Gene bank accessions are necessary for implementing many research and breeding projects. However, a great number of accessions are contaminated or confused. If such accessions are used, the results obtained from these projects are inaccurate and non-reproducible. There are methods that allow almost perfect genotype identification; nevertheless, they are relatively recent and results cannot be compared with the characteristics of the original accessions. Growing resistant cultivars is an environmentally safe and cheap way of disease management and knowledge of diverse resistance genes and their combinations can be used to identify varieties and verify their authenticity and homogeneity. For this purpose, all 172 accessions of the core collection (CC) of the Czech winter barley (Hordeum vulgare) gene bank, originating from 35 countries, were studied. For resistance tests, 51 reference isolates of Blumeria graminis f. sp. Hordei, collected in all nonpolar continents over a period of 63 years and representing the global virulence/avirulence diversity of the pathogen, were used. Only 25 barley accessions were homogeneous (genetically uniform), whereas 147 accessions were heterogeneous due to presence of different genotypes. In total, 17 resistance genes were found singly or in combinations; 76.3% of accessions with identified resistance genes carried alleles at the Mla locus. To purify the CC, progenies of individual plants must be multiplied and authenticity and homogeneity of the seed should be confirmed with resistance tests, and subsequently can be studied with more advanced methods.

Keywords: Blumeria graminis f. sp. Hordei; gene bank; Hordeum vulgare; pathogen isolates; infection response arrays; resistance gene postulation

1. Introduction

Biological diversity (biodiversity) of living organisms and their conservation is one of the basic preconditions for the development and well-being of mankind. Plants have a key role because they fix solar energy in their tissue and create the conditions necessary for other organisms, including humans. Cultivation of plant species, their selection and subsequently their breeding, resulted in a high diversity of crops represented by landraces, cultivars and other genotypes. These varieties, together with related wild species, comprise part of the available genetic resources. At present, many of these are maintained in gene banks, but there are numerous duplications of varieties [1]. Therefore, model collections—so-called core collections (CCs), which provide as much genetic diversity as possible in a limited number of genotypes—have been created [2–4]. Genetic resources are needed for improving crops, including their genetic resistance, which plays an essential role in disease management.

The genus barley (Hordeum) belongs to the grass family (Poaceae) and includes more than 30 species [5]. Hordeum vulgare L. is divided into two subspecies, wild (subsp. spontaneum) and cultivated barley (subsp. vulgare), an important cereal. Powdery mildew caused by the fungus Blumeria graminis f. sp. Hordei Marchal (Bgh) is a worldwide problem and one of the most frequent diseases of barley [6,7].

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Abstract: Gene bank accessions are necessary for implementing many research and breeding projects. However, a great number of accessions are contaminated or confused. If such accessions are used, the results obtained from these projects are inaccurate and non-reproducible. There are methods that allow almost perfect genotype identification; nevertheless, they are relatively recent and results cannot be compared with the characteristics of the original accessions. Growing resistant cultivars is an environmentally safe and cheap way of disease management and knowledge of diverse resistance genes and their combinations can be used to identify varieties and verify their authenticity and homogeneity. For this purpose, all 172 accessions of the core collection (CC) of the Czech winter barley (Hordeum vulgare) gene bank, originating from 35 countries, were studied. For resistance tests, 51 reference isolates of Blumeria graminis f. sp. Hordei, collected in all nonpolar continents over a period of 63 years and representing the global virulence/avirulence diversity of the pathogen, were used. Only 25 barley accessions were homogeneous (genetically uniform), whereas 147 accessions were heterogeneous due to presence of different genotypes. In total, 17 resistance genes were found singly or in combinations; 76.3% of accessions with identified resistance genes carried alleles at the Mla locus. To purify the CC, progenies of individual plants must be multiplied and authenticity and homogeneity of the seed should be confirmed with resistance tests, and subsequently can be studied with more advanced methods.

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Because of spontaneous mutations, genes of specific resistance against Bgh have occurred randomly in wild barley and barley landraces and have been used for the directed breeding of resistant varieties, first in Germany [8], and subsequently in other, mainly central and northwest European countries [9,10].

The Czech gene bank of winter barley includes about 2200 accessions and 172 of them have been selected for the CC. Cultivation of resistant cultivars is an environmentally safe and cheap way of crop protection and breeding barley resistant to powdery mildew has been traditionally based on use of specific genes. Even old cultivars and landraces can be characterized according to presence or absence of these genes. Changes in gene bank management provided an opportunity to reconsider the current state of conserved varieties. Therefore, the aims of this research were: (i) to test accessions of the Czech winter barley CC with a wide set of Bgh isolates; (ii) to detect seed homo/heterogeneity; and (iii) to postulate specific powdery mildew resistance genes in homogeneous accessions.

