

## Resistance to Powdery Mildew in Barley Recombinant Lines Derived from Crosses between *Hordeum vulgare* and *Hordeum bulbosum*

J.H. CZEMBOR<sup>1</sup>, A. PIETRUSIŃSKA<sup>2\*</sup>, U. PIECHOTA<sup>3</sup> and D. MAŃKOWSKI<sup>4</sup>

<sup>1</sup>Plant Breeding and Acclimatization Institute – National Research Institute,  
Independent Laboratory of Applied Biology, Radzikow, 05-870 Blonie, Poland

<sup>2</sup>Plant Breeding and Acclimatization Institute – National Research Institute,  
National Centre for Plant Genetic Resources: Polish Genebank, Radzikow, 05-870 Blonie, Poland

<sup>3</sup>Plant Breeding and Acclimatization Institute – National Research Institute,  
Plant Breeding and Genetics Department, Radzikow, 05-870 Blonie, Poland

<sup>4</sup>Plant Breeding and Acclimatization Institute – National Research Institute,  
Laboratory of Seed Production and Plant Breeding Economics, Department of Seed Science and Technology,  
Radzikow, 05-870 Blonie, Poland

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Barley recombinant lines obtained from crosses and backcrosses of common barley (*Hordeum vulgare* L.) cultivars Emir and Golden Promise with bulbosus barley grass (*H. bulbosum* L.) were tested against differential set of 14 *Blumeria graminis* D.C. Golovin ex Speer f. sp. *hordei* – synamorph *Erysiphe graminis* DC. f. sp. *hordei* Em Marchall isolates, pathogenic fungus causing powdery mildew. Powdery mildew resistance was found in 22 from 24 lines tested. Presence of unknown genes was postulated in 13 lines. In six of these lines the unknown genes were postulated present besides *Mla12* allele originated from *H. vulgare* parent (cv. Emir). The only line resistant to infection, from all isolates used, was 181P94/1/3/1/1-2. Four other lines could be considered highly resistant, because they were susceptible to only one powdery mildew isolate. The possibilities to use the hybrid lines with identified resistance to powdery mildew, especially line 181P94/1/3/1/1-2 in barley breeding programs were discussed.

**Keywords:** Barley, *Hordeum bulbosum*, powdery mildew, *Mla12*, resistance genes

### Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world, being principal crop in certain regions. Barley is grown in places where other cereals are not suitable for farming due to harsh environmental conditions such as high altitude, low rainfall (< 300 mm annually), or soil salinity (Bothmer et al. 2003a; Fischbeck 2003; Shrivastava and Kumar 2015). It is often attacked by barley powdery mildew fungus (*Blumeria graminis* DC. Golovin ex Speer f. sp. *hordei* – synamorph *Erysiphe graminis* DC. f. sp. *hordei* Em Marchall). Loss of yield caused by powdery mildew can reach up to

\*Corresponding author; E-mail: a.pietrusińska@ihar.edu.pl

30%, with average of 5–10% across all the regions (Ceccarelli et al. 1995; Gong et al. 2013; Tratwal and Bocianowski 2014). Besides powdery mildew damage is manifested as a lowering of grain quality, especially for malting purpose (Balkema-Boomstra and Masterbroek 1995).

Powdery mildew on barley is considered as one of the most clearly characterized system of host–pathogen genetic interactions. There are more than 100 mildew resistance alleles that have been identified in barley. Barley cultivars with effective genes for resistance to major pathogens have been an efficient means for controlling major diseases and preventing yield losses (Fischbeck 2003; Weibull et al. 2003; Burdon et al. 2014). Barley breeders commonly used in their breeding programmes such major resistance genes as: *Mla6*, *Mla7*, *Mla9*, *Mla12* and *Mla13* belonging to the *Mla* locus, and the resistance genes *MLk*, *MLg*, *MLLa*, *MLh* and *MLra*. However, virtually all of these genes were gradually overcome by virulent races within 4–5 years, when cultivars containing them were used on a large acreage. Exceptions are cultivars with the *Mlo*-resistance, carried by the recessive allele *mlo*, and resistance depends on *MLLa* resistance gene, which are efficient for long period use. Nevertheless, barley breeders, geneticists and plant pathologists are constantly looking for new efficient sources of powdery mildew resistance, in order to combine them with those already used in modern cultivars and to increase the resistance durability (Bonman et al. 2005; Shtaya et al. 2006c).

