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Review paper

Barley and *Blumeria graminis*. Introduction to the host – pathogen interaction

Jęczmień i *Blumeria graminis*. Wprowadzenie do charakterystyki układu gospodarz – patogen

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Barley (*Hordeum vulgare* L.) is one of the most economically important cereals and holds fourth place in the world by harvest area. Powdery mildew, caused by the pathogenic fungus *Blumeria graminis* f. sp. *hordei*, is one of the most important diseases that decrease the quantity and quality of the yield. Since there is a limited number of resistance genes present in cultivated crop varieties, there is a need to search for and identify new sources of resistance.

Key words: genepools, *Hordeum vulgare*, powdery mildew, resistance genes

Jęczmień (*Hordeum vulgare* L.) jest jednym z najważniejszych gospodarczo zbóż i zajmuje czwarte miejsce pod względem areału upraw na świecie. Mączniak prawdziwy, powodowany przez grzyb patogeniczny *Blumeria graminis* f. sp. *hordei*, jest jedną z najważniejszych chorób wpływających negatywnie na ilość i jakość plonu jęczmienia. Ograniczona pula genów odporności wykorzystywanych w odmianach uprawnych stwarza potrzebę poszukiwania i identyfikacji nowych źródeł odporności.

Słowa kluczowe: geny odporności, *Hordeum vulgare*, mączniak prawdziwy traw i zbóż, pule genowe

Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereals in terms of harvest area and yield, both in Poland and in the world (FAOSTAT 2019, GUS 2019). Fungal pathogens are an economically important factor limiting the quantity and quality of the yield (Singh et al. 2019). Powdery mildew, caused by *Blumeria graminis* f. sp. *hordei*, is one of the most important diseases with a negative effect on the yield (Savary et al. 2012, Walters et al. 2012). The widespread cultivation of spring and winter barley, as well as local climatic conditions, promote the persistence of this pathogen and the development of the disease. Extensive use of chemicals to protect crops is not socially accepted (Report on public consultations for the Strategy for Sustainable Rural Development, Agriculture and Fisheries 2030, 2019) and leads to the selection of fungicide-resistant pathogen strains (Lucas et al. 2015). Responsible use of chemicals and the cultivation of resistant varieties are in line with the main objectives of the European Union's Common Agricultural Policy for 2021-2027 (https://europa.eu/rapid/press-release_MEMO-18–3974_en.htm) and the 2030 Agenda for Sustainable Development, UN (http://www.un.org.pl/). The narrow genepool of the currently grown elite varieties of barley stimulates the need to search for new effective resistance genes in landraces and related wild species.

Barley

The genus barley (*Hordeum*) is taxonomically assigned to the *Poaceae* family and the *Triticeae* tribe (APG IV, 2016). This genus includes 32 species, most of which are diploid (von Bothmer et al. 2003a). About 200 botanical varieties of *H. vulgare* have been identified (Hanelt et al. 2001). Cultivated barley (*Hordeum vulgare* ssp. *vulgare* L.) originates from wild barley (*H. vulgare* ssp. *spontaneum* C. Koch). It was domesticated during the Neolithic revolution, about 13,000-11,000 years ago, in the area known as the Fertile Crescent, stretching from the Persian Gulf to the Nile valley and covering the lands of Iraq, Syria, Jordan, Lebanon, Palestine, Israel and Egypt (Salamini et al. 2002, Purugganan and Fuller 2009). DNA studies and the natural range of wild barley occurrence indicate a second independent domestication that took place at the eastern end of the Iranian Plateau in Pakistan

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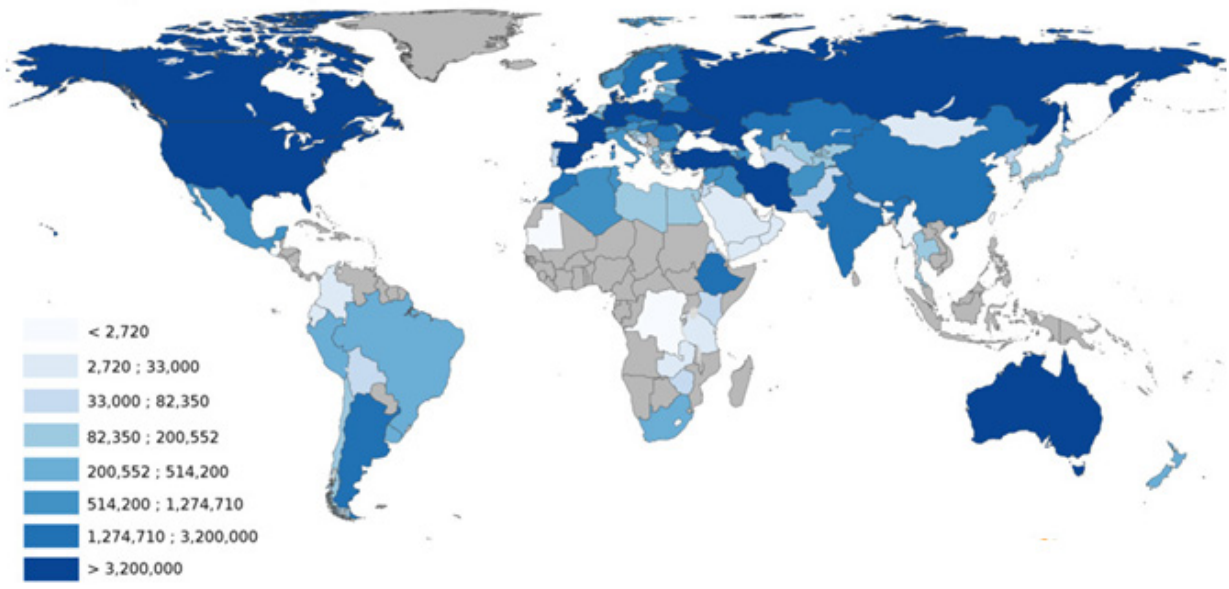
(Komatsuda 2014). Today, barley is one of the most popular cereals grown in the world. It owes its success to various and harsh environmental conditions. It is highly resistant to drought and soil salinity, as well as cold (von Bothmer et al. 2003a). Barley has a relatively short growth cycle, which is 60-90 days for spring forms (Agrometeorological Centre of Excellence, http://www.gov.mb.ca/agriculture/climate), and which can be completed before the onset of unfavourable conditions, i.e. summer drought and high temperatures.

