

Communication

# Mlo Resistance to Powdery Mildew (*Blumeria graminis* f. sp. *hordei*) in Barley Landraces Collected in Yemen

Jerzy H. Czembor \*  and Elżbieta Czembor 

Plant Breeding and Acclimatization Institute—National Research Institute (IHAR-PIB), Radzikow, 05-870 Błonie, Poland; e.czembor@ihar.edu.pl

\* Correspondence: j.h.czembor@ihar.edu.pl

**Abstract:** Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world. Powdery mildew on barley, which is caused by the pathogen *Blumeria graminis* f. sp. *hordei*, occurs world-wide and can result in severe yield loss. Thousands of barley accessions are stored in national gene banks, and their characterization for breeding purposes is needed. This study was conducted to determine the resistance to powdery mildew in 33 barley landraces from Yemen, which were obtained from the ICARDA gene bank. Twenty differential isolates of barley powdery mildew were used. Nine single plant lines were selected from five landraces, based on tests that were performed with 30 plants per landrace, after inoculation with the most avirulent isolate of barley powdery mildew available. Two of these landraces originated from the Al Bayda province in Yemen, and three others originated from Dhamar, Sanaa, and Taizz, respectively. Next, single plant lines were tested using a set of 20 differential isolates of powdery mildew. Two lines that were selected from landrace from the Al Bayda province in Yemen, showed disease reaction designated as 0(4), which is specific for the presence of Mlo resistance. The new source of highly effective Mlo powdery mildew resistance that is described in this study could be used in barley breeding programs.



**Citation:** Czembor, J.H.; Czembor, E. Mlo Resistance to Powdery Mildew (*Blumeria graminis* f. sp. *hordei*) in Barley Landraces Collected in Yemen. *Agronomy* **2021**, *11*, 1582. <https://doi.org/10.3390/agronomy11081582>

**Keywords:** *Hordeum vulgare*; *Blumeria graminis*; barley landraces; Mlo resistance; resistance genes; powdery mildew

Academic Editor: Karen Barry

Received: 5 July 2021

Accepted: 5 August 2021

Published: 9 August 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world. In many regions, barley is often grown in marginal agricultural areas with low annual precipitation (often less than 220 mm). Landraces in these areas are important, as they are often the only rain-fed crop possible and they are cultivated on mountain slopes, at elevations higher than other cereals. They are often grown not only for grain, but also for straw [1]. More than 485,000 accessions of the genus *Hordeum* are stored at more than 200 institutions worldwide [2]. These collections include new and old cultivars, landraces, mutants, breeding lines, and research and mapping plant materials of *H. vulgare* ssp. *vulgare* (299,165 accessions), wild barley *H. vulgare* ssp. *spontaneum* (32,385 accessions), and wild species of *Hordeum* (4681 accessions) [3]. Because of quarantine issues and safety reasons, many accessions are duplicated in gene banks. These genetic resources are of great value for breeding new cultivars that are well adapted to changing climate and weather anomalies, or more resistant to abiotic and biotic stresses [4–6].

The fungus *Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei* Em. Marchal is considered to be one of the most destructive foliar pathogens of barley in many regions of the world. In countries where mildew is a problem, yield losses in experimental tests usually exceed 25%. However, the average annual losses in barley production are lower, and in Central Europe they are estimated to be about 10% [7]. The best means of controlling powdery mildew was using resistance genes. However, the resistance that is conferred by most of these genes has not been maintained for more than a few years (5–10 years),

with one exception, which is Mlo resistance [8–11]. Mlo resistance is a unique type of resistance, because it is monogenic and non-race-specific. The recessive alleles at the *Mlo* locus condition the penetration resistance of the attacked epidermal cells, by rapid deposition of large callose-containing appositions (papillae) in the epidermal cell wall, directly subtending the attacking fungal appressoria. This resistance reaction is only characteristic for *mlo* alleles, and is designated as 0(4). The *mlo* locus was genetically mapped, and its molecular structure was described and patented. However, many factors, e.g., temperature, water stress or light intensity, may affect the expression of this gene. Negative pleiotropic effects that were common when *mlo* was used in earlier crosses have been overcome by recent breeding, and this type of resistance is presently utilized with increasing intensity in spring barley production. During the last 35 years, Mlo resistance has been deployed in many barley cultivars throughout Europe [12–16]. These investigations are conducted to increase the resistance durability [16–22].

Yemen is characterized by the presence of diverse agroecological zones and a long history of agriculture. Based on different landscapes, Yemen can be divided into the following five main geographical regions: the coastal plains (with rainfall below 50 mm/year), mountains and highlands (with rainfall on average 300–500 mm/year, but in some places more than 1 000 mm/year), the eastern plateau region (with rainfall below 100 mm), the desert, and the islands [23]. The cultivable land in Yemen is estimated to be about 7% of the total area. The largest part of the agricultural activities is in the Yemen highland and mountain area, in the western part of the country. The highlands of Yemen are characterized by their stone-wall terraces. These terraces represent over 40 percent of the country's arable land [24].

Cereals are grown on almost 60% of the total cultivated area, and barley is grown on about 50,000 hectares. In Yemen, barley is grown at 1800–3000 m above sea level [1,25].

