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Faculty of Science

Department of Cell Biology and Genetics

Master's thesis

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Mapping of powdery mildew race non-specific resistance gene from tetraploid wheat

Master's thesis

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Abstrakt	Tato práce byla zaměřená na genetické mapování nově objeveného genu rezistence zabezpečujícího totální rezistenci k padlí travní (*Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici*) identifikovaném v tetraploidní pšenici *Triticum turgidum* subsp. *dicoccum.* Tento genetický zdroj rezistence byl pojmenován jako GZ1. Za využití DArTseq markerů byla zkonstruována genetická mapa sestávající z 862 SNPs markerů. QTL analýza odhalila dva QTL ovlivňující resistenci. Homozygotně recesivní *QPm.GZ1-2A* umístěný na 2AL chromozomu a dominantní *QPm.GZ1-7A* umístěný na 7AL chromozomu. *QPm.GZ1-2A* a *QPm.GZ1-7A* QTLs překonaly LOD threshold s LOD skóre 14.51 a 8.91 a přispívají k variabilitě znaku 30% a 20%. Ukotvením obou lokusů na referenční genomovou sekvenci cv. Zavitan byly identifikovány kandidátní geny.
Klíčová slova	DArTseq markery, F_2 mapovací populace, fenotypování, genetická mapa, padlí travní, QTL analýza
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Abstract	The aim of this diploma thesis was genetic mapping of newly discovered resistance gene conferring total resistance against powdery mildew (*Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici*) identified in *Triticum turgidum* subsp. *dicoccum* wheat. The genetic source of resistance was named as GZ1. A genetic map using DArTseq markers was developed and consist of 862 SNPs markers. QTL analysis revealed two QTLs affecting the resistance. The homozygote recessive *QPm.GZ1-2A* located on 2AL chromosome and dominant *QPm.GZ1-7A* located on 7AL chromosome. The *QPm.GZ1-2A* and *QPm.GZ1-7A* QTLs surpassed LOD threshold reaching LOD score 14.51 and 8.91 and contributing to the trait variance by 30% and 20%, respectively. Both loci were anchored to the cv. Zavitan reference genome sequence and candidate genes were identified.
Keywords	DArTseq markers, F_2 mapping population, phenotyping, linkage map, powdery mildew, QTL analysis
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DECLARATION

I hereby declare that I elaborated this diploma thesis independently under the supervision of Mgr. Miroslav Valárik, Ph.D., using only information sources referred in the Literature chapter.

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LIST OF ABBREVAITIONS

ANOVA	Analysis of variance
Avr	Avirulant
CIM	Composite-interval mapping
DArTseq	Diversity arrays technology
DHLs	Double haploid lines
ETI	Effector-triggered immunity
GBS	Genotyping by sequencing
GWAS	Genome-wide association study
LD	Linkage disequilibrium
LOD	Logarithm of odds
LRR	Leucine-rich repeats domain
MAGIC	Multi-parental advanced generation intercross
MAS	Marker assisted selection
NB	Nucleotide-binding domain
NGS	Next generation sequencing
NILs	Nearly isogenic lines
PAMP	Pathogen-associated molecular pattern
PEV	Percentage of explained variance
PRR	Pattern recognition receptors
PTI	PAMP-triggered immunity
QTL	Quantitative trait loci
RAD-seq	Restriction site associate DNA sequencing
RILs	Recombinant inbred lines
SBS	Sequencing by synthesis
SIM	Single-interval mapping
SMA	Single-marker analysis
SNP	Single nucleotide polymorphism

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1 INTRODUCTION

Wheat is one of the most important agricultural crop cultivated worldwide as it represents the main source of energy for more than 40% of the human population. Wheat is considered as a major source of carbohydrate, but it also contains significant amount of other important nutrients including proteins, fibers, and minor components such as vitamins, minerals etc. Wheat production exceeds more than 750 million tonnes annually (http://www.fao.org). Demand for wheat is increasing due to the fast growing human population, which is expected to grow in the next 30 years up to 9 billion. To ensure food security, the wheat production must grow by 2 % per year. Nevertheless, the wheat yields could be endangered by pathogens and diseases.

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* is one of the most devastating fungal diseases of common wheat. Infected plants display white overlay on the leaves and stems. As a result, besides up taking nutrients from host cells, the fungus reduces photosynthesis on leaves and decreases leaf assimilation index. All this negatively affects grain yield components. Moreover, powdery mildew is able to spread by wind to a long distances. Heavy infestation of wheat with powdery mildew can decreases yield up to 40%. The most effective way to control powdery mildew infections is growing of resistant cultivars.

Selection of suitable germplasm and development of resistant cultivars is strongly dependent on identification of new resistant genes. This include search for new genetic sources with powdery mildew resistant genes and their chromosomal localization with molecular markers. Identification of molecular markers closely linked to the target resistant genes allows their use in breeding programs for crop improvement. Tightly linked markers are utilized in marker-assisted selection (MAS) for fast and effective introgression of new resistant genes to elite cultivars.

The utilization of resistant genes represents the most economical and environmentally safe approach to eliminate the use of fungicides. For instance, in 2017 the use of fungicides in the Czech Republic counted 1366 tonnes (http://www.fao.org). Recently, a total resistance against all powdery mildew races was identified in the GZ1 germplasm. This resistance, if characterized may help decreases the fungicide use and create more resistant wheat cultivars.

2 AIMS OF THE THESIS

- Theoretical review of the wheat importance, history and evolution of genome. Characterization of powdery mildew, its life-cycle and host-pathogen interaction. Characterization of genetic and QTL mapping.
- F_2 mapping population genotyping and phenotyping, construction of genetic map and QTL analysis.
- Results analysis and interpretation.

3 THE CURRENT STATE OF THE KNOWLEDGE

3.1 Wheat

Genus wheat (*Triticum*) is a member of the Pooideae subfamily of grasses of the Triticeae tribe, which besides comprises additional economically important genera such as barley (*Hordeum*) or rye (*Secale*) (Kellog, 2001). The genera belonging to the Triticae tribe evolved from a common ancestor about 11 million years ago (Huang *et al.,* 2002) and could be considered as closely related. This is supported by their crossability not only within the species but also within the tribe and it is used in breeding programs. Such wide crosses are used for wheat gene pool improvement for many important agronomical traits. Introductions of advantageous traits by breeders into current wheat cultivars from other species or genera have tended to be most effective when these species are close relatives or are even directly ancestral to it. An example is the so-called Triticale, which was derived from interspecific cross between wheat and rye. Genus *Triticum* includes 28 species (Kimber and Sears, 1987) with wild and domesticated varieties. Among the most cultivated species of domesticated wheats belong the common or bread wheat (*Triticum aestivum* subsp. *aestivum*) and durum, the pasta wheat (*Triticum turgidum* subsp. *durum*). Common wheat represents about 90% of the total wheat production and is used to produce bread, pastries, cookies, cakes, noodles or cereals, whereas durum wheat accounts for about 5% and is used to produce pasta and semolina products (Dixon *et al.,* 2009). The remaining amount counts for economically less significant varieties like spelta wheat.

3.1.1 Importance of wheat

Wheat belongs to the four (rice, wheat, corn, potatoes) worldwide most important agricultural crops. Wheat is grown in almost all areas and worldwide is ranging from 67°N in Scandinavia and Russia to 45°S in Argentina, including highlands in the tropics and subtropics (Feldman, 1995). Additionally the importance of wheat is emphasized by the fact that land sown to wheat counts more than 200 million ha and it is largest of all commercial crops. With more than 750 million tonnes of annual yield leads all cereal crops. Wheat is also, a major diet component as it covers 40% of staple food and provides 20% of proteins and fibers to the human diet (http://www.fao.org). In the Czech Republic it is the most produced food source with 4.7 million tonnes (as in 2017, http://www.fao.org).

3.1.2 History of wheat

Wheat was one of the first domesticated food crops. The beginning of its cultivation dates 10 000 years ago in the area of Fertile Crescent (Fig. 1) and overlaps with the transition humans from hunting and gathering of food to settled agriculture marked as the Neolithic Revolution (Dubcovsky and Dvořák, 2007). The earliest species that arose from the domestication of natural populations of wild wheats were einkorn and emmer wheat. These forms come from south-eastern Turkey and Levant based on their genetic relation with wild wheat ancestors still growing there (Heun *et al.,* 1997). The subsequent dissemination of wheats across Asia, Europe, and Africa enabled formation of contemporary lineages.

Figure 1. The fertile crescent. The dark green colour on the map shows the area of the Fertile Crescent representing today's Egypt, Israel, Jordan, Turkey, Syria, Iraq and Iran. (Adapted from Feuillet et al., 2008)

Wheat domestication was carried out by the selection for suite of traits improving yield, spike brightness and threshability. Originally, were these traits selected as morphological characters in cultivated lines as they were more attractive to the first farmers in comparison to their wild relatives. Nowadays, it is known that these traits are result of mutation at specific loci (Simons *et al.,* 2004). The most significant traits involved in domestication of common wheat are loss of spike shattering and conversion into free-threshing wheat.

The spike shattering is genetically determined by the *Br* (brittle rachis) loci localized on homoeologous group 3 chromosomes in *Triticeae*. Mutations at these loci

resulted in non-brittle rachis preventing the grains from disperse by wind (Fig 2.; Pourkheirandish *et al.,* 2018; Nalam *et al.,* 2006; Watanabe *et al.,* 2002). More complex is the transition of hulled wheat into free-threshing wheat since it is determined by quantitative loci. The free-threshing-related characteristics are predominantly affected by recessive mutations at the *Tg* (tenacious glume) loci causing the loss of tough glumes accompanied by modifying effects of the dominant mutation at the Q locus controlling threshability and mutations at several other loci (Jantasuriyarat *et al.,* 2004). The ability to identify significant traits at the genetic level has become a prerequisite in breeding and speed up the wheat improvement for desirable traits.

Figure 2. Differences between brittle rachis and non-brittle rachis of einkorn wheat. Brittle rachis of wild einkorn *Triticum boeticum* (left) and non-brittle rachis of domesticated einkorn *Triticum monococcum* (right). In the case of brittle rachis wheat grains are easily dispersed by wind, which is prevented in non-brittle rachis wheat. (Adapted from Pourkheirandish *et al.,* 2018).

The second bigger wheat improvement occurred between 1950s and 1960s in era called Green Revolution, often attributed to Norman Borlaug. At that time, the dwarfing *Rht1* and *Rht2* genes were introduced into already used cultivars and improved yield. *Rht* genes are responsible for reduced plant growth. The stems of tall plants were not strong enough to support the heavy spike of the high-yielding varieties and thus plants logged causing large yield losses compared to dwarfed plants, which are less prone to lodging.

The introduction of dwarfing traits into wheat in combination with the application of large amount of fertilizer and pesticides enabled doubling of production (Hedden, 2003).

3.1.3 Genome of wheat

As was stated above, wheat could be grown in many different environments and its genome shows high level of plasticity especially at higher ploidy level. The *Triticum* genome is organized into seven chromosomes (1x = 7) and several ploidy levels could be observed. The most common are the diploid (2n = 2x = 14), alotetraploid (2n = 4x = 28), and alohexaploid (2n = 6x = 42) wheats. The polyploid *Triticum* species are result of consecutive interspecific hybridization events (Fig. 3), which occurred at the distinct geographic areas and gave rise to Emmer and Timopheevi lineages comprising both tetraploid and hexaploid species.

The emmer lineage began with a hybridization occurred between *Triricum urartu* (genome AA) (Dvořák *et al.*, 1993) and the donor of BB genome, considered to be a close relative of goatgrass *Aegilops speltoides* (genome SS) (Killian *et al.,* 2007; Gornicki *et al.,* 2014) and gave rise to tetraploid wild emmer wheat *Triticum turgidum* subsp. *dicoccoides* (genome AABB). Domestication of this wheat led to evolve the domesticated form of wild emmer wheat *Triticum turgidum* subsp. *dicoccon* (genome AABB) and its subsequent cultivation the free-threshing *Triticum turgidum* subsp. *durum*, which gave rise to the widely grown pasta wheat cultivars of today (Dubcovsky and Dvořák, 2007). Since tetraploid wheat obtained genetic information from both ancestral diploids previously adapted to different environments, they are more vigorous and thus able to adapt to a wider range of environmental conditions than their progenitors (Dubcovsky and Dvořák, 2007; Feuillet *et al.,* 2008). This explains its dominance over the domesticated einkorn in the region of Diyarbakir in south-eastern Turkey and subsequent northeast expansion, which result in sympathry with *Aegilops tauschii*, a wild diploid species with a DD genome. The second hybridization event led to the formation of the hexaploid wheat *Triticum aestivum* with the genome AABBDD. With additional D subgenome hexaploid wheat obtain genes that have broader adaptability to different photoperiod and vernalization requirement, improved tolerance to salt, low pH, aluminium, and frost. It has increased resistance to some pests and diseases. Genome D also encodes proteins that affect the softness of grain endosperm and retain CO_2 during dough rising, and therefore flour made from bread wheat is particularly suitable for the bakery industry (Feuillet *et al.,* 2008).