2. Materials and Methods

2.1. Plant Material and Pathogen Isolates

All accessions of the CC of the Czech gene bank of winter barley were studied. The varieties originated from 35 countries, 80% of which were from Europe. The most frequent were those from Germany, including the former German Democratic Republic (DDR) (43 accessions), followed by the USA (13), the Czech Republic including the former Czechoslovakia (12), France (11) and the Soviet Union (10 accessions).

For resistance tests, 51 selected reference isolates of Bgh were used, which had been collected in 11 countries in all nonpolar continents over a period of 63 years (1953–2016) and representing the global virulence/avirulence diversity of the pathogen. Their responses on 35 standard barley genotypes, carrying different specific resistance genes, were presented in [11]. Before inoculation, all isolates were checked for their purity and their correct pathogenicity phenotypes were verified on standard barley lines [12]. The isolates were multiplied on leaf segments of susceptible Stirling [13].

2.2. Testing Procedure

About 50 seeds of each accession were sown in two pots (80 mm diameter) filled with a gardening peat substrate and placed in a mildew-proof greenhouse under natural daylight. The primary leaves were excised when the second leaves were emerging and leaf segments 20 mm long were cut from the middle part of healthy fully-expanded leaves. Three segments of each accession were placed on the surface of media (0.8% water agar containing 40 mg L⁻¹ of benzimidazole—a leaf senescence inhibitor) in a 150 mm Petri dish. Leaf segments were placed adjacently to each other along with four segments of Stirling oriented diagonally with their adaxial surfaces facing upward.

For inoculation, a cylindrical metal settling tower of 150 mm diameter and 415 mm in height was used and a dish with leaf segments was placed at the bottom of the tower. Conidia of each isolate, taken from a leaf segment of the susceptible cultivar with fully-developed pathogen colonies, were shaken onto a square piece (40 × 40 mm) of black paper to visually control the amount of inoculum deposited. Then the paper was rolled to form a blowpipe and conidia of the isolate were blown through a side hole of 13 mm diameter in the upper part of the settling tower over the Petri dish at a concentration of ca. 10 conidia mm⁻². The dishes with inoculated leaf segments were incubated at 20 ± 1 °C under artificial light (cool-white fluorescent lamps providing 12 h light at 30 ± 5 µmol m⁻² s⁻¹).

2.3. Evaluation

Seven days after inoculation, infection response (IR = phenotype of accession x isolate interaction) on the middle part of the adaxial side of leaf segments were scored on a scale 0–4, where 0 = no mycelium and sporulation, and 4 = strong mycelial growth and sporulation [14]. IRs 3, 3–4 and 4 were considered susceptible. Each accession was tested with a minimum of two replications. If there were significant differences in IRs between
replicates, additional tests were done. A set of 51 IRs provided an infection response array (IRA) for each accession. Based on the gene-for-gene model [15] the resistance genes in accessions were postulated by comparing the IRAs with previously determined IRAs of standard barley genotypes possessing known resistance genes.

Generally, IRs 0 to 2–3 were considered resistant, but a typical IR of each resistance gene was also taken into account; e.g., Mlra has a typical IR 0, but if IR 2–3 was found without detecting any other resistance gene then it was considered as a susceptible response [16].

3. Results

Of the 172 CC accessions of winter barley, only 25 were characterized by homogeneous IRAs demonstrating the genotypic uniformity of these single-line varieties. Resistance genes were identified in 20 of these accessions, while in five the IRAs did not correspond with the reported resistances and were therefore marked as unknown (u).

Heterogeneous IRAs, which revealed presence of multiple genotypes, were detected in 147 accessions, but in 59 of them the corresponding resistance genes were identified. In 42 of these, the genes were identified by excluding a low proportion of IRs that indicated an admixture with other genotypes. In 17 accessions, their resistances were deduced according to their being composed of two lines based on different homogeneous IRAs within each accession. Thirty-four IRAs obtained in resistance tests of accessions and representing identified specific genes and their combinations are shown in Table 1. In 88 accessions, resistance genes could not be identified due to the high heterogeneity of their IRAs.

A total of 76 accessions, phenotypes of 17 known specific resistance genes were obtained individually (13 genes) or in combinations, and in three accessions (Krakowski, Krusevacki and Opolski 152) no resistance genes were detected. Among the 17 Ml genes, the most frequent—aLo, a8, Ch, h and ra—were found in 25, 19, 12, 12 and 12 accessions, respectively. However, some genes, for example a8, Ch and ra, may also be present in other varieties because their phenotype is masked by resistance genes such as a6, a7, a12, a13 or g that were detected in 15 accessions. Besides the abovementioned three accessions, full susceptibility was found in one of two lines of four accessions. Fifty-eight of the 76 accessions with identified resistances (= 76.3%) carried genes located at the Mla locus (Table 2).
Table 1. Infection response arrays produced by nine *Blumeria graminis* f. sp. *hordei* isolates on 34 barley genotypes and their powdery mildew resistance genes.

