Identifying a novel powdery mildew resistance gene in a barley landrace from Morocco

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Abstract
Powdery mildew is a barley foliar disease that causes great loss in yield. Because of the limited number of effective resistance genes, efforts to identify new sources of resistance are frequently focused on genetically diversified landraces. The goal of this study was to characterise the powdery mildew resistance gene in barley line 2553-3 selected from the Moroccan landrace. Phytopathological testing against a set of differential pathogen isolates revealed different pattern responses of this gene from those of other known resistance genes. F2 and F2:3 (2553-3 × Manchuria) mapping populations were employed to investigate resistance inheritance. Two approaches were applied for the linkage analysis: in the first approach, 22 resistant and 21 susceptible homozygous F2 plants genotyped by the DArTseq platform (Diversity Arrays Technology, Pty. Ltd.) were used; in the second, 94 F2 plants were genotyped by converted DArTseq markers and SSRs. Both analyses delineated a new resistance gene on the short arm of chromosome 2H. The authors propose MlMor as a gene symbol for newly characterized powdery mildew resistance genes in barley line 255-3-3. The results presented herein provide a good foundation for the development of closer linkage markers and MAS breeding.

Keywords Barley (Hordeum vulgare L.) · DArTseq markers · Powdery mildew (Blumeria graminis f. sp. hordei) · Resistance gene · Linkage mapping

Introduction
Barley (Hordeum vulgare L.) is the second most important cereal according to harvest area in Europe (FAOSTAT 2016. http://www.fao.org/faostat) and is generally grown as fodder and for brewing purposes. Although barley has low environmental requirements and can be farmed under harsh conditions (Nevo 1992; Stanca et al. 1992; Newton et al. 2010; Honsdorf et al. 2014), fungal infections are a cause of concern. Blumeria graminis (D.C.) Golovin ex Speer f. sp. hordei Em. Marchal (Bgh) is an obligate Ascomycota pathogen that causes powdery mildew, one of the most widespread foliar diseases. Infection by this fungus leads to yield loss and decreased feed and malting quality. The two common approaches of controlling epidemics involve the use of fungicides and the cultivation of resistant crops. Chemical protection can provide positive selection for pathogen fungicide-resistant strains (Lucas et al. 2015), whereas the cultivation of resistant crop varieties is among the most economically effective and environmentally friendly methods of controlling disease.

Resistance genes for powdery mildew are widely distributed on the barley genome. Known resistance genes mapped on the barley genome include Mla - with almost 30 alleles, as well as five other genes—Mlf, MIGa, MlK, Mlnn, and Mlr—a on the chromosome 1H; MlLa on 2H; mlo, Mlg, and MlBo on 4H; Mlj on 5H; Mlh on 6H; and mlt and Mlf on 7H (Jørgensen and Wolfe 1994; Schönfeld et al. 1996). Except for mlo, all of these genes are race-specific, major resistance genes and subject to the “boom and bust effect,” with newly emerging pathotypes eventually overcoming resistance within a few years. The most effective and durable resistance depends on mlo, a recessive allele originating from Ethiopian landraces (Büschges et al. 1997) that has been widely introduced into European cultivars. However, the introduction of mlo can generate some negative outcomes: Mlo-resistant varieties are...
more susceptible to necrotrophic and hemibiotrophic pathogens (Brown and Rant 2013), and mlo has an impact on yield by decreasing the thousand-grain weight and yield from a given plot due to pleiotropic effects (Kjaer et al. 1990).

Because of the limited number of known resistance genes utilised to control barley powdery mildew, new potential resources need to be identified. Barley landraces originate from regions with traditional and primitive farming systems, which lack explicit crop progress (Camacho Villa et al. 2005). These materials are promising sources of genetic diversity. Landraces comprise heterogenic dynamic populations subject to natural selection. Long-term co-evolution of a host with the fungus that causes powdery mildew provides low pathogen pressure and increases durability and polymorphism at resistance gene loci. Previous screening studies on powdery mildew resistance in barley landraces have revealed novel resistant lines (Comadran et al. 2009). For example, Czembor (2000, 2002) investigated 131 barley lines originating from Moroccan landraces. During phytopathological testing, previously unknown powdery mildew resistance genes were found in 66 lines, which showed unique resistance reaction spectra that were distinguishable from those of other known genes. Among these lines, only 255-3-3 was resistant to all 23 Bgh isolates studied and it exhibited no visible infection symptoms to most (74%) of them (Czembor 2002). The aim of the present study was to identify resistance genes in barley line 255-3-3.