The Timopheevi lineage also began with the hybridization event between the donor of A genome, *Triticum urartu* (genome AA) and *Aegilops speltoides* (genome SS) or its close relative, providing the G genome (Gornicki *et al.,* 2014). This hybridization resulted in the formation of a tetraploid wheat *Triticum timopheevii* (genome AAGG). Subsequent hybridization event of *Triticum timopheevii* with cultivated *Triticum monococcum* (genome A^mA^m) was followed and gave arise of hexapolid wheat of this lineage *Triticum zhukovskyi* (genome AAGGA^mA^m; Jonson, 1968). For an unknown reason, this hexaploid wheat never gained more importance and therefore it was never cultivated as a significant crop.

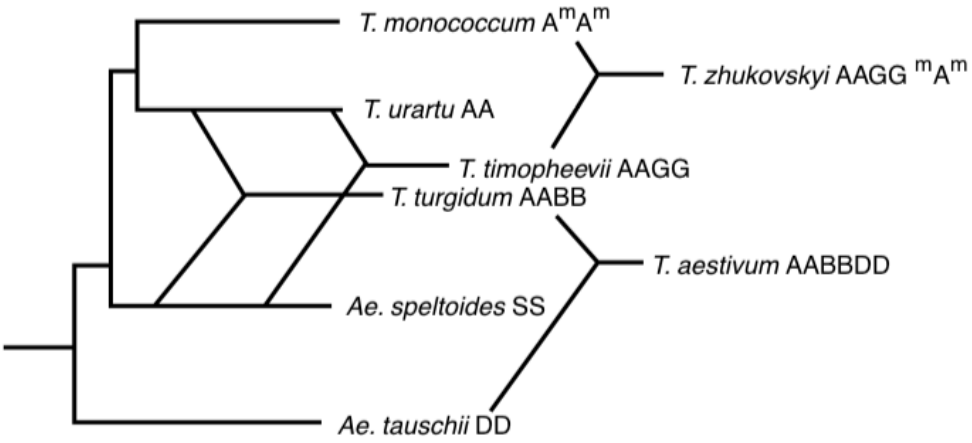

Figure 3. Phylogenetic evolution of wheat genome. Wheat has undergone several hybridization events during its development leading to an increase in the ploidy level of wheat. Two lineages arose, which are at the highest known ploidy level of wheat represented by *Triticum zhukovskyi* (AAGGA^mA^m) and *Triticum aestivum* (AABBDD) (Adapted from Dvořák, 2001).

3.2 Powdery mildew

Powdery mildew is general designation for obligate biotrophic fungal phytopathogens of wild and cultivated plants dependent on living host cells from which extract essential nutrients. This group of filamentous ascomycetes taxonomically belongs to the order Erysiphales of the family Erysiphaceae comprising 13 genera and more than 820 species infecting over 9000 angiosperms (Schulze-Lefert and Vogel 2000, Braun and Cook, 2012). The most significant is infection of economically important crops, such as grapevine, fruit trees, hop or cereals, which causes significant yield losses worldwide. For instance, yield losses caused by powdery mildew on wheat can reach 40% (Johnson *et al.,* 1979).

Powdery mildew of wheat is caused by fungus *Blumeria graminis* f. sp. *tritici*. The genus *Blumeria* comprises only single species *Blumeria graminis* further distributed into 8 individual subspecies (forme speciales, f. sp.) according to adaptation to a particular host. Among the most significant belongs *Blumeria graminis* f. sp. *tritici, hordei*, *secalis* or *avenae* (Braun and Cook, 2012) infecting cereals. Further within the individual subspecies exists lower degree of pathogenic specialization represented by pathotype or race resulting from host-pathogen interaction. This is only a phytopathogenic category using to determine pathogen genotype variability, respectively its isolate.

3.2.1 Host-pathogen interaction

There is a wide range of phytopathogens which cause infectious plant diseases. In order to defend against these biotic stress, plants possess effective defence system. After the pathogen invasion activation of inducible defence reaction is based on the plant ability to detect the presence of pathogen. This is the consequence of the interaction between the host genome and pathogen genome.

During the infection pathogen starts to produce effectors called pathogen-associated molecular patterns (PAMPs) to supress plant defence and modulate plant physiology to accommodate fungal invader and provide them with nutrients. These PAMPs are recognized through membrane-localized host pattern recognition receptors (PRRs), which trigger the first level of defence response designated PAMP-triggered immunity (PTI). The stimulation of PTI leads to activation of events resulting in callose deposition of the cell wall and thus preventing the pathogen from invasion. However, biotrophs fungi like powdery mildew, are able to overcome the PTI and as a counter mechanism, plants

develop second intracellular defence response called effector-triggered immunity (ETI). PAMPs that trigger ETI are usually recognized by the plant resistance genes products, which are conserved intracellular proteins containing the nucleotide-binding (NB) and leucine-rich repeats (LRR) domains. The NB-LRR proteins works as receptors with high variability within cytoplasmatic patterns recognition. Activation of ETI often leads to localized cell death response (Presti *et al.,* 2015).

Plant pathogens are usually adapted to a particular host circuit depending upon the nature of plant-pathogen interactions. These interactions are based on the mutual adaptation of a plant cell and a pathogen. Adapted pathogen and plant cell result in **host resistance**, while non-adapted pathogen and plant cell result in **non-host resistance**. Both host and non-host resistance are the outcomes of the plant immune response. In the case of non-host resistance, non-adapted pathogen does not possess genes encoding for PAMPs needed for interaction with plants cell PRRs and therefore this incompatibility of plant-pathogen interaction result in complete resistance of a plant. Host-resistance is a consequence of plant-pathogen compatibility. Adapted pathogen possess genes encoding for effectors that are able to interact with PRRs subvert PTI or ETI and therefore cause the infection. However, plants could possess effective resistance genes (R-genes), that can prevent them from the pathogen colonization (Gill *et al.,* 2015).

As stated earlier, individual subspecies of *Blumeria graminis* could be divided further into races in order to determine pathogen genotype. This is because plants may be resistant to a specific race or few races, while some may show resistance to a wide range of races. Based on this, host resistance is further divide into race specific resistance and race non-specific resistance (Crute and Pink, 1996).

3.2.1.1 Race specific resistance

Race specific resistance was first proposed by Flor (1971) as gene-for-gene concept and have been accepted till today. This type of resistance is governed by a specific interaction between the plant R-gene and the corresponding pathogen avirulence (avr) gene. For successful establishment of resistance after the pathogen invasion a gene pair matching of R and avr gene must be provided. If this is not accomplished, it will result in disease. In this case, the R gene is either inactive or absent and therefore avr gene of the pathogen become virulent (Flor, 1971). Thus, race-specific resistance is sufficient only against a particular genotype of the pathogen and is therefore more effective against a race of a pathogen characterized by this genotype.

Based on the genetic background, race specific resistance is usually determined by major resistant genes. During the co-evolution of host and pathogen multiple resistance genes and their relevant alleles have evolved. Currently, more than 100 major resistant genes and their alleles to powdery mildew have been identified across different species of wheat (McIntosh *et al.,* 2017; Li *et al.,* 2020).

Race-specific resistance to powdery mildew is generally short term and in case of cultivated crops tends to be effective about 3-5 years before it loses its efficiency (Wolfe and McDermott, 1994). Cultivation of varieties with specific resistance gene on a large area imposes strong selection pressure on the pathogen population and because of the resistance to only specific pathogen races it could be easily overcome due to the mutations at corresponding avirulence or resistance genes (Dreiseitl, 2003). In this case both avirulence and resistance genes lose their efficiency. However, in the same way new alleles of already existing resistance genes may be generated. Genetic mapping of novel genes or its new variants (alleles) allows their utilization in breeding programs for new cultivars already losing its resistance. In addition, combination of more than one resistant genes provides more genetically complex cultivars and hence broader spectrum of resistance (Laroche *et al.,* 2019).

3.2.1.2 Race non-specific resistance

Race non-specific resistance is usually considered as quantitative resistance based on the cumulative effects of several genes dispersed into multiple discrete loci know as quantitative trait loci (QTL). It usually provides durable, but partial resistance against wide-range of races (Vanderplank, 1963). Race non-specific resistance varies in a continuous way between the various phenotypes of the host population, from almost imperceptible (only a slight reduction in the growth) to quite strong (little or no growth of the pathogen). However, race non-specific resistance can be also provided by major genes as it is in case of Mildew resistance locus (*Mlo*) based resistance. Loss-of-function of the *Mlo* gene in barley was found to confer recessively inherited broad-spectrum resistance against the vast majority of powdery mildew isolates (Jorgensen, 1992). Mlo resistance provides complete resistance and is considered very durable. As a result, it has been successfully employed in agriculture for over 40 years.

The quantitative nature of non-specific resistance, determined by multiple minor resistance genes, makes it more difficult to handle in breeding programs (Kou and Wang, 2010). The main reason is problematic inheritance of all so-called minor genes together

after the crossbreeding. This can result in lower efficiency of the resistance. However, some QTLs possess differences in influence on phenotype. Based on this, they can be sorted into two groups as major QTLs with large effects and minor QTLs with slight effects (Kou and Wang, 2010). QTLs with large effects can confer very strong resistance and has been found to co-segregate even with a single locus, as shown for the *Lr34* (*Yr18/Pm38*) locus carrying pleiotropic gene conferring resistance to several fungal pathogens (Spielmeyer *et al.,* 2005) such as powdery mildew, leaf rust and stripe rust. In powdery mildew resistance, this locus explains up to 56% of the phenotypic variance of the trait (Lillemo *et. al,* 2008). QTLs with minor effects also contribute to resistance when combined additively with other QTLs but in breeding a combination of QTLs with strong effects is more preferred.

3.2.2 The life cycle of powdery mildew

The life cycle of powdery mildew is divided into sexual and asexual stages. During host growing season this pathogen is reproducing in an asexual way, in which a single colony is capable of producing up to 200 000 spores spreading by wind (Jorgensen, 1994; Zhang *et al.,* 2005). The sexual cycle is observed mainly before adverse periods, and developed structures enable pathogen to survive the unfavourable conditions.

For successful establishment of pathogenicity attachment to the plant surface, penetration, infection and colonization of the host has to occurred. The life cycle of powdery mildew (Fig. 5) is initiated when an ascospore or conidium lands on a host and starts to germinate. During this process a germ tube and appressorium is formed. The germ tube helps to attach the spore to the host surface. Thereafter appressorium produces a penetration peg that penetrates the epidermal cell and allows formation of haustorium inside the cell (Fig. 4 A; Glawe, 2008).

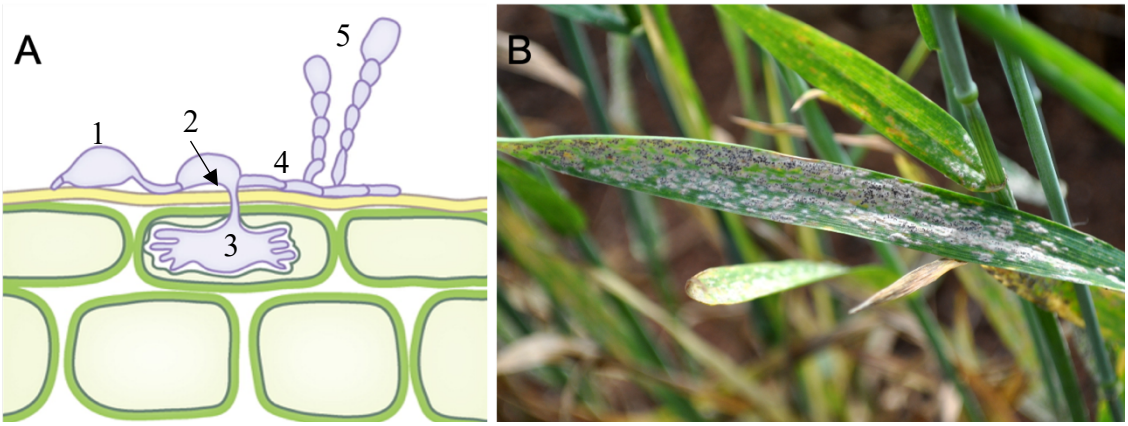

Figure 4. Different stages of the life cycle of powdery mildew. A. Haustorium formation – After the germination of ascospore or conidium appressorium is formed (1). Appressorium produces penetration peg (2), that penetrates into the epidermal cell and allows formation of haustorium (3). Multiple haustoria leads to the mycelium formation (4) from which conidiophores (or chasmothecia) can grow (5). **B. Mature powdery mildew** – Mature powdery mildew infections can appear grey or brown in color with black speckles. (Adapted from https://www.agric.wa.gov.au/spring/managing-powdery-mildew-wheat)

Haustorium is able to receive nutrient from the host cell and allows development of secondary hyphae that elongate and branch repeatedly. This result in formation of secondary appressoria which give arise another haustoria penetrating into the host cell. After 3-5 days, a white mycelium is clearly visible on the leaf surface (Fig. 4B). Mycelium might produce conidiophores or chasmothecia in response to environmental condition. In the early stages of development, most powdery mildew assemble conidiophores terminated by a chain of additional cells gradually maturing in conidia (asexual spores). The asexual stage of the infection serves to spread the disease and intensify its effects. Conidia also contain a large amount of water, which probably contributes to the ability to germinate in its absence (Glawe, 2008).