| No. | Ml Gene(s) | Race I | J-462 | EA30 | PF512 | C-132 | 3-33 | 65 | GH | 54 |
|-----|------------|--------|-------|------|-------|-------|------|----|----|----|
| 1   | none       | 4      | 4     | 4    | 4     | 4     | 4    | 4  | 4  | 4  |
| 2   | a6         | 0      | 4     | 2    | 4     | 4     | 0    | 0  | 0  | 0  |
| 3   | a6, h      | 0      | 4     | 2    | 4     | 1–2   | 0    | 0  | 0  | 0  |
| 4   | a6, h, ra  | 0      | 4     | 0–1  | 4     | 1–2   | 0    | 0  | 0  | 0  |
| 5   | a6, ra     | 0      | 4     | 0–1  | 4     | 4     | 0    | 0  | 0  | 0  |
| 6   | a7         | 0      | 0     | 1–2  | 4     | 4     | 0    | 0  | 1–2| 1–2|
| 7   | a7, h      | 0      | 0     | 1–2  | 4     | 1–2   | 0    | 0  | 1–2| 1–2|
| 8   | a8         | 0      | 4     | 4    | 4     | 4     | 4    | 4  | 4  | 4  |
| 9   | a8, Dr2, ra| 0      | 4     | 0–1  | 4     | 4     | 0–1  | 2  | 4  | 4  |
| 10  | a8, h      | 0      | 4     | 4    | 4     | 1–2   | 1–2  | 0  | 1–2| 1–2|
| 11  | a8, h, ra  | 0      | 4     | 0–1  | 4     | 1–2   | 1–2  | 0–1| 1–2| 1–2|
| 12  | a8, He2    | 0      | 4     | 4    | 4     | 4     | 4    | 4  | 4  | 2–3|
| 13  | a8, VIR    | 0      | 4     | 4    | 1     | 4     | 4    | 4  | 4  | 4  |
| 14  | a12        | 1      | 4     | 4    | 4     | 4     | 1    | 1  | 1  | 1  |
| 15  | a13        | 0      | 0     | 0    | 0     | 4     | 0    | 0  | 0  | 0  |
| 16  | aLo        | 0      | 0     | 4    | 4     | 4     | 4    | 4  | 4  | 4  |
| 17  | aLo, Dr2,  | 0      | 0     | 4    | 4     | 4     | 4    | 4  | 4  | 4  |
| 18  | aLo, h     | 0      | 0     | 4    | 4     | 1–2   | 1–2  | 4  | 1–2| 1–2|
| 19  | aLo, Lu    | 0      | 0     | 4    | 4     | 4     | 4    | 1–2| 1–2| 1–2|
| 20  | aLo, Lu, Ru2| 0    | 0     | 4    | 2–3   | 4     | 4    | 1–2| 1–2| 2–3|
| 21  | aLo, VIR   | 0      | 0     | 4    | 1     | 4     | 4    | 4  | 4  | 4  |
| 22  | a2         | 2      | 4     | 2    | 2     | 4     | 2    | 2  | 4  | 2  |
| 23  | at, h      | 2      | 4     | 2    | 2     | 4     | 1–2  | 2  | 2  | 1–2| 1–2|
| 24  | Dr2, ra    | 4      | 4     | 0–1  | 4     | 4     | 4    | 0–1| 2  | 4  |
| 25  | g          | 0      | 4     | 0    | 4     | 4     | 0    | 0  | 4  | 4  |
| 26  | h          | 4      | 4     | 4    | 4     | 1–2   | 1–2  | 4  | 1–2| 1–2|
| 27  | h, ra      | 4      | 4     | 0–1  | 4     | 1–2   | 1–2  | 0–1| 1–2| 1–2|
| 28  | Ch         | 2      | 4     | 4    | 4     | 4     | 4    | 4  | 4  | 4  |
| 29  | Ch, Dr2, ra| 2      | 4     | 0–1  | 4     | 4     | 4    | 0–1| 2  | 4  |
| 30  | Ch, ra     | 2      | 4     | 0–1  | 4     | 4     | 4    | 0–1| 4  | 4  |
| 31  | ra         | 4      | 4     | 0–1  | 4     | 4     | 4    | 0–1| 4  | 4  |
| 32  | Ru2        | 4      | 4     | 4    | 2–3   | 4     | 4    | 2–3| 2–3| 2–3|
| 33  | VIR        | 4      | 3     | 4    | 1     | 3     | 3    | 3  | 3  | 3  |
| 34  | Wo         | (2)    | (3)   | (3)  | (3)   | (3)   | (3)  | (3)  |

1. Phenotypes of host-pathogen interactions evaluated according to [14], where 0 = resistant and 4 = susceptible.
2. Genotype of standard variety Peruvian; Mlat was found only in combination with Mlh and response types of both these genes are similar (2 or 1–2). Parentheses indicate smaller number of colonies.

