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Proceeding Paper

Barley sources of resistance to the net form of net blotch (*Pyrenophora teres* f. *teres*) †

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Abstract: Barley is one of the economically important crop species and net form of net blotch (NFNB)caused by *Pyrenophora teres* f. *teres* has a significant impact on the quantity and quality of grain yield. Selection and inbreeding have resulted in a lack of genetic diversity in elite barley accessions. Old varieties often possess unique genetic traits that may have been lost in modern crop breeding. Therefore the aim of the current study was to identify sources of resistance to barley NFNB in the collection of European old varieties. In this study, 431 European barley accessions were evaluated phenotypically under field conditions scoring APR to NFNB and genotypically using DArTseq. The range of adult plant resistance (APR) variability at the HA growth stage was sufficient to determine marker-trait associations (MTAs). Net form of net blotch at the HA stage was scored with a range of 1.0 – 4.0 according to a 1 – 9 scale and GWAS identified 10 marker-trait associations (MTAs) for NFNB resistance. In the HA stage, two MTAs were identified on each chromosome 1H, 3H, 5H and 6H. Moreover, one on the chromosome 7H and un. One of these MTA is localized on chromosome 6H, corresponding with findings from other studies, and could contribute to the exploration of genetic resistance of barley to NFNB. Additionally, the results of this study will be utilized to establish a Polish Gene Bank platform for precise breeding programs.

Keywords: adult plant resistance; barley; DArTseq; *Hordeum vulgare*; net form of net blotch; *Pyrenophora teres* f. *teres*

1. Introduction

Barley is important in agriculture for several reasons, and its significance extends to both human and animal consumption as well as its role in sustainable farming systems. **It** is used in various food products and it is an essential component of animal feed. Moreover, barley is a primary ingredient in the production of malt, a crucial component in the brewing industry. Due to climate change, it is important that barley is often used as a cover crop or as part of crop rotation systems. Because it has relatively low water and fertilizer requirements compared to some other cereal crops, making it a more sustainable option in regions with limited resources or concerns about environmental impact [1]. It is ranked fourth in terms of the most cultivated crop (by area) in the world, following wheat, maize, and rice. Almost half of the world's barley-growing area is in Europe, including Poland, where it is the second most cultivated crop after wheat.

Net form of net blotch is a common foliar disease that affects barley, a cereal grain crop. It is caused by fungal pathogens belonging to the *Pyrenophora* genus, specifically *Pyrenophora teres*. *Pyrenophora teres* is further classified into two forms: *P. teres* f. *teres* (Drechsler) (*Ptt*) and *P. teres* f. *maculata* (Smedegaard-Petersen) (*Ptm*). Both forms of net blotch can weaken the barley plant, reduce photosynthesis, and ultimately lead to decreased grain yield and quality if left untreated. *Ptt* causes net form net blotch (NFNB) whereas *Ptm* causes spot form net blotch (SFNB). In the case of NFNB, net-like patterns

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typically appears as elongated, rectangular lesions with a net-like appearance on the leaves of barley plants. These lesions can be brown to grayish-green in color and can coalesce as the disease progresses. It primarily affects the lower leaves of the plant. The symptoms of SFNB appears as small, round to irregularly shaped lesions on the leaves of barley. Unlike the net-like lesions of the more common form, this type of NFNB creates discrete, darker spots with a defined margin [2,3].

The potential yield loss due to diseases is influenced by multiple factors. Maximized yield losses are often observed when these diseases manifest themselves before the heading stage of plant development. This critical period serves as a crucial juncture for the timely identification of early-activated pathogen-associated molecular patterns. These patterns hold the potential to elicit non-specific defense cascades within the plant, thereby highlighting the significance of early detection during this developmental phase.

Conversely, resistance mechanisms that come into play during late growth stages, such as the milky-waxy stage, become essential for identifying key resistance proteins. To summarize, effective disease management during the growth period relies heavily on timely detection and precise identification of pathogens. Proactive measures at this stage significantly contribute to successful disease control strategies. Moreover, this data can guide the selection of future varieties and the integration of these genes into breeding programs. [4].