Powdery mildew is heterothallic filamentous fungi. During unfavorable conditions mycelia forms morphologically different gametangia – female ascogonia and male antheridia. Connection between the two gametangia cells leads in their fusion and formation of dikaryotic maternal cells with subsequent fusion of nuclei and formation of zygote. Zygote grows into thick-walled and dark-pigmented ascocarp of the closed type called chasmothecium, where meiotic division result in asci with haploid ascospores (sexual spores). Chasmothecia allow the fungus to survive in adverse condition, forming mainly at the end of the host growing season.

Due to its obligatory biotrophic nature, powdery mildew must be able to survive during period of cold winters or hot and dry summers but also without presence of susceptible host tissue for infection. Powdery mildew is able to survive such condition within dormant buds of diverse crop and landscapes plants and as dormant mycelia that persist on hosts with persistent leaves. These infected parts of the host can be the source of primary inoculum that can initiate further infection after improved conditions (Glawe, 2008).

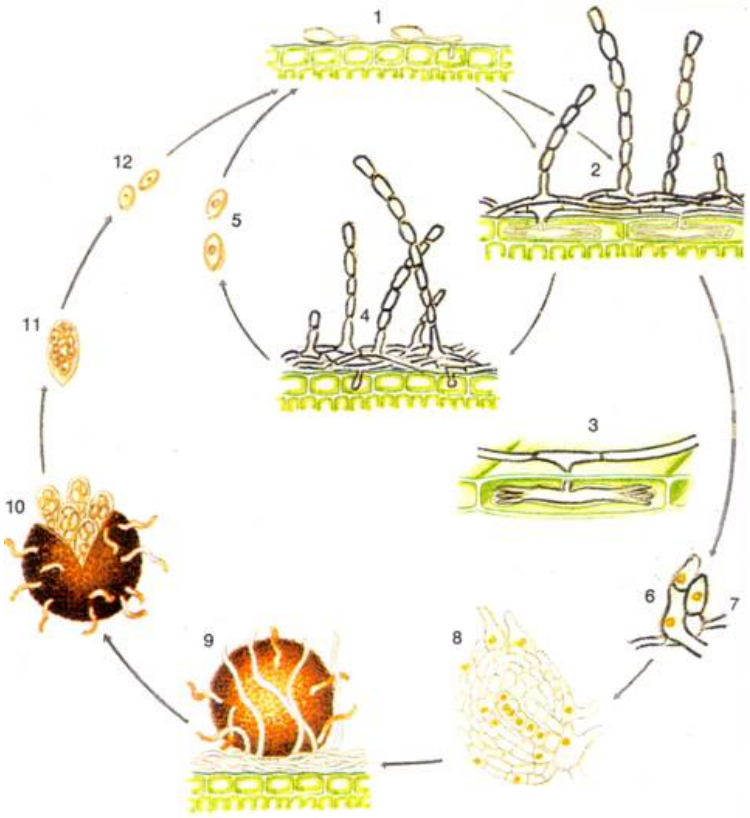

Figure 5. The life cycle of powdery mildew. 1 – gemination of haploid ascospore or conidia; 2, 4 – conidiophores with conidia; 3 – haustoria; 5 – conidia; 6 – ascogonia; 7 – antheridia; 8 – formation of ascocarp; 9, 10 – chasmothecium; 11 – ascus with haploid ascospores; 12 – ascospores. (Adapted from https://www.scritub.com/biologie/botanica/BOLILE-GRAULUI1211191616.php)

3.3 Genetic mapping of agronomically significant traits

All traits, including agronomically significant traits, are determined by major genes or group of genes contributing to the trait (QTLs). Identifying their location enables their further study and manipulation. For instance, among the important quantitative traits belong yield, grain quality or resistance to a various diseases and pests (Börner *et al.,* 2002; Cuthbert *et al.,* 2008; Grotewold *et al.,* 2015). Knowledge of both major genes and QTLs is crucial for increasing the rate of selective breeding to improve agronomically important crops.

Genetic mapping is used to identify and record the location of a specific gene to a particular region of a chromosome. The first step in trait mapping is construction of genetic maps using mapping populations and markers. The oldest and frequently used are F_2 mapping populations derived from bi-parental crosses (Schneider, 2005). The most common and reliable markers are based on DNA molecule polymorphism. DNA markers may be based on both coding (genes) and non-coding DNA sequences (for example intergenic regions or repetitive sequences). Genetic map shows the linear arrangements of the markers on the chromosomes. In the most common and accessible maps are their order and distances established by probability of recombination between them (linkage maps). Therefore, genetic maps do not show the real physical distances between markers, but rather their relative positions. To obtain the real position of DNA markers in genome, the linkage maps are anchored with physical maps for specific loci or whole genome physical maps. In physical maps distances between markers is measured in base pair (bp) and the maps offer the highest resolution, however, their construction is pricy and time consuming especially for large and complex genomes (O'Rourke, 2014)

As it was stated above most of the agriculturally significant traits are quantitatively determined, i.e by multiple genes with different contributions to the phenotypic trait variability. Thus, these traits exhibit continuous variation in a population and individuals cannot be categorized into distinct phenotypic classes as molecular markers. For the quantitative trait there is a continuum of allelic effects from small to large. This allows dividing quantitative trait loci into minor and major QTLs (Xin *et al.,* 2020). Major QTLs contribute to the total phenotypic variation with large effects which may segregate as Mendelian variants, while minor QTLs contribute with small effects and segregate as quantitative genetic variation. So their implementation to the map has to be handled differentially and QTL or Whole Genome Association Studies (GWAS) are used.

3.3.1 Development of mapping population

In order to map quantitative traits, it is essential to establish proper mapping population. In genetic mapping several types of mapping populations could be recognized depending on approaches for their development. Generally are mapping populations developed by intercrossing two or more individuals resulting in bi-parental or multi-parental mapping populations. Individuals exploit in development of mapping population must provide sufficient polymorphism of their DNA sequences and traits of interest. Mapping populations derived from bi-parental crosses are usually used for linkage mapping, while multi-parental mapping populations are desirably developed for association mapping. In recent years, also natural population are used in association mapping (Xu *et al.,* 2017) .

Bi-parental mapping populations are developed by crosses of parents contrasting in phenotype with the most distant genetic kinship. The result of this bi-parental cross is heterozygous F_1 hybrids from which further various types of mapping population may be derived such as F2 (Fig. 6A) or BC (backcross; Fig. 6B) populations, RILs (recombinant inbred lines; Fig. 6C), NILs (nearly isogenic lines) and DH (double haploid) lines (Schneider, 2005). The minimum size of bi-parental mapping population for primary mapping analysis is 100-200 individuals (Young, 2000). This ensure enough resolution and with minimising labour and cost. Increased number (thousands) of individuals improve mapping resolution which is required for fine mapping or cloning gene underlining the traits (Keller, 2005). Bi-parental populations provide high power to detect QTLs although it is limited on QTLs that contribute with large effect to the total phenotypic variation. This is due to a few recombination events occurring during its development, which at the same time allows the localization QTL to only 10-20 cM intervals (Xu *et al.,* 2017). Therefore, further fine-mapping is required before QTL can be cloned or used for MAS.

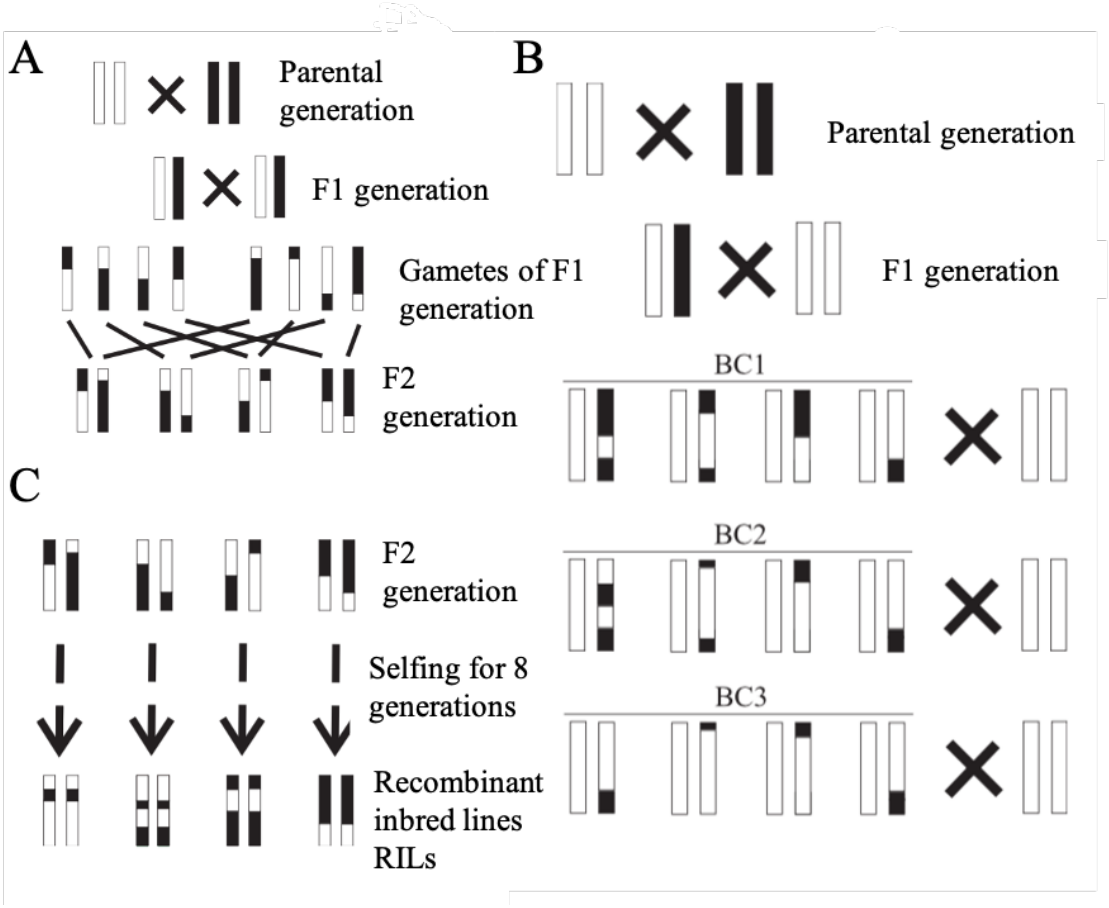

Figure 6. Bi-parental mapping populations. A. F2 mapping population – Two homozygous parental lines are crossed and give arise heterozygous F1 population. Subsequent self-cross of F1 generation provides recombinant gametes and result in the formation of recombinant F2 generation.; **B. Backcross population** – the F1 plants are backcrossed to one of the parents multiple times. It is based on the crosses between the donor and recurrent parent with selection on the desired trait. Donor parent provides the desired trait of our interest, but may not perform well in other areas. Recurrent parent is usually an improved or elite line into which we want to bring the favourable trait from donor parent. Backcrossing events with the recurrent parent reduces the proportion of the donor parent genome until a new line is almost identical to the recurrent parent, but with the desired trait from the donor parent. This result in the formation of Nearly Isogenic Lines (NILs.; **C. Recombinant inbred lines** – Recombinant inbred lines are commonly obtained by single-seed descent method - by self-pollinating F2 individuals and their descendants for multiple times. Multiple generations of RILs increases the number of recombination events that occur between markers. Thus, it is possible to order markers with stronger linkage. (Adapted with modifications from Schneider, 2005).

Multi-parental mapping populations are developed by crossing of multiple individuals. Such an example may be the MAGIC (multi-parental advanced generation intercross; Fig. 7) population first developed in *Arabidopsis thaliana* (Kover *et al.,* 2009). MAGIC populations are develop by inter-crossing multiple inbred founders several times in a well-defined order to combine the genetic material of all founders in a single line

(Cavanagh *et al.,* 2008). Increasing the number of accessions for development of MAGIC population leads to increased recombination frequency and therefore greater precision in QTL location (Huang *et al.,* 2012).

