



Article

Sources of Resistance to Powdery Mildew in Wild Barley (*Hordeum vulgare* subsp. *spontaneum*) Collected in Jordan, Lebanon, and Libya

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Abstract: Barley powdery mildew (BPM) is caused by the pathogen *Blumeria hordei* (*Bh*) and can lead to severe yield loss. Plant pathologists are looking for new sources of resistance to BPM. Barley accessions, including the wild subspecies *Hordeum vulgare* subsp. *spontaneum* (*Hvs*), are stored in many gene banks and are often a valuable source of economically important characteristics. The wild barley *Hvs* could be a valuable resistance source for BPM. The aim of the presented investigation was to detect new sources of BPM resistance in 81 accessions of *Hvs* collected in Jordan (46), Lebanon (24), and Libya (11). European differential isolates of BPM were used, and resistant single plant lines were selected for use from fifteen accessions from Jordan and Libya. These resistant single plant lines were tested for the presence of specific resistance genes using a differential set of *Bh* isolates. Hypotheses about the presence of specific resistance genes were made by comparing the reaction spectra of the tested lines with those of differential lines. After an analysis of the obtained results, it was concluded that all 31 tested single plant lines of *Hvs* had genes for resistance that are not represented in the barley differential set for resistance genes to *Bh*. Twenty-six lines of *Hvs* selected from accessions originated in Jordan and Libya showed resistance reactions to all isolates used. These lines will be further tested as new sources of effective resistance and used in barley prebreeding programs.

Keywords: *Blumeria hordei*; resistance genes; resistance; germplasm; gene bank; biodiversity; plant breeding; plant genetic resources; crop wild relatives; prebreeding



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1. Introduction

Among cereals, barley (*Hordeum vulgare* L.) is the fourth-most important in the world. It is used for feed, malt, and food. Recently, the use of barley as a food has become more and more popular due to its health properties [1–5].

Barley's primary gene pool includes two subspecies: domesticated barley (*Hordeum vulgare* subsp. *vulgare*) and wild barley (*Hordeum vulgare* subsp. *spontaneum*) (*Hvs*). Wild barley differs from cultivated barley in several traits, including a brittle rachis, and it is considered a progenitor of cultivated barley [3,6]. *Hvs* occurs in Southwest Asia and, most probably due to human activities, populations of *Hvs* are present in Morocco, Ethiopia, and Tibet [1,2].

Blumeria graminis (DC.) Golovin ex Speer f.sp. *hordei* Em. Marchal (*Bh*) is a fungus that causes barley powdery mildew (BPM). It is considered to be one of the most economically important pathogens acting on barley, and can cause significant yield losses. Many studies have shown that *Bh* is rapidly developing many new races and that its spores are dispersed by wind over long distances [7–17]. It occurs in many barley-growing regions of the world, but it is especially important in Europe. This is due to the maritime climate in most areas of Europe being suitable for the development of BPM. In addition, barley is grown in Europe in large areas and more than 60% of the world's production of barley originates from this continent [13,14]. The average annual losses caused by this disease in barley production

in Central Europe are estimated at about 10%. However, in many experiments, barley yield losses due to the occurrence of heavy infestation by BPM exceeded 25%. The grain yield obtained from barley fields where BPM was present was very often characterized by lower-quality characteristics important in malt production, such as higher grain protein content and lack of proper grain size uniformity [18–20].

Chemical control and agronomic practices are used to reduce BPM incidence. However, these methods are often not effective, and, in addition, there is a growing emergence of BPM resistance to fungicides [21,22]. A commonly used method to control BPM is the incorporation of new effective genes for powdery mildew resistance into barley cultivars [13,23]. This is the most effective and environmentally safe method to control this disease. Effective resistance not only protects the cultivated varieties but also reduces the production of inoculum and the spread of the pathogen to larger areas, leading to epiphytosis [12–14,24–26]. In most cases of growing cereals, including barley, in agricultural practice, control against fungal pathogens is based on integrated pest management (IPM) principles [23,27,28]. This approach to managing pests is based on combining biological, cultural, physical, and chemical methods to minimize economic, health, and environmental risks. Recently, the use of genetic resistance as a component of IPM has become more important due to the implementation of more environmentally friendly agricultural policies in many countries of the world [28,29].

In the last century, many gene banks were established because of increasing crop erosion. The main goal of gene banks is to preserve key plant genetic resources in order to meet current and future needs concerning food production [30–33]. This is achieved by introducing them into breeding programs to achieve biological progress and for use in direct production. To achieve this effectively, there is a need for phenotyping and genotyping data for major gene bank collections [6,30–35]. There are large collections of the genus *Hordeum* stored in many gene banks worldwide. It is estimated that about 485,000 accessions of this genus are stored at more than 200 institutions worldwide. These collections include *H. vulgare* ssp. *vulgare* (299,165 accessions), wild barley *Hvs* (32,385 accessions), and wild species of *Hordeum* (4681 accessions) [31,36]. However, these collections are very often duplicated and not properly characterized, especially concerning data about effective resistance to major pathogens.