Materials and methods

Plant materials

Spring barley (H. vulgare) line 255-3-3 (National Centre for Plant Genetic Resources Plant Breeding and Acclimatization Institute, Poland, https://bankgenow.edu.pl, ID number 17100361) was used for identifying powdery mildew resistance genes. This line was selected from Moroccan landrace 255 (ICARDA No. ICB 31956) and showed broad-spectrum resistance to powdery mildew in a previous study (Czembor 2002).

The mapping population was derived from a cross of 255-3-3 as the female parent with susceptible cv. Manchuria as the male parent.

An F2 population derived from a cross of 255-3-3 with P23 (MILa) was used for allelic testing.

A set of 30 differential varieties (DV) carrying various known powdery mildew resistance genes was used for the phytopathological tests. This set contained cv. Pallas, 21 Pallas near-isogenic lines (Kølster et al. 1986) and 8 selected cultivars (Table 1). This DV set represented most of the major resistance genes used in European cultivars.

Plants were grown in a control environment under a 19 °C/15 °C (16-h day/8-h night) regime. For Bgh propagation and the phytopathological tests, the plants were grown in transparent boxes to prevent mildew contamination.

Phytopathological tests

A set of 25 Bgh isolates collected in Poland in 2010, 2015 and 2017 were obtained from the collection of Plant Breeding and Acclimatization Institute, Poland. Bgh isolates were selected to achieve differences in virulence spectra and to specify the presence of resistance genes among the DV (Table 1).

Fungal inoculum was freshly propagated on susceptible cv. Manchuria. Ten-day-old seedlings with fully expanded first leaves were inoculated by shaking conidia from diseased plants. On the 8th day after inoculation, infection types (ITs) were scored on a 0–4-point scale (Mains and Dietz 1930), where 0, 1, and 2 indicate resistance, and 3 and 4 indicate susceptibility; extended by 0(4) level indicates Mlo resistance.

To determine the resistance of 255-3-3, the ITs of this line after inoculation with the set of Bgh isolates were compared with those obtained for the DV set. Tests were conducted with ca. 15 seedlings per line in two repetitions.

To determine the inheritance of resistance of 255-3-3, 190 F2 255-3-3 × Manchuria plants and 128 F2,1 families (25 individuals per family) were inoculated with the isolate Bgh27 avirulent to 255-3-3.

For allelic testing, 315 F2 255-3-3 × P23 plants were inoculated with isolate Bgh1-26 avirulent to both parental lines.

The numbers of resistant and susceptible plants were compared to those expected based on the theoretical Mendelian segregation ratio by the chi-square ($\chi^2$) test ($p = 0.05$).

Molecular analysis

Genomic DNA from 94 plants from F2 255-3-3 × Manchuria and the parental lines was used for the molecular analysis. DNA was extracted from a single plant leaf using the CTAB method (Murray and Thompson 1980). Samples from 43 homozygous F2 plants (22 homozygous resistant and 21 homozygous susceptible) and from the parental lines were genotyped using the DArTseq platform (Diversity Arrays Technology, Pty. Ltd.) (Von Cruz et al. 2013), and the DArTseq data were used for linkage analysis. For that purpose, DArTseq markers that were low quality, homozygous and had >20% missing calls were removed. The remaining markers were assessed for compatibility with the resistance/susceptibility trait. Markers with >80% goodness of fit were evaluated by Fisher’s exact test on 2 × 2 count tables using R (www.r-project.com). The null hypothesis was a random distribution of DArTseq marker variants within resistant and susceptible plants. Significant markers according to F-test results were assigned to a genetic location by BLASTN.
Table 1  Infection types of differential varieties and the 255-3-3 line after inoculation by with a set of Blumeria graminis f. sp. hordei isolates according to the Mains and Dietz scale (1930) extended by 0(4) point indicates Mlo resistance