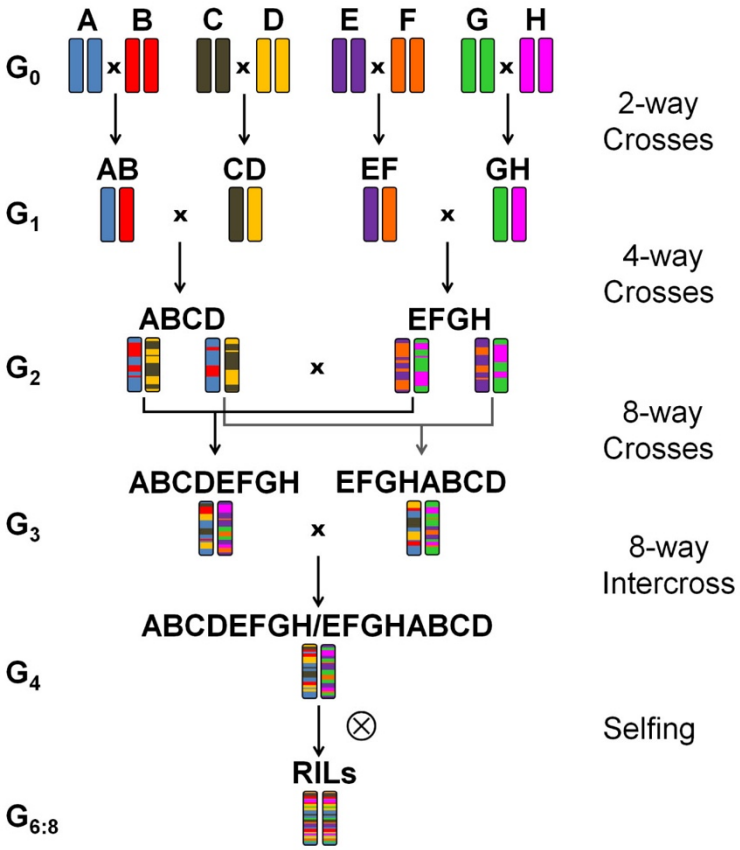

Figure 7. Multiparent advanced mapping population. Crossing scheme is based on the eight-founder crosses in predefined pattern. The inbred founders are paired and inter-mated known as a funnel. This result in a set of lines whose genome comprise a contribution from each of founder. This heterogenous stock provides multiple number of recombination events in population. (Adapted from Stadlmeier *et al.,* 2018).

3.3.2 Genotyping and Phenotyping

Mapping of traits and map construction requires genotyping and phenotyping all individuals of the mapping population. By genotyping, genetic markers are obtained and are used for construction of a genetic map. Phenotyping is performed according to the characteristics of the trait. Genetic map together with phenotypic data are used for mapping of QTLs of desired traits.

Nowadays, genotyping is based on use of high-throughput sequence-based Next generation sequencing (NGS) platforms. These methods allows obtaining genotype information of thousands of polymorphic loci in single analysis. The most common type of

polymorphism is single nucleotide polymorphism (SNPs), whose discovery is based on sequence information. Single nucleotide polymorphism represents variation in a single nucleotide that occurs at a specific position in the genome among individuals, where a variation at a specific position has to exceed a frequency of 1% (Johnson, 2010). It is the most frequent type of genetic polymorphism evenly distributed across the genome. For instance, in the wheat genome the SNP density ranges from 1 to 3 SNPs per 15 kb (Rimbert *et al.,* 2018). It provides differences between related sequences, both within an individual and between individuals within a population and may therefore provide a high density of markers near a locus of interest. Several genotyping methods involving NGS have been developed to detect alternative SNP alleles. All of them, RAD-seq (Miller *et al.,* 2007), DArTseq (Kilian *et al.,* 2012) or genotyping by sequencing (GBS; Elshire *et al.,* 2011) are similar in nature and allows to analyse genotypes of individuals of the mapping population for a large number of markers at once, which greatly accelerate the rough mapping phase.

3.3.3 Construction of linkage map

Genetic map indicates the order of the markers on the chromosome and their relative distances in cM and is essential for further QTLs mapping. The easiest way to calculate distance between two markers is by calculation of recombination frequency between them. Two genetic markers that are physically close to each other on the chromosome are unlikely to be separate during the crossing-over. They co-segregate into the same gametes and are considered to be in complete linkage. Conversely, two markers that are physically distant on the chromosome are rather to be separate during the crossing-over. They segregate separately into the gametes and are considered to be in incomplete linkage. Markers located on different chromosome are perfectly unlinked. The linkage between markers is determined by recombination frequency between them using data from mapping populations. Recombination frequency is calculated by recombination fraction, which is the ratio of the number of recombined gametes to the total number of studied gametes (Morgan, 1915; Sturtevant, 1913). Limited amount of markers in the map can lead in inability to identified all recombination events and double crossovers. To overcome this inaccuracies during the construction of genetic map, different genetic mapping functions could be used such as Haldane mapping function (Haldane, 1919) or Kosambi mapping function (Kosambi, 1943). Kosambi mapping function, in addition, models the effect of

positive interference, where one crossover event deters the occurrence of a second in close proximity to the first. A more accurate way of estimating the distances between two markers is the maximum likelihood estimation. It is an estimate of the parameter value – the value selected for the parameter is the most likely for a certain set of observations. Likelihood-based methods are nowadays widely used by computer programs for genetic maps construction such as for instance MapMaker (Lander *et al.,* 1987) or MultiPoint (Ronin *et al.,* 2017).

If a multiple number of markers segregate in a mapping population, they are first divided into linkage groups. The presence of a genetic linkage between the markers is statistically estimated by pairwise recombination fraction. The alternative hypothesis (the linkage exists between the markers) is compared with the null hypothesis (the linkage does not exist). Likelihood of odds of these hypotheses is evaluated with LOD score (logarithm of odds; Morton, 1955). LOD score estimate of whether two markers (loci) are likely to be located near each other on a chromosome and are therefore likely to be inherited together. A convenient rule is that all markers with pairwise LOD scores greater than 3 are classified into the same linkage group. A LOD score of 3 means the odds are a thousand to one that two markers are linked, and therefore inherited together. This is done for all pairs of markers and thereafter followed by further aligning of marker in the linkage group. Thus, markers are aligned to the developing linkage group in the correspondence of already linked markers (Xu, 2013). Markers in linkage belong to the same linkage group. Each linkage group should represent different chromosome and thus correspond to the basic chromosomal number of the species.

3.4 Quantitative trait loci mapping

Continuous variation in phenotype values for traits controlled by multiple genes prevent categorization of individual members of mapping population into distinct phenotypic classes and use of Mendelian laws for mapping the traits. Two main statistical method QTL and GWAS analysis are used to identify multiple loci contributing to the phenotype variation. Generally, QTLs mapping consist of four main steps (1) development of a mapping population; (2) genotyping and phenotyping of the mapping population; (3) construction of genetic map; and (4) detection of QTL using a statistical method.

3.4.1 QTL analysis

Detection of quantitative trait loci using the QTL analysis is based on interconnection of acquired data from genotyping and phenotyping. The main goal of QTL analysis is both to detect number of loci determining the trait and their power to the phenotype. Markers are used to divide the mapping population into different genotypic groups. The presence or absence of a particular marker locus determines whether significant differences exist between groups with respect to the trait being measured (Sehgal *et. al,* 2016). The individuals with different marker locus genotypes for QTL will have different mean values of the quantitative trait. The basic procedure of QTL analysis is based on likelihood statistic method. In analogy with the genetic mapping, two hypotheses are tested. The null hypothesis (QTL does not exist or does exist, but it is not associated with markers) is compared with the alternative hypothesis, which assumes existence of QTL and marker association. Likelihood of odds of these hypotheses is evaluate with LOD score (Morton, 1955). Subsequently, regions on the genome that show significant values of the test statistic are identified.

In QTL analysis, markers that are genetically linked to a QTL influencing the trait of our interest will segregate more frequently with trait values, whereas unlinked markers will not show significant association with phenotype. The easiest way to identified QTL is using **single-marker analysis** (SMA). Single marker analysis compute whether phenotype values differ among genotypes for a given marker. Associated marker is given as position of the QTL. Statistical methods used for single marker analysis are, for example, t-test (Gosset, 1908), analysis of variance (ANOVA); or linear regression (Kearsey and Hyne, 1994). However, single marker analysis brings some disadvantages. QTL mapping may be underestimated due to the recombination between the marker and QTL (Collard *et al.,* 2005). Therefore, another approaches have been developed such as **single-interval mapping** (SIM; Lander and Botstein, 1989) or **composite-interval mapping** (CIM; Jansen 1994; Zeng 1994). Single-interval mapping uses maximum-likelihood parameter estimation and a pair of markers in linkage to identified QTL located on the chromosome in the interval between them. Composite-interval mapping combines SIM with multiple statistical analysis of linear regression. CIM detects QTL in multiple marker intervals using other molecular markers as covariates to control for other QTL and thus increases the precision of QTL detection. Another extension of interval mapping represents **multiple**

interval mapping (MIM; Kao *et al.,* 1999). MIM is quite similar to CIM, but uses multiple marker intervals simultaneously to searching for multiple QTLs.

3.4.2 GWAS

In Genome-Wide Association Study (GWAS) are preferentially used for analysis of multi-parental populations or wide natural populations. GWAS enables the QTL analysis by associations between hundreds of thousands of single nucleotide polymorphism and specific trait (Gali *et al.,* 2019). It is based on the existence of haplotypes and linkage disequilibrium (LD). Haplotype represent allelic constitution of two or more loci in a particular region of a chromosome called haplotype block. Between loci within the haplotype block minimal recombination events occurs and therefore these loci segregate as a single allele. Thus, haplotype blocks represent a combination of alleles, which may exhibit certain polymorphism, most commonly SNPs and provide markers in tight linkage. Linkage disequilibrium is based on a probability of seeing alleles within the haplotype block more frequently together in a population as a result of minimal recombination events between them. Association mapping utilizes linkage disequilibrium to determine the presence of a statistical association between allelic variants to a particular trait within a population due to the history of recombination events. Each recombination leads to the narrowing area of a haplotype block and thus providing the smallest possible haplotype block region and higher resolution compared to linkage mapping. A multiple number of recombination events are therefore required to obtain such narrow area. GWAS tests DNA polymorphisms obtained from hundreds of natural accessions for genetic variations like SNPs. SNPs that are found significantly more frequently in group of genotypes of accessions with a certain trait of interest than in the general populations is likely to be associated with the trait. Association mapping takes advantage of historic recombination events accumulated over hundreds generations (Zhu *et al.,* 2008). The GWAS is represented by a Manhattan plot with significance p-value (the probability was observed by chance) on the y-axis and the genomic position in the x-axis (Choudhury *et al.,* 2019).

3.5 Verification of QTL mapping

Owing to the all factors of QTL mapping, identified QTL should be independently confirmed or validated before its further use (Collard, 2005). This is achieved by repeating the experiment. In bi-parental population, validation involves independent population

construction from the same parental genotype with a trait of interest used in the primary QTL mapping study. The conserved detected QTL, most likely the QTL having strong genetic effect, are further chosen as a region to focus for further analysis. This includes formation of an advanced mapping population and its subsequent testing for new markers. QTL verification is required to confirm the relationship between marker and trait (Lander and Kruglyak; 1995).

4 MATERIALS AND METHODS

4.1 Materials

4.1.1 Biological materials

4.1.1.1 Plant materials

The F_2 mapping population (125 plants) from cross of GZ1 x EBL created by doc. RNDr. Švec, CSc. UK Bratislava, SR was used. The spring resistant line GZ1, the nickname of the line DIM140, was collected during expedition in Sobotiště na Myjave in Slovakia and provided by Ing. Masár. The winter line Eichenbarlebener (nickname EBL) was obtained from Gene Bank of Slovak Republic, Piešťany. The specification of the plant material used for development of mapping population is shown in Table 1.

Table 1. Plant material used for genetic mapping

Material	GB code	Taxonomy	Type	Origin
GZ1	DIM140	*T. turgidum* subsp. *dicoccum*	spring	Slovakia
EBL	Eichenbarlebener	*T. turgidum* subsp. *dicoccum*	winter	Germany

4.1.1.2 *Blumeria graminis* materials

Blumeria graminis (DC) E.O. Speer f. sp. *tritici* (*Bgt*) used for phenotyping of mapping population were isolates **A17**, **A24** and **A3ab**. Isolates used for inoculation were selected based on their infect aggressiveness i.e., shortest time from spore inoculation of plant material to conidiophores formation. All of these isolates come from a collection of *Bgt* from the Research institute of plant production in Piešťany.

4.1.2 Chemicals and kits

4.1.2.1 Chemicals

- Agar (HiMedia)
- Benizimidazol (Sigma-Aldrich)

4.1.2.2 Kits

- NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany)

4.1.2.3 List of equipments

- NanoDrop (ND1000 spectrophotometer, Thermo Scientific)

- Water bath (J 18 BAIN UNIV, Thermo Electron Industries SAS)
- Mixer mill (MM301, Retsch)
- Centrifuge (5415 Eppendorf)
- Microwave (KOR-6C2B, DAEWOO; Soul, KOR)

4.1.3 List of solutions

Table 2. List of solutions for phenotyping

Solution for preparation of 0,5% agar medium	
200 ml	dH_2O
1 g	Agar
11,6 ml	862 $mg.l^{-1}$ Benzimidazol
Stock solution of benzimidazol (862 $mg.l^{-1}$)	
1 l	dH_2O
862 mg	Benzimidazol
Stored in a freezer (-20 °C)	

4.2 Methods

4.2.1 DNA extraction and purification

Total genomic DNA was extracted from all individuals of the mapping population. DNA was extracted from ca 2 cm long young leaf segments. The collected plant material was dried at 2 ml microtube at 37 °C for at least 24 hours. Dry leaf segments were homogenized in the presence of two glass balls (5 mm) at 27 Hz for 3 minutes. DNA extraction and purification was performed using the commercial NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany) according to instructions of the supplier with certain modifications as follow.