Countries in West Asia and North Africa (WANA), including Jordan, Lebanon, and Libya, have diverse agroecological zones and different types of agriculture, varying in intensity. Wild barley is a widespread species in this region, and genetically diverse populations of *Hvs* are reported to be collected [37–40]. Currently, there is also increasing interest in the study of both landraces and *Hvs* as potential sources of economically important characteristics to breed cultivars resistant to abiotic and biotic stresses, well adapted to changing climate conditions [41–48].

Hvs has been used since the very beginning of genetic studies on the resistance of barley to BPM. The very first study was conducted by Biffen, in 1907, who analyzed the mode of inheritance of BPM resistance in progenies created by crossing *H. vulgare* with *Hvs* [49]. Since that time, race-specific resistance genes have mainly been identified in cultivated barley landraces [50–65] and wild barley [66–77] mostly originating from the WANA region [7,9,13]. Based on genetic studies, many specific resistant genes were described in wild barley, including *Mla16-Mla21*, *Mla25-Mla29*, *Mla32*, *MlaLv*, *Mlf*, *Mlj*, *mlt*, *Ml(Ro)*, and *Ml(Ve)* [32,68,75]. Barley breeders used many BPM resistance genes, especially in the *Mla* locus and *Mlra*, *Mlk*, *MILa*, *Mlg*, and *Mlh* [7,9,13]. However, many of these genes have lost their effectiveness as a result of pathogen adaptation and the emergence of virulent races to these genes [7–14]. In the last 40 years, only barley cultivars with *Mlo* resistance have been characterized as those with durable resistance to BPM, because no known virulence for *mlo* genes has been identified. This type of resistance to BPM was identified in barley mutants and in landraces, but not in *Hvs* [13,78–80].

Many studies have proved that work on the genetics of resistance to BPM using a differential set of BPM isolates can be successfully used for investigations to determine the presence of specific resistance genes in barley genetic resources [7,13,14]. New, efficient sources of resistance to BPM for proper crosses in breeding programs are crucial to conducting resistance breeding [9,13,24–26]. The use of seedlings in studies conducted to postulate specific BPM resistance genes using a differential set of *Bh* isolates was proven to be an effective and sufficient method. This method is commonly used for the characterization of barley germplasm, concerning its BPM resistance [33,50,53,60–62,64,65].

The presented investigation goal was to detect new sources of BPM resistance in accessions of *Hvs* collected in Jordan, Lebanon, and Libya.

2. Materials and Method

2.1. Plant Material

Eighty-one accessions of wild barley (*H. vulgare* subsp. *spontaneum*) (*Hvs*) collected in Jordan, Lebanon, and Libya were obtained from the ICARDA gene bank. These accessions were collected in 10 expeditions (LBY81, LBY82, LBY90, LBN92-2, LBN93, LBN94-1, JOR81-2, JOR85, JOR88-1, and JOR95) during the period 1981–1995 (Supplementary Materials Table S1).

2.2. Pathogen

Twenty *B. graminis* f. sp. *hordei* Em Marschal (*Bh*) isolates were used to determine the resistance genes present in the tested accessions. They were selected from our collection of isolates to have possessed virulence genes corresponding to the most known resistance genes used in barley resistance programs in Europe (Table 1). Isolates 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 (PBAI-NRI) IHAR-PIB Radzików, Poland. The isolates were chosen according to differences in virulence spectra that were observed on the Pallas isolines differential set and on additional cultivars with resistance genes not present in Pallas isolines [75,81]. Each of them represented a different pathotype, determined using the selected set of 20 differential Pallas isolines. Isolate Bgh33 was the most avirulent isolate in the collection.

They were purified using 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 ensure the purity of isolates throughout the experiment.

Table 1. *Blumeria hordei* isolates used for artificial inoculation and their virulence spectra against resistance genes on the differential set of Pallas near-isogenic lines.

