

Powdery mildew resistance in selections from barley landraces collected from Turkey

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Mehltauresistenz in Selektionen von Gerste-Landsorten aus der Türkei

1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world, after wheat, maize and rice. In West Asia, including Turkey, barley is often grown in marginal

agricultural areas with low annual precipitation (often less than 220 mm). Landraces in this area 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 (CECCARELLI et al., 2000).

Zusammenfassung

101 Herkünfte in der Türkei gesammelter Gerste-Landsorten wurden auf Resistenz gegen Mehltau geprüft. Die Landsorten stammten aus der Genbank des Bundesamts für Züchtungsforschung (BAZ) Braunschweig, Deutschland. Vier getestete Landsorten (4023, 4061, 4097 und 4124) zeigten nach Beimpfung mit Mehltau Resistenz. Von diesen Landsorten wurden neun Einzelpflanzenlinien selektiert. Diese Linien wurden mit 19 verschiedenen Isolaten des Mehltaus geprüft. Diese Isolate wurden entsprechend ihrer Virulenzspektren ausgewählt, welche an den Differentialsortimenten aus ‚Pallas‘-Isolinien und aus 7 zusätzlichen Differenzierungssorten beobachtet wurden. Diese Isolate repräsentierten zusammen die Virulenzen gegenüber allen Hauptresistenzgenen, die in der Vergangenheit und gegenwärtig in Europa Verwendung finden. Vier von der Landsorte 4124 abstammende Linien (BGRC38917) zeigten Resistenz gegenüber allen verwendeten Isolaten und stellen eine hochwirksame Resistenzquelle dar. Die Resistenz dieser Linien ist entweder eine Mlo-Resistenz oder eine hocheffektive partielle Resistenz, die die Verbreitung des Pathogens bestimmt. In den verbliebenen fünf Linien war es unmöglich, die spezifischen Allele oder Allele für die Resistenz festzustellen. Die Verwendung von neuen Resistenzquellen (unbestimmte Gene, Mlo oder partielle Resistenzen) von türkischen Landsorten für die Diversifikation von Resistenzgenen in Gerstesorten wird diskutiert.

Schlagworte: Mehltau, *Blumeria graminis*, Kulturgerste.

Summary

One hundred one accessions of barley landraces collected from Turkey were screened for resistance to powdery mildew. Landraces originated from the Federal Centre for Breeding, Research on Cultivated Plants (BAZ) Gene Bank, Braunschweig, Germany. Four tested landraces (4023, 4061, 4097 and 4124) showed resistance after inoculation with powdery mildew. From these landraces 9 single plant lines were selected. These lines were tested with 19 differential isolates of powdery mildew. The isolates were chosen according to their virulence spectra, which were observed on the 'Pallas' isolines differential set and 7 additional differential cultivars. These isolates represented collectively virulences to all major resistance genes used in the past and currently in Europe. Four lines derived from landrace 4124 (BGRC38917) showed resistance to all isolates used and represent highly effective source of resistance. Resistance of these lines is either Mlo or highly effective partial resistance that involve prevention of pathogen penetration. In remaining 5 lines, it was impossible to postulate which specific allele or alleles for resistance are present. Use of new sources of resistance (unknown genes, Mlo or partial resistance) from Turkish barley landraces for diversification of resistance genes for powdery mildew in barley varieties is discussed.

Key words: *Blumeria graminis* (Erysiphe graminis), *Hordeum vulgare*.

Blumeria graminis (DC.) Golovin ex Speer f.sp. *hordei* Em. Marchal (synmorph *Erysiphe graminis* DC. f.sp. *hordei* Em. Marchal.) is important pathogen of barley (ZWATZ, 1987; CZEMBOR, 1996). The use of resistant cultivars is the most effective method to control powdery mildew and the incorporation of new genes for resistance to powdery mildew into barley cultivars has been very useful in combating powdery mildew. However, the resistance conferred by most of these new genes has not been maintained for more than a few years with one exception, which is the Mlo resistance (JØRGENSEN, 1994; HOVMOLLER et al., 2000). The Mlo resistance is a unique type of resistance because it is monogenic, non-race-specific and durable. Recessive alleles at the *mlo* locus condition penetration resistance of barley of attacked epidermal cells of barley due to rapid deposition of large callose-containing appositions (papillae) in the epidermal cell wall, directly subtending the attacking fungal appressoria (SCHWARZBACH, 1998; LYNKJAER et al., 2000). Mlo resistance has become a very important source of powdery mildew resistance in barley because there is no known virulence for these genes. However many factors e.g. temperature, water stress or light intensity may affect the expression of this genes (SCHWARZBACH, 1998; LYNKJAER et al., 2000). 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. Since 1979 (registration of cultivar 'Atem') the Mlo resistance has been deployed in more than 100 cultivars throughout Europe in spring barley (SCHWARZBACH, 1998; SCHWARZBACH, 2001 personal communication). It is estimated that about 20% spring barley cultivars grown in Central Europe carry Mlo resistance (SCHWARZBACH, 1998; CZEMBOR and CZEMBOR, 2001).