Based on genetic studies, barley gene pool can be divided into three parts (Bothmer et al. 2003b). Common barley (*H. vulgare* L.) and wild barley (*Hordeum spontaneum* L.) belong to the primary gene pool. *H. spontaneum* was used in many breeding programmes to transfer new desirable traits, like disease resistance and tolerance to abiotic stress (Fischbeck 2003; Backes et al. 2003; Pickering and Johnston 2005). The only one species, which is bulbosus barley (*Hordeum bulbosum* L.), belongs to secondary gene pool (Pickering et al. 2004; Bothmer et al. 2003b; Pickering and Johnston 2005; Wendler et al. 2014; Wendler et al. 2015). Bulbosus barley is a perennial and occurs, both as diploid and autotetraploid cytotypes, in the Mediterranean region, West Asia, Caucasus Mountains and part of Central Asia including: Iran, Afghanistan, Turkmenistan, Uzbekistan, and Kazakhstan. (Bothmer et al. 1995). In the tertiary gene pool of barley there are 29 other *Hordeum* species. These species are diploid, tetraploid and hexaploid forms and they are found in North and South America, Europe, the Middle East, Central Asia and South Africa (Bothmer et al. 1995). In some breeding programmes attempts have been made to use these species in crosses with *H. vulgare* but with limited success, due to strong crossability barriers (Bothmer et al. 2003; Pickering and Johnston 2005). Some of these barriers can be solved overcome by careful selection of parental genotypes and the environment in which crosses are being carried out. Pickering and co-workers described *H. vulgare* × *H. bulbosum* hybrids and their backcrossing to *H. vulgare* (Pickering et al. 2000). Several recombinant lines showed improved resistance to major barley pathogens, including leaf rust (Pickering et al. 2000).

Bulbosus barley grass, in the breeding programmes, has been used mainly to obtain doubled haploids (Pickering and Johnston 2005). In several reports *H. bulbosum* was described as a species with very high level of resistance to barley pathogens with pow-

dery mildew amongst them (Johnston et al. 2013; Johnston et al. 2015; Pickering et al. 2004; Pickering et al. 2006; Pickering and Johnston 2005; Shtaya et al. 2007; Walther et al. 2000). Despite of the mentioned observations, the number of reports on *H. bulbosum* genetic investigations and on successful transfer of resistance to major pathogens resistance from *H. bulbosum* to *H. vulgare* is very limited (Zhang et al. 2001; Pickering et al. 2004; Pickering and Johnston 2005). In these reports hybrid lines of *H. bulbosum* × *H. vulgare* expressed resistance to such diseases as leaf rust, stem rust, powdery mildew, scald, septoria speckled leaf blotch, BaYMV/BaMMV (Pickering et al. 2000, 2006; Walther et al. 2000; Shtaya et al. 2007).

The objective of this study was to investigate powdery mildew resistance in recombinant lines obtained from crosses of *H. bulbosum* with *H. vulgare*. Each of analyzed line was derived from different cross so had potential to carried variable introgressed elements and genes.

## Materials and Methods

### *Plant material*

Twenty-four recombinant lines obtained from single crosses and backcrosses of common barley cultivars Emir and Golden Promise, with accessions of *H. bulbosum*, were tested (Table S1\*). Recombinant lines were obtained at New Zealand Institute for Crop and Food Research, New Zealand (Pickering et al. 2000). In addition, the parental cultivars Emir and Golden Promise, were tested. The cv. Pallas, 24 Pallas near-isogenic lines (Kølster et al. 1986) and eight other cultivars, each carrying various known powdery mildew resistance genes, were used to determine virulence spectra of *B. graminis* f. sp. *hordei* isolates. Susceptible cv. Manchuria was used as a positive inoculation control (Table S3).

### *Pathogen*

Fourteen different isolates of *B. graminis* f. sp. *hordei* were used (Table S3), originated from the collections in Risø National Laboratory, Roskilde, Denmark; Danish Institute for Plant and Soil Science, Lyngby, Denmark; Edigenossische Technische Hochschule – ETH, Zurich, Switzerland and Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, Poland. The isolates were chosen according to differences in virulence spectra that were observed on the Pallas isolines differential set (Kølster et al. 1986) (Table S3), provided from Royal Agricultural and Veterinary University, Copenhagen, Denmark. Pathogen isolates were purified by single pustule isolation and were maintained and propagated on young seedlings of the powdery mildew susceptible cultivar Manchuria (CI 2330). Frequent virulence checks were made to assure the purity of isolates throughout the experiment.