Barley is the fourth cereal in the world, after wheat, maize and rice, in terms of harvest area, which is approx. 48 million ha (FAOSTAT, 2018). Almost half of the world's barley is grown in Europe (23 million ha), where this cereal ranks second after wheat (FAOSTAT, 2018) in terms of harvest area. The European Union is the leader in barley exports, which in 2016 amounted to over 8.5 million tonnes. World barley production by country is presented in Figure 1.

Poland ranks seventh among European countries in terms of barley harvest area. In 2019, it was over 1 million ha, which is approx. 13% of the total area of land under cereal crops, and in third place, after wheat and triticale (GUS, 2019). Considering the total yield of barley in Poland, which in 2017 was less than 305 million tonnes, this cereal ranks fourth, after wheat, triticale and maize (GUS, 2017).

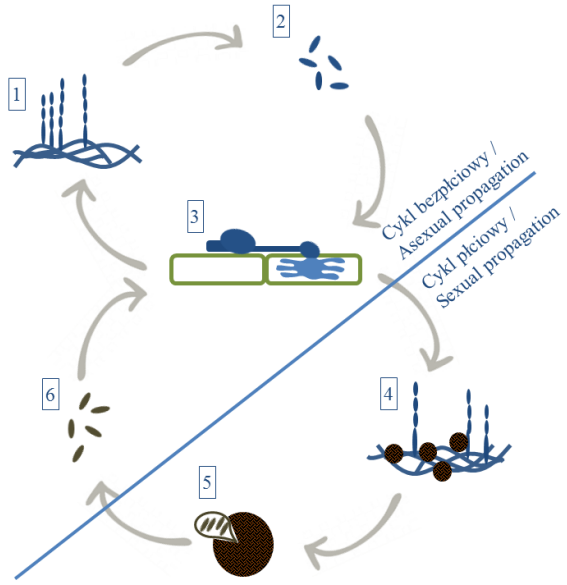
The use of barley changed depending on the historic period and culture. In Ancient Rome, barley grain was an important component of the human diet (Giraldo et al. 2019). The natural fermentation of grain during storage resulted in the discovery of alcoholic beverages. Barley beer was produced in Ancient Egypt over 5,000 years ago (Giraldo et al. 2019). Today barley is grown mainly for the production of feed for cattle and pigs. Fodder barley accounts for 85% of global production. Another 15% of harvested barley is used for food and seed. In the human diet barley grain is a rich source of β-glucans that normalize cholesterol and blood glucose levels (Collins et al. 2010). In the food industry, barley is mainly used to produce beer and whisky, flour and flakes. In 2014, global beer consumption amounted to almost 2 billion hectolitres, and over 21 million tonnes of barley were used by the brewing industry (http://e-malt.com/ after Giraldo et al. 2019).

The International Barley Sequencing Consortium elaborated a physical map and the complete sequence of the barley genome (The International Barley Genome Sequencing Consortium 2012). The haploid barley genome has seven chromosomes with a total length of approximately 5.3 Gbp. It is one of the largest genomes of all crops



Rys. 1. Światowa produkcja jęczmienia wyrażona w tonach (Actualitix 2019, https://en.actualitix.com/, źródło danych: FAOSTAT 2014).

Fig. 1. World barley production in tonnes (Actualitix 2019, https://en.actualitix.com/, data source: FAOSTAT 2014).



Rys. 2. Schemat cyklu życiowego Blumeria graminis, na podstawie Ridout i in. (2006), zmienione.

Fig. 2. The lifecycle scheme of Blumeria graminis, based on Ridout et al. (2006), modificated.