Genetic studies on resistance to diseases, including barley powdery mildew, is valuable for breeding programs and characterization of germplasm collections [26–28]. Identification of powdery mildew resistance genes, based on tests performed on seedlings, using isolates with different virulence spectra, is effective and sufficient for breeders and pathologist needs [22,23,29]. This study aimed at detecting new sources of powdery mildew resistance in barley landraces that were collected from Yemen, based on the results obtained during testing lines that were selected from these landraces with a set of powdery mildew isolates with different virulence.

## 2. Materials and Method

### 2.1. Plant Material

Thirty-three seed samples of barley landraces were provided by Dr. J. Valkoun, J. Konopka and Prof. S. Ceccarelli (International Center for Agricultural Research in the Dry Areas—ICARDA). These landraces were collected in Yemen, mostly in central mountains area of the country in 7 provinces (10 landraces from Dhamar, 6—Taizz, 6—Sanaa, 2—Sadah, 4—Al Bayda, 2—Al Hudayah and 3—Ibb) (Table 1).

### 2.2. Pathogen

To determine the R-genes present in landraces lines, 20 Bgh (*B. graminis* f. sp. *hordei* Em. Marschal) isolates, with virulence genes corresponding to known resistance genes, were used (Table 2). 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, provided kindly by Dr. H.J. Schaerer (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, provided by Dr. L. Munk (Royal Agricultural and Veterinary University, Copenhagen, Denmark) and on 12 additional cultivars. Each of them represent different pathotypes, determined using a selected set of 20 Pallas isolines differential and Triumph

and Gunnar cultivars. Isolate Bgh 33 was the most avirulent one. It was avirulent to resistance genes or their combinations, such as the following: Mla1, Mla3, Mla6 + Mla14, Mla7 + Mlk + ?, Mla7 + ?, Mla7 + MILG2, Mla9 + Mlk, Mla9, Mla12, Mla13 + MIRu3, Mla22, Mla23, MlRu2, Mlk, Mlp, Mlat, and present in additional cultivars included into a differential set, as follows: Benedicte (Mla9, MI (IM9)), Lenka (Mla13, MI (Ab)), Gunnar (Mla3, MI (Tu2)), Steffi (MI (St1)), MI (St2)), Kredit MI (Kr). Isolates Bgh 3, Bgh 1, Bgh 29, Bgh 51 belong to the most virulent group.

**Table 1.** Collection data of 33 landraces collected in Yemen.

No.	ICARDA IG	IHAR No.	Date	Longitude	Latitude	Altitude	Province	Collection Site
1	37313	5369	1977.10.–	4302-E	1506-N	0	Al Hudaydah	Turba
2	37314	5370	1977.19.–	4302-E	1506-N	0	Al Hudaydah	Turba
3	37316	5371	1977.11.26	4414-E	1524-N	2250	Sana'a	Sana'a
4	37319	5372	nd *	4402-E	1335-N	1400	Ta'izz	Ta'izz
5	37320	5373	nd	4414-E	1524-N	2200	Sana'a	Bani Husshaysh, NE of Sana'a
6	38520	5375	1980.06.08	4427-E	1423-N	2350	Dhamar	19 km S of Dhamar
7	38526	5376	1980.06.14	4405-E	1518-N	2500	Sana'a	15 km W from Sana'a
8	113096	5445	1988.–.–	4545-E	1452-N	nd	Al Bayda'	nd
9	113228	5448	1988.–.–	4430-E	1433-N	nd	Dhamar	nd
10	37601	5589	1980.05.–	4505-E	1415-N	2500	Al Bayda'	Al Sharaf, store
11	37603	5590	1980.05.–	4455-E	1415-N	2560	Al Bayda'	Agaba
12	37609	5591	1980.05.–	4548-E	1415-N	1950	Al Bayda'	Al Soumaa
13	37612	5592	1980.05.–	4345-E	1655-N	1970	Sa'dah	Al Mahazer
14	37613	5593	1980.05.–	4405-E	1330-N	2300	Ta'izz	Jabal Sabir
15	37615	5594	1980.05.–	4405-E	1330-N	2300	Ta'izz	Jabal Sabir
16	37620	5595	1980.05.–	4410-E	1350-N	2000	Ibb	Shiban
17	37623	5596	1980.06.–	4422-E	1418-N	2600	Ibb	Yarim market
18	37625	5597	1980.06.–	4412-E	1352-N	2500	Ibb	Jabal Al Kadra
19	37633	5601	1980.06.06	4348-E	1541-N	2400	Sana'a	Dharhan
20	37639	5602	1980.06.08	4427-E	1423-N	2350	Dhamar	19 km S of Dhamar
21	37642	5603	1980.06.08	4427-E	1425-N	2200	Dhamar	Dhumara, threshing floor
22	37645	5604	1980.06.09	4425-E	1433-N	2200	Dhamar	1 km E of Ghamar
23	37646	5605	1980.06.10	4426-E	1432-N	2200	Dhamar	4 km E of Dhamar
24	37651	5606	1980.06.10	4417-E	1453-N	2200	Dhamar	10 km N of Ma'bar
25	37656	5607	1980.06.11	4401-E	1528-N	2500	Sana'a	14 km from Shibam in direction to Sana'a
26	37660	5608	1980.06.14	4422-E	1343-N	2500	Ta'izz	Dar Mutera
27	37670	5609	1980.06.17	4415-E	1515-N	2200	Sana'a	14 km S of Sana'a
28	37673	5610	1980.06.20	4359-E	1336-N	1400	Ta'izz	Ta'izz market
29	38519	5612	1980.05.–	4345-E	1655-N	1970	Sa'dah	Al Mahazer
30	38521	5613	1980.06.08	4427-E	1425-N	2200	Dhamar	Dhumara, threshing floor
31	38522	5614	1980.06.09	4425-E	1433-N	2200	Dhamar	1 km E of Ghamar
32	38523	5615	1980.06.10	4417-E	1453-N	2200	Dhamar	10 km N of Ma'bar
33	38528	5616	1980.06.–	4359-E	1336-N	1400	Ta'izz	Ta'izz market