\*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

### Resistance tests

Five to 10 plants from each recombinant line were tested with 14 isolates of powdery mildew. Testing was conducted in the greenhouse. Plants were grown with at 16 h of light photoperiod and the temperature range of 16–22 °C. Inoculation was carried out when plants were 10–12 days old (2 leaf stage) by shaking or brushing conidia from diseased plants. After 8–10 days of incubation, the infection types were scored with use of 0–4 scale, developed by Mains and Dietz (1930) (Table S4), where 0(4) score indicates Mlo-resistance. Plants scored 0–2 were included into the resistant group and plants scored 3 and 4 were included into the susceptible group.

### Postulation of resistance alleles

Hypothesis about presence of the specific resistance genes was done on the basis of the gene for gene hypothesis (Flor 1956). Comparison of the reaction spectra of the tested lines with those of differential Pallas isolines after inoculation of differential set of 14 *B. graminis* f. sp. *hordei* isolates was conducted. Identification of resistance genes was made by eliminating resistance genes not present in tested lines. Next step was determining the postulated and possible resistance genes.

### Statistical analysis

In order to describe the differences between the tested lines and *B. graminis* f. sp. *hordei* isolates, a two-way ANOVA main effects analysis was carried out together with the Tukey's multiple comparison procedure. Data on the reaction of the tested lines and cultivars to the isolates, before the analysis, were subjected to square root transformation  $x' = \sqrt{x + 0.5}$  (Maccaferri et al. 2010). In order to assess better the differentiation of the tested lines in terms of the total response to individual isolates, a multivariable cluster analysis was done, using the Square Euclidean Distance and Ward's Agglomerative Clustering Method. Statistical calculations were performed with Statistica 13.1 program (Dell Inc. 2016).

## Results

Powdery mildew resistance genes were found in 22 from 24 lines tested (Table S2). Among them, nine lines (65F17/4/2, 102C2/14/3/1, 102C2/16/2, 102C2/70/1/3, 102C2/97/1, 119Y4/4/5/10, 169P15/8, 181P138/2 and 181P156/3) revealed *Mla12* resistance gene, the same as in the *H. vulgare* backcross parent cv. Emir. In other six lines (36L53/1/3-7/2/1, 177L6/2/9, 200A3, 216L1, 36L50/3/5/1 and 38P18/8/1/10) *Mla12* gene together with unknown resistance genes were postulated to be present. Unknown resistance genes only, were postulated in 7 lines (177L20/6/2-8/1/1-14, 181P94/1/3/1/1-2, 200A16/5/3, 216U3, 219W4, 36L36/4/1/7-17 and 182Q20/10).

The line 181P94/1/3/1/1/1-2 showed broad resistance spectrum. It was resistant to all isolates of *Blumeria graminis* f. sp. *hordei* used. This line expressed three different reactions for inoculation with specific isolates: 0, 1 and 2. Four other lines (177L20/6/2-8/1/1-14, 200A16/5/3, 216U3 and 219W4) can be considered highly resistant to powdery mildew, because of their resistance to 13 powdery mildew isolates.

Plants of two resistant lines (200A3 and 65F17/4/2) showed heterogeneous reaction after inoculation with some of the powdery mildew isolates. Among 24 lines used in research, two lines (219W4 and 36L36/4/1/7-17) revealed susceptibility to all used isolates. Barley cultivars used as back crossing parents showed lack of powdery mildew resistance (cv. Golden Promise) or resistance determined by gene *Mla12* (cv. Emir) (Table S2). The only line resistant to infection with all isolates used was 181P94/1/3/1/1/1-2. Plants of this resistant line expressed three different resistance reactions to inoculation with specific isolates: 0, 1 and 2. Four other lines (177L20/6/2-8/1/1-14, 200A16/5/3, 216U3 and 219W4) could be considered highly resistant to powdery mildew because they were susceptible after infection with just one powdery mildew isolate. Plants of two lines (200A3 and 65F17/4/2) showed heterogeneous reaction after inoculation with some of powdery mildew isolates used. Barley cultivars used as back crossing parents showed lack of powdery mildew resistance (cultivar Golden Promise) or resistance determined by gene *Mla12* (cultivar Emir) (Table S2).

