 that exhibited a peak of mRNA abundance at 12 h.a.i.

TIGS OF ASSOCIATED CGs

Ten RNAi silencing (TIGS) or over-expression constructs were obtained for the CGs significantly associated with Bgh interaction. These were used to test a phenotypic effect by TIGS or by transient over-expression in the barley-Bgh interaction. Because TIGS constructs were found to induce a weak but non-specific reduction of susceptibility of bombarded epidermal cells, data were tested for statistical significance using the median susceptibility index (normalized to corresponding empty-vector controls) of more than 1,000 constructs bombarded in different projects as more critical null-hypothetical value, rather than the value of the internal empty-vector control (Figure A5 in Appendix). Table 4 shows that TIGS of three candidates (HvWRKY2, vacuolar targeting receptor and an unknown protein) significantly increased resistance (reflected by reduced relative susceptibility index) whereas one (chorismate synthase) increased resistance upon transient over-expression. The rather low number of genes with a significant TIGS effect might be surprising because such an effect was one major initial selection criterion for our CGs. However, as discussed above, the initial set of candidates was compiled based on TIGS data that were tested with less stringent statistics. We cannot exclude, though, that the median-centered control caused false-negative results to some extent because (i) the obligate biotrophic pathogen Bgh might indeed be highly sensitive to
FIGURE 3 | Population stratification within the customized barley collection used for association of allelic variants with traits. (A) Principal component analysis (PCA) was performed by using 42 SSR markers. Minor SSR alleles (frequency < 0.05) were eliminated prior to analysis. For display of PC3 and 4 see Figure A3 in Appendix. (B) Population subgroups were determined as proposed by Evanno et al. (2005) by using the software STRUCTURE (Pritchard et al., 2000) and the same 42 SSR markers as for PCA. Green, accessions mostly from Near East and Central Asia; blue, accessions mostly from East Asia and USA; yellow, accessions mostly from Balkans and USA; red, accessions mostly from Europe and Ethiopia.

FIGURE 4 | Control for spurious associations of SNPs with two traits using four statistical models. p-Values of all identified SNPs are plotted in ascending order, and would lie on the diagonal if randomly distributed. Deviation of the distributions from the diagonal toward lower p-values indicates insufficiently corrected population structure or non-random selection of genes. No data for the trait “row number” using the general-linear model plus row number as covariate are shown because this caused statistical significance to collapse, as expected.

any RNAi-mediated disturbance of host–cell metabolism, homeostasis, or effector responsiveness, and because (ii) many of the candidates selected based on the preliminary TIGS data finally did obtain a CE score of three (out of a maximum of four) suggesting relevance for barley-Bgh interaction (see below).

CE SCORE FOR GENES AFFECTING Bgh INTERACTION

We assigned a CE score to the genes significantly associated with the Bgh interaction phenotype by assessing their localization inside QTL confidence intervals, their transcript regulation upon Bgh attack, and TIGS- or over-expression effects on resistance to Bgh (Table 5). This revealed one gene encoding HvWRKY2 (Con-tig4387_at) that obtained the maximum CE score of 4 and thus represents a prime candidate for future validation. Eight additional candidates were assigned a CE score of 3 and will also deserve further attention. The nine CGs with strong CE for a role in NR of barley to Bgh include five with a putative role in signal perception or defense- and cell-death regulation (HvWRKY2, Calreticulin, Cys-rich RLK, OPD-reductase, alpha/beta hydrolase) and one of each implicated in primary metabolism (chorismate synthase), transport (vacuolar targeting receptor), and stress response (HvWIR1a).

DISCUSSION

In this study we mined barley genetic resources for genes and alleles that may be relevant for NR to Bgh. Subsequently converging evidence for the identified genes was generated by integrating forward- and reverse-genetic datasets. As a result, 11 CGs were identified that exhibited significant SNP or haplotype associations with the Bgh-interaction phenotype being either associated with strong, race-non-specific seedling resistance or high susceptibility in a trait-customized worldwide collection of spring barley.

Several observations suggest that the seedling resistance to Bgh we selected for during the phenotypic screening of an initial set
FIGURE 5 | Candidate genes significantly associated with resistance to Bgh. Association of SNP and gene haplotypes was calculated for traits "Bgh interaction" and "row number" by using general-linear models that included population structure (Q-matrix, both traits) or population structure plus row number (RN) as covariate (only Bgh). The Vrs1 gene (highlighted in green) was included as positive control for association with row number. Genes highlighted by gray shading are located within confidence intervals of QTLs for resistance to Bgh. Please note that HvWIR1a showing also significant association with RN mapped close to RN gene Vrs2 (Pourkheirandish and Komatsuda, 2007). Yellow and orange shading indicates significant association with Bonferroni-corrected p < 0.05 and p < 0.01, respectively. Only the most significant SNP and haplotype per candidate gene and model is shown.

of 212 accessions was due to NR and distinct from race-specific resistance mediated by major R gene or from mlo-mediated race-NR: firstly, accessions likely to carry mlo-resistance alleles were excluded from the panel based on genotyping for mlo-11 and complementation by Mlo in a transient expression assay. Secondly, Mlo was extensively re-sequenced in the final population (almost the entire length of mRNA) without significant association to the "Bgh interaction" trait (Table S1 in Supplementary Material). Thirdly, re-sequencing of Mla did also not reveal significant association with "Bgh interaction," indicating that resistance was probably not...
caused by unexploited Mla alleles such as Mla16–Mla20 derived from H. vulgare ssp. spontaneum, against which virulence in European Bgh isolates might still be absent. Fourthly, testing of resistant accessions with four Bgh isolates from Israel collected near the center of diversity of H. vulgare ssp. spontaneum and differentiating between Mla alleles of wild barley, identified 14 genotypes to be at least partially resistant in a race-non-specific manner (Dreiseitl and Dinnoor, 2004). Finally, resistance of three F2 populations derived from crosses of resistant accessions (including two accessions exhibiting HR-type resistance) with cv Morex segregated in a quantitative manner indicating the action of several unlinked resistance QTL rather than major R genes. Indeed QTL mapping revealed 3–4 QTL in each of these populations. The sum of the additive QTL effects explained between 32.5 and 64.1% of the phenotypic variation in the individual populations suggesting a complex genetic setup of NR with additional loci of minor effect not detected here. Two of the resistance donors are cultivars (HOR 3726, cv “Nigrate” and HOR 3271, cv “Steffi”) and thus preferable for resistance-breeding purposes because they would introduce less linkage drag. Especially interesting in this respect would be the strong QTLs on the bottom of chromosome 2H and on the top of 7H explaining together 50% of phenotypic variation. Remarkably,
one of the CGs with a CE score of 3 encoding a calreticulin protein maps close to the QTL on the long arm of 2H. The donor of this QTL (HOR 3726) also carries the resistance-associated SNP12 in the calreticulin gene. Taken together, our results suggest the existence in cultivated barley of strong NR despite the presence of Mlo susceptibility alleles, which appears to be brought about by efficient allelic combinations of defense- or susceptibility-related host genes.