| Variety | Resistance genes | Blumeria graminis f. sp. hordei isolate accession numbers |
|---------|------------------|----------------------------------------------------------|
| 255-3-3 | u                | 1 2 2 2 2 1 1 2 2 0 2 2 2 2 1 1 2 2 2 2 1 1 1 1 1 |
| Manchuria | –    | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| Pallas  | Mlo8 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| P01     | Mlo1 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| P02     | Mlo3 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| P03     | Mlo6, Mlo14 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| P04B    | Mlo7, Mlo2 | 4 4 4 4 4 4 4 4 4 2 4 0 0 0 0 4 4 4 4 4 4 4 4 4 |
| P06     | Mlo7, Mlo2 | 4 4 4 4 4 4 4 4 4 0 0 0 0 0 0 4 4 2 4 4 2 4 4 4 |
| P08B    | Mlo9 | 4 4 4 4 0 0 0 0 0 4 4 0 0 0 0 4 0 4 4 4 0 0 0 0 |
| P09     | Mlo10, Mlo2 | 4 4 4 4 0 0 0 0 0 4 4 4 4 0 1 0 4 4 4 4 4 0 4 4 |
| P10     | Mlo12 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 4 4 4 4 4 4 4 4 |
| P11     | Mlo13, Mlo3 | 4 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 4 4 4 4 0 4 1 0 |
| P12     | Mlo22 | 4 0 0 0 4 4 0 0 0 0 0 4 4 4 0 4 0 0 4 4 4 1 0 4 |
| P13     | Mlo21 | 4 1 2 2 2 2 1 2 2 2 2 2 2 4 2 4 1 1 1 2 1 1 1 2 |
| P14     | Mlo8 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| P15     | Mlo2 | 2 4 2 2 4 2 2 4 2 4 2 4 2 4 4 4 4 4 4 4 2 4 4 4 |
| P17     | Mlo7 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 2 4 4 4 |
| P18     | Mlo6 | 4 4 4 4 4 4 4 4 4 2 4 2 2 4 4 4 4 4 4 4 2 4 2 4 |
| P19     | Mlo5 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| P20     | Mlo4 | 2 2 4 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| P21     | Mlo3, MloCP | 4 4 0 0 0 0 0 0 0 4 4 4 4 4 4 4 4 0 4 4 4 4 4 4 |
| P22     | mlo5 | 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 4 4 4 4 4 4 4 |
| P23     | Mlo5 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| P24     | Mlo6 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| Benedicte | Mlo9, Mlo5 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| Lenka   | Mlo6 | 0 4 0 2 2 0 2 0 2 0 2 0 2 2 2 2 2 2 2 2 2 2 2 2 |
| Gunnar  | Mlo7 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| Steffi  | Mlo5, MloCP | 4 4 0 0 0 0 0 0 0 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| Triumph | Mlo7 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| Borwina | Mlo5 | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| Iron    | Mlo5 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| Souleka | Mlo5 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |

*)Not available data
Genetic linkage maps were constructed using the JoinMap 4.0 software (Stam 1993) under the standard calculation settings: linkages with a recombination frequency smaller than 0.45 and an LOD score higher than 1; goodness-of-fit jump threshold for removing loci of 3 and performing a ripple after adding 3 loci and the Kosambi mapping function (Kosambi 1944). The phenotypic scores for the F$_2$ 255-3-3 × Manchuria population were converted to binary data according to the IT scores; specifically, 0, 1, and 2 (resistant) were recoded as 1, and 3 and 4 (susceptible) were recoded as 0. The converted scores were included in the analysis as resistance gene $RBgh255$. AS-DArT, SSR markers, and the $RBgh255$ gene were used for genetic map construction, and another map was generated for silicoDArT markers with physical positions on 2H. The map positions of the DArTseq markers were compared with a barley consensus 2H map from Barleymap database POPSEQ data (http://floresta.eead.csic.es/barleymap) (Cantalapiedra et al. 2015; Mascher et al. 2013) in MapChart software (http://www.joinmap.nl) (Voorrips 2002). Kruskal-Wallis analysis of associations between the DArTseq markers and resistance was carried out in MapQTL 6 software under standard conditions (van Ooijen 2009).