Homogenized material was processed with 400 µl Buffer PL1 and vortexed thoroughly. After that, 10 µl RNase A was added, mix thoroughly and incubated for 20 minutes at 65 °C. Lysate suspension was loaded onto the violet ring NucleoSpin Filter placed into a new 2 ml collection tube and centrifugated for 2 minutes at 11,000 g. Collected flow-through was treated with 450 µl of Buffer PC and vortexed thoroughly. A maximum volume of flow-through with Buffer PC was transferred onto new prepared green NucleoSpin Plant II column in new 2 ml collection tube and centrifugate for

2 minutes at 11,000x g. Flow-through was discarded and the green NucleoSpin column was washed three times – first with 400 µl Buffer PW, for the second time with 700 µl of Buffer PW2 and for the third time with 200 µl of Buffer PW2 with centrifugation between each step for 2 minutes at 11,000 g. Green NucleoSpin column with bind DNA was placed into 1,5 ml microcentrifuge tube (the 1,5 ml microcentrifuge tube is not provided, therefore 1,5 ml microtube with cut off lid was prepared) and eluted with Buffer PE twice. In each step 30 µl of elution buffer was added, incubated at 65 °C for 5 minutes and centrifugated for 2 minutes at 11,000 g. The obtained eluate was pipetted into a new 1,5 ml microtube. After the DNA extraction the concentration of extracted DNA was quantified with a NanoDrop spectrophotometer.

4.2.2 Genotyping

For the genotyping analysis, the DNA of 125 samples from mapping population GZ1xEBL were diluted to a range 50 – 60 ng/µl. DNA was analysed using DArTseq method (Kilian *et al.,* 2012) by the commercial provider, the Diversity Arrays Technology (Canberra, ACT, Australia; https://www.diversityarrays.com).

Obtained data were filtered before map construction. SNPs markers with more than 10 missing data-points and markers with large segregation distortion (over 30 %) were removed. The scores of all codominant polymorphic DArTseq markers were converted into genotype codes ("A"= GZ1; "B"=EBL; ; "H"=GZ1/EBL).

4.2.3 Phenotyping

Phenotyping was performed at the Faculty of Natural Science of Comenius University in Bratislava in cooperation with doc. RNDr. Švec, CSc. $F_{2:3}$ families of the GZ1xEBL mapping population were evaluated under controlled conditions for powdery mildew responses to *Bgt* isolates A17, A23 a A3ab.

In order to analyze resistance to *Bgt* isolates (causing powdery mildew) under the laboratory conditions 20 seedlings of each line were grown in plastic pots filled with peat. After 10 days, primary leaves were cut into 2.5 cm long segments and deposited on Petri dishes filled with 0.5% agar medium containing 862 $mg.l^{-1}$ benzimidazol. Leaf segments were inoculated with powdery mildew isolates (A17, A23, A3ab) that were cultured for 10 days on 3 cm long primary leaf segments of susceptible cultivar (EBL) cultivated on agar medium of the same composition mentioned above. The grown spores of powdery

mildew isolates were blown from the leaf segments using a syringe with a rubber hose extension into the inoculation tower inside which was an open Petri dish with the collected leaf segments of a single line. Inoculated leaf segments were incubated for 13 days in growth chamber under 24 hour of 800 lux light per day at 18-20 °C. Thereafter, the plant response to the powdery mildew was visually evaluated.

If all plants of a single genotype within one observation were covered with powdery mildew, the genotype was stated as susceptible (S). Otherwise, if all plants of a single genotype within one observation were not infected with powdery mildew, the genotype was stated as resistant (R). Mixture of these observation i.e. some plants were resistant and some plants were susceptible, the genotype was stated as heterozygous (H). Plants with obscure phenotype were not evaluated and in further analysis used as missing data ("-"). To evaluate the genetic inheritance for the deviation from Mendelian ratio of the GZ1 resistance to powdery mildew, the chi-square goodness of fit test (Agresti, 2007) was used (https://www.graphpad.com/quickcalcs/chisquared1.cfm.).

4.2.4 Genetic linkage map construction

The genetic linkage map was constructed using software MultiPoint version UltraDense (v4.1; multiqtl.com; Ronin *et al*., 2017). DArTseq SNPs markers, from preliminary treatment, were input into mapping software and were processed as a F2 population with default settings. Markers with more than four missing data points and $\chi 2$ value over 10 were eliminated. Markers were clustered into multiple linkage groups (LG) of ordered co-segregating markers, however, the minimum size, but not lower than 14 LG was selected for subsequent analysis due to the tetraploid character of *T. turgidum* subsp. *dicoccum*.

To obtain a stable skeleton map jackknife re-samplings were used to identify markers that caused unstable neighborhoods and disrupted the monotony of recombination changes. Such markers were verified for segregation ratios, linkage distances, missing data, and associations with other markers and, based on, were eventually removed to stabilize the linkage group until the value of global variation decreased below 1.1. Then, in case of long distances between markers, or for increasing density of linkage group, markers from Heap group was added to the linkage group locally within two markers or globally for whole linkage group. The order of markers was again checked for monotony distortion and map size enlargement and those causing disruption was also discarded. The

resulted LGs were exported to Microsoft Excel with recombination frequencies converted into centiMorgans (cM) using the Kosambi mapping function (Kosambi; 1943).

Individual linkage groups were assigned to a particular chromosome based on the wheat DArTseq consensus map by Diversity Arrays Technology (Canberra, ACT, Australia; https://www.diversityarrays.com). Visualization of exported LGs was performed by MapChart 2.32 (Voorrips, 2002).

4.2.5 Quantitative trait loci analysis

Quantitative trait loci (QTLs) were analyzed with MultiQTL (v2.6) software (multiqtl.com; Korol *et al*., 2001). For QTL analysis, skeleton linkage map together with phenotypic data was used to perform single-trait single-environment multiple interval mapping. Analysis was conducted for entire genome analysis as it was carried as multichromosome set. Prior to QTL analysis, phenotype data were transferred into numeric values (sensitive – 0; heterozygote – 0.5; resistant 1) or a symbol for a missing value (by default, the missing value symbol - $).

First analysis was carried as interval mapping with subsequent analysis by multiple interval mapping. LOD threshold values of QTL multiple interval mapping were determined by global permutation test with 1000 iterations. Significance LOD values was estimated by comparing hypotheses H1 (there is a QTL in the chromosome):H0 (no effect of the chromosome on the trait). The QTL was stated significant when their LOD scores exceeded the 95% ($p < 0.05$). Confidence interval was carried under the bootstrap analysis with 95 % confidence set (1000 iterations; Lebreton & Visscher, 1998). QTL effects were expressed as the percentage of explained variance (PEV) of the trait.

4.2.6 Anchoring of the resistance QTLs to the cv. Zavitan reference genome sequence and candidate gene identification

Candidate genes for GZ1 powdery mildew resistance were identified by the anchoring of the mapped QTLs to the cv. Zavitan reference genome sequence (Avni *et al.,* 2018). For the alignment with the reference sequence four peak flanking markers of each QTL (Table 3) were chosen. The marker sequences were Blast (Altschul *et al.,* 1990) aligned using blastn program with default parameters. Only best blast hits with E-value below e^{-100} were considered. Annotated genes from regions

delimited by the alignments were extracted from list of high confidence annotated genes (Avni *et al.,* 2018).

Table 3. Flanking markers used for the anchoring of the resistance QTLs to the cv. Zavitan reference genome sequence

QTL	Marker	Sequence
2A	1101086-26	TGCAGGTACAAGATCGCGCTCGGCCTGGGCTCCGCCTTGCGGTTCATCCACCCGAG ATCGGAAGAGCGG
	1147248-18	TGCAGATCGACAGTCATGCGGTGAGCAATCAAGTGGTGCAGATGGTAACCCAGAG TAGTGGCGCTAATA
	41420734-12	TGCAGCAGCTCGCCCTCATCCACGTGCCGAGATCGGAAGAGCGGTTCAGCAGGAA TGCCGAGACCGATC
	1053641-64	TGCAGATTGCGGTTACGGATTGAAGGCGGCGACACATCCTACCGTCCACGACAAA AGACGCCCATACAT
7A	1011397-23	TGCAGCAGCCCATGGAAGAGGCGCTGGCGGCGGCCAAGAGGAGAAGGTCGGAGC ACTACCACGGCGTCG
	2282553-59	TGCAGCTCGTCGATGACCGCGCGGCCGCCCGCGTGAATGCACAGGTGCTTGAATG CTGTCGAGAAGTCC
	1043460-61	TGCAGTAATGCTGCATGCATGCATGTCATGCAGTAAAGTTTGGGTGACATGCATTC ATGCATGGCGAAT
	1129987-36	TGCAGTATTCAAGGCGATGCTTGGGTTTATGTACGGCGACTCTCTGCCGAGATCGG AAGAGCGGTTCAG

5 RESULTS

5.1 Characterization of GZ1 resistance

Resistance of tetraploid wheat *Triticum turgidum* subsp. *dicoccum* (DIM140) was discovered by doc. RNDr. Švec, CSc. (UK Bratislava, Slovakia) and was named as GZ1. The identification of the genetic resistance of GZ1 to *Blumeria graminis* (DC) E.O. Speer f. sp. *tritici* preceded testing with a set of defined powdery mildew isolates that can differentiate lines possessing known resistance genes. GZ1 did not reveal the presence of any of these genes, since it showed resistance to a whole differentiation set. GZ1 was also tested with a collection of various powdery mildew populations obtained from the different Slovakia localities in 2006 and 2007 and showed resistance in all cases. Subsequently, non-specific resistance was also confirmed by inoculation with a mixture of different isolates. Further, GZ1 resistance was tested in field conditions, where exhibited total resistance to powdery mildew during the all growing stages. Thus, GZ1 represents a unique source of powdery mildew resistance with resistance non-specific level. In the F_1 generation of cross GZ1 line with a susceptible line, all plants showed a sensitive response to a mixture of powdery mildew isolates. This indicating a recessive type of inheritance of the trait. However, three phenotypic manifestation was observed in the $F_{2:3}$ generation. As it will be further explained, this is due to the quantitative character of the trait.

5.2 Powdery mildew resistance

A total of 125 $F_{2:3}$ lines of the mapping population derived from a cross between powdery mildew resistant GZ1 line and powdery mildew sensitive EBL line were inoculated with powdery mildew isolates (A17, A23 and A3ab), and evaluated for disease symptoms (Fig. 8). In susceptible plants, phenotypic variation was observed. In some cases, the infection was strong and leaf segments were completely covered with large colonies of powdery mildew, while in some other cases they were covered with small focused colonies only. (Fig. 8A and 8B). The resistant plants did not supported any colonies (Fig. 8C).

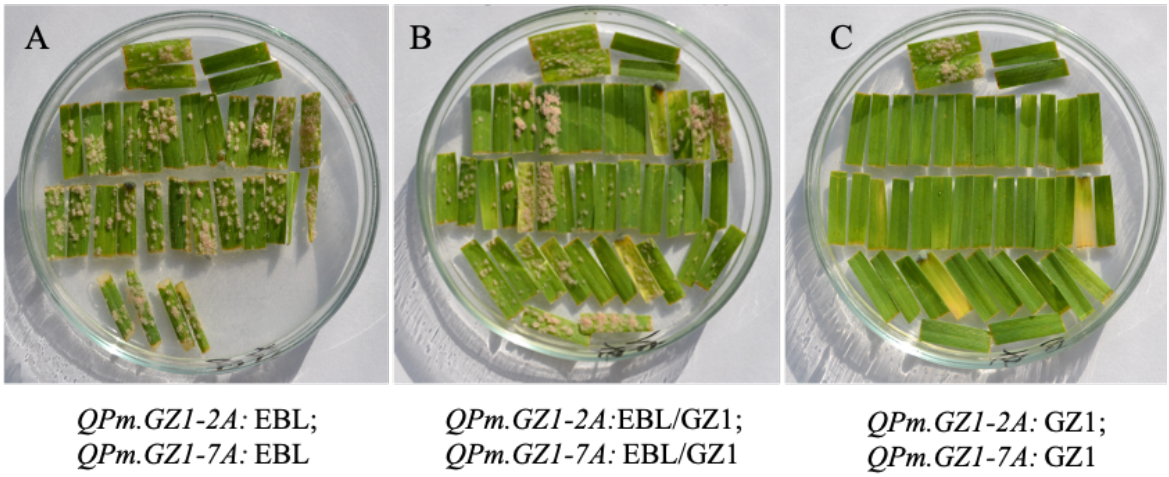

Figure 8. Phenotypic reaction of $F_{2:3}$ families to powdery mildew isolates A17, A23 and A3ab. In each figure, first line (horizontal) represents two segments of sensitive EBL line and two segments of resistant GZ1 line. The remaining segments (vertical) represent tested families, always two primary leaf segments of different plant of the same family. The description below states genotype constitution at the mapped QTLs. **A – Sensitive reaction of the genotype 84 GZ1xEBL families to powdery mildew.** All 14 plants were infected with powdery mildew with growth of large colonies. **B – Heterozygous reaction of genotype 72 GZ1xEBL families to powdery mildew.** Majority of the tested plants were susceptible with colonies except plants 3, 4, and 13 which were resistant. Interestingly, on the susceptible plants there were two types of colonies. The "small focused" colonies were seen on the plants 1, 7, 10, 11, 12, 13, 14, and 16. Sensitive plants with large colonies were the plants 2, 5, 6, 8, 9, 15, 17 and 19. **C – Resistant reaction of genotype 73 GZ1xEBL families to powdery mildew.** All 19 plants were resistant.