Table 2. Specific resistance genes against *Blumeria graminis* f. sp. *hordei* in 172 accessions of the Czech core collection of winter barley.

| Variety | State | Gene Bank No. | Het | Ml Gene(s) |
|---------|-------|---------------|-----|------------|
| Ager    | FRA   | 01C0500254    | H   | Ch, Dr2, ra|
| Agrilo  | DEU   | 01C0501570    | H   |            |
| Aizn Coiled Necn | JPN | 01C0500490 | H   |            |
| Alaska  | USA   | 01C0500491    | H   | aLo + aLo, h|
| Alissa  | DEU   | 01C0501710    | H   |            |
| Alterna | DDR   | 01C0500492    | a8  |            |
| Angela  | FRA   | 01C0501850    | H   |            |
| Anson   | GBR   | 01C0501355    | a8  |            |
| Antoninski | POL | 01C050048    | H   | aLo + Ch   |
| Argovia | CHE   | 01C0500891    | H   | a6, h, ra  |
| Aviron  | DEU   | 01C0501830    | H   | a6         |
| Babylone| FRA   | 01C0501571    |     |            |
Table 2. Cont.

| Variety                      | State   | Gene Bank No.      | Het  | MI Gene(s)          |
|------------------------------|---------|--------------------|------|--------------------|
| Bahadar                      | ETH     | 01C0500785         | H    |                    |
| Bankutti 14                  | HUN     | 01C0500257         | H    |                    |
| Beloruskij                   | SUN     | 01C0500747         | H    | aLo                |
| Boehmerwaelder               | DEU     | 01C0500112         | H    |                    |
| Bonanza                      | CAN     | 01C0500798         | H    |                    |
| Bora                         | AUT     | 01C0501848         | H    |                    |
| Bordia                       | BEL     | 01C0500047         | H    |                    |
| Borwina                      | DDR     | 01C0500836         | H    |                    |
| Breustedts Atlas             | DEU     | 01C0500174         | H    | aLo                |
| Breustedts Schladener        | DEU     | 01C0500025         | H    | aLo                |
| Brucker Vierzeilige No. 4    | AUT     | 01C0500175         | H    |                    |
| Brucker Zweizeilige No. 34   | AUT     | 01C0500176         | H    | a8 + a8, He2       |
| Camera                       | GBR     | 01C0501807         | H    | a7, h              |
| Capri                        | BEL     | 01C0500771         | H    |                    |
| Carola                       | DEU     | 01C0501703         | H    | u4                 |
| Carstens Zweizeilige         | DEU     | 01C0500130         | aLo  |                    |
| Carstenuv dvourady           | DEU     | 01C0500040         | aLo  |                    |
| Cenad 450                    | ROM     | 01C0500505         | H    | aLo                |
| Cenader Sechszeilige Typ B   | DDR     | 01C0500053         | H    | Ch                 |
| Cirpan 5652                  | BGR     | 01C0500189         | H    |                    |
| Clerix                       | FRA     | 01C0501556         | ra   |                    |
| Condorcor                    | DZA     | 01C0500749         | u    |                    |
| Cyklon                       | SUN     | 01C0500981         | H    |                    |
| Dagestanskij (Samuricum 293) | AZE     | 01C0500036         | H    | aLo + none         |
| Dana                         | ROM     | 01C0501501         | H    |                    |
| Decatur                      | USA     | 01C0500372         | H    |                    |
| Dover                        | CAN     | 01C0500369         | H    |                    |
| Drop                         | FRA     | 01C0501816         | H    |                    |
| Duet                         | GBR     | 01C0501683         | H    |                    |
| Eckendorfer Glatta           | DEU     | 01C0500277         | H    |                    |
| Eckendorfer                  | DEU     | 01C0500026         | H    |                    |
| Eckendorfer Vulkan           | DEU     | 01C0500420         | H    |                    |
| Engelens Dea                 | DEU     | 01C0500197         | H    |                    |
| Erfa                         | DDR     | 01C0500806         | aLo, | Lu                 |
| Esther                       | DEU     | 01C0500989         | H    |                    |
| Fimbull II                   | SWE     | 01C0500212         | H    | Ch                 |
| Firlbecks Astrid             | DEU     | 01C0500196         | H    |                    |
| Franger                      | USA     | 01C0500894         | H    |                    |
| Freya                        | DEU     | 01C0500918         | a6   |                    |
| Friedrichswerther Berg       | DEU     | 01C0500003         | aLo, | Dr2                |
| Frolic                       | GBR     | 01C0501399         | g    |                    |
| Frost                        | SWE     | 01C0501400         | H    | a6, h              |
| Gerum                        | BGR     | 01C0501491         | H    |                    |
| GK Eszter                    | HUN     | 01C0501812         | H    |                    |
| GK Metal                     | HUN     | 01C0501814         | H    |                    |
| Gloria                       | ROM     | 01C0500868         | a8   |                    |
| Groninger                    | DEU     | 01C0500518         | aLo  | Ch                 |
| Grosier                      | GBR     | 01C0501362         | a12  |                    |
| Guadiana                     | ESP     | 01C0500914         | H    |                    |
| Hardy                        | AUT     | 01C0501758         | H    |                    |
| Hatif de Grignon             | FRA     | 01C0500274         | aLo  | aLo, Dr2           |
| Hatvanit 377                 | HUN     | 01C0500677         | a8   | Ch                 |
Table 2. Cont.