**Results**

**Phytopathological tests**

To determine the possible resistance genes present in the 255-3-3 line, we assessed resistance against a diverse collection of 25 Bgh isolates and compared the results with the ITs of the DV set carrying various resistance genes and with susceptible cultivar Manchuria as a control (Table 1). The 255-3-3 line exhibited distinctive disease response patterns, with IT scores of 0, 1, and 2 according to the Mains’ and Dietz’s 5-level scale (1930). The line was resistant to all Bgh isolates.

Evaluation of the Bgh-inoculated F$_2$ 255-3-3 × Manchuria population revealed both susceptible and resistant individuals. Furthermore, phytopathological tests of F$_{2:3}$ families showed segregating heterozygous and non-segregating homozygous F$_2$ plants. The results of chi-squared tests confirmed the expectations of 3:1 for the F$_2$ population and 1:2:1 for F$_{2:3}$ ($p = 0.05$) (Table 2).

**Genotyping and genetic mapping**

DArTseq analysis of 43 homozygous F$_2$ 255-3-3 × Manchuria plants identified 3544 codominant DArTSNP markers and 8711 dominant silicoDArT markers. Of these DArTseq markers, 33 were selected for conversion to AS-DArT markers and used to genotype 94 F$_2$ individuals. Based on

| Population       | Bgh isolate | Number of plants/families | Predicted ratio | $\chi^2$   | $p$ value ($p = 0.05$) |
|------------------|-------------|---------------------------|-----------------|-----------|-----------------------|
|                  |             | Res | Seg | Sus |           |                        |
| 255-3-3 × Manchuria | F$_2$         | Bgh27 | 145 | – | 45 | 3:1 | 0.1754 | 0.6753 |
|                  | F$_{2:3}$     | Bgh27 | 40  | 58 | 30 | 1:2:1 | 2.6875 | 0.2609 |
| 255-3-3 × P23 (MILA) | F$_2$        | Bgh1–26 | 237 | – | 78 | 15:1 | 184.2305 | 0.0000 |
Table 3  DArTseq markers assembled into one linkage group with RBgh255

| AlleleID. | Trimmed sequence | $F$ test $p$ value | BLASTN results$^2$ | BLASTN $E$ value | AS-Dart primer sequences |
|----------|------------------|--------------------|---------------------|-----------------|--------------------------|
| 5258111  | 5258111|F|0-31:A>C-31:A>C TGCAGCCACCTTGCGGATTCAGAG TGCAGACGTTCTCTCTGCCG | 6.831E−09 | chr2H:3653981−3654025 | 3E−14 | F:GGATTCACGAGCCGGA R:CAACGTCGCTGTCAGCTT |
| 5335346  | 5335346|F|0-37:G>A-37:G>A TGCAGGCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAGGCTGCCTCGTACGCGTACAGCCTCAG... |
BLASTN alignment to the barley reference genome, each is located on the 2H chromosome.

Linkage analysis for AS-DArT (Table 3), SSR and RBgh255 assembled 14 markers into a single 36.59-cM group containing nine AS-DArTs, four SSRs, and RBgh255 (Fig. 1). Based on the known position of these markers, RBgh255 was mapped to the distal end of chromosome 2H, 5.50 cM distal to the nearest marker 3262153.

The linkage map for the silicoDArT markers from 2H and RBgh255 assembled 82 markers into one linkage 10.67-cM group (Fig. 1). RBgh255 was mapped to 3.65 cM, 0.91 cM proximal to 5249632 and 3257525 and 0.16 cM distal to 3256284.

The Kruskal-Wallis analysis demonstrated that only DArTseq markers mapping to the distal end of chromosome 2HL were significantly associated with RBgh255 (Fig. 1); the analysis did not reveal other significant loci in the genome (data not shown). The highest K value (α = 0.05) was for 3255919. The physical position of these sequences is chr2H:1839535:1839604:1 according to the barley reference genome (Table 4). These markers were previously mapped to 2.12 cM on the 2H consensus barley map (Fig. 1). With reference to the highest K value marker 3255919, RBgh255 mapped 0.32 cM distal on the silicoDArT map and 8.36 cM distal on the AS-DArT map.

Comparative map analysis revealed eight common DArTseq markers between the AS-DArT and silicoDArT maps and 12 between the silicoDArT and consensus 2H maps (Fig. 1).