Out of 125 $F_{2:3}$ GZ1xEBL plants, 16 were susceptible, 63 were heterozygous and 34 were resistant. The remaining 12 did not provide reliable phenotype and were used as missing data (Table 4). Chi-square value of segregation of $F_{2:3}$ mapping population was 7.09 with a P=0.0289, which indicate significant distortion from 1:2:1 segregation.

Table 4. Phenotypic evaluation of $F_{2:3}$ families of the mapping population inoculated with powdery mildew isolates A17, A23 and A3ab.

Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype
GZ1xEBL - 1	S	GZ1xEBL - 27	H	GZ1xEBL - 54	H	GZ1xEBL - 79	-	GZ1xEBL - 107	H
GZ1xEBL - 2	H	GZ1xEBL - 28	H	GZ1xEBL - 55	H	GZ1xEBL - 80	R	GZ1xEBL - 108	H
GZ1xEBL - 3	H	GZ1xEBL - 29	H	GZ1xEBL - 56	H	GZ1xEBL - 81	R	GZ1xEBL - 109	H
GZ1xEBL - 4	S	GZ1xEBL - 30	R	GZ1xEBL - 57	H	GZ1xEBL - 82	-	GZ1xEBL - 110	R
GZ1xEBL - 5	R	GZ1xEBL - 31	R	GZ1xEBL - 58	H	GZ1xEBL - 83	H	GZ1xEBL - 111	S
GZ1xEBL - 6	S	GZ1xEBL - 32	H	GZ1xEBL - 59	H	GZ1xEBL - 84	S	GZ1xEBL - 112	S
GZ1xEBL - 7	R	GZ1xEBL - 33	H	GZ1xEBL - 60	H	GZ1xEBL - 85	R	GZ1xEBL - 113	-
GZ1xEBL - 8	H	GZ1xEBL - 34	S	GZ1xEBL - 61	-	GZ1xEBL - 86	R	GZ1xEBL - 115	-
GZ1xEBL - 9	R	GZ1xEBL - 35	H	GZ1xEBL - 62	H	GZ1xEBL - 87	H	GZ1xEBL - 116	H
GZ1xEBL - 10	H	GZ1xEBL - 36	-	GZ1xEBL - 63	R	GZ1xEBL - 88	H	GZ1xEBL - 117	-
GZ1xEBL - 11	H	GZ1xEBL - 37	H	GZ1xEBL - 64	R	GZ1xEBL - 89	H	GZ1xEBL - 118	H
GZ1xEBL - 12	H	GZ1xEBL - 39	S	GZ1xEBL - 65	R	GZ1xEBL - 91	H	GZ1xEBL - 119	H
GZ1xEBL - 13	R	GZ1xEBL - 41	H	GZ1xEBL - 66	H	GZ1xEBL - 92	R	GZ1xEBL - 120	R
GZ1xEBL - 14	S	GZ1xEBL - 42	H	GZ1xEBL - 67	H	GZ1xEBL - 93	R	GZ1xEBL - 121	S
GZ1xEBL - 15	H	GZ1xEBL - 43	H	GZ1xEBL - 68	R	GZ1xEBL - 94	H	GZ1xEBL - 123	-
GZ1xEBL - 17	H	GZ1xEBL - 44	H	GZ1xEBL - 69	R	GZ1xEBL - 95	R	GZ1xEBL - 124	H
GZ1xEBL - 18	R	GZ1xEBL - 45	R	GZ1xEBL - 70	H	GZ1xEBL - 96	R	GZ1xEBL - 125	S
GZ1xEBL - 19	H	GZ1xEBL - 46	R	GZ1xEBL - 71	R	GZ1xEBL - 97	H	GZ1xEBL - 126	H
GZ1xEBL - 20	R	GZ1xEBL - 47	H	GZ1xEBL - 72	H	GZ1xEBL - 98	R	GZ1xEBL - 127	R
GZ1xEBL - 21	R	GZ1xEBL - 48	S	GZ1xEBL - 73	R	GZ1xEBL - 99	H	GZ1xEBL - 128	-
GZ1xEBL - 22	-	GZ1xEBL - 49	S	GZ1xEBL - 74	H	GZ1xEBL - 100	H	GZ1xEBL - 129	H
GZ1xEBL - 23	S	GZ1xEBL - 50	R	GZ1xEBL - 75	H	GZ1xEBL - 101	H	GZ1xEBL - 130	H
GZ1xEBL - 24	H	GZ1xEBL - 51	H	GZ1xEBL - 76	-	GZ1xEBL - 103	H	GZ1xEBL - 131	H
GZ1xEBL - 25	S	GZ1xEBL - 52	H	GZ1xEBL - 77	H	GZ1xEBL - 104	R	GZ1xEBL - 132	R
GZ1xEBL - 26	R	GZ1xEBL - 53	H	GZ1xEBL - 78	-	GZ1xEBL - 105	S	GZ1xEBL - 133	H

S – susceptible (green); **H** – heterozygous (yellow); **R** – resistant (red); "**-**" missing data (white)

5.3 Genetic map

A total of 23,012 SNPs markers were identified in DArTseq analysis. After filtering on quality (missing datapoints) and normal segregation ($\chi 2$) a set of 7,985 SNPs markers were used for the construction of genetic map. Two types of genetic linkage map were generated – skeleton linkage map and global linkage map. Skeleton linkage map comprises of only a representative (skeleton) markers. Global linkage map is enriched with attached markers from bound together markers. Bound together markers are part of a group of co-segregating markers for which a representative marker (skeleton marker) has been selected and attached to the skeleton linkage map. For QTL analysis only skeleton map was used (Table 5).

The skeleton map spanned a genetic length of 2865.04 cM with an average marker interval 3.73 cM. The length of chromosomes ranged from 186.99 to 262.11 cM with an average of 204.65 cM. The map was constructed from 862 SNPs markers. Marker numbers

per chromosome ranged from 38 to 88 with an average of 61 markers. The highest number of markers (88) was assigned to the chromosome 7A, whereas chromosome 4B contained the lowest number of markers (38). The lowest and highest marker density were observed for chromosome 4B (4.92 cM/marker) and chromosome 2B (2.64 cM/marker), respectively. The average marker density for whole skeleton linkage map was 3.69 cM/marker. Only chromosomes with mapped QTL were visualized (Fig. 9).

Table 5. Skeleton linkage map information

Chromosome	Genetic length [cM]	Average marker interval [cM]	Marker density [cM/marker]	Number of markers
1A	195.52	3.24	3.76	52
2A	220.58	2.64	3.80	58
3A	242.33	4.33	4.33	56
4A	188.82	2.98	3.56	53
5A	251.52	3.76	4.12	61
6A	202.18	3.80	4.04	50
7A	262.11	3.50	2.98	88
1B	192.85	4.92	3.21	60
2B	192.50	3.56	2.64	73
3B	255.68	3.21	3.50	73
4B	186.99	4.92	4.92	38
5B	237.50	4.04	4.09	58
6B	243.12	3.24	3.24	75
7B	230.84	4.09	3.45	67
Total	1563.06	52.25	51.65	862
Avarage	111.65	3.73	3.69	62

5.4 QTL detection and identification

Quantitative trait loci analysis using MIM method revealed two genomic regions associated with the resistance to powdery mildew located on the chromosome 2A (denominated as *QPm.GZ1-2A*) and chromosome 7A (denominated as *QPm.GZ1-7A*) as they exceed the estimated threshold value and therefore was considered as significant.

The *QPm.GZ1-2A* was located within an interval from 155.59 cM to 163.48 cM on 2A chromosome (Fig. 9) with LOD score of 14.51 and p-value 0.00093. The LOD significantly exceeded the estimated LOD threshold of 3.627. The bootstrap analysis identified the sample mean at the position 159.54 cM and with the 95% confidence set the confidence interval from 151.8 cM to 167.3 cM. The effect of *QPm.GZ1-2A* QTL explained up to 31% of the total phenotypic variance observed for powdery mildew resistance (Table 6).

The *QPm.GZ1-7A* was located within and interval from 233.71 cM to 262 cM on chromosome 7A (Fig. 9). The LOD score 8.91 also significantly exceeded threshold of 3.803 with p-value 0.00093. The sample mean for the dataset was calculated at the position 248.13 with confidence interval from 219.9 cM to 262 cM at the 95 % confidence. The *QPm.GZ1-7A* explained up to 20% of the total phenotypic variance observed for powdery mildew resistance (Table 6).

Table 6. The powdery mildew resistance related QTLs

Chromosome	LOD treshold	Max LOD	P value	Loci position (cM)	Confidence interval	Mean value (cM)	PEV (%)
2A	3.62	14.51	0,00093	155.6-163.5	151.8- 67.3	159	31
7A	3.80	8.91	0,00093	233.7-262.0	219.9 - 262	248	20

PEV – percentage of explained variance of the trait

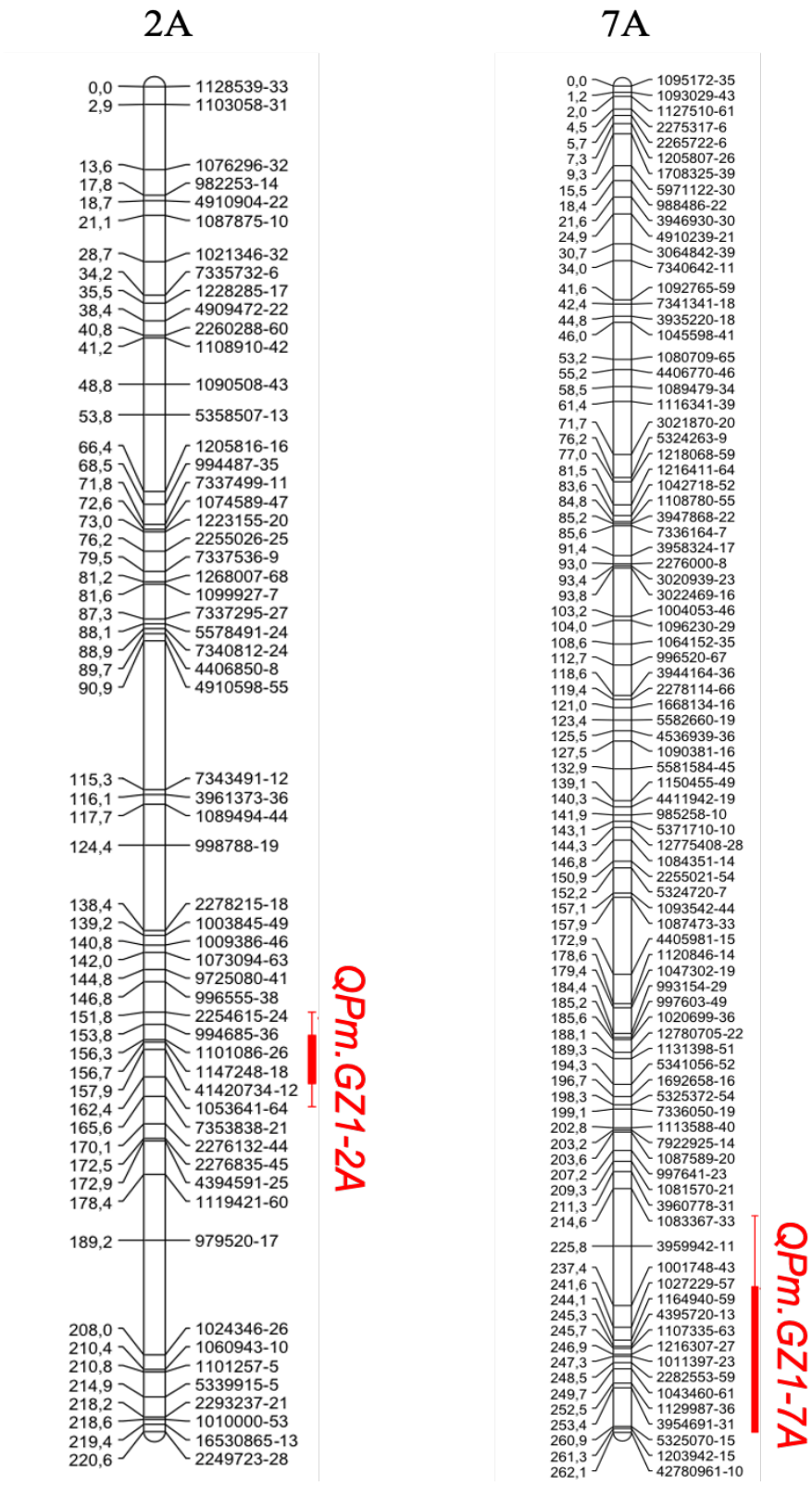

Figure 9. The high-density genetic linkage map of chromosome 2A and 7A with QTLs associated with powdery mildew resistance. Black line of each chromosome indicates a DArTseq codominant marker with the position in cM on the left and the marker name on the right. The red bar of each chromosome indicates the position of QTL for powdery mildew resistance. The red thin line represents confidence interval of the QTL.