| Variety                  | State | Gene Bank No. | Het | MI Gene(s) |
|--------------------------|-------|---------------|-----|------------|
| Hauters Wintergerste    | DEU   | 01C0500199    | H   |            |
| Hokkaidou Hadaka         | JPN   | 01C0501937    | H   |            |
| Hooded 10                | USA   | 01C0500525    | H   |            |
| Cordzay 18               | TJK   | 01C0500297    | H   | a8         |
| Ibiza                    | BEL   | 01C0501762    | H   |            |
| Intensiv 2               | ROM   | 01C0500752    | H   | a8, h, ra  |
| Iskra                    | SUN   | 01C0500871    | H   |            |
| Jolante                  | DEU   | 01C0501704    | a6  |            |
| Jubilej 100              | BCR   | 01C0501381    | H   | a8 + Ch    |
| Jubilejnij               | UKR   | 01C0500299    | H   |            |
| Juduraki                 | JPN   | 01C0500533    | H   |            |
| Jura                     | DEU   | 01C0501646    | a7  |            |
| Jutta                    | DDR   | 01C0500084    | H   | a8         |
| Kamil                    | CSK   | 01C0501070    | aLo, Lu, Ru2 |
| Karcagi 1039             | HUN   | 01C0500168    | Ch  |            |
| Karnobat                 | BGR   | 01C0501367    | H   | Ch         |
| KIM M3 53/54             | CSK   | 01C0500221    | aLo |            |
| Kirgizskij 247           | KGZ   | 01C0500188    | aLo |            |
| Kleinwanzlebener Record  | DDR   | 01C0500007    | H   | Ch + Ch, ra|
| Kompolti 4               | HUN   | 01C0501836    | H   | Ru2        |
| Konjicski                | SUN   | 01C0500304    | H   |            |
| Kostek                   | POL   | 01C0501813    | a8 + aLo none |
| Krakowski                | POL   | 01C0501814    | H   |            |
| Krasnodarskij 2929       | SUN   | 01C0500034    | H   |            |
| Kromir                   | CSK   | 01C0501131    | H   |            |
| Kromoz                   | CSK   | 01C0501106    | H   |            |
| Kruglik 21               | SUN   | 01C0501000    | H   | Ch         |
| Krusevacki               | YUG   | 01C0500543    | H   | none       |
| Kujawiak III             | POL   | 01C0501815    | H   | aLo + aLo, h |
| Ledeci Beta              | HUN   | 01C0500246    | H   | a8 + Ch    |
| Leon                     | NLD   | 01C0500310    | H   |            |
| Local (Balkan)           | GRC   | 01C0500225    | H   |            |
| Local (Merkez-Kaza)      | TUR   | 01C0500291    | H   | at, h, aLo, VIR |
| Lomerit                  | DEU   | 01C0501835    | H   |            |
| Lunet                    | CSK   | 01C0501016    | H   |            |
| Luran                    | CZE   | 01C0501538    | H   | u          |
| Luxor                    | CSK   | 01C0501234    | H   |            |
| Maguelone                | FRA   | 01C0500782    | H   |            |
| Marconee                 | USA   | 01C0500545    | H   | u          |
| Marinka                  | NLD   | 01C0501081    | H   | a7         |
| Marjorie                 | FRA   | 01C0501820    | H   | a8, h      |
| Marna                    | FRA   | 01C0501572    | H   | ra         |
| Martha                   | AUT   | 01C0500770    | H   | u          |
| Mc Nair 601              | USA   | 01C0500434    | H   |            |
| Merlot                   | DEU   | 01C0501890    | H   | a6, h, ra  |
| Michigan Winter          | USA   | 01C0500312    | H   | aLo        |
| Mijana                   | MDA   | 01C0501672    | H   | aLo        |
| Miraj 1                   | ROM   | 01C0500869    | H   |            |
| Mironovskij 82           | UKR   | 01C0501676    | H   |            |
| Monaco                   | FRA   | 01C0501289    | H   | ra         |
| Muellers                 | DEU   | 01C0500135    | H   |            |
| Boehmmerwaelder          | DEU   | 01C0500135    | H   |            |
| Nachicivandany           | AZE   | 01C0500098    | H   |            |
| Nakaizumi Zairai         | JPN   | 01C0500548    | H   | a8 + none  |
| Nelly                    | DEU   | 01C0501709    | H   |            |
| Noveta                   | DNK   | 01C0501495    | H   |            |
| Novosadski 703           | YUG   | 01C0501569    | H   |            |
| O.A.C. Halton            | CAN   | 01C0501045    | H   |            |
| Odesskij 2095            | UKR   | 01C0501097    | H   |            |
Table 2. Cont.