South East Turkey is considered as a part of the Fertile Crescent which is assumed to be the original area of cultivation and the center of origin of *Hordeum vulgare* L. The concept of correlated host-pathogen evolution implies that genetic diversity in the populations of barley is matched by diversity in populations of *B. graminis* f.sp. *hordei* (WOLFE, 1988). Considering this, barley landraces collected from Turkey may possess resistance to powdery mildew resulting from the long co-evolution with populations of the pathogen.

This study aimed at detecting new effective sources of powdery mildew resistance in barley landraces from Turkey.

2. Materials and methods

2.1 Plant material

Seed samples of 101 *H. vulgare* L. landraces (BGRC No.: 38720–38797, 38798, 38881, 38884–38887, 38890–38892, 38894, 38900, 38902, 38906–38909, 38911–38917, 38919) were obtained from the Federal Centre for Breeding, Research on Cultivated Plants (BAZ) Gene Bank, Braunschweig, Germany. Seed samples of these landraces were transferred to BAZ Gene Bank in 1984 from Max-Planck-Institute (MPI), Köln (Cologne), Germany. They were collected in Turkey before 1972 in 12 areas: Elma Dag (7), Eskishehir (53), Iki-Hiran Tosya (1), Karakoese (1), Konia (18), Muttalip (3), Cankiri (12), Taurus Mt. (2), Van Vil Van (1), Angora (1), Corum (1) and Pollati Bei Ang (1). The majority of these landraces (97%) was collected in Anatolia. All of them were of a spring growth type and had hulled kernels. In Polish conditions they showed low resistance for lodging and were intermediate in heading date.

2.2 Pathogen

Nineteen isolates of *B. graminis* f. sp. *hordei* were used. They originated from the collections in Risø National Laboratory, Roskilde, Denmark; Danish Institute for Plant and Soil Science, Lyngby, Denmark; ETH Zürich, Switzerland and IHAR Radzików, Poland. The isolates were chosen according to their virulence spectra which were observed on the 'Pallas' isolines differential set (KØLSTER et al., 1986) and 7 additional differential cultivars. Young seedlings of the cultivar 'Manchuria' (CI 2330) were used to maintain all isolates and frequent virulence checks were made to assure the purity of isolates throughout the experiment.

2.3 Inoculation and disease assessment

The inoculation was carried out when plants were 10–12 days old (two leaf stage) by shaking or brushing conidia from diseased plants. After 8–10 days of incubation, the disease reaction types were scored on the primary leaf of the seedlings. This scoring was done according to a 0–4 scale adapted from MAINS and DIETZ (1930). This scale was broadened by including score 0(4) describing the infection type characteristic for gene *mlo* (JENSEN et al., 1992). This modification was done because *mlo* gene was not known

before 1942 (JØRGENSEN, 1994). If 0(4) infection type was observed, additional microscopic investigations were conducted to indicate cells from which colonies originated.

2.4 Resistance tests

This investigation was conducted during 1997–2000. In winter 1997/98 about thirty plants per landrace were evaluated in the greenhouse with the R 303a isolate of *B. graminis* f. sp. *hordei*. R 303a represented the most avirulent isolate available allowing the expression of a maximum number of resistance genes. The cultivar 'Manchuria' was used as a susceptible control.

Four of the tested 101 landraces showed resistance reactions. Each of these landraces was collected in different provinces: BGRC38721 (Elma Dagħ – South Central Anatolia), BGRC38759 (Eskishehir – North West Anatolia), BRGC38795 (Cankiri – North Central Anatolia) and BGRC38917 (Konia – South Central Anatolia). From each landrace, one to five resistant plants were selected. These plants were grown in the greenhouse to obtain their seed. Progeny of these plants were again tested with the R 303a isolate and 9 lines showing resistance reaction were obtained. These lines were tested with 19 isolates of powdery mildew during the winter 1999/2000 (Table 1). The plants were grown at 16–22 °C with 16 h light.