The results of the statistical analysis confirmed the results of powdery mildew resistance. The analysis of variance, carried out for the reactions of the tested lines and varieties to the set of isolates, allowed to find significant differences in reactions of genotypes tested ( $MS = 1.4810$ ,  $MSE = 0.1054$ ,  $F\text{-statistic} = 14.052$ ,  $p < 0.0001$ ) as well as in the pathogenicity of isolates used in the studies ( $MS = 6.304$ ,  $MSE = 0.105$ ,  $F\text{-statistic} = 59.818$ ,  $p < 0.0001$ ). In order to better characterize better the differences in the reactions of the tested lines and varieties to the set of isolates, homogeneous groups were determined, based on the Tukey's test (Fig. S1). The tested lines and varieties were divided into 4 homogeneous groups. The cv. Golden Promise and lines: 38U4 / 1/3/8/1, 38U20 / 3/4/5/1, 182Q20 / 1 and 36L36 / 4/1 / 7-17 of the affected by powdery mildew formed the largest group. However, the rates of infection for 200A16/5/3, 219W4, 177L20/6/2-8/1/1-14, 216U3, 181P94/1/3/1/1/1-2, 177L6/2/9, 200A3, 216L1, 36L53/1/3-7/2/1, 38P18/8/1/10, 36L50/3/5/1 lines are relatively low. The 200A16/5/3 and 219W4 lines were characterized by significantly lower paralysis than Emir cultivar. The 200A16/5/3 and 219W4 lines were characterized by significantly lower infection than cv. Emir.

Three main groups of the tested objects were obtained, with use of cluster analysis of variation of multiple experimental reactions of tested varieties and lines, into individual isolates (Fig. S2).

The first group, along with the cv. Golden Promise, is a group of barley lines characterized by the strongest reaction to individual isolates. The second group with the cv. Emir is a group of barley lines characterized by a weak interaction, with reference to particular isolates separately. The third group of barley lines expressed the weakest reaction to the isolates. The analysis of variance indicated that the pathogenicity of tested isolates was

characterized by quite large differences ( $MS = 1.186$ ,  $MSE = 0.206$ ,  $F\text{-statistic} = 5.744$ ,  $p < 0.0001$ ). The results presented in Table S5 confirm the division into three homogeneous groups.

### Discussion

Wild crop relatives are a rich source of genetic diversity. It is a good source of resistance to biotic stress, including diseases (Pickering et al. 2000; Walther et al. 2000; Thomas 2003). Wild species are more heterogenic than crops because were not subjected to strong breeding selection. In contrast to crops, wild relatives were subjected to natural selection evolution at the pathogen pressure. This process maintains much more genetic differentiation in resistance loci, than popularly applied breeding procedures, where intense artificial selection leads to genetic erosion. Bulbosus grass (*H. bulbosum* L.) is a wild species that belongs to barley secondary gene pool, which means presence of interspecific reproductive isolation. Pickering (2000) described the technique that broke cross-breeding barriers between barley and the bulbosus grass and allows introducing new genes to barley cultivars. From this time bulbosus grass an approachable source of new traits and genes for breeders. *H. bulbosum* was disclosed as a donor of some resistance genes, for example for fungal diseases: *Rynchosporium secalis* (Pickering et al. 2006), rust (Walther et al. 2000). It is also a donor of *MIHb*, resistance gene to powdery mildew. Pickering and co-workers (1995) located *MIHb* on 2I (Introgressed) chromosome after introgressing to *H. vulgare*. Disclosing novel genes is very promising because powdery mildew is highly mobile and much variable fungus (Limpert et al. 1999). Limited number of known and used genes of powdery mildew resistance generates the need to search for new effective donors of resistance. Especially the known genes, excluding partial resistance *mlo* recessive allele, are race-specific and code vertical resistance. Almost all of these genes were overcome by the appearance of new aggressive pathotypes of the pathogen.

In the presented study seedling resistance test was used in order to describe infection types, expressed by barley recombinant lines after inoculation with differentiated isolates of *B. graminis* f. sp. *hordei*. This kind of testing is sufficient for disease resistance screening and is used commonly in breeding programmes, to postulate the presence of genes in modern cultivars and to screen for new sources of effective resistance (Brooks et al. 2000; Shtaya et al. 2006b). However, this kind of tests are not very useful to identify and describe partial resistance. For description of this kind of resistance there is a need to conduct measurements of resistance characteristics, additional to the infection type. Furthermore, partial resistance is generally better expressed at the adult plant stage (Martinez et al. 2001; Shtaya et al. 2006a; Ochoa and Parlevliet 2007; Wang et al. 2010). Final determination of the number of resistance genes and the type of their action in tested hybrid lines, may be established with the use of crosses and backcrosses among appropriate genotypes (Derevnina et al. 2015).