Modern barley breeding programs often prioritize the development of varieties with improved disease resistance, including resistance to NFNB . The Polish Gene Bank, in conjunction with other gene banks, plays a two-fold role: preserving plant genetic resources and acting as a reservoir of new genetic variations. As a result, they serve as invaluable reservoirs of genetic material crucial for essential traits in breeding programs [5]. Without meticulously curated genetic collections, the potential of the genetic material stored in gene banks remains untapped [6]. Therefore, the long-term strategies of conventional gene banks should shift towards becoming comprehensive biological resource centers, offering access to the wealth of metadata associated with their holdings [7-9]. Old varieties often possess unique genetic traits and the process of incorporating new alleles into elite cultivars is more straightforward and efficient when sourcing from old cultivars and landraces, as opposed to wild relatives [10]. Given these factors, it is imperative to extensively explore old barley cultivars and landraces gathered from European countries in the quest for novel genes [11-14].

Recent progress in next-generation sequencing has empowered plant scientists to produce numerous single nucleotide polymorphism (SNP) markers and create precise genetic maps. The availability of cost-effective sequencing platforms has allowed researchers to conduct genome-wide association studies, enhancing their ability to map quantitative trait loci (QTL) related to agronomic traits and disease resistance.

Multiple studies mapping sources of NFNB resistance have been conducted in barley using linkage mapping [15-20] and association mapping approaches [20-25]. Numerous quantitative trait loci (QTL) have been documented to contribute to the resistance against the net form of net blotch, demonstrating minor effects during both the seedling and adult plant stages. These QTLs have been precisely identified and positioned on chromosomes 1H, 3H, 4H, 5H, and 7H. Additionally, various previous studies have specifically documented QTL on chromosome 6H. Despite its importance, the broad expanse of the identified genomic region makes it less favorable for direct integration into breeding programs targeting NFNB.

The objective of this study was to establish associations between genetic loci and adult plant resistance (APR) against the net form of net blotch (NFNB) at both the heading and seed's milky-waxy plant development stages. To accomplish this, we conducted a GWAS analysis using DArTseq-derived markers and phenotypic data for 431 barley accessions segregating for these specific disease resistance traits.

2. Materials and Methods

The plant material used in the preset study was characterized in terms of adult powdery mildew and rusts resistance and agronomic traits under field conditions 2018 – 2019 in a was described by authors in the previous study [8,9].

2.1. Plant material

In summary, a collection of 431 barley accessions, including landraces and old cultivars, stored at the Polish Gene Bank (National Centre for Plant Genetic Resources: NCPGR) were phenotyped and evaluated using DArTseq: 137 POL, 67 DEU, 38 SWE, 35 CSK, 34 FRA, 27 GBR, 25 DNK, 21 NLD, 12 AUT, 8 SUN, 6 NOR, 4 FIN, 3 IRL, 3 CAN, 2 USA, 2 HUN and 1 each from UKR, TUR, PRK, NZL, JPN, BEL and one of unknown origin [9].

2.2. Field experiment and phenotypic evaluation

In 2019, field experiments were meticulously carried out at the Plant Breeding and Acclimatization Institute-National Research Institute (PBAI-NRI) in Radzikow, situated near Warsaw, Poland. Notably, no specific permissions were deemed necessary. The experimental setup comprised two replications, each consisting of two rows with a row length of 2.0 meters. The planting configuration included a plant spacing of 4.0 centimeters and a row spacing of 20.0 centimeters [8,9]. .

Net form of net blotch (NFNB) was scored according to IPGRI descriptors (https://cropgenebank.sgrp.cgiar.org/index.php/learning-space-mainmenu-454/manuals-and-handbooks-mainmenu-533/descriptors-mainmenu-547 (accessed on 29 October 2021), using a 1–9 scale, where 1 means no symptoms of the disease (immune reaction). Measurements were undertaken during the heading stage (HA), precisely when half of the heads had emerged for 50% of the plants within a plot (identified as Z55 on the Zadoks growth scale). Subsequently, the assessment was repeated two weeks later, precisely during the early milky-waxy seed maturity stage (MW; designated as Z75 on the Zadoks growth scale).

2.3. Statistical analysis

Due to the limited number of seeds accessible for each accession in the NCPGR, the experiment could only be conducted in a single environment and during one specific year. To ensure comprehensive and reliable results, it is advisable to replicate the experiment and perform phenotypic assessments across multiple environments Basic analysis of phenotypic data was performed with the Statistica software and Excel 2019. It was used to obtain the range, mean, standard deviation (SD), coefficient of variation (CV) and frequency distribution of barley accessions for NFNB resistance. .