2.5 Postulation of resistance alleles

Hypotheses about the specific resistance genes present were made from the comparison of the reaction spectra of the

tested lines with those of differential lines and cultivars. The next step was postulation of resistance genes present and was done based on the gene for gene hypothesis. If a compatible reaction (scores 3 and 4) was observed with one given isolate, it meant that the cultivar did not possess the resistance alleles for which the isolate was avirulent. Incompatible reactions (scores 0–2) with isolates possessing only one avirulence allele among the remaining possible resistance alleles made it possible to postulate that the matching resistance allele was present (BROWN and JØRGENSEN, 1991; CZEMBOR, 1996).

3. Results

All 9 tested lines possessed resistance allele or alleles for powdery mildew of barley. It was impossible to postulate which specific allele or alleles for resistance are present in 5 (4023-1-2, 4023-2-5, 4061-1-1, 4097-1-2, 4097-2-2) of the tested lines. Four lines originated from landrace 4124 (4124-1-2, 4124-2-3, 4124-3-3, 4124-5-4) showed reaction 0(4). These colonies were also about half of size in comparison to susceptible control. Young leaves of these lines were also investigated under microscope. It was observed that mildew colonies originated from successful infection in the subsidiary cells next to the stomata on the barley epidermis. However on 2 occasions (line 4124-3-3) it was observed that colony originated from short cell in contact with stomata. Based on the observations the possibility of *mlo* allele presence in selections from landrace 4124 was postulated. Because these lines showed also resistance reaction type 0 additionally to allele *mlo* the presence of unknown allele or alleles characterized by infection type 0 is

Table 1: Resistance alleles and infection types of 9 lines to infection with 19 isolates of *B. graminis* f. sp. *hordei*.
Tabelle 1: Resistenzallele und Infektionstypen von 9 Linien gegenüber einer Infektion mit 19 Isolat von Mehltau

| No. | Line | Isolates | | | | | | | | | | | | | | | | | | Postulated resistance alleles | |
|-----|----------|----------|-------|--------|----------|----------|--------|---------|--------|--------|----|---------|------|--------|-----------|----------|----------|---------|--------|-------------------------------|----------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | | 19 |
| | | 58-74.1 | 59-12 | D 17-1 | Em A30.1 | HL 3/5.c | MH 1-3 | R 189.1 | R 303a | Ru 3.2 | TR | En1/2-2 | R A1 | E 303. | 59-2 92-1 | SZ/ 11.2 | Ra7 C10a | Ra 10-2 | Ra 16a | Ra 22-2 | |
| 1 | 4023-1-2 | 0 | 0 | 0 | 4 | 4 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 2 | 0 | 4 | 4 | 4 | 4 | 4 | ?A |
| 2 | 4023-2-5 | 0 | 0 | 0 | 4 | 4 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 2 | 0 | 4 | 4 | 4 | 4 | 4 | ? |
| 3 | 4061-1-1 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | ? |
| 4 | 4097-1-2 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 4 | ? |
| 5 | 4097-2-2 | 0 | 0 | 0 | 4 | 4 | 4 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 4 | 1 | 4 | ? |
| 6 | 4124-1-2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0(4) | 0 | 0 | 0(4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <i>mlo</i> , ? |
| 7 | 4124-2-3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0(4) | 0 | 0 | 0(4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <i>mlo</i> , ? |
| 8 | 4124-3-3 | 0 | 0 | 0 | 0(4) | 0(4) | 0 | 0 | 0 | 0(4) | 0 | 0 | 0(4) | 0 | 0 | 0 | 0 | 0 | 0(4) | 0 | <i>mlo</i> , ? |
| 9 | 4124-5-4 | 0 | 0 | 0 | 0(4) | 0 | 0 | 0 | 0 | 0(4) | 0 | 0 | 0(4) | 0 | 0 | 0 | 0 | 0 | 0(4) | 0(4) | <i>mlo</i> , ? |

^A Resistance alleles which were not eliminated from the reactions of susceptibility and could not be confirmed with the reactions of resistance

considered. Alternatively, these observations may indicate presence of effective partial resistance, which involve highly effective resistance against pathogen penetration instead the presence of *mlo* gene.

4. Discussion

Breeding for resistance, as an alternative approach to chemical control of powdery mildew, has been very successful, inexpensive (no need for training of farmers and special equipment) and environmentally safe (FINCKH et al., 1999). Barley breeders are constantly looking for new sources of effective powdery mildew resistance (DREISEITL and JØRGENSEN, 2000; CZEMBOR, 2001). This study demonstrates that in barley landraces from the Mediterranean region (including Turkey), if tested with large number of differentiating isolates, may be identified new effective sources of resistance. Additional advantage for breeders is that these new sources are available and characterised as single plant lines with homogenous resistance reactions. Among 101 investigated landraces from Turkey four (4%) showed resistance for *B. graminis* f. sp. *hordei*. The frequency of powdery mildew resistant landraces in the present study is similar or smaller than assessed in other investigations (NEGASSA, 1985; LEUR et al., 1989; JØRGENSEN and JENSEN, 1997; CZEMBOR, 2000, 2001).