Test of allelism

An allelic test was performed between the barley 255-3-3 resistance gene and MLa, a powdery mildew resistant gene previously mapped to chromosome 2H (Giese et al. 1993; Hoseinzadeh et al. 2019). A phytopathological test of 315 F2 255-3-3 × P23 (MLa) individuals revealed resistant and susceptible plants. The chi-squared test did not support a 15:1 segregating ratio (Table 2). Analysis of Res (resistant) and Sus (susceptible) AS-PCR variants of MWG097 marker linkages with MLa (Mohler and Jahoor 1996) revealed polymorphism between the parental lines (Fig. 2). Amplification of a Sus variant was obtained for Manchuria and 255-3-3, and amplification of a Res variant was obtained for P23 (MLa).

Discussion

Investigation of barley landrace genetic resources can broaden the available gene pool. The aim of the present study was to characterise a new source of barley resistance to powdery mildew. The barley line 255-3-3, which was previously described as having broad-spectrum resistance, originates from the Moroccan landrace (Czembor 2002). Czembor (2002) showed that the 255-3-3 line was resistant to all 23 Bgh isolates assessed, and exhibited an IT score of 0 according to the Mains and Dietz scale (1930) after inoculation with 17 (81%) isolates. In the present phytopathological tests, 255-3-3 exhibited resistance to all 25 Bgh isolates tested (Table 1). Nonetheless, due to the distinctiveness of the 255-3-3 IT spectrum from the spectra of the DV set, representing the majority of known powdery mildew resistance genes introduced into European crops (Dreiseitl 2014), the IT data did not indicate any commonly known resistance genes. This result indicates that 255-3-3 carries unique resistance genes that are not represented in the DV set.

Phenotypic analysis of F2 and F2:3 progeny of 255-3-3 × Manchuria showed a segregation rate indicating a single dominant resistance gene in the 255-3-3 line.

To reveal the linkage between the resistant gene and genetic location, efforts were focused on homozygous F2 individuals. Extreme segregants analysis on the DArTseq platform is an economical and useful method to search for significantly associated markers. DArTseq data processing allowed the construction of two linkage maps. The genetic distance of the RBgh255 resistance locus to the nearest markers is 5.5 cM on the AS-DArT map and 0.16 cM on the silicoDArT map (Fig. 1). As the genotyping populations comprised 94 and 43 plants, the resulting map densities are satisfactory for determining the RBgh255 locus. The results provide strong evidence for the presence of RBgh255 on the distal end of chromosome 2HS, despite divergence between the distances and the order of markers located on the analysis maps, this inconsistency was caused by the small size of the mapping population. In general, a population size ranging from 50 to 250 individuals is sufficient for preliminary genetic mapping (Mohan et al. 1997), although larger mapping populations may close the gap with markers having high linkage and producing more accurate genetic maps (Ferrera et al. 2006).

There are 13 known powdery mildew race-specific resistance genes and one partial resistance mlo gene of known position in the barley genome. Among them, only MLa has been mapped to 2H, on the distal end of the long arm (Hoseinzadeh et al. 2019). This powdery mildew resistance gene, which originates from the botanical variety Laevigatum (Giese et al. 1993), was introduced to the Dutch cultivar ‘Vada’ in the 1950s. There is no known major resistance gene for powdery mildew on the short arm of chromosome 2H originating from cultivated barley (Jørgensen and Wolfe 1994; Schönfeld et al. 1996).
| Locus      | Group | Position | K value$^1$ | D$^2$ | Sequence                                                                 | Physical position$^3$                       | E value$^3$  |
|------------|-------|----------|-------------|-------|--------------------------------------------------------------------------|---------------------------------------------|--------------|
| 3255919    | 2H    | 2.120    | 35.75       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3258971    | 2H    | 2.200    | 32.21       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3272514    | 2H    | 5.520    | 28.97       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3267127    | 2H    | 6.370    | 28.97       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3256485    | 2H    | 6.520    | 24.21       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3261154    | 2H    | 6.520    | 26.36       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 325533     | 2H    | 7.220    | 22.70       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3269567    | 2H    | 7.790    | 24.21       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3255748    | 2H    | 7.930    | 23.34       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3259865    | 2H    | 7.930    | 24.72       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3262153    | 2H    | 8.290    | 25.28       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3258828    | 2H    | 9.350    | 24.21       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3262763    | 2H    | 12.110   | 16.88       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
| 3255012    | 2H    | 12.180   | 19.06       | 2     | TGCAGCTAGGCGCTAAGCACTGACAAACCAGG GACGTCACCATGATGTTGACTATCATCTTT AATACGACG | chr2H:1839535:1839604:1                     | 1.1E−30      |
An allelic test between RBgh255 and MlLa revealed segregation in the F2 generation, with susceptible individuals proving that MlLa and RBgh255 are not allelic variants. The ratio of resistant to susceptible plants within the population deviated from the expected 15:1 for two independent dominant genes, which may be due to some error in the IT rating. MlLa is known to confer moderate resistance, corresponding to scores of 2–3 on Mains’ and Dietz’s scale (Marcel et al. 2007), and this level of resistance may cause misclassification of resistant plants as susceptible. Res and Sus variants of the marker MWG097 (Mohler and Jahoor 1996) confirmed different variants at MlLa carried by 255-3-3 and P23 (MlLa) (Fig. 2). The different IT in interaction with the set of Bgh isolates utilised indicated that the resistance gene carried by 255-3-3 is not allelic to MlLa (Table 1).