| No. | Near-Isogenic Lines/Cultivars | Resistance Genes | Isolates | | | | | | | | | | | | | | | | | | | |
|-----|-------------------------------|-------------------------|----------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| | | | Bgh 1 | Bgh 2 | Bgh 3 | Bgh 4 | Bgh 8 | Bgh 9 | Bgh 11 | Bgh 13 | Bgh 14 | Bgh 24 | Bgh 28 | Bgh 29 | Bgh 31 | Bgh 33 | Bgh 36 | Bgh 40 | Bgh 48 | Bgh 51 | Bgh 57 | Bgh 63 |
| 1 | Pallas | <i>Mla8</i> | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | |
| 2 | P1 | <i>Mla1</i> | 0 | 0 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | |
| 3 | P2 | <i>Mla3</i> | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | |
| 4 | P3 | <i>Mla6, Mla14</i> | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 0 | 4 | 4 | 4 | 4 | 4 | 4 | |
| 5 | P4A | <i>Mla7, Mlk, +?</i> | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 4 | 0 | 2 | 2 | 2 | 4 | 4 | 2 | |
| 6 | P4B | <i>Mla7, +?</i> | 4 | 4 | 4 | 1 | 0 | 2 | 2 | 4 | 4 | 0 | 2 | 4 | 4 | 1 | 4 | 4 | 1 | 4 | 4 | |
| 7 | P6 | <i>Mla7, MILG2</i> | 4 | 4 | - * | 0 | 0 | 2 | 1 | 2 | 4 | 0 | 2 | 2 | 4 | 0 | 4 | 2 | 0 | 4 | 4 | |
| 8 | P7 | <i>Mla9, Mlk</i> | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | |
| 9 | P8A | <i>Mla9, Mlk</i> | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | |
| 10 | P8B | <i>Mla9</i> | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | |
| 11 | P9 | <i>Mla10, MIDu2</i> | 4 | 4 | 4 | 0 | 1 | 4 | 0 | 4 | 0 | 2 | 0 | 4 | 4 | 4 | 4 | 0 | 0 | 4 | 4 | |
| 12 | P10 | <i>Mla12</i> | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 4 | 0 | 4 | 4 | 0 | 4 | 0 | |
| 13 | P11 | <i>Mla13, MIRu3</i> | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | |
| 14 | P12 | <i>Mla22</i> | 4 | 4 | 0 | 4 | 4 | 0 | 4 | 0 | 4 | 4 | 4 | 4 | 0 | 0 | 4 | 4 | 4 | 0 | 0 | |
| 15 | P13 | <i>Mla23</i> | 1 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| 16 | P14 | <i>Mlra</i> | 4 | 4 | 4 | 0 | 4 | 4 | 4 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | |
| 17 | P15 | <i>MI(Ru2)</i> | 4 | 4 | 4 | 4 | 3 | 4 | 2 | 4 | 4 | 2 | 0 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | |
| 18 | P17 | <i>Mlk</i> | 4 | 4 | 4 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 0 | 4 | 4 | 2 | 4 | 2 | 2 | 4 | 4 | |
| 19 | P18 | <i>Mlnn</i> | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | |
| 20 | P19 | <i>Mlp</i> | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | |
| 21 | P20 | <i>Mlat</i> | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 4 | 2 | 2 | |
| 22 | P21 | <i>Mlg, MI(CP)</i> | 4 | 4 | 4 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | 0 | 4 | 0 | 4 | |
| 23 | P22 | <i>mlo5</i> | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 3 | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | 0(4) | |
| 24 | P23 | <i>MI(La)</i> | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | |
| 25 | P24 | <i>Mlh</i> | 4 | 4 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | |
| 26 | Benedicte | <i>Mla9, MI(IM9)</i> | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 4 | 0 | 4 | 4 | 0 | 4 | 4 | |
| 27 | Lenka | <i>Mla13, MI(Ab)</i> | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | |
| 28 | Gunnar | <i>Mla3, MI(Tu2)</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 29 | Steffi | <i>MI(St1), MI(St2)</i> | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 2 | 3 | 0 | 4 | 2 | 0 | 2 | 4 | |

Table 1. Cont.

| No. | Near-Isogenic Lines/Cultivars | Resistance Genes | Isolates | | | | | | | | | | | | | | | | | | | |
|-----|-------------------------------|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| | | | <i>Bgh 1</i> | <i>Bgh 2</i> | <i>Bgh 3</i> | <i>Bgh 4</i> | <i>Bgh 8</i> | <i>Bgh 9</i> | <i>Bgh 11</i> | <i>Bgh 13</i> | <i>Bgh 14</i> | <i>Bgh 24</i> | <i>Bgh 28</i> | <i>Bgh 29</i> | <i>Bgh 31</i> | <i>Bgh 33</i> | <i>Bgh 36</i> | <i>Bgh 40</i> | <i>Bgh 48</i> | <i>Bgh 51</i> | <i>Bgh 57</i> | <i>Bgh 63</i> |
| 30 | Kredit | <i>MI(Kr)</i> | 4 | 2 | 4 | 0 | 2 | 0 | 0 | 2 | 4 | 4 | 4 | 2 | 4 | 0 | 4 | 2 | 2 | 4 | 4 | 4 |
| 31 | Jarek | <i>MI(Kr), +?</i> | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 2 | 4 | 4 |
| 32 | Trumph | <i>Mla7, MI(Ab)</i> | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 33 | Borwina | <i>MI(Bw)</i> | 4 | 3 | 3 | 0 | 4 | 0 | 4 | 4 | 4 | 2 | 2 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 2 | 2 |
| 34 | Manchurian | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

* No data.

2.3. Populations and Single Plant Lines Resistance Tests

In the preliminary study, thirty plants per accession were evaluated with the *Bgh33* isolate. Next, the selected single plant lines represented by five plants were tested with 20 differential isolates of *Bh*.

All these tests were conducted under controlled conditions with a 16/8 h day/night photoperiod and a 22/16 °C temperature regime. In all tests, the cultivar Manchuria CI 2330 was used as a susceptible control.

Seedlings with a fully expanded first leaf were inoculated with *Bh* by shaking conidia from the susceptible cv. Manchuria CI 2330. After 8–10 days, the reaction type (RT) of plants to infection by *Bh* was scored. A five-point RT scale was used: 0, no visible symptoms; 0(4), sparse, small colonies originating from the stomatal subsidiary cells (Mlo resistance); 1, minute necrotic flecks, no mycelial growth, and no sporulation; 2, frequent chlorosis, reduced mycelial growth, and no or very scarce sporulation; 3, moderate mycelial growth, moderate sporulation, and occasional chlorosis; 4, profuse sporulation of well-developed colonies (Figure 1) [52,82]. Plants with RT of 0, 0(4), and 1 were classified as highly resistant (R), plants that scored 2 as moderately resistant (M), and plants with ratings of 3 and 4 as susceptible and very susceptible.