Unlike proposals by other studies of NR or mlo-mediated, race-NR of barley (Schulze-Lefert, 2004), most of the resistant genotypes did not exhibit papilla-associated penetration resistance but responded to Bgh isolates by triggering HR at early or later stages during the interaction. In the latter case, macroscopically visible, darkly pigmented leaf flecks became visible on Bgh-attacked leaves. The absence of rapidly acting (penetration) resistance is also reflected by substantial haustorium formation in Bgh-attacked epidermal peels (see also Figure 7).

We also tested further components of cell-death pathways such as bax-inhibitor 1 or EDS1 (Falk et al., 1999; Huckelhoven et al., 2003). Whereas EDS1 turned out to be monomorphic in the customized collection, Bax-inhibitor 1 might be also be associated with Bgh-interaction phenotype although the most strongly associated SNP [−Log(10)p = 2.9] was not significant after Bonferroni correction (p = 0.105). Several of the CGs associated with the Bgh-interaction phenotype or close paralogs had previously been discussed with respect to plant-pathogen interactions: the identical calreticulin (Contig1903_at) was found to be upregulated at the transcript level in Bgh-attacked barley leaves and to affect NR in transient over-expression and silencing experiments (Hu et al., 2009). The enzyme occupies an important branch point downstream of the shikimate pathway leading to the synthesis of the aromatic amino acids phenylalanine and tryptophan, precursors of lignin-like materials accumulating in attacked cell walls and indole alkaloids such as gramine exhibiting antimicrobial activity in barley, respectively (Matsuo et al., 2001). Another candidate, calreticulin (Contig1903_at), is the closest and second closest homolog to CRT1 and CRT3 of A. thaliana, respectively, that were reported as important components of defense-signaling during ETI and receptor-quality control during PTI (Saijo et al., 2009; Kang et al., 2010). Finally, a RLK containing a N-terminal cysteine-rich domain distinct from the DUF26-motif might be involved in PAMP perception (Jones and Dangl, 2006). Two genes (HvWIR1a and Cys-rich RLK) were found to co-localize inside the meta-QTL for resistance to Bgh on chromosome 5HS and both were significantly associated with the Bgh-interaction phenotype. This can be explained either by a complex genetic setup of the QTL with several underlying genes or by LD between the associated candidates and a nearby causative gene. We found that the Cys-rich RLK gene was not polymorphic between Dom and Rec, the parents of the OWB population that contributed one of the QTL to the meta-QTL and...
thus probably not responsible for the resistance QTL, at least not in this population. By contrast, different (rare) alleles of HvWIR1a are present in Dom and Rec leaving HvWIR1a as the more likely candidate for the causative gene for NR, as previously suggested (Douchkov et al., 2011). However, we neither found a significant TIGS effect of HvWIR1a nor of the Cys-rich RLK. The negative result of HvWIR1a silencing differs from a previously reported result (Douchkov et al., 2011) and can be attributed to the fact that we applied a more stringent statistical analysis here, as described above. In conclusion it appears quite possible that another, yet undiscovered gene located in the region is responsible for the observed, robust meta-QTL effect against Bgh. Unlike the initial assumption that association-genetic analyses result in narrower peaks of significant markers compared to biparental QTL analysis, due to many more meiosis events having broken down genetic linkage, genomic regions of markers significantly associated with a given trait can be wide and blurry, sometimes even without a clear maximum. This phenomenon has been discussed in terms of two factors confounding the analysis and sometimes prohibiting the identification of the causative genes: firstly, co-selection between nearby genes can occur if they together influence a given trait and secondly, population structure can cause “fixed” loci of low genetic diversity (Atwell et al., 2010). Therefore, saturating the resistance QTL confidence interval on barley chromosome 5HS with genes and performing systematic functional analysis to provide CE might be a more promising approach to identify the causative gene(s).

HvWRKY2, which was the only gene with maximum CE score in this study, was proposed earlier as an important factor of ETS as well as Mla10-mediated resistance (Shen et al., 2007). According to the current model HvWRKY2 is a transcriptional repressor of defense-related genes, and sequestration of the repressor by a Mla10/Avra10 complex results in the triggering of hypersensitive cell death (Shen et al., 2007). It remains open whether HvWRKY2 is directly targeted by an effector protein (e.g., AvrMla10) of Bgh triggering either its transcriptional up-regulation or its protein stabilization or activation. Here we report on natural allelic diversity of HvWRKY2 that was associated with Bgh-interaction phenotypes by unlocking worldwide barley genetic resources. This result lends the first direct genetic evidence that HvWRKY2 is indeed participating in plant–pathogen co-evolution, at least in the barley–Bgh system, and that valuable alleles might be identified and exploited for breeding. Interest-ingly, HvWRKY2 was one of the few examples where we could identify a resistance- (and not susceptibility-) associated haplotype that was defined by a non-synonymous exonic SNP (Figure A4 in Appendix).