Turkey is characterised by big contrast in its natural conditions because of their mountainous topography and differences in climate of the Mediterranean winter-rain zone and the Anatolian Plateau. These environmental conditions allow for the expression of a wide array of genes and a wide diversity of wild and domesticated barley (NEGASSA, 1985; ALEMAYEHU, 1995). Collection missions in Mediterranean countries are recommended because landraces of major crops in these countries are subject to genetic erosion due to drought and desertification (VALKOUN et al., 1995; HAMMER et al., 1996).

The most interesting findings in this study were results suggesting the presence of spontaneous Mlo resistance in barley landrace 4124 (BGRC38917) collected near Konya (south-central Anatolia). However, final evidence of the presence of Mlo resistance in 4 lines selected from landrace 4124 (BGRC38917) can be established only through crosses and backcrosses among appropriate genotypes and/or by using molecular markers or sequence analysis (CZEMBOR, 1996; MANNINEN et al., 1997). If such studies confirm results described in this paper it will be the first discovery of

spontaneous Mlo resistance in barley landrace originating from a country other than Ethiopia and Libya (JØRGENSEN, 1971; NEGASSA, 1985; SCHWARZBACH, 1997; CZEMBOR, 2000). However, another interpretation of data concerning infection type and microscopic observation is possible. These results may indicate presence of partial resistance that involves resistance against penetration of pathogen. Subsidiary cell infection is a commonly described phenomenon in barley with highly effective partial resistance (HIRATA, 1967; CARVER, 1975, 1986). The possibility of presence of high level of partial resistance in tested lines is also very interesting and valuable trait for barley breeders. By using this type of resistance barley breeders may ensure more stable resistance of barley cultivars to powdery mildew (PARLEVLIT, 1993). In both cases, presence of Mlo or partial resistance, these 4 lines represent highly effective resistance which is recommended to be used in barley breeding programs aiming in creation new cultivars of barley giving high and stable yield.

Twenty-five different *mlo* resistance alleles are known. This resistance was first identified in 1942, in an X-ray induced mutant (M66) of the German variety 'Haisa' (JØRGENSEN, 1971). Since 1942, Mlo resistance was more than 150 times independently induced by mutation. About 1970 it was also discovered as a spontaneous gene in barley landraces from Ethiopia collected by German expeditions in the 1930s and in 1985 it was described in barley landraces collected in southern Ethiopia (JØRGENSEN, 1971, 1994; SCHWARZBACH, 1997; NEGASSA, 1985). All *mlo* alleles with exception of *mlo11* were produced by mutagenesis. The first in Europe Mlo resistant barley variety was 'Atem', released in The Netherlands in 1979, derived its resistance (*mlo11*) from the Ethiopian landrace L92. Almost all barley varieties with Mlo resistance have the same allele *mlo11* with the important exception: 'Alexis' (*mlo9*) (SCHWARZBACH, 1997; JØRGENSEN, 1994). This proves that in contrary to the mutants, barley landraces were the most important sources of Mlo resistance. Perhaps a just important role may play the possible new sources of this resistance described in the present study.

Some cultivars with the *mlo* gene show complete resistance. Probably this is due to a gene or genes causing resistance in the stomatal subsidiary cells. Good example of this is 'Atem' which possess resistance allele *mlo* and *MLLa* (SCHWARZBACH, 1997). In this study three lines originating from landrace 4124 (BGRC38917) showed not only reaction type 0(4) but also reaction type 0. Based on these results the presence of an unknown allele or alleles in addi-

tion to *mlo* allele was postulated. Further studies of these lines are needed to determine if Mlo or partial resistance is present and to determine if and what kind of other resistance allele or alleles are present.

This study confirmed findings of other investigators that many barley landraces possess mildew resistance genes different from genes present in cultivated varieties (NEGASSA, 1985; LEUR et al., 1989; JØRGENSEN and JENSEN, 1997; CZEMBOR, 2000, 2001). New sources of resistance identified in this investigation (unknown genes, Mlo or partial resistance), especially those highly effective against major virulence genes, may increase the diversity of the powdery mildew resistance genes present in barley cultivars in Europe. Future work will concentrate on the genetics study of resistance occurring in selections from landrace 4124 (BGRC38917) using appropriate crosses and/or molecular markers or sequence analysis.

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