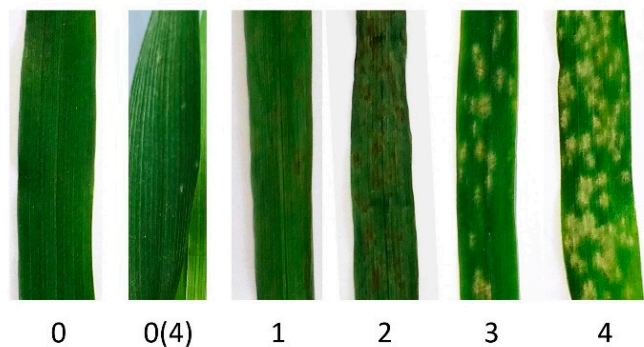


Figure 1. Range of barley seedling infection types for the *Blumeria hordei* interactions (image by Jerzy H. Czembor). Each infection type is based on a 0–4 scale, where 0 (the immune reaction) is no visible symptoms; 0(4), sparse small colonies originating from the stomatal subsidiary cells (Mlo resistance); 1 (resistant), minute necrotic flecks, no mycelial growth, and no sporulation; 2 (moderately resistant), frequent chlorosis, reduced mycelial growth, and no or very scarce sporulation; 3 (susceptible), moderate mycelial growth, moderate sporulation, and occasional chlorosis; and 4 (very susceptible), profuse sporulation of well-developed colonies. Infection types 3 and 4 are considered to be compatible (i.e., virulent pathogen/susceptible host).

2.4. Postulation of Resistance Alleles

The postulation of the presence of resistant genes was based on a comparison of reaction spectra observed on tested accessions and the barley differential set (Table 1). This was undertaken based on the gene-for-gene hypothesis [83]. The RT observed on each accession was compared with the *Bh* virulence spectrum on the barley differential set.

3. Results

In the preliminary study, among 81 tested accessions of *H. vulgare* subsp. *spontaneum* collected in Jordan (46), Lebanon (24), and Libya (11), 15 accessions expressed resistance to isolate Bgh33 of *Bh* (Table 2, Figure 2). Eleven of them originated from Jordan (23.9% evaluated) and four from Libya (36.4% evaluated).

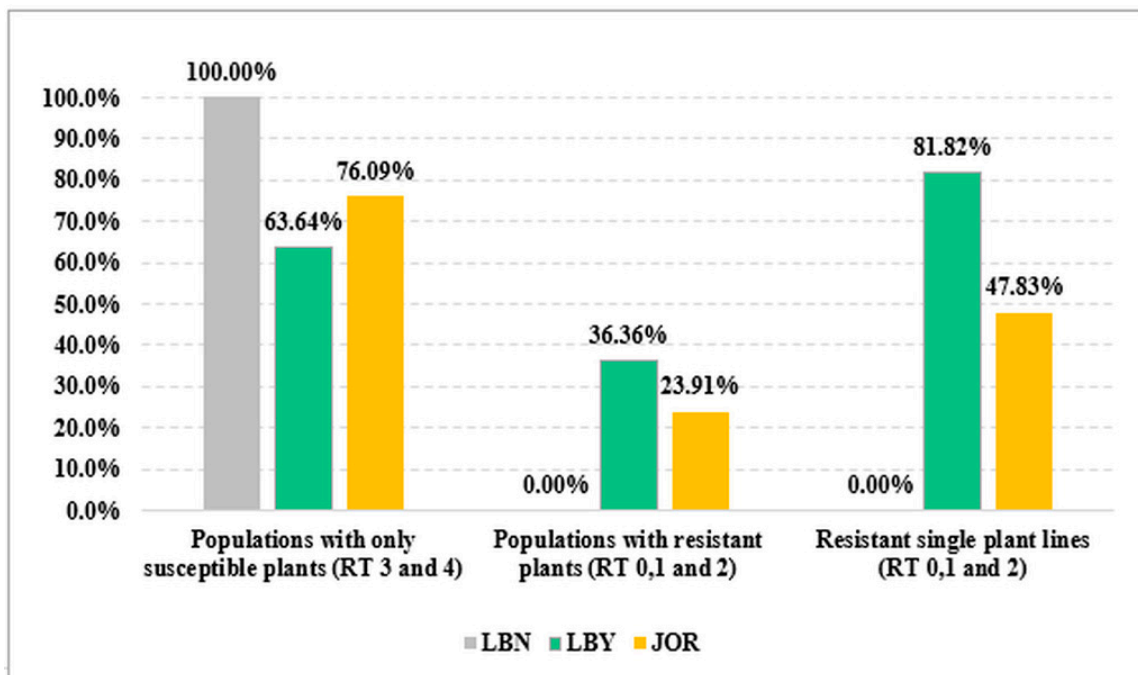


Figure 2. The frequency distribution histogram presents the percentage of populations with only susceptible plants and with resistant plants of *Hvs* originating from Lebanon (LBN), Jordan (JOR), and Libya (LBY) tested with isolate Bgh33 and the percentage of selected resistant single plant lines from *Hvs* populations from these countries.