To conclude, by searching for natural allelic diversity associated either with susceptibility or NR to Bgh in a trait-customized collection of barley genotypes we identified 11 genes that were further analyzed in an approach of converging evidence. This produced robust information for a number of candidates likely to explain at least part of the phenotypic variation observed in barley interacting with Bgh. Five out of nine top candidates with CE score of 3–4 (Cys-rich RLK, Calreticulin, OPD-reductase, HvWRKY2, chorismate synthase) have been implicated in PTI supporting the notion that the observed NR is based on PTI, despite the fact that resistance was primarily associated with cell-death responses and not papilla formation. We are currently using several of these candidates for marker-assisted introgression and outcrossing of favorable and unfavorable alleles, respectively, in elite spring barley lines suffering from susceptibility to the disease. This should allow conclusions about their value for improving durable and broadly acting resistance against Bgh – and maybe one or several other fungal diseases – by a knowledge-based approach.

MATERIALS AND METHODS

POWDERY MILDEW RESISTANCE SCORING

Barley plants used for association of allelic variants with traits were grown in 7 × 11 multipot trays containing compost soil from IPK nursery without fertilization in a greenhouse at 17–20°C with supplemental light from sodium halogen lamps to reach a photoperiod of 16 h. Hypervirulent B. graminis (DC.) E. O. Speer f. sp. hordei, isolates 78P and D12–12 were used for resistant screening and cultivated by weekly inoculation of 7-day-old seedlings of barley cv “Golden Promise.” Second leaves of 2-week-old seedlings were inoculated with Bgh 78P or D12–12 in a detached leaf assay, and Bgh symptoms were scored 7 days after inoculation as described (Altpeter et al., 2005). For resistance scoring of segregating F2 populations, isolate CH4.8 was used, and disease severity on second leaves was determined by estimating the percentage of the area covered by pustules (rounded to the nearest multiple of 10, e.g., 30 or 70%). Additional inoculation experiments in the detached leaf assay were performed with four powdery mildew isolates (H-148, H-289, Y-035, and Y-069) from Israel described as virulent against different Mla alleles present in wild barley H. vulgare ssp. spontaneum (Dreiseitl and Dinoor, 2004). Powderly mildew symptoms were also rated on plants grown under high infection pressure in double rows in the field at one location (KWS LOCHOW GmbH, Wohlde, Germany) in years 2007 and 2008.

SCREENING FOR mlo-ALLELES

Segments of second leaves of 14-day-old powdery mildew-resistant accessions were co-bombarded with a mixture of GUS-reporter plasmid pUbiGUS (Schweizer et al., 1999) and BAC F15, carrying wildtype Mlo (Buschges et al., 1997). Two days after the bombardment leaf segments were inoculated with Bgh isolate D12–12 at a density of approx. 60 conidia mm⁻², and haustoria in GUS-stained (transformed) epidermal cells were counted 48 h after infection. The susceptibility index was calculated as (ΣGUS-stained epidermal cells containing at least one haustorium/ΣGUS-stained epidermal cells). Each accession was bombarded with or without BAC F15, and complementation of susceptibility by a factor of at least five was taken as indication of mlo-mediated resistance. For genotyping of mlo-11 isolated DNA of powdery mildew-resistant accessions was used for PCR-based screening as described by (Piffanelli et al., 2004).

INHERITANCE OF RESISTANCE – F2-ANALYSIS

Crosses between resistant accession and susceptible cultivar Morex were performed using Morex as female parent. Ten-50 single F1 plants were self pollinated to establish F2 populations. One to two F2 populations per cross, each containing 108 F2 individuals, were
used for phenotyping, and 92 individuals per population were used for QTL mapping.

**QTL MAPPING**

Genetic linkage maps were generated using JoinMap 3.0 (Van Ooijen and Voorrips, 2001) assuming Haldane’s mapping function (Haldane, 1919). Markers were assigned to linkage groups at logarithm of odds (LOD) ≥ 3.0 with a maximum recombination fraction of 0.4. The maps in the respectively mapping populations were developed based on the “barley VeraCode (Illumina) 1” set of 384 highly polymorphic SNPs derived from barley OPA1 and OPA2 marker sets (Close et al., 2009).

Quantitative trait loci analysis was performed using composite interval mapping (CIM) with the program package PLABMQTL QTL (Utz and Melchinger, 1996). For detection of QTL LOD threshold was set to 3.0. After QTL detection, critical LOD scores were determined for all traits in all populations based on 1,000 permutations (α = 10%) as recommended by Churchill and Doerge (1994).

**CG Re-SEQUENCING AND DETERMINATION OF POLYMORPHISMS**

Genomic isolation was done using the “DNeasy 96 Plant Kit” (Qiagen, Hilden, Germany) in a collection of 61 spring barley genotypes that differed strongly in susceptibility or resistance to Bgh. DNA Fragments of CGs were amplified by PCR from genomic DNA. PCR reactions contained (in a volume of 10 μl) 30 ng DNA template, 1 μM each of forward and reverse primer (Table S6 in Supplementary Material), 5 μl Taq PCR master mix (Qiagen, Hilden, Germany), and were used for 40 cycles at 94/97°C (30 s at each temperature; N, annealing temp.), followed by a final elongation step at 72°C for 7 min. PCR Fragments were purified using PCR clean-up NucleoFast® 96PCR plates (Macherey-Nagel, Düren, Germany) before Sanger sequencing on an ABI 3730 instrument. Lasergene7/SeqMan (DNAstar Inc., Madison, USA) software was used for sequence analysis. SCF Trace files of sequences were aligned and SNPs were identified. All identified SNP and the corresponding haplotypes were exported to Excel tables.

**POPULATION STRUCTURE AND KINSHIP MATRIX**

For the detection of population structure, 42 SSR markers equally distributed over the seven barley chromosomes were used (Haseneyer et al., 2010a). The resulting SSR allelic patterns were used to calculate the Q-matrix for population stratification by STRUCTURE 2.1 software (Pritchard et al., 2000). The admixture model was used and burn in of 1,000,000 followed by 5,000,000 iterations. The most likely number (K) of subgroups was chosen on the basis of second-order-rate change of the likelihood function as described by Evanno et al. (2005). K-matrix for kinship was determined by using the software package SPAGeDi1.2 (Hardy and Vekemans, 2002). Negative kinship values were set to zero following Yu et al. (2006).