| Variety              | State | Gene Bank No. | Het | MI Gene(s) |
|----------------------|-------|---------------|-----|------------|
| Okal                 | CSK   | 01C0501032    | H   |            |
| Okayama Mitsuki      | JPN   | 01C0501946    | H   |            |
| Hadaka               |       |               |     |            |
| Oksamyt              | UKR   | 01C0500881    | H   | aLo        |
| Oma                  | USA   | 01C0500326    | H   |            |
| Opolski 152          | POL   | 01C0500760    | H   | none       |
| Pallidium 310/1      | AZE   | 01C0501099    | H   |            |
| Pallidium 728/15     | SUN   | 01C0501102    | H   |            |
| Pamina               | DDR   | 01C0500744    | H   |            |
| Pavlovicky           | CSK   | 01C0500002    |     | a8         |
| Peragis Mittelfruehe | DEU   | 01C0500006    | a8  |            |
| Perga                | DEU   | 01C0500202    | H   |            |
| Persikum 64          | SUN   | 01C0500332    | H   |            |
| Poljarnyj 14         | SUN   | 01C0500563    | H   |            |
| Po-ri                | PRK   | 01C0500018    | H   |            |
| Probsdorfer Robusta  | AUT   | 01C0500866    | H   |            |
| Protidor             | ITA   | 01C0500973    | H   |            |
| Ragusa 34-40         | YUG   | 01C0500335    | H   |            |
| Rapidan              | USA   | 01C0500902    | H   | a8, VIR    |
| Rengapolbordi        | PRK   | 01C0500800    | H   |            |
| Reni                 | DEU   | 01C0501843    | H   | Ch, ra + ra|
| Rozen                | BGR   | 01C0501377    | H   |            |
| Russe 85             | BGR   | 01C0500008    | H   |             |
| Scorpio              | BEL   | 01C0501623    | H   |            |
| Schwarze             | DEU   | 01C0500123    | aLo|            |
| Wintergerste         | DEU   | 01C0500917    | H   | Dr2, ra    |
| Sigra                | DEU   | 01C0501797    | H   | a6, ra     |
| Silke                | DEU   | 01C0500030    | H   | aLo + ra   |
| Sirvandany 30        | AZE   | 01C0500012    | H   | aLo         |
| Slaski Il            | POL   | 01C0501038    | H   |             |
| Sorra                | DDR   | 01C0500271    | H   |             |
| Strengs Dura         | DEU   | 01C0500042    | H   |             |
| Stupicky dvouradry   | CSK   | 01C0500001    | H   |             |
| Stupicky sestirady   | CSK   | 01C0500100    | H   |             |
| Sumovský             | CSK   | 01C0500101    | aLo|             |
| Tamaris              | FRA   | 01C0501714    | H   | h, ra      |
| Tiffany              | DEU   | 01C0501580    | H   | a7          |
| Traminer             | DEU   | 01C0501889    |     |             |
| Tschermaks           | AUT   | 01C0500348    | H   | a8          |
| Vierzellige Glatte   | TKM   | 01C0500352    | H   |             |
| Uzen-czjan 64        | ITA   | 01C0500585    | H   |             |
| Ventitrite           | NLD   | 01C0501849    | H   |             |
| Vilna                | ARM   | 01C0500355    | VIR|             |
| VIR 6139             | NLD   | 01C0501815    | H   |             |
| Virgo                | ARM   | 01C0500945    | H   |             |
| Vogelsanger Gold     | USA   | 01C0500586    | H   |             |
| Volbar               | USA   | 01C0500373    | H   | a7 + a7, h |
| Wade                 | USA   | 01C0500219    | H   | Wo          |
| Will                 | USA   | 01C0500102    | H   |             |
| Zalarinec            | SUN   | 01C0500591    | H   |             |
| Zend                 | TUR   | 01C0500592    | a8  |             |
| Zenit                | BGR   | 01C0501382    | a13 |             |