None of the plants with accessions from Lebanon showed powdery mildew resistance in preliminary testing with isolate Bgh33. Twelve of the tested accessions in which plants were resistant to isolate Bgh33 showed heterogenous RT to powdery mildew: three of them showed only one type of reaction, eleven showed two different types, and one showed three types (0, 2, and 4).

Among the scored resistance RTs in tested lines with 20 differential isolates, the most common reaction was 0 (immunity) (Table 3, Figure 2). This RT was observed in all tested lines, with a frequency of 65.5%. The remaining RTs occurred with frequencies of 1—0.96%, 2—31.1%, and 4—2.4%. Reaction types 3 and 0(4) were not observed. In total, 97.6 observed reactions represented resistance RTs (0, 1, and 2). The analysis was conducted, comparing the spectrum of RTs of 31 tested lines to infection with 20 differential isolates, with results observed on a differential set of barley. Based on this analysis, it was concluded that all of the tested lines had an unknown gene or genes for resistance which were not represented in the differential set. Twenty-six of these lines (83.9%) showed resistance RTs to all isolates used. After the analysis of the obtained results, it was concluded that all selected 31 single plant lines of wild barley had unknown genes for resistance, which were not represented in the Pallas isolines differential set.

Table 2. Resistance of lines selected from accessions *H. vulgare* subsp. *spontaneum* from Jordan (JOR), Lebanon (LBN), and Libya (LBY) to *B. hordei* to isolate Bgh33 after inoculation at the seedling stage.

| No. | ICARDA | | | OTHERNUMB *** (IHAR No.) | Isolate <i>Bh</i> <i>Bgh</i> 33 | No. | ICARDA | | | OTHERNUMB (IHAR No.) | <i>Bgh</i> 33 |
|-----|--------|---------|-----------|-----------------------------|------------------------------------|-----|--------|---------|--------|-------------------------|---------------|
| | IG * | Crop No | ORI ID ** | | | | IG | Crop Nr | ORI ID | | |
| 1 | 38616 | 180007 | JOR | 1075 | 4 | 41 | 40177 | 181568 | LBN | 1116 | 4 |
| 2 | 38617 | 180008 | JOR | 1076 | 4 | 42 | 40178 | 181569 | LBN | 1117 | 4 |
| 3 | 38618 | 180009 | JOR | 1077 | 4 | 43 | 40179 | 181570 | LBN | 1118 | 4 |
| 4 | 38619 | 180010 | JOR | 1078 | 4 | 44 | 40180 | 181571 | LBN | 1119 | 4 |
| 5 | 38620 | 180011 | JOR | 1079 | 4 | 45 | 40181 | 181572 | LBN | 1120 | 4 |
| 6 | 38621 | 180012 | JOR | 1080 | 4 | 46 | 40182 | 181573 | LBN | 1121 | 4 |
| 7 | 38622 | 180013 | JOR | 1081 | 4 | 47 | 40183 | 181574 | LBN | 1122 | 4 |
| 8 | 38623 | 180014 | JOR | 1082 | 4 | 48 | 40184 | 181575 | LBN | 1124 | 4 |
| 9 | 38624 | 180015 | JOR | 1083 | 0, 4 | 49 | 40185 | 181576 | LBN | 1125 | 4 |
| 10 | 38625 | 180016 | JOR | 1084 | 0, 4 | 50 | 40186 | 181577 | LBN | 1126 | 4 |
| 11 | 38626 | 180017 | JOR | 1085 | 4 | 51 | 40187 | 181578 | LBN | 1127 | 4 |
| 12 | 38627 | 180018 | JOR | 1086 | 0 | 52 | 40188 | 181579 | LBN | 1128 | 4 |
| 13 | 38628 | 180019 | JOR | 1087 | 4 | 53 | 40189 | 181580 | LBN | 1129 | 4 |
| 14 | 38629 | 180020 | JOR | 1088 | 4 | 54 | 40190 | 181581 | LBN | 1130 | 4 |
| 15 | 38630 | 180021 | JOR | 1089 | 0, 4 | 55 | 40191 | 181582 | LBN | 1131 | 4 |
| 16 | 38631 | 180022 | JOR | 1090 | 4 | 56 | 40193 | 181584 | LBN | 1132 | 4 |
| 17 | 38632 | 180023 | JOR | 1091 | 4 | 57 | 40194 | 181585 | LBN | 1133 | 4 |
| 18 | 38633 | 180024 | JOR | 1092 | 4 | 58 | 112846 | 181657 | LBY | 1134 | 0, 2, 4 |
| 19 | 39398 | 180789 | JOR | 1093 | 4 | 59 | 112847 | 181658 | LBY | 1135 | 4 |
| 20 | 39821 | 181212 | JOR | 1094 | 2, 4 | 60 | 110816 | 181628 | LBN | 1136 | 4 |
| 21 | 39822 | 181213 | JOR | 1095 | 4 | 61 | 110819 | 181629 | LBN | 1137 | 4 |
| 22 | 39823 | 181214 | JOR | 1096 | 4 | 62 | 110823 | 181630 | LBN | 1138 | 4 |
| 23 | 39824 | 181215 | JOR | 1097 | 4 | 63 | 110831 | 181631 | LBN | 1139 | 4 |
| 24 | 39825 | 181216 | JOR | 1098 | 4 | 64 | 110833 | 181632 | LBN | 1140 | 4 |
| 25 | 39826 | 181217 | JOR | 1099 | 4 | 65 | 116004 | 181639 | LBY | 1141 | 4 |
| 26 | 39827 | 181218 | JOR | 1100 | 4 | 66 | 116005 | 181640 | LBY | 1142 | 0, 4 |
| 27 | 39828 | 181219 | JOR | 1101 | 2, 4 | 67 | 115780 | 181660 | JOR | 1143 | 4 |