**ASSOCIATION OF ALLELIC VARIANTS WITH TRAITS**

Association of SNP and haplotypes with traits “Bgh interaction” and “row number” was calculated by using the TASSEL 2.0.1 software and two general-linear models (GLM1 = genotype + Q + trait; GLM2 = genotype + Q + row_number + trait). GLM2 was not used for trait “row number.” In addition, a mixed-linear model was calculated (MLM = genotype + Q + K + trait) but data are not shown here because they deviated only very slightly from GLM1.

**CALCULATION OF LD**

Linkage disequilibrium between loci was calculated using the TASSEL 2.0.1 software with 1000 permutations. For LD-description, significance level (p-value) and correlation coefficient (R²-value) were chosen.

**GENETIC MAPPING OF CGs**

Genetic map positions were derived from the single “QSM” population of Q21861 × SM89010 (Moscou et al., 2011), or from consensus maps “Barley, OPA23-2008, Consensus” (Close et al., 2009), “Barley, Integrated, Marcel 2009” (Aghnoum et al., 2010), and in silico mapping by synteny-based gene-order prediction in grasses (Mayer et al., 2011). All map positions of CGs were projected onto the “Barley, Integrated, Marcel 2009” map as described (Schweizer and Stein, 2011), except for positions derived from the “QSM” population: here local offset of approximately 4.5 cM was manually corrected by using a number of common markers with “Barley, Integrated, Marcel 2009” at the QTL region on chromosome 5HS.

**TRANSIENT-INDUCED GENE SILENCING**

cDNA Fragments of approximately 500bp were PCR amplified from barley EST clones by using primers 5‘-GGG GCA CTC GTC TCC GGG and 5‘-TGA ATT AAA ATT TCT TTT CTG AAC C (Contig4387_at); 5‘-CTC ACT AAA GGG AAC AAA AGC TGG AG and 5‘-CCC GAA CAT ATC GCT CGT A (Contig12036_at); 5‘-CTC ACT AAA GGG AAC AAA AGC TGG AG and 5‘-GGC CAG GTA CAT AAC GAA CG (Contig4636_at); 5‘-GAT TGA TCA GTA CTT AAA GG and ATC GGA GAG GTA GAA AGT AT (Contig5146_at); 5‘-TTG TCA GAG CAC GTT ATC AT and 5‘-ACA TCT GAA TAC AGG ATC ATC CAT AAC TGG AG and 5‘-TGG-3‘ (Contig5108_s_at); 5‘-CTC ACT AAA GGG AAC AAA AGC TGG AG and 5‘-TCT TGC TTT CCA AAA GGA TCA ACA G (Contig2169_at); 5‘-CTC ACT AAA GGG AAC AAA AGC TGG AG and 5‘-GAG TAT AGG CCG CCC GTT C (Contig5974_s_at); 5‘-GAG GGA GCA GTT ATC CAT CT and 5‘-CTG TGG AAA TTC TCT AAA CGA (Contig21490_at); 5‘-TGG TCA ATT TTA TAC CAC ACA GG and TTC CAT TGG TGG TAC ATC TTT ACA A (Contig2354_at). The resulting DNA fragments were used for the generation of RNAi constructs, and TIGS experiments were performed by particle bombardment as described (Douchkov et al., 2005). Three days after the bombardment leaf segments were inoculated with Bgh isolate CH4.8 at a density of 150–200 conidia mm⁻², and GUS-stained (transformed) epidermal cells as well as haustoria-containing transformed (susceptible) cells were counted 48 h after inoculation. The susceptibility index was calculated relative to the empty-vector control pIPKTA30, and values

2http://www.maizegenetics.net/
were log(2)-transformed in order to normalize their distribution for statistical analysis by a one-sample t-test. This test was performed against the hypothetical relative susceptibility-index value of 0.355 corresponding to the observed median of more than 1,000 RNAi constructs and reflecting a non-specific TIGS effect.

**TRANSCRIPT-PROFILING**

Seven-day-old barley plants of cv. Vada were inoculated with Bgh (isolate CH4.8) and Bgt (Swiss field isolate FAL92315), and the abaxial epidermis of inoculated primary leaves or from non-inoculated control leaves was peeled at 6–74 h after inoculation, as described (Zellerhoff et al., 2010). Total RNA was extracted by using the RNassey plant mini kit (Qiagen, Hilden, Germany), checked for quantity and quality by using an Agilent Bioanlyser 2100 (Agilent Technologies Inc.), and hybridized to a 44K Agilent oligonucleotide array as described (Chen et al., 2011). Single-channel array processing was utilized followed by data normalization with default parameters, and significant transcript-regulation events were determined by using GeneSpring GX (v11.5.1) software (Agilent technologies Inc.). Transcripts were assumed to be significantly regulated if p-values corrected for false-positive rate (FDR, Benjamini–Hochberg method) were smaller than 0.05. All primary data of the analyzed CGs are shown in Table S5 in Supplementary Material.

**CE SCORE**

The CE score of selected CGs was obtained by assigning each of the following statistically significant results one point, and by adding up points: SNP or haplotype association with the trait “Bgh response”; localization inside QTL confidence interval for resistance to Bgh; transcript regulation in epidermal peels; and altered Bgh interaction upon TIGS or transient over-expression.

**REFERENCES**

Agnhoun, R., Marcel, T. C., Johrde, A., Pecchioni, N., Schweizer, P., and Niks, R. E. (2010). Basal host resistance of barley to powdery mildew: connecting quantitative trait loci and candidate genes. Mol. Plant Microbe Interact. 23, 91–102.