\* nd—no data.

**Table 2.** *B. graminis* f. sp. *hordei* isolates used for artificial inoculation and their virulence spectra against resistance genes on differential set of Pallas near-isogenic lines and 11 cultivars.

No.	Pallas Isolines and Cultivars	Gene	Bgh Isolates																			
			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	P1	<i>Mla1</i>	0	0	4	4	4	0	0	0	0	0	0	4	0	0	4	0	0	4	0	0
2	P2	<i>Mla3</i>	1	0	0	0	0	0	0	0	0	0	0	4	0	0	4	4	0	0	0	0
3	P3	<i>Mla6, Mla14</i>	0	0	0	0	0	0	4	0	4	0	4	0	0	0	4	4	4	4	4	4
4	P4B	<i>Mla7, + ?</i>	4	4	4	1	0	2	2	4	4	0	2	4	4	1	4	4	1	4	4	4
5	P8B	<i>Mla9</i>	4	0	4	0	0	0	4	0	4	0	4	0	0	0	0	0	0	0	4	0
6	P10	<i>Mla12</i>	0	0	4	0	0	4	0	0	4	0	0	4	4	0	4	4	0	4	0	4
7	P11	<i>Mla13, MlRu3</i>	4	0	4	0	0	0	0	4	4	0	0	4	0	0	0	0	0	4	0	4
8	P12	<i>Mla22</i>	4	4	0	4	4	0	4	0	4	4	4	4	4	0	0	4	4	4	0	0
9	P13	<i>Mla23</i>	1	1	2	1	2	1	2	1	1	1	1	2	1	1	1	1	1	1	1	1
10	P14	<i>Mlra</i>	4	4	4	0	4	4	4	4	0	4	4	4	4	4	4	4	4	4	4	4
11	P15	<i>Ml (Ru2)</i>	4	4	4	4	3	4	2	4	4	2	0	4	4	2	4	4	4	4	4	4
12	P17	<i>Mlk</i>	4	4	4	2	2	2	2	4	2	2	0	4	4	2	4	2	2	4	4	4
13	P18	<i>Mlnn</i>	4	4	4	4	4	2	4	4	4	2	2	4	4	4	4	4	4	4	2	2
14	P19	<i>Mlp</i>	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
15	P20	<i>Mlat</i>	2	2	2	4	2	2	2	4	2	2	2	2	2	2	2	4	2	4	2	2
16	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)	0(4)
17	P23	<i>Ml (La)</i>	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4
18	P24	<i>Mlh</i>	4	4	4	0	4	4	4	4	4	4	4	4	4	4	0	4	4	4	4	4
19	Trumph	<i>Mla7, Ml (Ab)</i>	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
20	P21	<i>Mlg, Ml (CP)</i>	4	4	4	0	0	0	4	0	4	0	4	4	4	4	4	4	0	4	0	4
21	Pallas	<i>Mla8</i>	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
22	Gunnar	<i>Mla3, Ml (Tu2)</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	P4A	<i>Mla7, Mlk, + ?</i>	2	2	2	2	2	2	2	2	2	2	4	2	4	0	2	2	2	4	4	2
24	P6	<i>Mla7, MILG2</i>	4	4		0	0	2	1	2	4	0	2	2	4	0	4	2	0	4	4	4



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.

### 2.3. Landraces and Single Plant Lines Resistance Tests

Approximately 30 plants per landrace were evaluated with the Bgh 33 isolate, under controlled chamber conditions with a 16/8 h day/night photoperiod and a 22/16 °C temperature regime.

Seedlings with a fully expanded first leaf (two-leaf stage) were inoculated with powdery mildew by shaking conidia from the susceptible cv. Manchuria. After 8–10 days, infection types were scored. A five-point (0 to 4) reaction type (RT) scale was used, as follows: 0, no visible symptoms; 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, 0(4) sparse small colonies originating from the stomatal subsidiary cells [29–32]. Plants with disease scores of 0 to 1 were classified as highly resistant (R), plants that scored 2 as a moderately resistant (M), and rating of 3 or 4 as susceptible and very susceptible. Plants scored 0(4) possess the *mlo5* gene. The cultivar Manchuria was used as a susceptible control.

In the group of plant material from Yemen there were 6 single plant lines selected from 5 landraces, and which were classified as highly resistant and moderately resistant to powdery mildew. They were grown in greenhouse conditions to obtain seeds for future evaluations using a set of 20 Bgh isolates. Postulation of resistant genes was based on a comparison of reaction spectra designated on landraces lines and the barley differential set (Table 2).