Three of analyzed recombinant lines (200A3, 216U3, 219W4) were previously described by Shtaya and co-workers (2007). Their research revealed high resistance level to powdery mildew in these three lines. These lines exhibited mainly scores: 0, 1 and 2 after



infection, the same as in our study. In this work, postulation of resistance gene was expanded for resistance genes not-represented in Pallas isolines, like Ml(St1) and Ml(St2) carried by cv. Steffi or Ml(Bw) – Borwina, Ml(Kr) – Kredit and Ml(1192) – Jarek.

The durability of the powdery mildew resistance effects presented in barley cultivars may be increased by using many different strategies. These strategies are: combining partial (minor genes) and race-specific (major) resistance genes, multiline cultivars, using partial resistance, combining different race-specific resistance genes into one cultivar and deploying many cultivars with different resistance genes in space (e.g. cultivar mixtures) or time (winter versus spring barley) (Finckh et al. 2000; Brown and Hovmøller 2002; McDonald and Linde 2002; Boyd et al. 2013). Very important for durability of resistance to powdery mildew is the proper use of new sources of resistance to this pathogen (Brown and Hovmøller 2002; McDonald and Linde 2002). It is expected that the new genes described in this paper will find (proper) application in this process.

Wild barley *H. bulbosum* is an important genetic resource for resistance to biotic and abiotic stress. Many scientists state that genepool of cultivated varieties is limited and that breeders are restricted to crossing within the primary genepool, which consists of *H. vulgare* (in form of modern cultivars and landraces) and its closest diploid relative, *H. spontaneum* (Russell et al. 2000; Pickering and Johnston 2005). However, presented study confirms the opinion that secondary barley gene pool can be a source of very valuable characteristics for barley breeding. Broadening the genepool of cultivated barley varieties and description of new sources of resistance are important for many reasons. Future strategies for the control of barley pathogens will have to focus on more ecologically acceptable pest control methods. Criticism of chemicals usage (pesticides, fungicides, herbicides, and mineral fertilizers) in agriculture is increasing in developed countries societies. Breeding for resistance represents an ecologically safe method. In addition to ecological, there are also economic arguments (use of fuel, labor, and special machines) and the argument about fungicide resistance development in strains of powdery mildew, which are in favour of breeding for resistance compared to instead of chemical control (Walters et al. 2012, 2013; Wulff et al. 2011; Ney et al. 2013).

Hybrid lines with identified resistance to powdery mildew originated from *H. bulbosum*, especially the line 181P94/1/3/1/1-2 resistant to all isolates used, should be used in breeding programmes to provide cultivars with highly effective resistance to this disease. In addition, *H. bulbosum* is a valuable source of resistance genes not only to fungal diseases, like powdery mildew and leaf rust, but also to barley mosaic viruses (Walther et al. 2000; Ruge et al. 2000; Ruge-Wehling et al. 2006). Based on the available literature it can be concluded, that breeding importance of *H. bulbosum* is constantly increasing due to its usefulness in inducing haploid plants as well as an effective source of resistance to biotic and abiotic stress.

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### Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <http://www.akademai.com/content/120427/>

Electronic Supplementary *Table S1*. Recombinant *H. vulgare* × *H. bulbosum* lines, their parentages and chromosome location of *H. bulbosum* introgression

Electronic Supplementary *Table S2*. Reaction of 24 *H. vulgare* × *H. bulbosum* recombinant lines and parental cultivars to infection with 14 differential isolates of *Blumeria graminis* f. sp. *hordei* according to Mains' and Dietz's (1930) scale

Electronic Supplementary *Table S3*. Differential Pallas isolines and resistant cultivars, and their infection types for the differential set of *B. graminis* f. sp. *Hordei*, according to Mains' and Dietz's (1930) scale. The 0(4) score indicates Mlo-resistance

Electronic Supplementary *Table S4*. Description of infection types and codes used (Mains and Dietz, 1930)

Electronic Supplementary *Table S5*. The average reactions of recombinant *H. vulgare* × *H. bulbosum* lines to particular *B. graminis* f. sp. *hordei* isolates with homogeneous groups

Electronic Supplementary *Figure S1*. The average reactions of the *H. vulgare* × *H. bulbosum* recombinant lines and parental cultivars to a differential set of *Blumeria graminis* f. sp. *hordei* isolates, divided into homogeneous groups

Electronic Supplementary *Figure S2*. Dendrogram of cluster analysis of the *H. vulgare* × *H. bulbosum* recombinant lines and parental cultivars resistance to *B. graminis* f. sp. *hordei* received. The Square Euclidean Distance and Ward's Agglomerative Clustering Method