Alt peter, F., Varshney, A., Abderhalden, O., Douchikov, D., Sautter, C., Kume lehn, J., Dudler, R., and Schweizer, P. (2005). Stable expression of a defense-related gene in wheat epidermis under transcriptional control of a novel promoter confers pathogen resistance. Plant Mol. Biol. 57, 271–283.

Atwell, S., Huang, Y. S., Vilhjalmsson, B. J., Willems, G., Horton, M., Li, Y., Meng, D. Z., Platt, A., Tarone, A. M., Hu, T. T., Jiang, R., Muliyati, N. W., Zhang, X., Amer, M. A., Baxter, I., Brachi, B., Chory, J., Dean, C., Debieu, M., de Meaux, J., Ecker, J. R., Faure, N., Kniskern, J. M., Jones, J. D. G., Michael, T., Nemri, A., Roux, F., Salt, D. E., Tang, C. L., Todesco, M., Traw, M. B., Weigel, D., Marjoram, P., Borevitz, J. O., Bergelson, J., and Nordborg, M. (2010). Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465, 627–631.

Bindschedler, L. V., Burgis, T. A., Mills, D. J. S., Ho, J. T. C., Cramer, R., and Spand, P. U. (2009). In planta proteomics and proteogenomics of the biotrophic barley fungal pathogen Blumeria graminis f. sp. hordei. Cell. Proteomics 8, 2368–2381.

Brown, J. K. M., Simpson, C. G., and Wolfe, M. S. (1993). Adaptation of barley powdery mildew populations in England to varieties with 2 resistance genes. Plant Pathol. 42, 108–115.

Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., Van, D. R., Van, D. L. T., Diergarde, P., Groenendijk, J., Tossch, S., Vos, P., Salamini, F., and Schulze, L. P. (1997). The barley Mlo gene: a novel control element of plant pathogen resistance. Cell 88, 695–705.

Cao, A. H., Xing, L. P., Wang, X. Y., Yang, X. M., Wang, W., Sun, Y. L., Qian, C., Ni, J. L., Chen, Y. P., Liu, D. I., Wang, X., and Chen, P. D. (2011). Serine/threonine kinase gene StpK-V, a key member of powdery mildew resistance gene Pm21, confers powdery mildew resistance in wheat. Proc. Natl. Acad. Sci. U.S.A. 108, 7727–7732.

Chen, H., Hedley, P. E., Morris, J., Liu, H., Niks, R. E., and Waugh, R. (2011). Combining genetic genomics and bulked segregant analysis-based differential expression: an approach to gene localization. Theor. Appl. Genet. 122, 1375–1383.

Churchill, G. A., and Doerge, R. W. (1994). Empirical threshold values for quantitative trait map-ping. Genetics 138, 963–971.

Close, T. J., Bhat, P. R., Lonardi, S., Wu, Y. H., Rostoks, N., Ramsay, L., Druka, A., Stein, N., Svenson, J. T., Wananmaker, S., Boudag, S., Roose, M. L., Moscou, M. J., Chao, S. A. M., Varshney, R. K., Szucs, P., Sato, K., Hayes, P. M., Matthews, D. E., Klein-hofs, A., Muehlbauer, G. J., De Yongou, J., Marshall, D. F., Madishetty, K., Fenton, R. D., Condamine, P., Graner, A., and Waugh, R. (2009). Development and implementation of high-throughput SNP genotyping in barley. BMC Genomics 10, 582. doi:10.1186/1471-2164-10-582.

Collins, N. C., Sadanandom, A., and Schulze-Lefert, P. (2002). “Genes and molecular mechanisms controlling powdery mildew resistance in barley,” in The Powdery Mildews eds R.

**ACKNOWLEDGMENTS**

We would like to acknowledge excellent technical support by Manuela Knauff and Jenny Morris. We thank Drs. Amos Dinoor, Kerstin Flath and Adrian Broda for the kind gift of Bgh isolates and for performing field trials. We are grateful to Malthe Schmidt, KWS LOCHOW GMBH for kind help during QTL analysis. This work was supported by project BIOEXPLOIT (EU FP6, to Patrick Schweizer and Viktor Korzun).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Plant-Microbe_Interaction/10.3389/fpls.2011.00113/abstract

Table S1 | Summary of association of allelic variants of polymorphic CGs with the Bgh-interaction phenotype and with row number.

Table S2 | Kinship (K) matrix of the customized barley collection based on 42 SSR markers. Software SPAGeDi1.2 (Hardy and Vekemans, 2002) was used to calculate kinship.

Table S3 | Structure (Q) matrix of the customized barley collection based on 42 SSR markers. Software STRUCTURE (Pritchard et al., 2000) was used to calculate population structure.

Table S4 | Correlation between row number of barley genotypes and resistance to Bgh.

Table S5 | Normalized and log(2) transformed signal intensity data (single-channel analysis) of selected transcripts corresponding to genes associated with resistance to Bgh. RNA was extracted from epidermal peels of barley after inoculation with either Bgh or the non-host pathogen B. graminis f. sp. tritici (Bgt).

Table S6 | Primers used for the re-sequencing of CGs. Please note that the lower case letters correspond to adapters containing T3 or T7 primer binding sites used for Sanger sequencing of amplified DNA fragments on an ABI 3730 sequencer.
R. Belanger, W. R. Bushnell, A. J. Dick, and T. L. W. Carver (St. Paul: APS Press), 134–145.

Dietrich, R. A., Richberg, M. H., Schmidt, R. Dean, C., and Dangl, J. L. (1997). A novel zinc finger protein is encoded by the Arabidopsis Lsd1 gene and functions as a negative regulator of plant cell death. Cell 88, 685–694.

Douchkov, D., Johorde, A., Nowara, D., Himmelbach, A., Luend, S., Niks, R., and Schweiz, P. (2011). Convergent evidence for a role of WRI1 proteins during the interaction of barley with the powdery mildew fungus Blumeria graminis. J. Plant Physiol. 168, 20–29.

