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

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**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 worldwide 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.

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

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 Riso 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 + Mk + ?, Mla7 + ?, Mla7 + MlLG2, Mla9 + Mk, Mla9, Mla12, Mla13 + MlRu3, Mla22, Mla23, MlRu2, Mk, Mlp, MlAt, and present in additional cultivars included into a differential set, as follows: Benedicte (Mla9, Ml (IM9)), Lenka (Mla13, Ml (Ab)), Gunnar (Mla3, Ml (Tu2)), Steffi (Ml (St1)), Ml (St2)), Kredit Ml (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 Hussays, NE of Sana’a             |
| 6   | 38520      | 5375     | 1980.06.08 | 4427-E    | 1423-N   | 2350      | Dhamar          | nd                                     |
| 7   | 38526      | 5376     | 1980.06.14 | 4405-E    | 1518-N   | 2500      | Sana’a          | 15 km W from Sana’a                    |
| 8   | 113096     | 5445     | nd         | 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’da’          | 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’da’          | 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 | 4357-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 | Virulence | 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 36 | Bgh 40 | Bgh 48 | Bgh 51 | Bgh 57 | Bgh 63 |
| 1   | P1 Mla1                        | 0 0 4 4 4 4 0 0 0 0 0 4 0 0 4 0 0 4 0 0 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| 2   | P2 Mla3                        | 1 0 0 0 0 0 0 0 0 0 0 0 4 0 0 0 0 0 0 0 0 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| 3   | P3 Mla6, Mla14                 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| 4   | P4B Mla7, + ?                  | 4 4 4 4 1 0 2 2 4 4 0 2 4 4 1 4 4 4 1 4 4 4 4 4 |
| 5   | P8B Mla9                       | 4 0 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| 6   | P10 Mla12                      | 0 0 4 0 0 0 4 0 0 0 4 0 0 4 0 0 4 0 0 4 0 0 0 0 |
| 7   | P11 Mla13, MLRu3               | 4 0 4 0 0 0 0 0 0 0 4 4 0 0 4 0 0 0 0 0 0 0 0 4 |
| 8   | P12 Mla22                      | 4 4 0 4 4 0 4 0 4 4 4 4 4 4 4 4 0 0 4 4 4 4 0 0 |
| 9   | P13 Mla23                      | 1 1 2 1 2 1 2 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 |
| 10  | P14 Mla7                       | 4 4 4 0 4 4 4 4 4 4 4 0 4 4 4 4 4 4 4 4 4 4 4 4 |
| 11  | P15 Ml (Ru2)                   | 4 4 4 4 3 4 2 4 4 4 2 0 4 4 2 4 4 4 4 4 4 4 4 4 |
| 12  | P17 Mlk                        | 4 4 4 0 2 2 2 2 4 4 2 2 0 4 4 2 4 2 4 2 4 4 4 4 |
| 13  | P18 Minn                       | 4 4 4 4 4 4 2 4 4 4 2 2 4 4 4 4 4 4 4 4 4 4 4 4 |
| 14  | P19 Mlp                        | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 |
| 15  | P20 Mlat                       | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 4 2 2 2 |
| 16  | P22 mlo5                       | 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) 0(4) |
| 17  | P23 Ml (La)                    | 4 4 4 4 4 4 2 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| 18  | P24 Mlh                        | 4 4 4 0 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| 19  | Triumph Mla7, Ml (Ab)          | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| 20  | P21 Mlg, Ml (CP)               | 4 4 4 0 0 0 4 0 4 0 4 0 4 4 4 4 4 4 4 4 4 4 4 4 |
| 21  | Pallas Mla8                    | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 |
| 22  | Gunnar Mla3, Ml (Tu2)          | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| 23  | P4A Mla7, Mlk, + ?             | 2 2 2 2 2 2 2 2 2 2 2 2 2 4 2 4 0 2 2 2 4 2 4 2 |
| 24  | P6 Mla7, MlLG2                 | 4 4 4 0 0 2 2 1 2 4 0 2 2 4 0 4 2 0 4 2 4 4 4 4 |
| No. | Pallas Isolines and Cultivars | Virulence | 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 36 Bgh 40 Bgh 48 Bgh 51 Bgh 57 Bgh 63 |
| 25  | P7 Mla9, Mlk                |           | 4 0 4 0 0 0 0 0 4 0 0 0 4 0 0 0 0 0 0 0 0 0 4 0 |
| 26  | P8A Mla9, Mlk               |           | 4 0 4 0 0 0 0 0 4 0 0 0 4 0 0 0 4 0 0 0 0 0 0 0 4 0 |
| 27  | P9 Mla10, MlDu2             |           | 4 4 4 0 1 4 0 4 0 2 0 4 4 4 4 4 0 0 4 4 4 4 4 4 |
| 28  | Benedicte Mla9, Ml (IM9)    |           | 0 0 4 0 0 0 0 0 4 0 0 0 4 0 0 4 4 0 4 4 0 4 0 4 |
| 29  | Lenka Mla13, Ml (Ab)        |           | 4 0 4 0 0 0 0 0 4 0 0 0 4 0 0 0 4 0 0 0 0 4 0 4 |
| 30  | Steffi Ml (St1), Ml (St2)   |           | 2 2 2 0 0 0 0 0 4 0 0 2 3 0 4 2 0 2 0 2 0 4 |
| 31  | Kredit Ml (Kr)              |           | 4 2 4 0 2 0 0 2 4 4 4 2 4 0 4 2 2 4 4 4 4 |
| 32  | Jarek Ml (Kr), + ?          |           | 4 4 4 4 4 2 4 4 4 4 4 4 4 4 4 2 4 4 2 4 4 |
| 33  | Borwina Ml (Bw)             |           | 4 3 3 0 4 0 4 4 4 2 2 3 4 4 3 4 4 2 2 |
| 34  | Manchuria -                 |           | 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 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 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 Manchuriian. 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 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 | Postulated Resistance Gene |
|-----|-----------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----------------|
| 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)  | mlo   |
| 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)  | mlo   |
| 3   | -         | 5598 1 1 | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 2     | 4     | 0     | 4     | 4     | 4     | 4     | 4     | nd    |
| 4   | -         | 5598 4 2 | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 4     | 2     | 4     | 2     | 4     | 2     | 4     | 4     | 4     | nd    |
| 5   | 37646     | 5605 1 1 | 2     | 4     | 4     | 4     | 4     | 2     | 4     | 4     | 4     | 4     | 4     | 2     | 4     | 2     | 2     | 4     | 4     | 4     | 4     | ?     |
| 6   | 37656     | 5607 1 1 | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 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     | 4     | 4     | 4     | 4     | 0     | 0     | Mla22 |
| 8   | 38528     | 5616 1 2 | 4     | 4     | 4     | 0     | 0     | 0     | 2     | 2     | 2     | 0     | 2     | 4     | 4     | 2     | 4     | 2     | 0     | 4     | 2     | Mla7, Ml (LG2) |
| 9   | 38528     | 5616 3 1 | 4     | 4     | 4     | 0     | 0     | 0     | 2     | 4     | 2     | 0     | 2     | 4     | 4     | 0     | 4     | 2     | 0     | 4     | 2     | Mla7, Ml (LG2) |

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 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.

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