Extensive research on cultivars, landraces and wild barley genotypes revealed resistance loci on 2H. MlHb, which is a resistance gene originating from H. bulbosum, was mapped to chromosome 2H(2HI) after introgression to H. vulgare (Pickering et al. 1995). A strong crossing barrier between bulbous and cultivated barley excludes natural transfer of MlHb to H. vulgare (Blattner 2018). Nevertheless, Comadran et al. (2009), analysed almost 200 barley accessions from the Mediterranean basin area and indicated coincidence of the Bgh resistance gene with the approximate MlHb location, which suggested the occurrence of an alternative resistance locus in cultivated barley located on 2H bins: 3, 4 and 5 of the Steptoe × Morex bin map (Kleinholds and Graner 2001; Druka et al. 2002; Cooper et al. 2004). The distal end of 2H bin 3 was mapped to the MWG878 marker with a physical location of approximately 11 Mb (chr2H:11119104–11119596) according to barley genome Hv_IBSC_PGSB_v2 on the EnsemblPlants database release 37 (www.plants.ensembl.org) (Aken et al. 2017). The RBgh255 resistance gene is located on 2H bin 1 according to the physical location of DArTseq marker 3255919 with the highest K-statistic (chr2H:1839535:1839604) (Table 4). The proximal end of 2H bin 1 is located on the ABG058 marker with a physical location of DArTseq marker 325938807–325938875.

### Table 4 (continued)

| Locus     | Group | Position | K value | Df  | Sequence                                      | Physical position    |
|-----------|-------|----------|---------|-----|-----------------------------------------------|----------------------|
| 32585982  | 2H    | 13.050   | -0.56   | 2   | TGCACAGGGTGGCTGCAAGTAATTATTAGGCACACATATTAGCT | chr2H:28914091–28914699:1 |
| 32585992  | 2H    | 17.850   | 8.40    | 2   | TGCACAGCTCAGTGGACGACGAGGCGGTGCAGATGAA         | chr2H:28610043–28610649:-1 |
| 3258637   | 2H    | 19.850   | 5.14    | 2   | TGCACAGGGCGGTCGTGGACGACGAGGCGGTGCAGATGAA     | chr2H:28914091–28914699:1 |
| 32585992  | 2H    | 19.850   | 5.14    | 2   | TGCACAGGGCGGTCGTGGACGACGAGGCGGTGCAGATGAA     | chr2H:28914091–28914699:1 |
| 3258637   | 2H    | 19.850   | 5.14    | 2   | TGCACAGGGCGGTCGTGGACGACGAGGCGGTGCAGATGAA     | chr2H:28914091–28914699:1 |

1 Kruskal-Wallis K-statistic value, α = 0.05; 2 number of degrees of freedom; 3 results for BLASTN against the barley genome Hv_IBSC_PGSB_v2 on EnsemblPlants database release 37 (www.plants.ensembl.org, accessed 28.11.2017).