Douchkov, D., Nowara, D., Zierold, U., and Schweiz, P. (2005). A high-throughput genotyping system for the functional assessment of defense-related genes in barley epidermal cells. Mol. Plant Microbe Interact. 18, 755–761.

Dreiselt, A., and Dinoor, A. (2004). Phenotypic diversity of barley powdery mildew resistance sources. Genet. Resour. Crop Evol. 51, 251–257.

Druka, A., Potokina, E., Luo, Z. W., Jiang, N., Chen, X. W., Kearsey, M., and Waugh, R. (2010). Expression quantitative trait loci analysis in plants. Plant Biotechnol. J. 8, 10–27.

Elliot, C., Zhou, F. S., Spielmenyer, W., Panstruga, R., and Schulze-Lefter, F. (2002). Functional conservation of wheat and rice Mlo orthologs in defense modulation to the powdery mildew fungus. Mol. Plant Microbe Interact. 15, 1069–1077.

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Falk, A., Feuillet, C., Langridge, P., and Waugh, R. (2008). Cereal breeding takes a walk on the wild side. Trends Genet. 24, 24–32.

Godfrey, D., Bohlenius, H., Peder sen, C., Zhang, Z. G., Emmens er, J., and Thordal-Christensen, H. (2010). Powdery mildew fungal effector candidates share N-terminal Y/F/WxC-motif. BMC Genomics 11, 317. doi:10.1186/1471-2164-11-317.

Haldane, J. B. S. (1919). The calculation of distances between the loci of linked factors. J. Genet. 9, 99–102.

Hardy, O. J., and Vekemans, X. (2002). SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2, 618–620.

Haseney, G., Stracke, S., Paul, C., Einfeld, C., Broda, A., Piepho, H. P., Graher, A., and Geiger, H. H. (2010a). Population structure and phenotypic variation of a spring barley world collection set up for association studies. Plant Breed. 129, 271–279.

Haseney, G., Stracke, S., Piepho, H. P., Sauer, S., Geiger, H. H., and Graher, A. (2010b). DNA polymorphisms and haplotype patterns of transcribed genes involved in barley endospore development are associated with key agronomic traits. BMC Plant Biol. 10, 5. doi:10.1186/1471-2229-10-5.

Haudry, A., Cenci, A., Ravel, C., Bataillon, T., Brunel, D., Poncet, C., Hochu, L., Poirier, S., Santoni, S., Glemm, S., and David, J. (2007). Grindig up wheat: a massive loss of nucleotide diversity since domestication. Mol. Biol. Evol. 24, 1506–1517.

Hu, P. S., Meng, Y., and Wise, R. P. (2009). Functional contribution of chiormate synthase, anthranilate synthase, and crominate mutase to penetration resistance in barley-powdery mildew interactions. Mol. Plant Microbe Interact. 22, 311–320.

Huckelhoven, R. (2007). Cell wall–associated mechanisms of disease resistance and susceptibility. Annu. Rev. Phytopathol. 45, 101–127.

Huckelhoven, R., Deichert, C., and Kogel, K. H. (2005). Overexpression of barley BAX inhibitor 1 induces breakdown of mlo-mediated penetration resistance to Blumeria graminis. Proc. Natl. Acad. Sci. U.S.A. 102, 5535–5540.

Hyten, D. L., Song, Q. J., Zhu, Z. F., Huang, H. Y., Liu, Y., Hu, S. N., and Sun, C. Q. (2009). Patterns of nucleotide diversity in wild and cultivated rice. Plant Syst. Evol. 281, 97–106.

Matsuo, H., Taniguchi, K., Hiramoto, T., Fujimura, T., Matsuoka, M., Matsumoto, T., and Yano, M. (2007). Six-rowed barley originated from a mutation in a homedomain-leucine zipper 1-class homeobox gene. Proc. Natl. Acad. Sci. U.S.A. 104, 1424–1429.

Kou, Y. J., and Wang, S. P. (2010). Broad-spectrum and durability: underst anding of quantitative disease resistance. Curr. Opin. Plant Biol. 13, 181–185.

Li, X. R., Tan, L. B., Zhu, Z. F., Huang, H. Y., Liu, Y., Hu, S. N., and Sun, C. Q. (2009). Patterns of nucleotide diversity in wild and cultivated rice. Plant Syst. Evol. 281, 97–106.

Matsuoka, H., Taniguchi, K., Hiramoto, T., Yamada, T., Ichinose, Y., Toyoda, K., Takeda, K., and Shiraiishi, T. (2001). Gramine increase associated with rapid and transient systemic resistance in barley seedlings induced by mechanical and biological stresses. Plant Cell Physiol. 42, 1103–1111.

Mayer, R. F. X., Martis, M., Hedly, P. E., Simkova, H., Liu, H., Morris, I. A., Steuer, B., Bau dien, S., Roessner, S., Gundlach, H., Kukalova, M., Suchankova, P., Murat, E., Felder, M., Nuss baumer, T., Graher, A., Salse, J., Endo, T., Sakai, H., Tanaka, T., Itoh, T., Sato, K., Pfister, M., Matsumoto, T., Scholz, U., Doele, J., Waugh, R., and Stein, N. (2011). Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23, 1249–1263.

Moscou, M. J., Lauter, N., Caldo, R. A., Nettleton, D., and Wise, R. P. (2011). Quantitative and temporal definition of the MrA transcriptional regulon during barley-powdery mildew interactions. Mol. Plant Microbe Interact. 24, 694–705.

Nowari, L., and Lehmann, C. O. (1972). 14. Prüfung von Sommergersten auf ihr Verhalten gegen Mehltau (Erysiphe graminis fsp. hordei March.) Kulturpflanze 20, 283–296.

Nowari, L., and Lehmann, C. O. (1973). 17. Prüfung von Sommergersten auf ihr Verhalten gegen Mehltau (Erysiphe graminis DC. f. sp. hordei Marchal). Kulturpflanze 21, 275–294.

Nowari, L., and Mansfeld, R. (1955). 1. Prüfung von Sommergersten auf ihr Verhalten gegen Erysiphe graminis DC. f. sp. hordei Marchal. Kulturpflanze 3, 105–113.