The *QPm.GZ1-2A* covered 7.9 cM and comprised of 4 markers - 1101086-26, 1147248-18, 41420734-12, 1053641-64 (Fig. 10). Alignment with a reference sequence of cv. Zavitan (Avni *et al*., 2007) revealed synteny with a 1,65 Mbp long region. The region in the cv. Zavitan comprised 18 genes, from which only one had relation to the plant resistance response (Table 7).

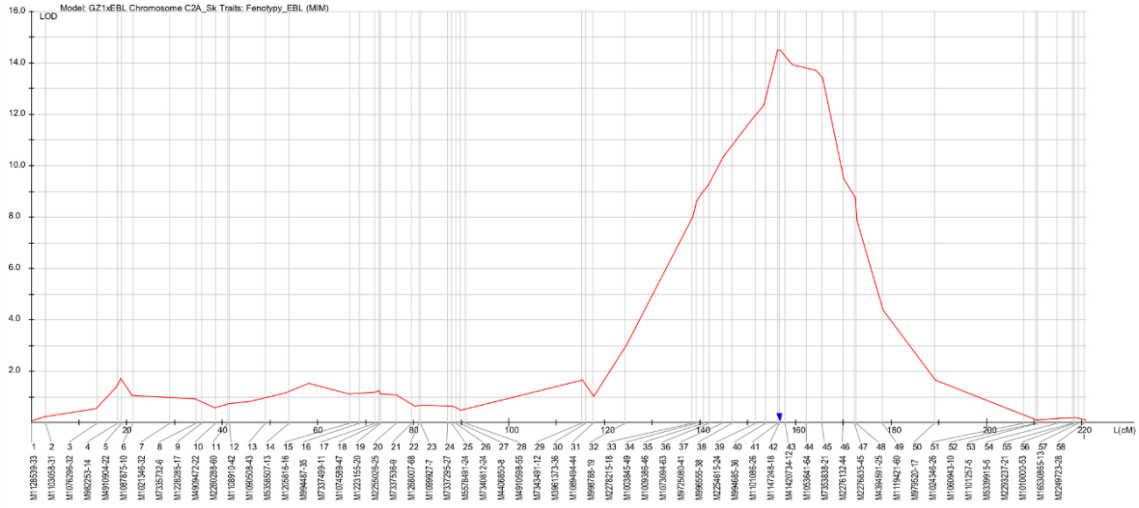

Figure 10. The *QPm.GZ1-2A* QTL associated with powdery mildew resistance on chromosome 2A. Marker positions are indicated in cM on the x-axis. LOD score is shown on y-axis. The QTL was located in the region from 155 – 163 cM. The peak reached the LOD maximum of 14.51 at the position 159.54 cM (blue arrowhead).

Table 7. The *QPm.GZ1-2A* syntenic region on cv. Zavitan chromosome 2A

Chromosome	Genome position	Length [bp]	Gene
2A	698059339 - 698061007	1668	Concanavalin A-like lectin protein kinase family protein
2A	698284784 - 698285136	352	myb-like transcription factor family protein
2A	698335525 - 698336964	1439	unknown function
2A	698500966 - 698501124	158	SET domain group 40
2A	698621623 - 698622296	673	Protein SRC2
2A	698623242 - 698623566	324	Retrovirus-related Pol polyprotein from type-1 retrotransposable element R2
2A	698656086 - 698656362	276	D-2-hydroxyglutarate dehydrogenase, mitochondrial
2A	698719674 - 698719712	38	D.melanogaster polytene
2A	699009509 - 699009520	11	expansin B2
2A	699011498 - 699012040	542	undescribed protein
2A	699075504 - 699078664	3160	Disease resistance protein
2A	699415781 - 699416190	409	imidazoleglycerol-phosphate dehydratase
2A	699595310 - 699595832	522	GPI mannosyltransferase 2

2A	699602143 - 699603336	1193	GRAS family transcription factor
2A	699715438 - 699715701	263	Transposon protein, putative, CACTA, En/Spm sub-class
2A	699751034 - 699751112	78	UDP-glucose 4-epimerase 5
2A	699776957 - 699777533	576	BLT14.2 protein
2A	699780048 - 699780865	817	Cold-regulated protein 2

Red highlight – gene with the relation to the plant resistance

QTL detected on the chromosome 7A covered a 28.3 cM region. This region contains 14 markers - 1001748-43, 1027229-57, 1164940-59, 4395720-13, 1107335-63, 1216307-27, 1011397-23, 2282553-59, 1043460-61, 1129987-36, 3954691-31, 5325070-15, 1203942-15, 42780961-10 (Fig. 11). Markers 1011397-23, 2282553-59, 1043460-61, 1129987-36 were flanking the QTL peak and in cv. Zavitan genome (Avni *et al.,* 2007) they delimited syntenic region of about 2 Mbp. The region comprised 21 genes, from which eight genes had relation to the plant resistance response. (Table 8).

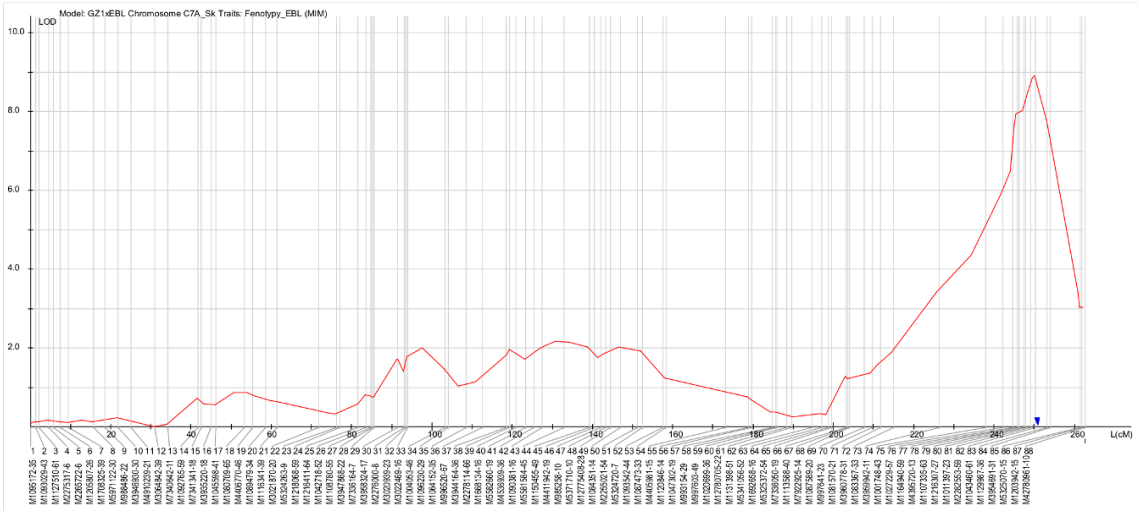

Figure 11. The *QPm.GZ1-7A* QTL associated with powdery mildew resistance on chromosome 7A. Marker positions are indicated in cM on the x-axis. LOD score is shown on y-axis. The QTL region was identified between 233 – 262 cM and the LOD maximum of 8.91 was reached at the position 248 cM (blue arrowhead).

Table 8. The *QPm.GZ1-7A* QTL syntenic region on cv. Zavitan chromosome 7A

Chromosome	Genome position	Length [bp]	Gene
7A	705143309 - 705143572	263	unknown function
7A	705150294 - 705150890	596	unknown function
7A	705222312 - 705222643	331	undescribed protein
7A	705295261 - 705295296	35	undescribed protein
7A	705414983 - 705416040	1057	Pseudouridine synthase family protein
7A	705511766 - 705511798	32	unknown function
7A	705591957 - 705592385	428	unknown function
7A	705644152 - 705644163	11	undescribed protein
7A	705647470 - 705647856	386	TTF-type zinc finger protein with HAT dimerisation domain
7A	705664664 - 705664948	284	Disease resistance protein (CC-NBS-LRR class) family
7A	705765822 - 705765833	11	unknown function
7A	705785417 - 705788311	2894	Powdery mildew resistance protein PM8
7A	705875557 - 705876315	758	undescribed protein
7A	705877842 - 705878618	776	Disease resistance protein (CC-NBS-LRR class) family
7A	705914261 - 705914659	398	Disease resistance protein RGA2
7A	705923382 - 705923438	56	Disease resistance protein
7A	705923592 - 705925000	1408	Disease resistance protein
7A	705952642 - 705953071	429	Pm3-like disease resistance protein
7A	705958597 - 705958813	216	undescribed protein
7A	705963761 - 705964381	620	Disease resistance-responsive (dirigent-like protein) family protein
7A	706674989 - 706675842	853	unknown function

Red highlight – genes with the relation to the plant resistance

Correlation between this two QTLs were examined by comparing the phenotype and genotype data obtained from DArTseq analysis at the mapped QTL region flanked by markers 1101086-26, 1053641-64 (*QPm.GZ1-2A*) and 1011397-23, 1129987-36 (*QPm.GZ1-7A*).

The homozygous constitution of GZ1 genotype on *QPm.GZ1-2A* was in all cases linked with total resistance to powdery mildew with no respect to the genotype constitution on *QPm.GZ1-7A* (Fig. 8B and C). The homozygous constitution of EBL genotype at both

QPm.GZ1-2A and *QPm.GZ1-7A* was linked with susceptibility to powdery mildew (Fig. 8A). *QPm.GZ1-2A* with homozygous EBL genotype and *QPm.GZ1-7A* with homozygous GZ1 genotype was linked with the formation of small colonies and, although not so frequently, with plants without visible colonies (Fig. 12A). Homozygous constitution of EBL on *QPm.GZ1-2A* with heterozygous constitution *QPm.GZ1-7A* resulted in segregation for large colonies, small colonies and also in plants with no visible colonies (Fig. 12B). Heterozygous constitution on *QPm.GZ1-2A* with homozygous constitution of GZ1 genotype on *QPm.GZ1-7A* resulted in resistant plants and plants with small colonies. Heterozygous constitution on *QPm.GZ1-2A* with the EBL genotype on *QPm.GZ1-7A* plants were resistant or full sensitive to the powdery mildew. If there was heterozygous constitution of genotypes at both identified QTL, all types of observable phenotypes were seen – large colonies, small colonies and resistant plants (Fig. 8B).

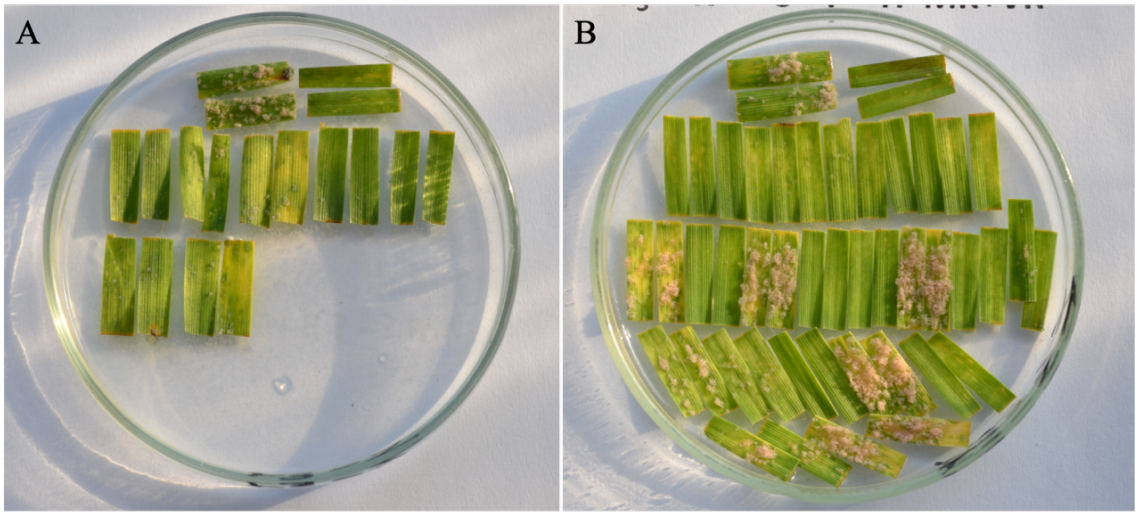

Figure 12. Phenotypic reaction of $F_{2:3}$ families to powdery mildew isolates A17, A23 and A3ab focusing on *QPm.GZ1-7A*. In each figure, first line (horizontal) represents two segments of sensitive EBL line and two segments of resistant GZ1 line. The remaining segments (vertical) represent tested families, always two primary leaf segments of different plant of the same family. The description below states genotype constitution at the mapped QTLs. **A – Phenotype reaction of the genotype 2 GZ1xEBL families with the homozygous constitution of EBL genotype at *QPm.GZ1-2A* and GZ1 genotype at *QPm.GZ1-7A*.** Majority of the tested plants were susceptible with small colonies except the plant 5 with no visible colonies. **B – Phenotype reaction of the genotype 47 GZ1xEBL families with the homozygous constitution of EBL genotype at *QPm.GZ1-2A* and heterozygous constitution at *QPm.GZ1-7A*.** Sensitive plants with the large colonies can be seen on the plants 7, 9, 12, 15, 18, 20 and 21. Plants with small colonies are plants 1, 2, 3, 4, 5, 8, 11, 14 and 16. Plants with no colonies are 6, 10, 13, 17 and 19.