1. Grzybnia z konidioforami / Mycelium with conidiophores.
2. Konidia / Conidia.
3. Zarodnik infekujący komórkę gospodarza / A spore infects host cell.
4. Grzybnia z klejstotecjami / Mycelium with cleistothecia.
5. Klejstotecjum z workami / Ascii in cleistothecium.
6. Askospory / Ascospores.

and the third largest cereal genome after triticale (21.3 Gbp) and wheat (14.5 Gbp). The complete sequence is deposited in the open EnsemblPlants repository (https://plants.ensembl.org/) (Aken et al. 2017). Barley is a model plant used in scientific research. By 2018 the term *barley* appeared in over 50,000 research papers indexed in the Elsevier Scopus database, and 2% of them were Polish publications (Giraldo et al. 2019).

The breeding of barley led to the creation of many varieties. These varieties are categorized in accordance with the OECD quality criteria (2004), depending on the vernalization requirements for spring and winter varieties, and starch composition and protein content in the grain for feed and malting cultivars. Barley breeding programmes focus on increasing the nutritional value and tolerance to biotic and abiotic stresses, especially in the context of global climate change (Riehl 2019). It is still challenging to control nearly 250 barley pathogens, which cause significant losses in yield and quality of grain (Singh et al. 2019). Powdery mildew caused by *Blumeria graminis* f. sp. *hordei,* is next to rust (*Puccinia hordei*) and scald (*Rynchosporium commune*), the most important disease in barley (Savary et al. 2012, Walters et al. 2012). It causes a 10-20% loss in yield on average, and up to 50% in favourable conditions (Tratwal and Weber, 2006). The cultivation of barley all year round, the use of spring and winter forms, as well as a long growing season and a moderate climate promote the development of this disease (Jørgensen and Wolfe 1994).

Blumeria graminis f. sp. hordei

Powdery mildew of grasses and cereals is a fungal disease caused by *Blumeria graminis*, from the order *Erysiphales*, class *Leotiomycetes,* phylum *Ascomycota*. The order *Erysiphales* includes only one family, *Erysiphaceae*. Molecular analyses of the internal transcribed spacer (ITS), a noncoding domain within the ribosomal DNA genes, contributed to the revision of the previously adopted taxonomy. The *Erysiphaceae* family was divided into tribes reflecting the origin and morphology of particular species. Powdery mildew of cereals and grasses is caused by *Blumeria graminis* (D.C.) Golovin ex Speer, the only species representing the *Blumerieae* tribe. Within this species there are special forms (*formae speciales*) adapted to interaction with a compatible host species (Wyand and Brown 2003). This classification, based on both molecular and phenotypic analyses, was presented in the publication by Braun (2011) and the textbook by Braun and Cook (2012), and is identical to the classification presented in the Species Fungorum database (http://www.speciesfungorum.org/; 10.2019, *Centre for Agriculture and Biosciences International*, UK).

Blumeria graminis is an obligate biotroph. Fungal propagules (conidia) are dispersed by wind (Figure 2). Conidia contain 75% of water and thus can germinate fast, even on dry leaves. Just a few minutes after landing on the leaf of the host plant, a conidium produces a short primary germ tube that is used for host recognition. A few hours later the conidium produces a secondary germ tube. This tube develops an appresorium which, through physical pressure and chemical degradation, penetrates the wall of host epidermal cells.

At the next stage of infection, the haustorium is produced inside host cells, a special structure for the exchange of metabolites between the pathogen and the host. During compatible colonization, secondary haustoria and vegetative hyphae are produced epiphytically. A few days after infection, the mycelium produces conidiophores that release conidia on the host surface. The macroscopic symptom of the disease is a powdery white,

grey to brown mycelium on the leaf surface. It can be accompanied by chlorosis, necrosis, wilting, and weakening. The complete asexual life cycle of the pathogen is seven to ten days long and is repeated almost all year round, causing host reinfections and disease progression.

At the end of the growing season, *B. graminis* propagates in a sexual cycle. Plasmogamy and karyogamy occur between compatible gametangia formed on fungal hyphae. Meiosis leads to the formation of ascospores. Black spots of fruiting bodies visible on the epiphytic mycelium are cleistothecia containing ascii with ascospores. Cleistothecia can survive in unfavourable environmental conditions during hot late summer, and winter. Under favourable conditions mature ascii release ascospores that infect a susceptible host. *B. graminis* can survive winter in the form of a vegetative mycelium and cleistothecia on winter varieties and volunteer host plants.