Table 2. Cont.

| No. | ICARDA | | | | OTHERNUMB *** (IHAR No.) | Isolate Bg Bgh 33 | No. | ICARDA | | | | OTHERNUMB (IHAR No.) | Bgh 33 |
|-----|--------|---------|-----------|--|-----------------------------|----------------------|-----|--------|---------|--------|------|-------------------------|--------|
| | IG * | Crop No | ORI ID ** | | | | | IG | Crop Nr | ORI ID | | | |
| 28 | 39829 | 181220 | JOR | | 1102 | 4 | 68 | 115781 | 181661 | JOR | 1144 | 0, 4 | |
| 29 | 39850 | 181241 | JOR | | 1103 | 4 | 69 | 115782 | 181662 | JOR | 1145 | 4 | |
| 30 | 39851 | 181242 | JOR | | 1104 | 4 | 70 | 115784 | 181664 | JOR | 1146 | 0 | |
| 31 | 39877 | 181268 | JOR | | 1105 | 4 | 71 | 115785 | 181665 | JOR | 1147 | 4 | |
| 32 | 39933 | 181324 | LBY | | 1107 | 4 | 72 | 115786 | 181666 | JOR | 1148 | 2, 4 | |
| 33 | 39934 | 181325 | LBY | | 1108 | 3 | 73 | 115787 | 181667 | JOR | 1149 | 4 | |
| 34 | 39935 | 181326 | LBY | | 1109 | 0, 2 | 74 | 115788 | 181668 | JOR | 1150 | 4 | |
| 35 | 39936 | 181327 | LBY | | 1110 | 4 | 75 | 115789 | 181669 | JOR | 1151 | 4 | |
| 36 | 39937 | 181328 | LBY | | 1111 | 0, 4 | 76 | 115790 | 181670 | JOR | 1152 | 0 | |
| 37 | 39938 | 181329 | LBY | | 1112 | 4 | 77 | 115791 | 181671 | JOR | 1153 | 4 | |
| 38 | 39939 | 181330 | LBY | | 1113 | 4 | 78 | 115792 | 181672 | JOR | 1154 | 4 | |
| 39 | 40156 | 181547 | LBN | | 1114 | 4 | 79 | 115793 | 181673 | JOR | 1155 | 0, 2 | |
| 40 | 40168 | 181559 | LBN | | 1115 | 4 | 80 | 115795 | 181674 | JOR | 1156 | 4 | |
| | | | | | | | 81 | 115796 | 181675 | JOR | 1157 | 4 | |

* ICARDA number of accession; ** Country of origin: Lebanon (LBN), Jordan (JOR), and Libya (LBY); *** Other number—IHAR number.

Table 3. Resistance of lines selected from accessions *H. vulgare* subsp. *spontaneum* to *B. hordei* isolates after inoculation at the seedling stage.

| No. | ICARDA | | | | | Othernumb (IHAR Project No.-Line) | Isolates | | | | | | | | | | | | | | | | | | | | Postulated Resis- tance Alleles |
|-----|---------------|-------------------|------------|-----------|--------|--|----------|---|------|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|------|-------|--|
| | ICARDA- IG | IG Line No. | Crop No | ORI ID | 1 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | Bgh 1 | |
| 1 | 38624 | 1 | 180015 | JOR | 1083-1 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | un * | | |
| 2 | 38625 | 1 | 180016 | JOR | 1084-1 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | un | | |
| 3 | 38625 | 2 | 180016 | JOR | 1084-3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | un | | |
| 4 | 38625 | 3 | 180016 | JOR | 1084-4 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | un | | |
| 5 | 38627 | 1 | 180018 | JOR | 1086-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | un | | |
| 6 | 38630 | 1 | 180021 | JOR | 1089-1 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | un | | |
| 7 | 39821 | 1 | 181212 | JOR | 1094-1 | 4 | 0 | 4 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 4 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | un | | |
| 8 | 39821 | 2 | 181212 | JOR | 1094-2 | 2 | 0 | 4 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 4 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 2 | un | | |
| 9 | 39828 | 1 | 181219 | JOR | 1101-1 | 2 | 0 | 2 | - ** | 0 | - | 2 | 0 | 0 | - | 2 | 0 | - | 0 | 0 | 0 | - | - | - | un | | |
| 10 | 39828 | 2 | 181219 | JOR | 1101-2 | 0 | 0 | 0 | - | 0 | 2 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | - | 0 | 0 | 0 | - | - | 0 | un | |