The possibility of the presence of resistance genes was concluded on the basis of the gene-for-gene hypothesis [33]. The infection response spectrum of each landrace was compared with the Bgh virulence spectrum previously found on the set of barley differential varieties.

## 3. Results

Among 33 landraces from Yemen, 7 (21,2%) expressed resistance to isolate Bgh 33 of *B. graminis* f. sp. *hordei* (Table 3). Two of these landraces (5589, 5590) originate from the Al Bayda province of Yemen, and three others originate from Dhamar (5605), Sanaa (5607), and Taizz (5616), respectively.

Among the landraces from Yemen, the plants belong to seven lines selected from, five landraces were classified as highly resistant, moderately resistant, and which were evaluated using set of 20 Bgh isolates, which possess virulence genes that correspond to known resistance genes (Table 3).

The seedling leaves of two lines, 5589-1-1 and 5590-1-1, showed no visible signs of infection, except for occasional infection type 4 (compatible) mildew colonies. These colonies were also about half of the size in comparison to those observed on the susceptible control cultivar Manchurian. Such a reaction was designated as 0(4). This reaction type is characteristic for Mlo resistance, and is described as follows: sparse small colonies originating from the stomatal subsidiary cells. In addition, the young leaves of these lines were investigated under a microscope. It was observed that, in almost all cases (with two exceptions), mildew colonies originated from a successful infection in the subsidiary cells next to the stomata on the barley epidermis. Based on these observations, the possibility of *mlo* allele presence in these selections was postulated. Another highly resistant line was line 5607-1-1, which showed resistance reaction zero after infection with two isolates. Three other lines were susceptible to infection with isolate three. Six tested lines were classified in four groups according to their resistance spectrum. It was not possible to postulate the presence of specific resistance genes in four lines (5598-1-1, 5598-4-2, 5605-1-1, and 5607-1-1).

**Table 3.** Set of barley (*H. vulgare* L.) landraces originated from Yemen, which revealed resistance to at least one *B. graminis* f. sp. *Hordei* isolate after inoculation at the seedling stage.

No.	ICARDA IG	IHAR No.	Bgh Isolates																			Postulated Resistance Gene		
			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	37601	5589 1 1	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)	0(4)	0(4)	<i>mlo</i>
2	37603	5590 1 1	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)	0(4)	0(4)	<i>mlo</i>
3	-	5598 1 1	4	4	4	4	4	4	4	4	4	4	4	2	4	0	4	4	4	4	4	nd	2	?
4	-	5598 4 2	4	4	4	4	4	4	4	4	2	4	4	4	2	2	4	2	4	4	4	nd	4	?
5	37646	5605 1 1	2	4	4	4	4	2	4	4	4	4	4	4	4	2	4	4	2	2	4	4	4	?
6	37656	5607 1 1	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	?
7	-	5611 2 2	4	4	0	4	4	0	4	4	4	4	4	4	4	0	0	4	4	4	4	0	0	<i>Mla22</i>
8	38528	5616 1 2	4	4	4	0	0	0	2	2	2	0	2	4	4	2	4	2	0	4	2	nd	<i>Mla7, Ml (LG2)</i>	
9	38528	5616 3 1	4	4	4	0	0	0	2	4	2	0	2	4	4	0	4	2	0	4	2	4	<i>Mla7, Ml (LG2)</i>	

nd—no data.

#### 4. Discussion

Landraces are the easiest to use directly in breeding programs, because there are no problems in conducting crosses. They are genetically heterogeneous, dynamic populations. They come from regions with traditional agricultural culture, where there are no active systemic breeding programs [34]. They are subject to natural selection without strong breeding pressure, and they are well adapted to local climatic conditions. The local varieties often display unique traits that have been driven out of the elite varieties in the selection process, and are considered crucial for resistance breeding and the restoration and expansion of the gene pool of cultivated forms [26].

This study shows the presence of spontaneous Mlo resistance in barley landraces (5589, 5590) that were collected in Yemen, in the province Al Bayda, in an area around Al Bayda city (also transliterated as Al-Baidah or Beida, anciently Nashqum), about 200 km south-east of Sanaa. In addition, it was observed that mildew colonies originated from successful infection in the subsidiary cells next to the stomata on the barley epidermis. Only two exceptions were observed, in which the powdery mildew colony originated from a short cell in contact with the stomata. This is in agreement with the observations concerning the origin of colonies on Mlo-resistant plants, made by other investigators [12,13,34].

Controlling of powdery mildew is possible by growing genetically resistant barley cultivars [6,10,16,35]. This is a relatively inexpensive and environmentally safe barley protection measure. It started to be used from the beginning of the application of modern, intensive methods in production. Currently, the powdery mildew of barley is one of the most common and most widespread disease of barley in Europe, causing significant yield losses. However, this disease that is opposite to leaf rust was, for a long time, not important in barley production [35,36].

The genetic diversity of barley landraces offer many traits for barley breeding, especially concerning their resistance to biotic and abiotic stresses [36–38]. The genetic heterogeneity within the barley landraces is due to a low level of outcrossing occurring in barley, and farmers mixing the seed of different landraces [39–41]. In some studies, more than 50% of tested landraces were found to be a genotypic mixture [42]. However, in some cases, phenotypic diversity does not reflect genetic diversity. For example, this occurs in barleys from Ethiopia, in comparison barleys originating from the Fertile Crescent [43,44].