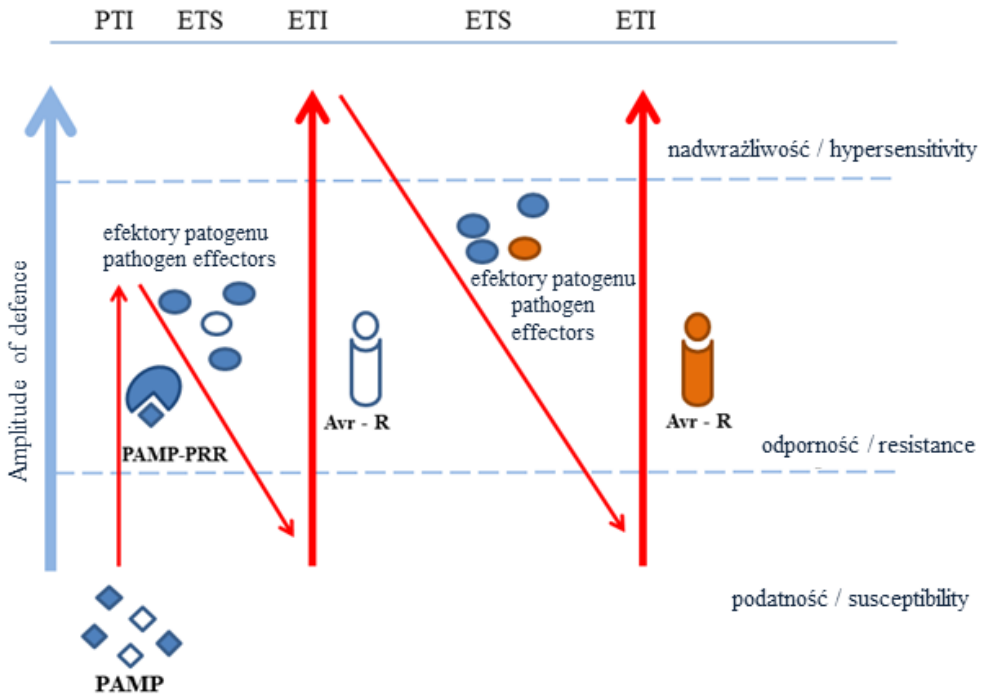
Blumeria graminis is the sixth of the ten most important fungal plant pathogens due to its economic and scientific importance, according to the experts collaborating with the Molecular Plant Pathology journal (Dean et al. 2012). According to the classification proposed by McDonald and Linde (2002), *B. graminis* is a high risk pathogen due to its high adaptability and very large population size. New races of this pathogen showing different virulence are produced in the sexual cycle, and the share of virulent races increases dramatically in the asexual cycle. When weather is favourable, sporulation begins just six days after infection. After ten days, up to 100,000 conidia are released from a single infection site. The spores easily spread to neighbouring plants, and can also be dispersed by wind for hundreds of kilometres (Jørgensen and Wolfe 1994). In addition, the high rate of spontaneous mutations, estimated at 1.3E-8 - 2.29E-9 per nucleotide per year (Oberhaensli et al. 2011, Hacquard et al. 2013), contributes to the creation of new races of this fungus.

Eight isolates of *B. graminis* have been sequenced, including four of *B. graminis* f. sp. *hordei* (A6, CC146, DH14, K1) (NCBI, 10.2019). The size of the fungus genome is estimated at 120–130 Mbp. This is three to four times more than the size of the genomes of other pathogenic fungi from the *Ascomycota* genus, for example *Magnaporthe oryzae* genome is 40 Mbp. The size of the *B. graminis* f. sp. *hordei* genome results from the large number of repetitive DNA and the presence of transposable elements. These sequences account for 64% of the whole genome (Spanu et al. 2010). The presence of transposable elements leads to large genomic rearrangements and the formation of physiological races with different virulence on various host genotypes.

Despite its large genome, *B. graminis* f. sp. *hordei* has a reduced number of genes encoding enzymes hydrolyzing the plant cell wall in the host. Two genes encoding cellulose hydrolase, four for hemicellulose and one for pectin, have been identified (Spanu et al. 2010). A similar reduced number of genes encoding proteins from these families was observed in the genomes of other obligate biotrophs, e.g. *Puccinia graminis* f. sp. *tritici*, in contrast to facultative biotrophs that have more than 100 genes encoding enzymes involved in the degradation of the cell wall of the host, like *Sclerotinia sclerotiorum* and *Colletotrichum higginsianum*. The genome of *B. graminis* f. sp. *hordei* contains 248 (Spanu et al. 2010), or 500 (Panstruga 2012) sequences potentially encoding virulence factors. So far, two genes encoding the effector factors *Avrk1* and *Avra10* have been identified (Ridout et al. 2006).

Resistance of plants to pathogens

Plants have developed various multi-level defence mechanisms of resistance to pathogens (Chen 2013, Zhang et al. 2013). The fundamental classical hypothesis of the resistance mechanism is Flor's gene-for-gene (1956), describing a direct interaction between the product of the host resistance gene *R* and the avirulence factor Avr of the pathogen. Most *R* genes are dominant and determine complete race-specific resistance (Kourelis and van der Hoorn 2018). In the course of further research, Flor's hypothesis was incorporated into the zigzag model developed by Jones and Dangl (2006) (Figure 3). This model illustrates the successive stages of pathogen infection and host response. According to the zigzag model, two mechanisms are involved in the recognition of the pathogen and the activation of defence mechanisms. The first one depends on pattern recognition receptors (PRR) recognizing pathogen-associated molecular patterns (PAMPs), e.g. chitin (Zipfel 2008, 2009; Schwessinger and Ronald 2012). The recognition of PAMPs leads to the activation of PAMP-triggered immunity (PTI). PTI is manifested, for example, by the induction of pathogenesis-related gene (PR) expression, cell wall apposition, and oxidative burst. PTI is potentially durable. A pathogen that evades or overcomes PTI initiates the secretion of virulence factors (effectors) into the host cells, which facilitate infection and cause effector-triggered susceptibility