Table 3. Cont.

| No. | ICARDA | | | | Othernumb (IHAR Project No-Line) | Isolates | | | | | | | | | | | | | | | | | | | | Postulated Resis- tance Alleles |
|-----|---------------|-------------------|------------|-----------|---|----------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--|
| | ICARDA- IG | IG Line No. | Crop No | ORI ID | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | |
| | | | | | | Bgh 1 | Bgh 2 | Bgh 3 | Bgh 4 | Bgh 8 | Bgh 9 | Bgh 11 | Bgh 13 | Bgh 14 | Bgh 24 | Bgh 28 | Bgh 29 | Bgh 31 | Bgh 33 | Bgh 36 | Bgh 40 | Bgh 48 | Bgh 51 | Bgh 57 | Bgh 63 | |
| 11 | 39828 | 3 | 181219 | JOR | 1101-3 | 2 | 0 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 12 | 39935 | 1 | 181326 | LBY | 1109-1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | |
| 13 | 39935 | 2 | 181326 | LBY | 1109-2 | 0 | 0 | 0 | - | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | - | 0 | 0 | 0 | - | - | 0 | |
| 14 | 39935 | 3 | 181326 | LBY | 1109-3 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 2 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 0 | |
| 15 | 39935 | 4 | 181326 | LBY | 1109-4 | 0 | 0 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | |
| 16 | 39937 | 1 | 181328 | LBY | 1111-1 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 4 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 0 | |
| 17 | 39937 | 2 | 181328 | LBY | 1111-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | |
| 18 | 112846 | 1 | 181657 | LBY | 1134-1 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 19 | 112846 | 2 | 181657 | LBY | 1134-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | |
| 20 | 116005 | 1 | 181640 | LBY | 1142-1 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 0 | |
| 21 | 115781 | 1 | 181661 | JOR | 1144-1 | 4 | 2 | 4 | - | 0 | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 0 | - | 2 | 4 | 0 | - | - | 2 | |
| 22 | 115781 | 2 | 181661 | JOR | 1144-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | |
| 23 | 115781 | 3 | 181661 | JOR | 1144-3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | |
| 24 | 115784 | 1 | 181664 | JOR | 1146-1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | |
| 25 | 115786 | 1 | 181666 | JOR | 1148-2 | 0 | 0 | 0 | 2 | 0 | - | 2 | 2 | 0 | 2 | - | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | - | |
| 26 | 115790 | 1 | 181670 | JOR | 1152-1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 27 | 115790 | 2 | 181670 | JOR | 1152-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 28 | 115793 | 1 | 181673 | JOR | 1155-1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 29 | 115793 | 2 | 181673 | JOR | 1155-3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | |
| 30 | 115793 | 3 | 181673 | JOR | 1155-3 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | |
| 31 | 115793 | 2 | 181673 | JOR | 1155-2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 4 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | |

* Unknown resistance gene. ** No data.

4. Discussion

In the presented study, new, well-characterized sources of highly effective BPM resistance for European conditions were identified. Such well-characterized single plant lines are crucial as genetic resources for barley resistance breeding and for applying genetic control of this fungus in agricultural practice. Two major strategies for BPM control are available. The first is to use genetic control by growing resistant cultivars, and the second is the application of fungicides [2,7,13,23,27–29]. However, in many countries, *Bh* races resistant to commonly used fungicides have been described [23,27–29]. In addition, the cost of fungicides and concerns about the environment have led many countries to restrict their use in disease control [27,28]. Taking this into account, BPM control using effective resistance genes is increasingly important in IPM strategies in Europe and other parts of the world. In addition, breeding for resistance as a strategy to control plant pests and diseases is increasingly understood and accepted by societies as ecologically safe.

In the present study, it was confirmed that *Hvs* accessions from Jordan, Lebanon, and Libya are diverse and possess valuable characteristics for breeding purposes. This diversity is due to the presence of many mountainous regions and different climate zones in these countries. Such conditions are favorable for the evolution of very diverse genotypes of plants, including wild barley [37–39]. Powdery mildew occurs commonly in this area on barley and wild barley. Because the area of the Fertile Crescent is considered the center of origin and diversification of barley, it is also the center of the presence of very diverse resistance genes to BPM [1,2,9,10,13]. This was confirmed in the present study, in which new sources of resistance to BPM in selections from accessions of *Hvs* from Jordan and Libya were identified.

Eleven of these selections originated from Jordan, four from Libya, and none from Lebanon. Twelve tested accessions showed heterogenous resistance reactions to powdery mildew: three of them showed only one type of reaction, eleven showed two types, and one showed three types (0, 2, and 4). Heterogenous reactions of *Hvs* accessions to powdery mildew were also reported in other studies [76,77]. In populations of *Hvs*, BPM did not develop to levels that significantly damage plants. This is the result of both the stabilizing effect of the genetic heterogeneity within the populations of *Hvs* and the presence of resistance sufficient to control limited disease development [75–77].