Yemen is characterized by big contrast in its natural conditions, because of its mountainous topography (Sarat Mountain ranges: Sarat al-Hajaz, Sarat 'Asir and Sarat al-Yemen) and big contrasts in climate because of the transitional location between the Red Sea, Gulf of Aden, and Arabian Sea on one side, and the Empty Quarter (Rub al Khali—the world's largest sand desert) on the other side. Because of its very different agro-climatic conditions, the expression of a wide array of genes, and a wide diversity of barley, is allowed. Collection missions in Yemen are recommended, because the landraces of major crops in this country are subject to genetic erosion, due to drought and desertification [23].

The most interesting finding in this study is the presence of spontaneous Mlo resistance in barley landraces (5589, 5590) that were collected in Yemen, in the province Al Bayda. Mlo resistance is associated with a negative pleiotropic effect that is expressed by increased susceptibility to necrotrophic and other pathogens, and lower yielding [36,45–50]. For the first time, Mlo resistance was identified in a local variety from Ethiopia [36,50,51]. This natural allele has been designated *mlo11*. The remaining variants were identified in barley-induced mutants. The Mlo gene encodes a transmembrane protein of unexplained function. Immunity is conditioned by loss-of-function mutants. Literature reports indicate that almost 50 *mlo* alleles have been identified [51]. All of them, with the exception of *mlo11*, were produced by mutagenesis [36,51]. The first in Europe was the Mlo-resistant barley variety 'Atem' (released in the Netherlands in 1979), which derived its resistance (*mlo11*) from the Ethiopian landrace L92. Almost all the barley varieties with Mlo resistance have the same allele, *mlo11*, with an important exception, 'Alexis' (*mlo9*). This proves that, in contrary to the mutants, barley landrace were the most important sources of Mlo resistance [12,14,36,51–53].

The presence of *mlo* resistance genes in barley collected in Yemen should be not be a very big surprise, because it was described previously in Ethiopia. These geographical regions are just separated by the strait Gate of Tears (Bab el Mandeb), which is 27.4 km wide, they are very similar on the agroecological level, and there are many records on the exchange of goods and people across this strait, from ancient times [25,54].

In the presented study, a seedling resistance test was used in order to describe the infection types that were expressed by barley recombinant lines after inoculation with the differentiated isolates of *B. graminis* f. sp. *hordei*. This kind of testing is sufficient for disease resistance screening, and is commonly used in breeding programs, to postulate the presence of genes in modern cultivars, and to screen for new sources of effective resistance [16,19,52,53]. However, these kinds of tests are not very useful to identify and describe partial resistance. For a 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 [55–57]. A final determination of the number of resistance genes, and their type of action in the tested hybrid lines, may be established with the use of crosses and backcrosses among appropriate genotypes.

## 5. Conclusions

The results of this study demonstrate the practical advantages of preserving the genetic diversity of barley in the form of landraces, to control barley powdery mildew by breeding for resistance.

This is the first report about the discovery of spontaneous Mlo resistance in barley landrace originating from Yemen, and in a country other than Ethiopia, Libya, and Turkey [12,15,16,32,58].

Future work will concentrate on the genetic study of Mlo resistance occurring in selections from the landraces 5589 and 5590, and resistance in landrace 5607. This work will include appropriate crosses and/or molecular markers. A pre-breeding effort is also needed, to introduce these alleles into elite cultivars of barley, to create initial materials for European breeding programs. This is a necessary step between the barley gene bank collections and the practical use of barley genetic resources in breeding programs.

In recent years, Mlo resistance has become a very important source of durable powdery mildew resistance in Europe [15,16]. Consequently, the new source of highly effective powdery mildew resistance that is described in this study, should be successfully used in barley breeding programs.

**Author Contributions:** Conceptualization, J.H.C.; methodology, J.H.C.; formal analysis, J.H.C. and E.C.; investigation, J.H.C.; resources, J.H.C.; writing—original draft preparation, J.H.C. and E.C.; visualization, E.C.; project administration, J.H.C.; funding acquisition, J.H.C. Both authors have read and agreed to the published version of the manuscript.

**Funding:** The results presented are based on evaluation carried out in the frame of the ECPGR-funded complementary module of the European Union project GENRES PL98-104 on barley genetic resources evaluation.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are available in tables. Contact person is Jerzy H. Czembor, IHAR-PIB Radzików.

**Acknowledgments:** Authors thank J. Valkoun, J. Konopka and S. Ceccarelli (ICARDA, Aleppo, Syria) for providing seed samples of barley landrace from Yemen, H. J. Schaerer (ETH, Zurich, Switzerland) for the powdery mildew isolates, L. Munk (Royal Agricultural and Veterinary University, Copenhagen, Denmark) for the near-isogenic lines of ‘Pallas’.

**Conflicts of Interest:** The authors declare no conflict of interest. Authors declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or

interpretation of reported research results. Any role of the funders in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results must be declared in this section.