Panstruga, R., Parker, J. E., and Schulze-Lefter, P. (2009). SnapShot: plant immune response pathways. Cell 136, U978–U978.

Piffanelli, P., Ramsay, L., Waugh, R., Benabdelmoumen, O., D’Hont, H., Hollrlikr, C., Jorgensen, J. H., Schulze-Lefter, P., and Panstruga, R. (2004). A barley cultivation-associated polymorphism conveys resistance to powdery mildew. Nature 430, 887–891.

Pourkheirandish, M., and Komatsuda, T. (2007). The importance of barley genetics and domestication in a global perspective. Ann. Bot. 100, 999–1008.

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multi-locus genotype data. Genetics 155, 945–959.

Reinbothe, C., Springer, A., Samol, L., and Reinbothe, S. (2009). Plant oxylipins: role of jasmonic acid during programmed cell death, defence and leaf senescence. FEBS J. 276, 4666–4681.

Rostoks, N., Ramsay, L., MacKenzie, K., Cardle, L., Bhat, P. R., Roose, M. L., Svensson, J. T., Stein, N., Varshney, R. K., Marshall, D. E., Graher, A., Close, T. J., and Waugh, R. (2006). Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc. Natl. Acad. Sci. U.S.A. 103, 18656–18661.

Rumbeug-Roos, P., and Saarma, M. (1998). Phytoalexin, a barley vacuolar aspartic proteainase, is highly expressed during autolysis of developing trachey cells and sieve cells. Plant J. 15, 139–145.

www.frontiersin.org
Race-non-specific pathogen resistance of barley

Saijo, Y., Tintor, N., Lu, X. L., Rauf, P., Pajerowska-Mukhtar, K., Haweker, H., Dong, X. N., Robotzki, S., and Schulze-Lefert, P. (2009). Receptor quality control in the endoplasmic reticulum for plant innate immunity. *EMBO J.* 28, 3439–3449.

Sato, K., Nankaku, N., and Takeda, K. (2009). A high-density transcript linkage map of barley derived from a single population. *Heredity* 103, 110–117.

Schulte, D., Close, T. J., Graner, A., Langridge, P., Matsumoto, T., Muehlbauer, G., Sato, K., Schulman, A. H., Waugh, R., Wise, R. P., and Stein, N. (2009). The international barley sequencing consortium-at the threshold of efficient access to the barley genome. *Plant Physiol.* 149, 142–147.

Schulze-Lefert, P. (2004). Knocking on heaven’s wall: pathogenesis of and innate immunity. *Curr. Opin. Plant Biol.* 7, 377–383.

Schweizer, P., Pokorny, J., Abderhalden, O., and Dudler, R. (1999). A transient assay system for the functional assessment of defense-related genes in wheat. *Mol. Plant Microbe Interact.* 12, 647–654.

Schweizer, P., and Stein, N. (2011). Large-scale data integration reveals co-localization of gene functional groups with meta-QTL for multiple disease resistance in barley. *Mol. Plant Microbe Interact.* 24, 1492–1501.

Shen, Q. H., Saijo, Y., Mauch, S., Biskup, C., Biert, S., Keller, B., Seki, H., Ulker, B., Somssich, I. E., and Schulze-Lefert, P. (2007). Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* 315, 1098–1103.

Spanu, P. D., Abbott, J. C., Arseme, J., Burgis, T. A., Soanes, D. M., Stuber, K., van Themaat, E. V. L., Brown, J. K. M., Butcher, S. A., Gurr, S. J., Lebrun, M. H., Ridout, C. J., Schulze-Lefert, P., Talbot, N. J., Ahmadinejad, N., Armet, C., Barton, G. R., Benjdia, M., Bidzinski, P., Bindschedler, L. V., Both, M., Brewer, M. T., Cadle-Davidson, L., Cadle-Davidson, M. M., Collemare, J., Cramer, R., Frenkel, O., Godfrey, D., Harriman, J., Hoede, C., King, B. C., Klages, S., Kleemann, J. Knoll, D., Koti, P. S., Kreplak, J., Lopez-Ruiz, F. J., Lu, X. L., Maekawa, T., Mahani, S., Micali, C., Milgroom, M. G., Montana, G., Noir, S., O’Connell, R. I., Oberhaensli, S., Parlang, F. Pedersen, C., Quesneville, H., Reinhardt, R., Rott, M., Sacristan, S., Schmidt, S. M., Schon, M., Skamnioti, P., Sommer, H., Stephens, A., Takahara, H., Thordal-Christensen, H., Vigouroux, M., Wesling, R., Wicker, T., and Panstruga, R. (2010). Genome expansion and gene loss in powdery mildew fungi reveal trade-offs in extreme parasitism. *Science* 330, 1543–1546.

Thomma, B., Nurnberger, T., and Joosten, M. (2011). Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* 23, 4–15.

Utz, H. F., and Melchinger, A. E. (1996). PLABQTL a program for composite interval mapping of QTL. *J. Agric. Genet.* 2, 1–5.

Van Ooijen, J. W., and Voorrips, R. E. (2001). JoinMap® 3.0, *Software for the Calculation of Genetic Linkage Maps*. Wageningen: Plant Research International.

Waugh, R., Jannink, J. L., Muehlbauer, G. J., and Ramsay, L. (2009). The emergence of whole genome association scans in barley. *Curr. Opin. Plant Biol.* 12, 218–222.

Wei, F. S., Wong, R. A., and Wise, R. P. (2002). Genome dynamics and evolution of the Mla (powdery mildew) resistance locus in barley. *Plant Cell* 14, 1903–1917.

Wise, R. P., Lauter, N., Szabo, L. J., and Schweizer, P. (2009). “Genomics of biotic interactions in the Triticeae,” in *Genetics and Genomics of the Triticeae*, eds G. J. Muehlbauer and C. Schweizer. *Plant Physiol.* 152, 2053–2066.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 October 2011; accepted: 22 December 2011; published online: 10 January 2012.