In tested lines with 20 differential BPM isolates, the most common RT was 0 (immunity). This kind of RT was observed in all tested lines and with 65.5% of all observed RTs. The remaining RTs occurred with frequencies of 1—0.96%, 2—31.1%, and 4—2.4%. Reaction types 3 and 0(4) were not observed. In total, 97.6 observed reactions represented resistance RTs (0, 1, and 2). Such a high percentage of resistance RTs showed that *Hvs* collected in Jordan and Libya are valuable sources of resistance for European barley breeding, which is in agreement with other studies [66,67,73–76].

In the presented study, new sources of BPM resistance were identified, which are very important for applying genetic control of this fungus, because *Bh* is characterized by a high level of genetic variability. It can develop new races in a short time that can spread across long distances [7–17]. This results in a rapidly reduced number of resistance genes effectively controlling the occurrence and spread of *Bh* being available for barley breeders [13,14]. At the same time, modern barley cultivars, which are grown in large areas across Europe, often have no partial type of host resistance due to breeding for a variant-specific type of BPM resistance in most modern breeding programs [13,24,25]. The presence of such resistance should be investigated in tested *Hvs* accessions in additional specific tests, and not only in the seedling stage conducted in the presented study. It will be especially interesting to test *Hvs* accessions in the adult stage of plant development to identify adult plant BPM resistance. The loss of these types of resistance during the breeding process was recognized a long time ago by plant pathologists and plant breeders, and several ways to increase the durability of race-specific resistance genes were proposed. Major strategies were proposed and implemented: the use of multiline cultivars, the combining (“pyramiding”) of different resistance genes into one variety, and the deployment of many

cultivars with different resistance genes in space (e.g., cultivar mixtures) or time (winter versus spring barley) [7,13,24,25]. However, for such BPM strategies for genetic control, it is very useful to introduce new and effective sources of resistance into breeding materials. Such newly identified sources of BPM resistance are still being found in barley landraces and wild relatives [13,60–65,75–77]. There are many examples of the new sources of resistance to BPM originating from *Hvs* populations being successfully used by barley breeders to develop new resistant cultivars. In most cases, these new resistance genes were deployed in new cultivars under different strategies to prolong the duration of their effectiveness against BPM [7,13,23,24]. The use of newly identified sources of resistance to BPM is important in breeding new varieties with effective resistance deployed in agriculture in various strategies. The additional advantage to barley breeders of using germplasm from *Hvs* is the possibility of introducing other desirable agronomic traits, e.g., tolerance to drought conditions and other biotic and abiotic stresses [41–47]. However, for many barley breeders, the heterogeneity of *Hvs* accessions is a problem because it complicates and prolongs the breeding process. Often, prebreeding activities resulting in well-characterized single plant lines of *Hvs* with many important economically traits, including resistance to BPM, are needed. The prebreeding activities presented here provide breeders with new BPM sources of resistance to be used in different breeding strategies [7,24,25]. The big advantage of the use of *Hvs* genetic resources in barley breeding is a lack of problems with sterility. Such problems are often present if *H. bulbosum* or mutants are used [7,13].

In the presented study, a test with a set of differential BPM isolates was used and the selection of single plant lines was conducted to identify new sources of resistance in wild barley accessions. This method was described in many studies to identify specific resistance genes in barley accessions and breeding lines [7,13,14]. In addition, it was successfully used in many studies to screen both landraces and wild barleys for new effective resistance genes [14,50–53,56–59,64,65,75–77]. However, for the description of the partial type of resistance to BPM, this kind of test is not sufficient. For the detection of this kind of resistance, there is a need to obtain, in addition to the RT, the measurements of resistance parameters in different stages of plant development (e.g., at the adult plant stage) [84,85]. The adult plant resistance of tested accessions should be investigated in additional specific tests because almost all wild barleys contain major specific resistance genes which very often mask minor resistance genes determining the partial resistance or the presence of adult resistance [7,13,84–91].

A very interesting genetic resource for the breeding of resistant barley is the 31 single plant lines of wild barley which have genes for resistance not represented in the BPM differential set. These newly identified sources of highly effective resistance to BPM in single plant lines of *Hvs* from Jordan and Libya will be used in the barley prebreeding program.

Further studies are needed to determine the mode of action of resistance genes in identified new sources of BPM resistance described in the presented study based on the results of testing hybrids resulting from crosses among appropriate genotypes [50,55,62]. In the future, some other available methods for the characterization of resistant lines will have to be used, especially those for the study of partial and adult resistance [84–91].

In addition, modern molecular methods have to be used for the further characterization of resistance genes identified to be efficiently used in barley breeding [3,86,92–94].

5. Conclusions

The *Hvs* populations from Jordan and Libya are valuable sources of BPM resistance. Selected single plant lines of *Hvs* may be used in prebreeding programs to provide barley breeders with new, well-characterized sources of BPM resistance. Future studies will concentrate on determining the genetic basis of resistance occurring in 31 *Hvs* selections. They will include the crosses of investigated selections with well-chosen parents and the development of molecular markers. To successfully introduce the described new sources of BPM resistance into barley elite cultivars, prebreeding work is needed in the creation of initial, well-characterized plant materials. A necessary step is to use barley germplasm

from gene banks, first in breeding programs and second in agricultural practice, as an elite cultivar.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13102462/s1>, Table S1. Collection data of 81 accessions of wild barley (*H. vulgare* subsp. *spontaneum*) collected in Jordan, Lebanon, and Lybia.

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