## References

- Al-Harazi, M. Barley in the Republic of Yemen. In Proceedings of the 9th International Barley Genetics Symposium, Brno, Czech Republic, 20–26 June 2004; p. 25.
- Anonymous; FAO. The second report on the state of the world's plant genetic resources for food and agriculture. In *Commission on Genetic Resources for Food and Agriculture*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2010; p. 370.
- Knupfer, R. Triticeae genetic resources in ex situ genebank collections. In *Genetic and Genomics of the Triticeae. Plant Genetics and Genomics: Crops and Models*; Muelbauer, G.J., Feuillet, C., Eds.; Springer Science: New York, NY, USA, 2009; Volume 7, pp. 31–80.
- Hickey, L.T.; Lawson, W.; Platz, G.J.; Fowler, R.A.; Arief, V.; Dieters, M.; German, S.; Fletcher, S.; Park, R.F.; Singh, D.; et al. Mapping quantitative trait loci for partial resistance to powdery mildew in an Australian barley population. *Crop. Sci.* **2012**, *52*, 1021–1032. [[CrossRef](#)]
- Douchkov, D.; Lück, S.; Johrde, A.; Nowara, D.; Himmelbach, A.; Rajaraman, J.; Stein, N.; Sharma, R.; Kilian, B.; Schweizer, P. Discovery of genes affecting resistance of barley to adapted and non-adapted powdery mildew fungi. *Genom. Biol.* **2014**, *15*, 518. [[CrossRef](#)] [[PubMed](#)]
- Brown, J.K.M. Durable resistance of crops to disease: A Darwinian perspective. *Ann. Rev. Phytopathol.* **2015**, *53*, 513–539. [[CrossRef](#)]
- Murray, G.M.; Brennan, J.P. Estimating disease losses to the Australian barley industry. *Australas. Plant Pathol.* **2010**, *39*, 85–96. [[CrossRef](#)]
- Finckh, M.R.; Gacek, E.S.; Czembor, H.J.; Wolfe, M.S. Host frequency and density effects on powdery mildew and yield in mixtures of barley cultivars. *Plant Pathol.* **1999**, *48*, 807–816. [[CrossRef](#)]
- Hovmoller, M.S.; Caffier, V.; Jalli, M.; Andersen, O.; Besenhofer, G.; Czembor, J.H.; Dreiseitl, A.; Flath, K.; Fleck, A.; Heinrichs, F.; et al. The European barley powdery mildew virulence survey and disease nursery 1993–1999. *Agonomie* **2000**, *20*, 729–743. [[CrossRef](#)]
- McDonald, B.A.; Linde, C. Pathogen population genetics, evolutionary potential, and durable resistance. *Ann. Rev. Phytopathol.* **2002**, *40*, 349–379. [[CrossRef](#)]
- Dreiseitl, A. Differences in powdery mildew epidemics in spring and winter barley based on 30-year variety trials. *Ann. Appl. Biol.* **2011**, *159*, 49–57. [[CrossRef](#)]
- Jørgensen, I.H. Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica* **1992**, *63*, 141–152. [[CrossRef](#)]
- Czembor, J.H.; Czembor, E. Mlo resistance of barley to powdery mildew (*Blumeria graminis* f. sp. *hordei*). Part I. Genetics, phenotype, mechanism and molecular investigations. *Biul. IHAR* **2003**, *230*, 335–354.
- Czembor, J.H.; Czembor, E. Mlo resistance of barley to powdery mildew [*Blumeria graminis* f. sp. *hordei*]. Part II. Sources of resistance and their use in barley breeding. *Biul. IHAR* **2003**, *230*, 355–374.
- Czembor, J.H.; Czembor, E. Mlo resistance of barley to powdery mildew (*Blumeria graminis* f. sp. *hordei*) Part III. Durability of resistance. *Biul. IHAR* **2003**, *230*, 375–386.
- Dreiseitl, A. Specific resistance of barley to powdery mildew, its use and beyond. A concise critical review. *Genes* **2020**, *11*, 971. [[CrossRef](#)]
- Czembor, J.H.; Johnston, M.R. Resistance to powdery mildew in selections from Tunisian landraces of barley. *Plant Breed.* **1999**, *118*, 503–509. [[CrossRef](#)]
- Czembor, J.H. Resistance to powdery mildew in selections from Moroccan barley landraces. *Euphytica* **2002**, *125*, 397–409. [[CrossRef](#)]
- Dreiseitl, A. A novel resistance against powdery mildew found in winter barley cultivars. *Plant Breed.* **2019**, *138*, 840–845. [[CrossRef](#)]
- Hoseinzadeh, P.; Zhou, R.; Mascher, M.; Himmelbach, A.; Niks, R.E.; Schweizer, P.; Stein, N. High Resolution Genetic and Physical Mapping of a Major Powdery Mildew Resistance Locus in Barley. *Front. Plant Sci.* **2019**, *10*, 146. [[CrossRef](#)] [[PubMed](#)]
- Piechota, U.; Czembor, P.C.; Słowacki, P.; Czembor, J.H. Identifying a novel powdery mildew resistance gene in a barley landrace from Morocco. *J. Appl. Genet.* **2019**, *60*, 243–254. [[CrossRef](#)] [[PubMed](#)]
- Piechota, U.; Czembor, P.C.; Czembor, J.H. Evaluating barley landraces collected in North Africa and the Middle East for powdery mildew infection at seedling and adult plant stages. *Cereal Res. Commun.* **2020**, *48*, 179–185. [[CrossRef](#)]
- Bawazir, A.A. Genetic variation in population of cereal crops in the southern coastal zone of Yemen. *Plant Genet. Resour. Newsl.* **2001**, *127*, 44–45.
- Aw-Hassan, A.; Alsanabani, M.; Bamatraf, A.R. *Impact of Land Tenure and Other Socioeconomic Factors on Mountain Terrace Maintenance in Yemen*; CAPRI Working Paper, No. 3; CGIAR, IFPRI: Washington, DC, USA, 2000; pp. 1–41.
- Muller, W.W. Outline of the history of ancient Southern Arabia. In *Yemen: 3000 Years of Art and Civilization in Arabia Felix*. Pinguin-Verlag (Innsbruck) and Umschau-Verlag (Frankfurt/Main); Daum, W., Ed.; Pinguin-Verlag: Innsbruck, Austria, 1987; pp. 49–54.