Citation: Spies A, Korzun V, Bayles R, Rajaraman J, Himmelbach A, Hedley PE and Schweizer P (2012) Allele mining in barley genetic resources reveals genes of race-non-specific powdery mildew resistance. *Front. Plant Sci.* 2:113. doi: 10.3389/fpls.2011.00113

This article was submitted to Frontiers in Plant-Microbe Interaction, a specialty of Frontiers in Plant Science. Copyright © 2012 Spies, Korzun, Bayles, Rajaraman, Himmelbach, Hedley and Schweizer. This is an open-access article distributed under the terms of the Creative Commons Attribution NonCommercial License, which permits non-commercial use, distribution, and reproduction in other forums, provided the original authors and source are credited.
## APPENDIX

| Barley differential | Resistance gene(s) | Disease rating |
|---------------------|--------------------|----------------|
|                     |                    | P78 | D12/12 |
| Hord. spontaneum    | Mla16              | 0   | 0     |
| Hord. spontaneum    | Mla17              | 1-2 | 2     |
| Hord. spontaneum    | Mla18              | 0   | 0     |
| Hord. spontaneum    | Mla19              | 0   | 0     |
| Hord. spontaneum    | Mla20              | 1-2 | 0     |
| P13                 | Mla23              | 2   | 2     |
| Hord. spontaneum    | Mla26              | 1-2 | 0     |
| Hord. spontaneum    | Mfi                | 2   | 1     |
| Apex                | mlo11, Mlg, Ml(CP) | 0   | 0     |
| P22                 | mlo5               | 0   | 0     |
| Alexis              | mlo9               | 0   | 0     |
| Aura                | Mla6, Mla14, Mlg, Ml(CP) | 3 | 0 |
| Meltan              | U, Mla13, Ml(Imp8), Ml(Hu4) | 3 | 0 |
| P12                 | Mla22              | 0   | 3     |
| Gunnar              | Mla3, Ml(Tu2)      | 0   | 2-3   |
| Hord. spontaneum    | Mla28              | 2-3 | 0     |
| Hord. spontaneum    | Mla27              | 0   | 4     |
| Olga                | U                  | 4   | 0     |
| P03                 | Mla6, Mla14        | 4   | 0     |
| P06                 | Mla7, Ml(LG2)      | 4   | 0     |
| P08B                | Mla9               | 4   | 0     |
| P09                 | Mla10, Ml(Du2)     | 4   | 0     |
| P11                 | Mla13, Ml(Ru3)     | 4   | 0     |
| Teo                 | Mla7, Mlg, Ml(CP), Mlk, MLa | 3 | 1 |
| Goldi               | U, Mla12, MlLa     | 3-4 | 0     |
| Lerche              | Mlg, Ml(CP), Mla7  | 3   | 2     |
| P01                 | Mla1               | 1-2 | 3     |
| P02                 | Mla3               | 1-2 | 3     |
| Bonwina             | Ml(BW1,2)          | 2-3 | 2     |
| Amazone             | Mlg, U             | 2   | 4     |
| Camilia             | U                  | 2   | 4     |
| P21                 | Mlg, Ml(CP)        | 3   | 3     |
| Maresi              | Mla12, Ml(AB), Mlg, Ml(CP) | 2-3 | 3 |
| P15                 | Ml(Ru2)            | 2-3 | 3     |
| P20                 | Mlat               | 2   | 3-4   |
| Thuringia           | Ml(S1,2), U        | 2   | 3-4   |
| Hord. spontaneum    | Mlj                | 2-3 | 2-3   |
| Stefi               | Ml(S1,2)           | 1-2 | 3-4   |
| Katharina           | U                  | 3   | 4     |
| P18                 | Mlnn               | 4   | 3     |
| P23                 | Ml(La)             | 4   | 3     |
| Kredit              | Ml(Kr)             | 4   | 2-3   |
| Marinka             | Mlg, Ml(CP), Mla7  | 4   | 2-3   |
| P17                 | Mlk                | 4   | 2-3   |
|etta                | Ml(AB)             | 3-4 | 3     |
| P19                 | Mlp                | 2-3 | 3-4   |
| Banteng             | Ml(Ba)             | 4   | 4     |
| P04 B               | Mla7, U            | 4   | 4     |
| P10                 | Mla12              | 4   | 4     |
| Dura                | Ml(Dr)             | 4   | 3-4   |
| Hellas              | Ha                 | 4   | 3-4   |
| P14                 | Mlra               | 4   | 3-4   |
| P24                 | Mlh                | 4   | 3-4   |
| Sissys              | Mla12, Mlg, Ml(CP) | 4   | 3-4   |
| Trumpf              | Mla7, Ml(AB)       | 4   | 3-4   |

**FIGURE A1** | Virulence spectrum of two polyvirulent German *Bgh* isolates. Disease symptoms were scored from 0 (resistant) to 4 (fully susceptible) using the scoring system as described (Jensen et al., 1992).
FIGURE A2 | Genotyping of resistant accessions for the presence of the *mlo-11* allele mediating resistance to *Bgh*. Presence of *mlo-11* results in a PCR fragment of 1100 bp (dominant marker, highlighted by red circles).
FIGURE A3 | Population structure determined by PCA of SSR data (42 SSR markers). PC3 and 4 are shown, for PC1 and 2 see Figure 3.
FIGURE A4 | Continued
FIGURE A4 | Continued
FIGURE A4 | Graphical SNP- and haplotype tables of genes significantly associated with resistance to Bgh. Amino-acid changes caused by non-synonymous SNP or in/dels located within coding regions of CGs are shown below tables. Vrs1 is shown as example of the gene most significantly associated with row number. H1–H14, haplotypes Nr. 1–14 per CG.

FIGURE A5 | Distribution of relative SI values (relative to the empty-vector control pIPKTA30) of 1084 RNAi constructs obtained in first-round TIGS screenings. The median value of this distribution was used for the null hypothesis in one-sample t-tests for significance of TIGS effects.