1 [17]. 2 ARM Armenia, AUT Austria, AZE Azerbaijan, BEL Belgium, BGR Bulgaria, CAN Canada, CSK Czechoslovakia, CZE Czech Republic, DDR German Democratic Republic, DEU Deutschland, DNK Denmark, DZA Algeria, ESP España, FRA France, GBR Great Britain, GRC Greece, HUN Hungary, CHE Switzerland, ITA Italy, JPN Japan, KGŻ Kyrgyzstan, MDA Moldavia, NLD Netherlands, POL Poland, PRK Democratic People’s Republic of Korea, ROM Romania, SUN Soviet Union, SWE Sweden, TJK Tajikistan, TKM Turkmenistan, TUR Turkey, UKR Ukraine, USA United States of America, YUG Yugoslavia. 3 Heterogeneous—the variety is composed of two or more genotypes with different resistance genes. 4 Unknown.
4. Discussion

The heterogeneity of accessions in the collection is high and can have several causes [18], including methods of breeding cultivars or collecting landraces, out-crossing and mechanical admixtures. The frequency of heterogeneous accessions may be even higher, especially in the groups of accessions with identical resistances (in this study mainly MlaLo and Mla8) possibly resulting from cross-contamination. For example, a mixture of Belorusskij and Breustedts Atlas, which both contain MlaLo, would be homogeneous in resistance tests and its heterogeneity through contamination of these varieties would not be revealed.

A second possibility for incomplete detection of heterogeneity is that for each of $M_l$ genes $a8$, Ch, Dr2, Ho2 and VIR there was only one avirulent isolate available. Since accessions were represented by only three leaf segments in the tests, it is possible that this small sample might not have detected underlying heterogeneity.

Another possibility of not detecting possible heterogeneity is the overlap of IRAs of resistance genes. For example, Mla8 can only be detected by the avirulent isolate Race I, which is also avirulent on accessions containing the unlinked resistance gene Mlg (characterized by IR 0); and hence, Mla8 cannot be detected in the presence of Mlg.

Brown and Jørgensen [19] compiled published results of specific resistance genes in European barley cultivars. The catalog includes 699 barley varieties, almost all grown in the 20th century and among them are 117 winter barleys. The spring Haisa II and Union, carrying Mlg derived from Pflugs Intensiv, and the winter varieties Dea and Hauters Wintergerste derived from Ragusa b, are among the first varieties with powdery mildew resistance genes introduced in breeding programs (Table 3). In the present report, Mlh and Mlra were the most frequent genes used by breeders, whereas Mlg was found only in Frolic. However, evolution of the pathogen in winter and spring cultivars has occurred over a long period [20] and the virulence frequencies to these genes in a central European population increased to almost 100% in 2002 [21], although later the virulence frequency to Mlg decreased to 84.3% in 2017 [22]. The three $M_l$ genes ($g$, $h$ and $ra$) have no practical value in grown cultivars but can be used for characterizing barley genotypes.

Table 3. First recorded use of specific resistance genes to powdery mildew in the breeding of European barley cultivars.

| $M_l$ Gene(s) | Cultivar       | Growth Habit | Year of Registration |
|--------------|---------------|--------------|---------------------|
| $g$          | Haisa II      | S            | 1950 $^2$           |
| $g$          | Union         | S            | 1955 $^3$           |
| $g$          | Tipper        | W            | 1980 $^3$           |
| $ra$         | Dea           | W            | 1953 $^2$           |
| $h$, $ra$    | Hauters Wintergerste | W            | 1953 $^3$           |
| $a6$         | Maris Badger  | S            | 1963 $^3$           |
| $a6$         | Vogelsanger Gold | W            | 1965 $^3$           |
| $Wo$         | Doris, Ogra   | W            | 1974 $^3$           |
| $Wo$, $ra$   | Hexa          | W            | unknown $^3$        |
| $a12$        | Maris Trojan  | W            | 1975 $^3$           |
| $a13$        | Rupal         | S            | 1972 $^3$           |
| $a13$        | NR 468        | W            | 1977 $^4$           |
| $a13$, $g$   | Koral         | S            | 1978 $^5$           |
| $a13$        | Zenit         | S            | 1985 $^5$           |
| $a13$        | Zenit         | W            | 1987 $^6$           |
| $a13$        | Pipkin        | W            | 1983 $^3$           |
| $a7$         | Marinka       | W            | 1985 $^3$ $^4$      |
| $a7$         | Ola           | W            | unknown $^3$        |

$^1$ S spring, W winter. $^2$ [17]. $^3$ [19]. $^4$ [23]. $^5$ [24]. $^6$ This contribution.