2.4. Genotyping and Data Filtering Process

Genotyped by Diversity Arrays Technology (DArT) were 454 accession (431 evaluated under field conditions and 25 additional, used as a controls). SNP calls were made against IBSC Barley Morex v2 assembly [26]. The Barley GBS 1.0 platform DArT genotyping service returned 28,530 in-silico DArT-seq markers. DArT data was handled in the same manner as described previously [27,28]. That is, we used the dartR v1.1.11 package [29] in the R programming language. SNPs and genotypes were removed if SNP markers contained >5% missing data and genotypes contained >10% missing data, respectively. SNPs with a reproducibility score (RepAvg) <100% were removed. Non-informative monomorphic SNPs were removed, so too were rare SNPs with a minor allele frequency of <1%. After filtering, 453 (1 individual was removed due to having >10% missing calls) and 10,153 SNP's were retained for further analysis.

2.5. Genome-wide association studies (GWAS)

The GWAS analysis followed the methodology described by authors [8,9,14,27,28]. We utilized the GAPIT v2018.08.18 R package for the analysis. Our study employed the

recently developed Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) model, known for minimizing false positives, enhancing true positive identification, and its capacity to handle large datasets [28]. Markers' physical genome positions were obtained from the DArTseq SNP genotype file. Considering that GAPIT requires complete data, only markers with a physical position on one of the chromosomes and zero missing data were included in the GWAS analysis. GWAS for NFNB was performed to assess disease resistance scoring at the heading and milky-waxy seed stages. Additionally, Manhattan plots were generated to visualize the distribution of SNPs across the chromosomes.

3. Results and Discussion

Collection of 431 accessions was evaluated under field conditions for net form of net blotch (NFNB) resistance at heading (HA) and milky-waxy (MW) stages. Range of adult plant resistance (APR) variability at plants HA and MW growth stages were sufficient to determine marker-trait associations (MTAs). NFNB at HA stage was scored with a range of 1.0 – 4.0 according to 1 – 9 scale with standard deviation (SD) 0.54 and coefficient variation 0.29% . Phenotypic variation of disease severity at HA stage are presented on the Figure 1.