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![Fig. 2 Allele-specific PCR products of Res and Sus variants of marker MWG097 and original MWG097 as a positive control (K+); (K_H2O)—not template control](image-url)
position of 3.2 Mb (chr2H:3239540–3239624). Considering an approximate distance of 10 Mb between RBgh255 and the candidate genes described by Comadran et al. (2009), there is strong evidence that these loci are different. Spies et al. (2012) mapped candidate genes for resistance to Bgh on 2H in barley cv. Steffi. This variety was included in the DV set used in this study and showed distinctive resistance spectra from 255-3-3 line after inoculation with the Bgh set (Table 1). Genetic analysis of Steffi resistance (Spies et al. 2012) indicated quantitative trait segregation and polygenic inheritance that was opposite to the qualitative and monogenic resistance of 255-3-3 line. Previous reports have also revealed Bgh resistance loci in wild barley (H. vulgare ssp. spontaneum) accessions on 2H (Répková et al. 2009; Tuterová et al. 2010; Ames et al. 2015). Analysis of H. spontaneum lines PI282605 (Répková et al. 2009) and PI466197 (Tuterová et al. 2010) indicated quantitative and semi-dominant loci opposite to those of the qualitative fully dominant RBgh255. Furthermore, both described QTL were mapped proximal to RBgh255, with the highest associations with Bma0134 (chr2H:4010391–4010488) (Tuterová et al. 2010) and cMWG682 (chr2H:3326342–3326919) (Répková et al. 2009) located on 2H bin 2. Since RBgh255 is located on 2H bin 1, this gene is very unlikely to be the same as the loci described by Répková et al. (2009) and Tuterová et al. (2010). The powdery mildew QTL described by Ames et al. (2015) was collocated with the lang1031QPm.S42-2H.a field resistance QTL (von Korff et al. 2005) and mapped near the HVM36 marker with a physical location of 22 Mb (chr2H:22074463–22074562) on 2H bin 3, which was apart from the RBgh255 resistance gene. Moreover, QTL corresponding to 2H (Aghnoum et al. 2010) are involved in seedling and adult plant resistance to Bgh under field and controlled conditions. Among them, Rbgq7 carries seedling resistance in controlled conditions but has been mapped on 2H bin 4. These works showed many loci corresponding to 2H as a significant and prospective source for Bgh resistance. Differences in phenotypes, inheritances, and location on 2H between RBgh255 and other described loci indicate that RBgh255 is probably a distinctive and newly described resistance gene.

Resistance genes have been previously reported in landraces (Comadran et al. 2009; Czembor 2000, 2002; Newton et al. 2000; Spies et al. 2012), and have been successfully introduced into elite germplasms. These genes include Mlo originating from the German landrace Weißenstephan, Mla3 from the Uruguayan landrace Ricardo, Mla12 from Arabische and the durable resistance gene mlo, originating from Ethiopian landraces. The RBgh255 gene is potentially valuable to breeders for breeding resistance to powdery mildew, and rare broad-spectrum resistance is promising for growers and interesting for scientists. According to the statement “Only when a pathogen isolate with virulence corresponding to that resistance is found can the resistance gene, according to terminology, be classified as race-specific” (Jørgensen and Wolfe 1994), a Bgh isolate virulent to 255-3-3 has not been found, in either the previous research by Czembor (2002) or this study. In this work, phytopathological tests were conducted with a Bgh set covering 21 common and known resistance genes and 13 Mla alleles. However, a limitation of the present study is that it focused only on Polish Bgh isolates; conversely, Czembor’s studies, which were conducted more than 15 years ago, utilised isolates from central Europe emerging at that time. New promising resistance genes should correspond to virulence genes in the pathogen population; therefore phytopathological tests were performed using Bgh collected from Poland in recent years. The study of markers linked to the identified gene provides a good basis for the development of more useful MAS markers closer to RBgh255.

This study revealed and characterised a novel powdery mildew resistance gene in barley line 255-3-3 selected from the Moroccan landrace. In accordance with nomenclature recommendations (Jørgensen 1987), the authors propose MIMor as a gene symbol for the resistance described.

Authors’ contributions Piechota U. conducted experiments, analysed data, wrote manuscript; Czembor P.C. conceptualised study, reviewed manuscript; Slowacki P. isolated DNA, carried out PCRs; Czembor J.H. acquired funds, reviewed manuscript; all authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals The research involved neither human participants nor animals.

Informed consent Not applicable.

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