Completely ineffective genes with no positive effect on resistance in agricultural cultivation were uncovered. These included first, Mla8, often present in old and mainly spring barleys [25,26], second, MlaLo, so far found only in winter barleys [27,28] and
third, *MlCh*, found in both winter and spring varieties [29]. These genes must have been contained in barley varieties for a long time since the global pathogen population has completely adapted to them, because no corresponding avirulent pathotypes have been found in cultivated barley over almost seven decades. Therefore, these genes could only be revealed through resistance tests using avirulent old Japanese isolates (*Mla8* and *MlCh*) or isolates collected from wild barley in Israel (*MlaL*). In these tests, these three genes and especially *MlaL* were the most frequent. A new gene, *MlVIR*, initially found in this CC and reported recently [29], was resistant to only one of 51 isolates used. This gene, similar to the above three genes discussed in this paragraph, can also be used for genetic characterization of barley varieties. A current complete list of barley powdery mildew resistance genes also can be found in [29].

We found *Mla7* in Marinka, which confirmed the result of Dreiseitl [23]. However, in the European catalog, a combination of *Mla7* and *Mlg* genes is listed [19]. A possible explanation is that some authors reported the identified resistance genes regardless of whether they were a single-line variety or a variety consisting of two or more similar genotypes. As an example, Alaska tested here is composed of two genotypes, one carrying *MlaL* and the other with the same resistance (*MlaL*) supplemented by another gene (*Mlh*). Therefore, during maintenance breeding, one of these lines may be inadvertently selected.

Of the set of 79 accessions with an identified resistance, Zenit from Bulgaria was the only one carrying *Mla13*. This gene was first used in Swedish spring barley varieties Rupal (1972) and Seru (1973) and its source was the Indian landrace Rupee [19]. The same allele, derived from the Balkan landrace Imunne 25 [30], became the most important gene of specific resistance in barley, especially in the former Czechoslovakia where it was first transferred into the high-quality spring malting varieties Koral and Safir registered in 1978 [24]. *Mla13* was at the time fully effective in the field and present in several new varieties. These cultivars contributed to 57% of the crop’s area in 1983 and until 1985 they had been grown on 1.5 Mha [31]. In 1985, a strong *Bgh* epidemic occurred especially in these varieties and the “Czechoslovakian” pathogen population was the cause of overcoming the resistance; it resulted in reduced yield and quality and led to changes in the varietal composition of barley in much of Europe [32].

It was subsequently found that *Mla13*, derived from the breeding strain Platen 49–49 bred in Germany, was used in the Bulgarian winter barley NR 468 registered in that country one year before the registration of Koral in the Czech Republic [23]. Zenit, tested in our study, was registered in Bulgaria 10 years after NR 468, but is still among the first winter varieties carrying *Mla13*. It is likely that the donor of this allele was also Platen 49–49.

In the Czech Republic, Zenit was registered in 1985 and also carries *Mla13* [24]. When the same and formerly rarely encountered allele, especially in winter barley, was found in Bulgarian Zenit, the first conclusion drawn was that Bulgarian and Czech Zenit cultivars were identical. Nevertheless, although they had the same names and resistance alleles and similar years of registration, the Bulgarian Zenit is six-rowed and has a winter habit whereas the Czech Zenit is a spring type and two-rowed. As mentioned above, specific resistance genes can be useful to characterize varieties and confirm their authenticity and pedigree [33]. However, the unique case of both Zenit varieties shows the contrary.

In gene banks, the resistance of accessions to pathogens is assessed during their multiplication in the field [34,35]. As has been shown here, most accessions are heterogeneous with different resistance of individual components (genotypes). Almost all accessions carry specific resistance genes, the efficacy of which on small plots of gene banks can be significant, while the same specific resistance in large commercial fields can be negligible. Genetic resources are used primarily for breeding new varieties and for further research. From this point of view, it is especially important to exploit non-specific resistance caused by minor genes [29]. However, because of the masking effects of major genes, evaluation of gene bank accessions in the field may not allow the recognition of minor genes which could be pyramided to provide valuable durability.
5. Conclusions

- Crop genetic resources are among the basic preconditions for the development and well-being of mankind and they are maintained in gene banks. However, many accessions are not genetically uniform, mostly due to mechanical admixtures of other genotypes.
- Growing resistant cultivars is an environmentally safe and cheap way of disease management but knowledge of diverse resistance genes and their combinations, together with knowing the history of individual genes, can be also used to identify varieties and verify their authenticity and homogeneity.
- In 172 accessions of the core collection (CC) of the Czech winter barley gene bank, resistance genes against powdery mildew were studied; however, 147 accessions were heterogeneous due to presence of different genotypes.
- In total, 17 resistance genes were found singly or in combinations; 76.3% of accessions with identified resistance genes carried alleles at the \( Mla \) locus.
- For purifying the accessions, progenies of individual plants are multiplied and authenticity and homogeneity of the seed will be confirmed with resistance tests. Subsequently the accessions can be used without risk of false results and can be studied with more advanced methods.

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