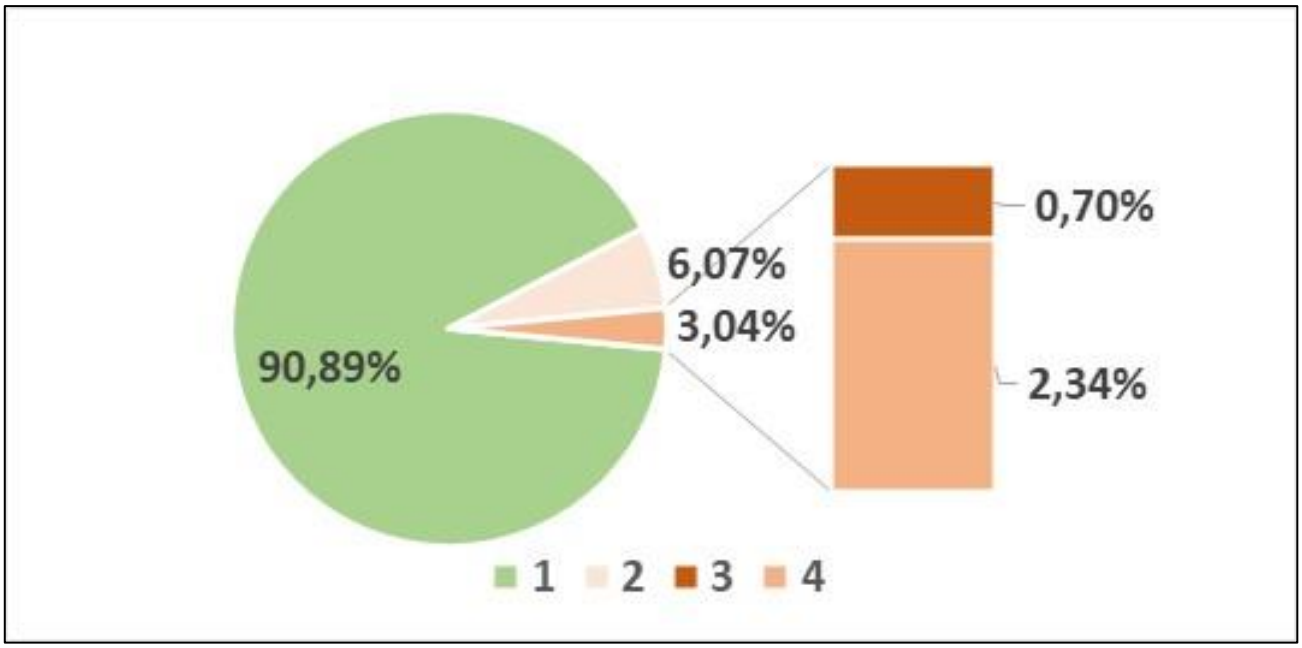

Figure 1. The frequency distribution histogram presents the percentage of old varieties scored for net form of net blotch (NFNB) at heading stage (HA) using 1 – 9 scale (1 = no symptoms of the disease).

Based on the number in Polish Gene bank database accessions with resistance for NFNB scored for 4.0 originated from DEU/DNK (43672, 19I00614, 43727, 43727), POL (19I00609, 42388, 19I00601, 43319), FRA (43760), GBR (43689), IRL (40388), AUT (43778), SUN (43778), TUR (43779) (https://wyszukiwarka.ihar.edu.pl/en). At MW stage the severity of the disease was lower than at HA stage and only few genotypes have symptoms of the disease scored for 3.

At HA stage, GWAS identified 10 marker-trait associations (MTAs) for NFNB resistance (Table 1, Figure 3). Specifically, two MTAs were identified on each chromosomes 1H, 3H, 5H, 6H (Table 1 and Figure 2). Moreover, one on the chromosome 7H and un.

Table 1. Significant marker-trait associations (MTAs) associated with net form of net blotch (NFNB) at heading (HA) stage.

Chromo-some	SNP Position	SNP ID	Adj p-value	Ref	Alt	Flanking Sequence
1H	555850679	3665728-48-G/C	0,0039	G	C	TGCAGCTCCAGCAAACGCAGGCGCCGCTCATGCCAAGCAGGTTCATGT[G>C]CTCCCGCCAGCTCTCCGAGA
1H	525028066	4185419-21-T/G	0,0039	T	G	TGCAGCACTGGCCGTGCTAGT[T>G]CATAGTACACACACCACAACAAGCGCTGCTGCGGGGGGGAGCACCAC
3H	10806146	3922813-26-C/T	0,0000	C	T	TGCAGAGCTTGCAGGTAGCAGCGCAG[C>T]TGTCGACCACGTCCTCGCAGGTGCACGCCG
3H	644804966	3666438-26-G/A	0,0039	G	A	TGCAGGTGCAGCCACGAGAGCTGGCC[G>A]CTAACGAGGAGTCGTCGTTCAGGCCCGTGCCTCCG
5H	503899956	3665444-43-T/G	0,0039	T	G	TGCAGCTGTTTGCCAAGTTGGACGGAGATGTCCCTGTTTTAGT[T>G]GTGTCGACGCTGAATTCCCCCAAGA
5H	17105715	3255282-16-G/A	0,0109	G	A	TGCAGGGAGATTACTG[G>A]TTTCAGTCTGCCTTAAATCCAGAGTCTCCAGATAGCGCAATCCCCTGATTTC
6H	388486233	3432352-13-G/T	0,0000	G	T	TGCAGCATTCCTT[G>T]TACTGATACAGTGATGACATGACGGTTGGGCCG
6H	210765795	4414028-24-G/C	0,0039	G	C	TGCAGCTTGAGCTCGTTGTCCATG[G>C]CCTTGAATGCATTGGTGCAGGCCTCCGTCCACTCCTCCAGCATC
7H	207567692	7223598-17-C/G	0,0000	C	G	TGCAGCTCTTCATCTGG[C>G]AGACGTAGCTGCGCCG
Un	103707884	6272935-22-T/C	0,0041	T	C	TGCAGTAGTTTCTTCTCTCTTT[T>C]TAGTGTATTTTCTACTGCTAGAACCG