6 DISCUSSION

Tetraploid wheat *Triticum turgidum* subsp. *dicoccum* GZ1 was studied for its broad-range resistance to *Blumeria graminis* (DC) E.O. Speer f. sp. *tritici*. For this purpose, F_2 mapping population was derived from a cross between the resistant GZ1 line and susceptible line EBL. The preliminary analysis of the F_1 hybrids indicated that the powdery mildew resistance is controlled by one recessive gene as indicated by the sensitive reaction of all individuals. Testing of the F_2 population showed segregation of the phenotype close to 3:1 with prevalence of sensitive plants. This confirmed that the resistance is controlled by gene in homozygote recessive stage. Since the resistance reaction was conducted on the primary leaf there is a little material for enough repetitions and the results from F_2 lines was inconclusive (data not shown). So, to increase reliability of phenotype analysis, 20 plants of the $F_{2:3}$ families were used to reconstruct and verify phenotypes of the F_2 lines. The $F_{2:3}$ phenotype analysis confirmed that the resistance is controlled by the recessive gene. For example, in $F_{2:3}$ family of heterozygous F_2 line 72 only 3 of 19 plants were resistant (Figure 8B). Up to date, more than 100 powdery mildew resistance genes/alleles at 63 loci (*Pm1-Pm66*) have been described in common wheat and its wild relatives (McIntosh *et al.,* 2017; Li *et al.,* 2020; Lu *et al.,* 2020). Most of these genes are dominant R-genes of large effect and only *Pm5*, *Pm9* and *Pm26* are recessive. (Hsam *et al.,* 2001; Schneider et al., 1991; Rong *et al.,* 2000) In cereals, the most famous recessive resistance gene is the *Mlo* from barley (Buschges *et al.,* 1997). The *Mlo* gene also confers race non-specific resistance in all developmental stages, similarly as for GZ1. *Mlo* resistance is characterized by the cell wall appositions and papillae formation at the encounter sites of the pathogen (Bayles *et al*., 1990). In some cases, local cell-wall apposition can be accompanied by necrotic leaf spots (Piffanelli *et al.,* 2002, Makepeace *et al.,* 2007). Although, the GZ1 resistance mechanism has not been studied here, no necrotic leaf spots were observed. Homozygote recessive character of GZ1 may indicate analogous gene to *mlo*. However, the *Mlo* gene was mapped on chromosome 4H (Simons *et al.,* 1997) which is not syntenic to *QPm.GZ1-2A* mapped on the 2A chromosome. The fact that no necrotic spots were observed and different location of genes may indicate difference from the *Mlo* gene.

In our case, susceptible plants showed strong infection, leaf segments were almost completely covered by powdery mildew. However, light infection symptoms were also observed in many cases as some leaf segments were spotted with small colonies, only (Fig.

8B, 12A and B). Phenotypic data of $F_{2:3}$ families also revealed distortion from Mendelian segregation as they result in 16 sensitive, 63 heterozygous and 34 resistant individuals. Calculated chi-square value 7.09 correspond the p-value 0.0289. Since the p-value is less than the 0.05, the difference between the observed and expected data are considered to be statistically significant and therefore the segregation of observed data does not fit with Mendelian ratios 1:2:1. The number of resistant plants is exceeding the number of sensitive plants for almost 18 individuals causing segregation shift towards the resistant one. This shift in segregation and two distinct phenotypic reactions indicated existence of polygenic character of the resistance. Most of resistance genes are usually mapped as "mendelian" traits which means they are coded by dominant R-genes of large effect (reviewed in Eli *et al.,* 2014). However, polygenic inheritance of resistance has been reported as well, but with much lower frequency (Jakobson *et al*., 2006). Since the R genes are usually responsible for race specific resistance a combination of multiple R-genes is widely used in breeding to prolongate durability of resistance, known as gene pyramiding (Laroche *et al.,* 2019). On the other hand, some genes confer race non-specific resistance, but frequently only partial. These genes also shows long term durability.

F_2 mapping population was genotyped using the DArTseq markers and only SNPs markers were used for the construction of genetic map. Two types of genetic linkage map were generated (global genetic linkage map and skeleton linkage map). However, skeleton markers are considered to be most informative and reliable compared to attached markers of global genetic linkage map. Therefore skeleton linkage map was used for the QTL analysis. The skeleton linkage map comprises of 862 SNPs markers and spanned a genetic length of 2865.04 cM with an average marker density 3.69 cM/marker. The length of genetic map constructed here is comparable with published durum genetic maps (Table 9; Colasuonno *et al.,* 2014; Prat *et al.,* 2016; Marone *et al.,* 2012; Alsaleh *et al.,* 2014).

Table 9. Comparison of developed linkage map with published durum maps

Type of the population	Map length [cM]	Number of markers	Marker density [cM/marker]	Marker types	Citation
RIL (Svevo × Ciccio)	1773	5670	0.3	SNPs, DArTs, SSR	Colasuonno *et al.,* 2014
RIL (DBC-480xDurobonus)	1781	1052	1.7	DArTseq, SSR	Prat *et al.,* 2016
RIL (DBC-480xSZD1029K)	2219	1006	2.2	DArTseq, SSR	Prat *et al.,* 2016
RIL (DBC-480xKarur)	2806	1609	1.7	DArTseq, SSR	Prat *et al.,* 2016
F2 (GZ1xEBL)	2865	862	3.6	DArTseq - SNPs	The present study
RIL (CresoxPedroso, OfantoxCappelli, Cirillo×Neodur, Ciccio×Svevo, Messapia×MG4343); F2-F3 (Latino×Primadur)	3058	1898	1.6	DArT, SSR, EST, RFLP, TRAP, biochemical and morphological loci	Marone *et al.,* 2012
RIL	4853	395	12.3	SSR, AFLPs, SPP	Alsaleh *et al.,* 2014

Yellow highlight – genetic linkage map from the present study

SSR – simple sequence repeats; EST – expressed sequence tags; RFLP – random fragment length polymorphism; TRAP – target region amplification polymorphism; AFLP – amplified fragment length polymorphism; DArT/DArTseq – Diversity Arrays Technology; SPP – seed storage protein; RIL – Recombinant inbred line

As was indicated from the phenotypes the QTL analysis confirmed polygenic determination of the trait and identified two loci contributing to the resistance. The powdery mildew resistance of GZ1 is controlled by two loci the *QPm.GZ1-2A* on chromosome 2A with LOD score 14.5 (Fig. 10) and the *QPm.GZ1-7A* with LOD score 8.9 on chromosome 7A (Fig. 11). QTLs are stated as significant when exceeded estimated LOD threshold of 3 (Lander and Botstein, 1989) and a p-value <0.05. The *QPm.GZ1-2A* QTL explained 30 % to total phenotypic variance. The *QPm.GZ1-7A* QTL explained 20% of total phenotypic variance. Even the *QPm.GZ1-2A* QTL explains only 30% of the total resistance variance the plants with locus in homozygote recessive constitution confers total resistance. This contradiction between the PEV and phenotype of the *QPm.GZ1-2A* may be affected by the contribution of the *QPm.GZ1-7A* QTL and effect of environment in the statistical analysis.

Also phenotypic manifestation of both loci seems to be different. In case of dominant powdery mildew loci at 2A and 7A chromosomes, symptoms are manifested as small colonies or no visible colonies (Fig. 12A and B). Heterozygosity in both loci results in all kind of symptoms, large and small colonies and also resistant plants (Fig 8B). Therefore, it is assumed that QTL associated with 7A chromosome is dominant and has additive effect. Verification of effect of each locus separately would need "mendelisation" of both QTLs and independent phenotypic characterization.

To identify candidate genes for the *QPm.GZ1-2A* and *QPm.GZ1-7A* loci the flanking markers were used to anchor the regions to cv. Zavitan reference genome sequence (Avni *et al*., 2018). The alignment revealed syntenic position of the *QPm.GZ1-2A* at long arm of the Zavitan chromosome 2A. Quite a few powdery mildew resistant genes have been identified on the 2AL chromosome. These include formally named *Pm4* gene (Briggle, 1966) and tentatively designated genes for powdery mildew such as *PmLK906* (Niu *et al*., 2008), *PmHNK54* (Xu *et al*. 2011), *PmPs5A* (Zhu *et al*., 2005) and *PmY66* (Hu *et al*., 2008). Moreover, *Pm4* consists of four resistance alleles *Pm4a*, *Pm4b*, *Pm4c* and *Pm4d* (The *et al*., 1979, Hao *et al*., 2008, Schmolke *et al*., 2010). Despite the knowledge of considerable number of genes/alleles only one of the named genes shows resistance in recessive stage. *PmLK906* recessive resistant gene was mapped in a common wheat line known as 'Lankao 90(6)', where the source of recessive resistance was hexaploid triticale 'Mzalenod Beer'. 'Lankao 90(6)' lines have maintained their resistance to powdery mildew for more than 15 years and two commercial high-yielding wheat cultivars, 'Yumai 66' and 'Lankaoaizao 8' were derived from 'Lankao 90(6)'. However, this gene was found coding race-specific resistance to powdery mildew (Niu *et al*., 2008), whereas GZ1 resistance was so far characterized as race non-specific resistance. The syntenic region in cv. Zavitan contains only single gene related to resistance similar to *RPP13* resistance gene coding protein that recognize effector proteins from the oomycete pathogens (Borhan *et al*., 2004; Allen *et al*., 2004). This supports it as candidate, but recessive manner of *QPm.GZ1-2A* is contradicting it. If this is the gene or if it is some other gene missing in the Zavitan genome is a question. Cloning of the *QPm.GZ1-2A* gene could solve the question.

Similarly the *QPm.GZ1-7A* QTL was found syntenic to the long arm of the chromosome 7A (7AL) of cv. Zavitan. *Pm1* (Hsam *et al*., 1998) and *Pm9* (Schneider *et al*., 1991) genes together with a temporarily designated *PmG16* (Ben-David *et al*., 2010), *MlIw72* (Ji *et al*., 2008), *Mlm2033* (Yao *et al*., 2007), *Mlm80* (Yao *et al*., 2007) and

mlRd30 (Singrun *et al*., 2004) were also identified on the 7AL chromosome. In the case of Pm1 locus, five different alleles *Pm1a, Pm1b, Pm1c (Pm18), Pm1d,* and *Pm1e (Pm22)* have been found (Sears and Briggle, 1969; Hsam *et al*., 1998; Singrun *et al*., 2002). Two of the above mentioned genes/alleles *Pm9* and *mlRd30* show recessive inheritance (mlRd30 might represent allelic variant of Pm9 gene, when allelism test for *Pm9* have not been carried out; Shingrun *et al*., 2002). The rest of the mentioned genes/alleles show dominant inheritance, which is also the case of the *QPm.GZ1-7A* QTL. Additionally, the syntenic region in Zavitan genome comprise cluster of eight resistance related genes including two *NBS-LRR* related genes and *Pm8* and *Pm3-lik*e genes. This suggests high chance of *QPm.GZ1-7A* gene being a classical R gene.

The resistance of GZ1 may be conferred by the same loci/alleles mentioned above for both 2AL QTL and 7AL QTL or by different loci of the powdery mildew resistance. For this purpose, additional studies are necessary to conduct to differentiate between already designated powdery mildew resistance genes and GZ1 resistance. Even genes conferring the GZ1 resistance are unknown, GZ1 already represents a unique source of broad-spectrum resistance and the QTLs flanking markers could be used for utilization of the resistance in breeding programs. Additionally, the interesting genetics of the *QPm.GZ1-2A* locus makes gene underlining the resistance an attractive target for gene cloning and additional scientific study.

7 CONCLUSION

The aim of this diploma thesis was genetic mapping of newly discovered resistance gene providing resistance to powdery mildew at non-specific level identified in GZ1 line. F_2 mapping population was developed from a cross between the resistant GZ1 line and susceptible EBL line and genotyped with DArTseq markers. Codominant DArTseq markers were used for the construction of high density linkage map. $F_{2:3}$ families F_2 lines were phenotype in order to analyse response to powdery mildew. Phenotypic data together with genetic linkage map were used for QTL analysis, which revealed two QTLs affecting the resistance on 2AL and 7AL chromosome. These QTLs were temporarily designated as *QPm.GZ1-2A* and *QPm.GZ1-7A*, respectively. The homozygote recessive *QPm.GZ1-2A* results in total resistance to powdery mildew and overlaps the effect of dominant *QPm.GZ1-7A*, which manifests with partial resistance. Both loci were anchored to the cv. Zavitan reference genome sequence and revealed candidate genes. Of these, one has relation to plant resistance response in syntenic region to *QPm.GZ1-2A* and eight in syntenic region to *QPm.GZ1-7A.* Further studies will be necessary to verify the identified QTLs and characterized them.

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