26. Newton, A.C.; Akar, T.; Baresel, J.P.; Bebeli, P.J.; Bettencourt, E.; Bladenopoulos, K.V.; Czembor, J.H.; Fasoula, D.A.; Katsiotis, A.; Koutis, K.; et al. Cereal landraces for sustainable agriculture. A review. *Agron. Sustain. Dev.* **2010**, *30*, 237–269. [[CrossRef](#)]
27. Jørgensen, L.N.; Hovmøller, M.; Hansen, J.G.; Lassen, P.; Clark, B.; Bayles, R.; Rodemann, B.; Flath, K.; Jahn, M.; Goral, T.; et al. IPM Strategies and Their Dilemmas Including an Introduction to [www.eurowheat.org](http://www.eurowheat.org). *J. Integr. Agric.* **2014**, *13*, 265–281. [[CrossRef](#)]
28. Lamichhane, J.R.; Arseniuk, E.; Boonekamp, P.; Czembor, J.H.; Decroocq, V.; Enjalbert, J.; Finckh, M.; Korbin, M.; Koppel, M.; Kudsk, P.; et al. Advocating a need for suitable breeding approaches to boost integrated pest management: A European perspective. *Pest Manag. Sci.* **2017**, *74*, 1219–1227. [[CrossRef](#)]
29. Mains, E.B.; Dietz, S.M. Physiologic forms of barley mildew, *Erysiphe graminis hordei* Marchal. *Phytopathology* **1930**, *20*, 229–239.
30. Czembor, J.H.; Czembor, H.J. Resistance to powdery mildew in barley landraces collected from Jordan. *Plant Breed. Seed Sci.* **1998**, *43*, 65–80.
31. Dreiseitl, A. Genotype Heterogeneity in Accessions of a Winter Barley Core Collection Assessed on Postulated Specific Powdery Mildew Resistance Genes. *Agronomy* **2021**, *11*, 513. [[CrossRef](#)]
32. Czembor, J.H. Sources of resistance to powdery mildew in barley landraces from Libya. *Sver. Utsadesf. Tidskrift J. Swedish Seed Assoc.* **2000**, *110*, 232–248.
33. Flor, H.H. The complementary genetic systems in flax and flax rust. *Adv. Genet.* **1956**, *8*, 29–54.
34. Camacho Villa, T.C.; Maxted, N.; Scholten, M.A.; Ford-Lloyd, B.V. Defining and identifying crop landraces. *Plant Genet. Res. Characteriz. Utiliz.* **2005**, *3*, 373–384. [[CrossRef](#)]
35. Schwarzbach, E. The mlo based resistance of barley to mildew and the response of mildew populations to the use of varieties with the mlo gene. *Czech J. Genet. Plant Breed.* **1998**, *34*, 3–10.
36. Lyngkjær, M.; Newton, A.C.; Atzema, J.L.; Baker, S.J. The Barley mlo-gene: An important powdery mildew resistance source. *Agronomy* **2000**, *20*, 745–756. [[CrossRef](#)]
37. Czembor, J.H.; Czembor, H.J. Inheritance of Resistance to Powdery Mildew (*Blumeria graminis* f. sp. *hordei*) in Selections from Moroccan Landraces of Barley. *Cereal Res. Commun.* **2001**, *29*, 281–288. [[CrossRef](#)]
38. Ceccarelli, S.; Grando, S.; van Leur, J.A.G. Barley landraces of the fertile crescent offer new breeding options for stress environments. *Diversity* **1995**, *11*, 112–113.
39. Amezrou, R.; Gyawali, S.; Belqadi, L.; Chao, S.; Arbaoui, M.; Mamidi, S.; Rehman, S.; Sreedasyam, A.; Verma, R.P.S. Molecular and phenotypic diversity of ICARDA spring barley (*Hordeum vulgare* L.) collection. *Genet. Resour. Crop. Evol.* **2017**, *65*, 255–269. [[CrossRef](#)]
40. Milner, S.G.; Jost, M.; Taketa, S.; Mazón, E.R.; Himmelbach, A.; Oppermann, M.; Weise, S.; Knüpfper, H.; Basterrechea, M.; König, P.; et al. Genebank genomics highlights the diversity of a global barley collection. *Nat. Genet.* **2018**, *51*, 319–326. [[CrossRef](#)] [[PubMed](#)]
41. Parzies, H.K.; Spoor, W.; Ennos, R.A. Outcrossing rates of barley landraces from Syria. *Plant Breed.* **2000**, *119*, 520–522. [[CrossRef](#)]
42. Leur van, J.A.G.; Gebre, H. Diversity between some Ethiopian farmer's varieties of barley and within these varieties among seed sources. *Genet. Res. Crop Evol.* **2003**, *50*, 351–357. [[CrossRef](#)]
43. Abdel-Ghani, A.H.; Parzies, H.K.; Omary, A.; Geiger, H.H. Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. *Theor. Appl. Genet.* **2004**, *109*, 588–595. [[CrossRef](#)]
44. Bothmer von, R.; Sato, K.; Komatsuda, T.; Yasuda, S.; Fischbeck, G. The domestication of cultivated barley. In *Diversity in Barley (Hordeum vulgare)*; Bothmer von, R., Hintum van, T., Knüpfper, H., Sato, K., Eds.; Elsevier Science B.V.: Amsterdam, The Netherlands, 2003; pp. 9–28.
45. Chabane, K.; Valkoun, J. Characterisation of Genetic Diversity in ICARDA Core Collection of Cultivated Barley (*Hordeum vulgare* L.). *Czech J. Genet. Plant Breed.* **2011**, *40*, 134–136. [[CrossRef](#)]
46. Dreiseitl, A. Heterogeneity of Powdery Mildew Resistance Revealed in Accessions of the ICARDA Wild Barley Collection. *Front. Plant Sci.* **2017**, *8*, 202. [[CrossRef](#)]
47. Jarosch, B.; Kogel, K.-H.; Schaffrath, U. The Ambivalence of the Barley Mlo Locus: Mutations Conferring Resistance against Powdery Mildew (*Blumeria graminis* f. sp. *hordei*) Enhance Susceptibility to the Rice Blast Fungus *Magnaporthe grisea*. *Mol. Plant Microb. Interact.* **1999**, *12*, 508–514. [[CrossRef](#)]
48. Kumar, J.; Hüchelhoven, R.; Beckhove, U.; Nagarajan, S.; Kogel, K.-H. A Compromised Mlo Pathway Affects the Response of Barley to the Necrotrophic Fungus *Bipolaris sorokiniana* (Teleomorph: *Cochliobolus sativus*) and Its Toxins. *Phytopathology* **2001**, *91*, 127–133. [[CrossRef](#)] [[PubMed](#)]
49. Brown, J.K.M.; Rant, J.C. Fitness costs and trade-offs of disease resistance and their consequences for breeding arable crops. *Plant Pathol.* **2013**, *62*, 83–95. [[CrossRef](#)]
50. Jensen, H.P.; Jensen, J. Associations between three ml-o powdery mildew resistance genes and agronomic traits in barley. *Euphytica* **1990**, *46*, 185–193. [[CrossRef](#)]
51. Jørgensen, J.H.; Wolfe, M. Genetics of Powdery Mildew Resistance in Barley. *Crit. Rev. Plant Sci.* **1994**, *13*, 97–119. [[CrossRef](#)]
52. Büschges, R.; Hollricher, K.; Panstruga, R.; Simons, G.; Wolter, M.; Frijters, A.; van Daelen, R.; van der Lee, T.; Diergaarde, P.; Groenendijk, J.; et al. The Barley Mlo Gene: A Novel Control Element of Plant Pathogen Resistance. *Cell* **1997**, *88*, 695–705. [[CrossRef](#)]