Rys. 3. Schemat modelu zig-zag odporności roślin; na podstawie Jones i Dangl (2006), zmienione. Rozpoznanie PAMP przez receptory PRR powoduje aktywację odporności PTI. Sekrecja efektorów patogenu przełamuje PTI i indukuje podatność ETS. Gdy specyficzny czynnik Avr zostanie rozpoznany przez roślinne białko R, następuje aktywacja odporności ETI, która wyraża się reakcją nadwrażliwości. W wyniku presji selekcyjnej, patogen traci Avr i indukuje podatność ETS. Powstają nowe białka R uczestniczące w ETI.

Fig. 3. The zig-zag model of plant immune system, based on Jones and Dangl (2006), modificated. Plants detect PAMP via PRRs to trigger PTI immunity. Pathogens deliver effectors that interfere with PTI, resulting ETS susceptibility. One Avr effector is recognized by an R protein, activating ETI immunity and induction of hypersensitive reaction. Pathogen is selected that have lost Avr and induce ETS susceptibility. New R proteins are developed, resulting in ETI.

(ETS). If a specific effector (Avr factor) is recognized by the *R* resistance gene product, effector-triggered immunity (ETI) is induced (Jones and Dangl 2006). ETI leads to a hypersensitivity reaction, i.e. programmed host cell death and arrest of pathogen development. As a result of selective pressure, the pathogen overcomes the host's response through the loss of the Avr factor. The emergence of new virulent races favours the selection of new R proteins binding the effectors produced by the virulent isolate. Binding the Avr and R factors may be direct in accordance with the gene-for-gene model, or indirect through a guard protein (Dangl and Jones 2001) or a decoy protein (van der Hoorn and Kamoun, 2008). In a detailed study, Kourelis and van der Hoorn (2018) distinguished nine mechanisms of action of R proteins.

The pathogen-host interaction depends on three components: the genetic background of both organisms and the environmental conditions in which the interaction takes place. Plants vary in terms of their susceptibility and resistance, while pathogens vary in their virulence. The result of this interaction depends on the long pathogen-host coevolution: plants evolve towards recognizing the pathogen, and the pathogen evolves towards avoiding or overcoming the host's immunity (Stukenbrock and McDonald 2009). Models are simplified concepts of a complex sum of interactions. The actual host response does not strictly follow each of the mechanisms included in the zigzag model, but fluctuates smoothly between the PTI and ETI. As the phylogenetic distance between the potential host and the specialized host increases and the degree of pathogen specialization decreases, the share of ETI in the overall plant response in favor of PTI decreases (Schulze-Lefert and Panstruga 2011). The mechanism and outcome of infection depends on the spectrum of factors determining the pathogen's virulence and host response involved in both types of resistance, as well as the degree of pathogen specialization and host compatibility.

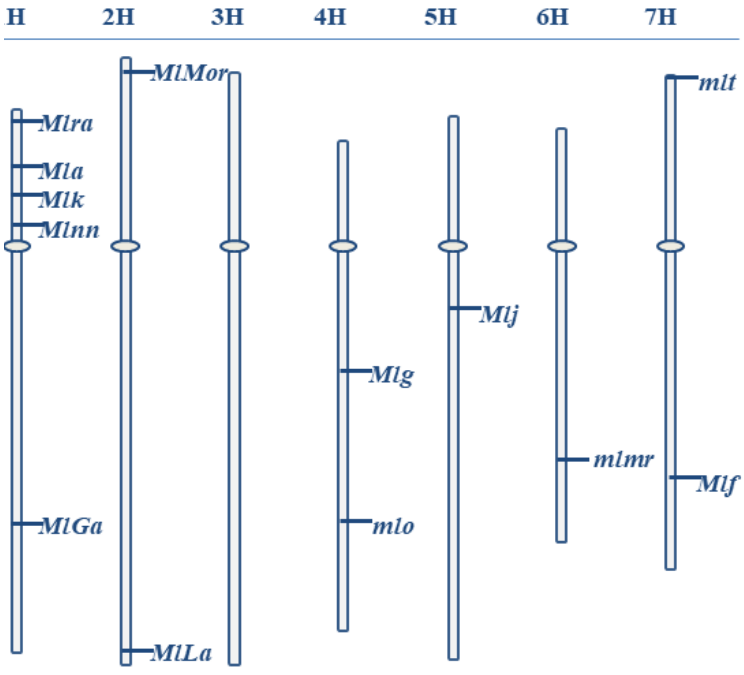
The interaction between barley and *B. graminis* f. sp. *hordei* is one of the best investigated and modelled plant-pathogen systems (Panstruga and Dodds 2009, Spanu et al. 2010). During

infection, barley rapidly identifies the pathogen. Transcriptome profiles in the host change as early as four to six hours after inoculation. The quick response indicates the recognition of the PAMP signal and induction of PTI. After overcoming PTI response, the pathogen secretes effectors into the host cells. About 500 candidate genes for potential effector proteins have been identified in the genome of *B. graminis* f. sp. *hordei* (Panstruga 2012). These proteins can be bound by a range of R proteins in barley triggering ETI.

Resistance genes to powdery mildew in barley

Barley race-specific resistance to powdery mildew has been investigated since the 1930s (Jørgensen and Wolfe 1994). Barley genes determining resistance to mildew are called *Ml-* (*Mildew locus*) (Jørgensen 1987, Franckowiak and Lundqvist 2009). Information about resistance genes is published in the Barley Genetic Newsletter http://wheat.pw.usda.gov/ggpages/bgn) (Jørgensen 1993). A review paper by Jørgensen and Wolfe (1994) mentions 28 alleles in locus *Mla*, 16 genes closely linked to locus *Mla* and 41 other genes for race-specific resistance. Jørgensen and Wolfe (1994) relied on reports from the 1970s, 1960s and 1950s, and studies in the field of classical genetics and phytopathology. They indicated that some of the listed genes were identified based on inconclusive findings. In some cases, researchers identifying these genes did not provide any data on which they based their reports, and for example, after the revision of data, the *mld* and *Mlp* genes originally assigned to chromosome 1H(5) were removed from the barley genetic map (Jensen 1990). In a summary of mapped barley resistance genes, Ordon (2009) presented a list of 11 major genes for powdery mildew resistance.

There are 11 resistance genes on the barley consensus genetic map (Figure 4). *Mla, MlGa, Mlk, Mlnn* and *Mlra* are located on the chromosome 1H, *MlLa* and *MlMor* on the chromosome 2H, *mlo, Mlg* on 4H, *Mlj* on 5H, *mlmr* on 6H, and *mlt* and *Mlf* on 7H (Jørgensen and Wolfe 1994, Schönfeld et al. 1996, Chełkowski et al. 2003, Piechota et al. 2019, 2020). These genes come from barley landraces as well as from the *H. spontaneum*. One gene (*MlLa*)



Rys. 4. Konsensusowa mapa genetyczna jęczmienia (H. vulgare) z naniesionymi genami odporności na B. graminis f. sp. hordei, na podstawie Chełkowski i in. (2003), zmienione.

Fig. 4. The barley (Hordeum vulgare) consensus genetic map with resistance genes to B. graminis f. sp. hordei, based on Chełkowski et al. (2003), modificated.

comes from the botanical variety *Leavigatum*. All of these are major genes. Most of them, except *mlf* and *mlo*, are dominant. Apart from *mlo*, these genes determine race-specific resistance. The molecular background of resistance determined by these resistance genes has been poorly investigated.