53. Panstruga, R.; Molina-Cano, J.L.; Reinstadler, A.; Mueller, J. Molecular characterization of mlo mutants in North American two- and six-rowed malting barley cultivars. *Mol. Plant Pathol.* **2005**, *6*, 315–320. [[CrossRef](#)]
54. Kivisild, T.; Reidla, M.; Metspalu, E.; Rosa, A.; Brehm, A.; Pennarun, E.; Parik, J.; Geberhiwot, T.; Usanga, E.; Villems, R. Ethiopian Mitochondrial DNA Heritage: Tracking Gene Flow across and around the Gate of Tears. *Am. J. Hum. Genet.* **2004**, *75*, 752–770. [[CrossRef](#)]
55. Shtaya, M.J.Y.; Sillero, J.C.; Rubiales, D. Search of partial resistance against *Puccinia hordei* in barley landraces from the Fertile Crescent. *Plant Breed.* **2006**, *125*, 343–346. [[CrossRef](#)]
56. Shtaya, M.J.Y.; Marcel, T.C.; Sillero, J.C.; Niks, R.E.; Rubiales, D. Identification of QTL for powdery mildew and scald resistance in barley. *Euphytica* **2006**, *151*, 421–429. [[CrossRef](#)]
57. Aghnoum, R.; Marcel, T.C.; Johrde, A.; Pecchioni, N.; Schweizer, P.; Niks, R.E. Basal host resistance of barley to powdery mildew: Connecting quantitative trait loci and candidate genes. *Mol. Plant Microb. Interact.* **2010**, *23*, 91–102. [[CrossRef](#)] [[PubMed](#)]
58. Czembor, J.H.; Frese, L. Powdery mildew resistance in selections from barley landraces collected from Turkey. *Die Bodenkultur* **2003**, *54*, 35–40.