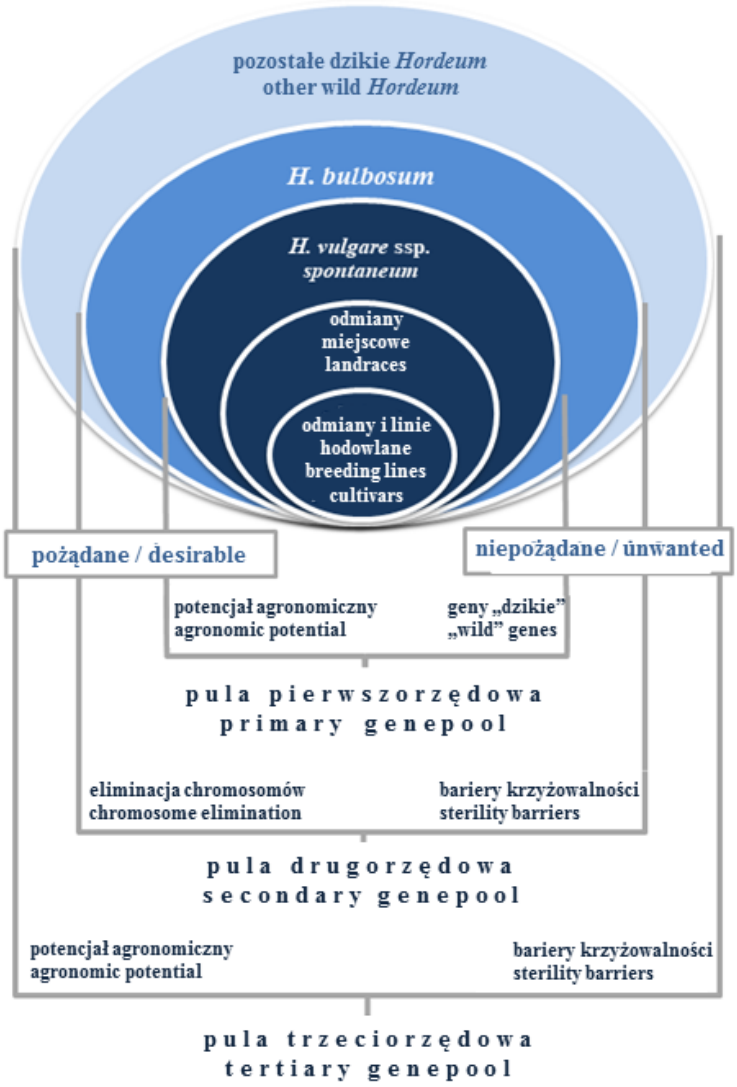
One of the identified resistance genes is the recessive allele *mlo* (Jørgensen 1992, Reinstädler et al. 2010). Mlo-based resistance is manifested by the presence of single small of *B. graminis* f. sp. *hordei* pustules on the host leaves. Penetration of the pathogen is stopped because of the epidermal cell walls apposition and the formation of papillae, local protective structures in the cell wall on the side of the cell membrane. Mlo confers a partial resistance because various expression of mlo gene in particular types of epidermal cells. Papillae are formed spontaneously, even in the absence of the pathogen in the short epidermal cells which are resistant. Long cells are still susceptible to infection. Mlo-based resistance is race-nonspecific. It also does not generate selection pressure on the population of *B. graminis* f. sp. *hordei* population. It is also associated with a negative pleiotropic effect manifested by an increased susceptibility to necrotrophic and hemibiotrophic pathogens (Jarosch et al. 1999, Kumar et al. 2001, Brown and Rant 2013) and lower yielding (Kjær et al. 1990). Mlo-based resistance was first identified in a barley landrace from Ethiopia (Büschges et al. 1997). This natural allele was designated *mlo11*. Other variants of this gene were identified in barley after artificial mutagenesis. The *Mlo* gene encodes a transmembrane protein of unexplained function. Resistance is determined by the loss of function mutations. The substitution of aminoacids in the MLO protein determining resistance have also been identified. Four of them are cysteine exposed outside the cell membrane (Reinstädler et al. 2010, Appiano et al. 2015). Although almost 50 *mlo* alleles have been reported, 13 variants of barley MLO proteins are deposited in the UniProt database (https://www.uniprot.org/, access: 10.2019) (The UniProt Consortium 2019), while the InterPro database (https://www.ebi.ac.uk/interpro/, access: 10.2019) (Mitchell et al. 2019) contains 215 MLO-like proteins also identified in barley. MLO variants determine different levels of resistance and different degrees of negative pleiotropic effect. *mlo11* is most commonly used in barley cultivars.

The second identified gene of resistance to *B. graminis* f. sp. *hordei* is a multiallelic locus *Mla*. Approximately 30 variants of the *Mla* sequence have been identified. The NCBI database (access: 10.2019) includes 29 coding sequences. New variants are still being disclosed (Maekawa et al. 2019). The length of the *Mla* locus is more than 260 kbp. *Mla* is located on the short arm of the 1H chromosome, at a position of about 8.5 Mbp. Approximately 30 open reading frames have been identified at the *Mla* locus, concentrated on three gene islands separated by transposable elements. The eight genes identified at this locus potentially encode the CC-NBS-LRR or MLA proteins from three families: RGH1, RGH2 and RGH3. Known functional *Mla* alleles belong to the RGH1 family and are homologs of RGH1bcd, a pseudogene identified in a susceptible cultivar Morex (Brabham et al. 2017). The expression of *Mla* is induced by the presence of the pathogen and only occurs in an incompatible interaction (Halterman et al. 2003). Because of its high complexity and variability, the *Mla* locus is an important source of resistance in breeding programmes.

In a recent study, Hoseinzadeh et al. (2019) located the *M1La-H* gene identical to *MlLa* on chromosome 2HL and identified markers flanking this locus. The researchers selected four candidate genes from the NBS-LRR class. They also identified a mutation in one of the candidate genes that was associated with resistance to powdery mildew.

Studies on the search for and identification of powdery mildew resistance genes are still being published. Examples of newly described genes include *Ml(Ve),* identified in 2018 in cultivar Venezia (Dreiseitl 2018) and *Ml(Lu,)* identified in 2019 in a number of varieties of winter barley cultivars (Dreiseitl 2019). Identification of these genes relied on phytopathological tests. The above-mentioned publications did not indicate the location of these genes in the barley genome, and no other genetic analyses were performed to demonstrate their uniqueness. Many of the resistance genes used in breeding studies have only been identified based on the infection profiles after the differential set of *B. graminis* f. sp. *hordei* inoculation.

Most of the modern spring barley cultivars posses Mlo-based resistance (Dreiseitl 2017). In winter barley, the pyramids of the major resistance genes are used. Resistance genes introduced into European cultivars and the durability of the resistance determined by them were described by Dreiseitl (2014a, 2017). Dreiseitl listed 38 genes/alleles present in barley cultivars from Central Europe (Dreiseitl 2014a). Most cultivars registered in 2011-2015 contained the *mlo* allele (present in 27 out of 67 tested varieties). In the remaining barleys, Dreiseitl identified two- to six-gene pyramids, and he



Rys. 5. Schemat pul genowych jęczmienia, pierwszo- drugo- i trzeciorzędowej, na podstawie von Bothmer i in. (2003b), zmienione.

Fig 5. The scheme of barley primary, secondary and tertiary gene-pools, based on von Bothmer et al. (2003b), modificated.

indicated the presence of an unknown resistance gene in three of them (Dreiseitl 2017). The *mlo* allele was detected in most of the spring barley cultivars from the 2019 COBORU Descriptive List of Agricultural Plant Varieties (50 out of 61 analysed). In 31 analysed winter cultivars, single major genes or two-gene pyramids were identified.

Barley genetic resources

Currently grown barley cultivars have been created as a result of long and strongly selective breeding pressure. The ongoing selection of varieties to improve the parameters of agronomic traits has narrowed their genepool and led to a loss of genetic diversity (Tanksley and McCouch 1997, Buckler et al. 2001). This process has significantly reduced the plasticity of varieties in adapting to biotic and abiotic stresses, and especially to climate change. This problem can be solved by expanding the genepool using old varieties and landraces, as well as wild relatives (McCouch et al. 2013).

Barley genetic resources include cultivars, landraces, breeding lines, wild species of the genus *Hordeum,* and materials deposited in gene banks. These resources can be classified based on the concept of primary, secondary and tertiary genepools (Figure 5) (von Bothmer et al. 2003b). The primary barley genepool includes all forms of cultivated barley and its wild ancestor, *H. vulgare* ssp. *spontaneum*. The genetic material is transferred easily within the primary pool by artifitial crossing. There are no postzygotic barriers to crossability. Barley landraces carry desirable agronomic traits,

including many unidentified alleles determining resistance to powdery mildew (Czembor 2000a, 2000b, 2002). Also wild barley is a source of resistance with potential utility for breeding (Dreiseitl 2014b).

The secondary genepool contains only one species - bulbous barley (*H. bulbosum* L.). Hybridization of *H. vulgare* with *H. bulbosum* is difficult because it leads to the elimination of *H. bulbosum* chromosomes. This process was used in the *H. bulbosum* method for the creation of doubled haploids of barley.

The barley lines with *H. bulbosum* introgressions are a valuable source of variation in cultivated barley (Czembor et al. 2019). *H. bulbosum* is a source of resistance to *B. graminis* f. sp. *hordei* determined by the *MlHb* gene (Pickering et al. 1995).

The tertiary genepool includes all other species of the genus *Hordeum*. Transfer of genetic material by crossing is almost impossible. The potential of this genepool can be utilized by means of chromosomal and genetic engineering techniques.

Powdery mildew resistance genes identified in barley landraces include, for example, *Mlg* identified in the German landrace Weihenstephan; *Mla3* – in the Ricardo landrace from Uruguay, and *Mla12* in Arabische landrace (Jørgensen and Wolfe 1994). Landraces originating from the regions where cultivated barley was isolated and domesticated, i.e. North Africa and the Middle East, show a large variability of resistance loci. This results from a long coevolution with specific pathogens such as *B. graminis* f. sp. *hordei*. These varieties are subject to weaker pressure from the pathogen and the resistance carried by them is relatively more durable (Camacho Villa et al. 2005, Morrell and Clegg 2007). The analysis of the African population of *B. graminis* f. sp. *hordei* revealed that barley landraces originating from Africa are highly diversified in terms of resistance to powdery mildew (Dreiseitl and Kosman 2013, Jensen et al. 2013). For example, studies on barley landraces from Jordan or Morocco allowed for the selection of 160 and 133 lines, respectively, resistant to powdery mildew (Czembor 2000a, 2000b, 2002, Abdel-Ghani et al. 2008).

Of all the described genetic resources, landraces are the easiest to use directly in breeding programmes. Landraces are heterogeneous and genetically dynamic populations. They come from regions where traditional agriculture persisted, and no active systemic breeding programmes are implemented (Camacho Villa et al. 2005). They undergo natural selection without strong breeding pressure. They are also adapted to local climatic conditions. Landraces carry unique traits that have been eliminated from the elite cultivars in the strong selection process and are considered essential for resistance breeding and for the restoration and extension of the genepool of cultivated barley forms (Akem et al. 2000).

Concluding remarks

Goal 2 of the UN 2030 Agenda is to "End hunger, achieve food security and improved nutrition, and promote sustainable agriculture" (http://www.un.org.pl/). Advances in plant breeding are fundamental for improving food security and sustainable production. These advances require the availability of a rich genepool, which would allow breeders to blend important positive traits with the genetic background of cultivars. Local and old crop varieties carry many desirable traits. Research is needed to recreate such varieties and evaluate the possibility of their adaptation. Contemporary molecular biology offers a wide range of techniques and tools which, together with the available complete reference sequence of the barley genome, can significantly contribute to identifying genes responsible for these traits and help breeders introduce these genes to elite cultivars. The use of resistant varieties in integrated pest management is also recommended in Directive 2009/128/EC of the European Parliament and of the Council establishing a framework for Community action for the sustainable use of pesticides.

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