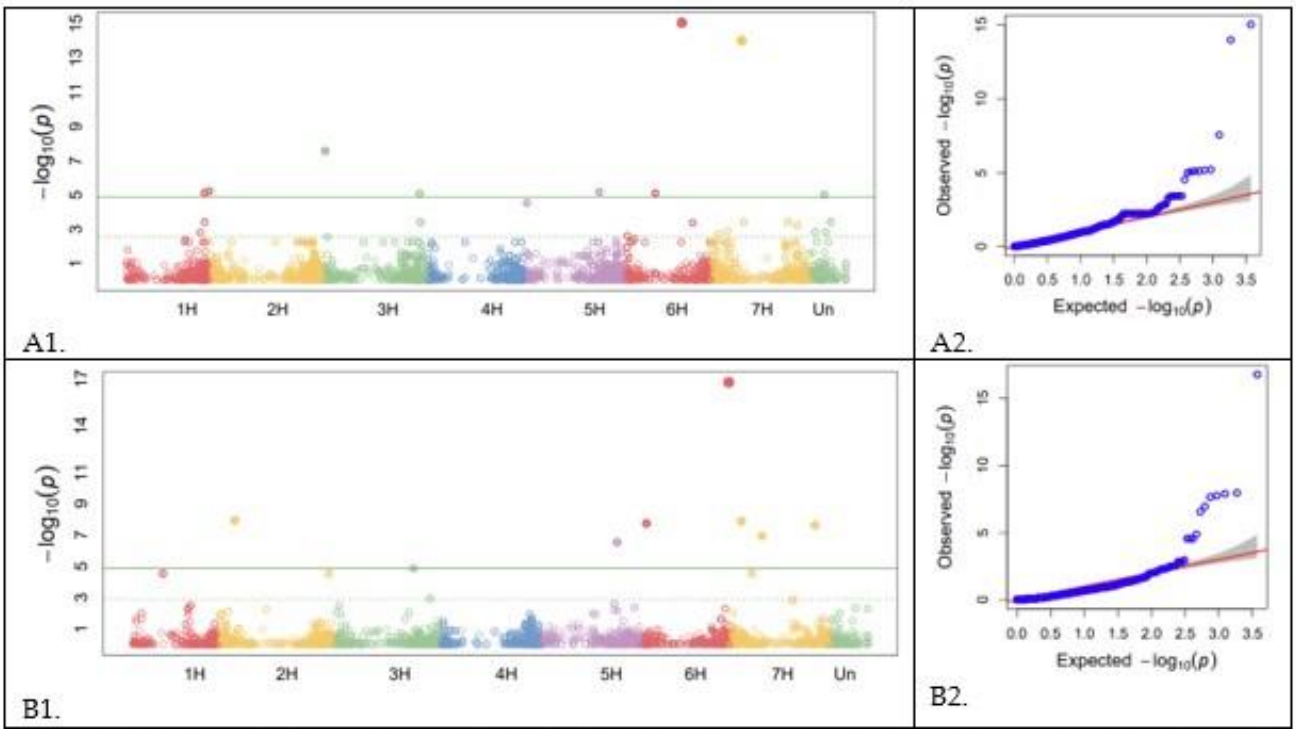

Figure 2. Single nucleotide polymorphism (SNP) significantly associated with net form of net blotch (NFNB) resistance in barley identified by genome-wide association study (GWAS) with BLINK model. Manhattan plot and Quantile-quantile plot for heading stage (A1 and A2); Manhattan plot and Quantile-quantile plot for milky-waxy stage (B1 and B2).

It is important that two MTAs identify at the HA stage were situated on chromosome 6H closely located each other 3432352-13-G/T and 4414028-24-G/C. At the MW stage, one, two, one, and three significant trait-associated markers (MTAs) were identified on chromosomes 1H, 2H, 5H, and 7H, respectively (Table 2, Figure 2). Since the severity of the disease was low at the MW stage, specific MTAs can only provide limited support for the interpretation of barley genetic resistance determination for NFNB.

Table 2. Significant marker-trait associations (MTAs) associated with net form of net blotch (NFNB) at milky-waxy (MW) stage.

Chromosome	SNP Position	SNP ID	Adj p-value	Ref	Alt	Flanking Sequence
1H	198280215	6272702-14-A/C	0,0096	A	C	TGCAGCGGGGCGCT[**A>C**]GCAGCTGTTTCATGGGCCG
2H	112361626	4184432-64-C/A	0,0000	C	A	TGCAGAGGTTGCGGGTAGGTCATCAGTACCTTGGCCTTGGGGATGACCTTGACGGCGACGGCGT[**C>A**]CCCC
2H	729416254	6281659-7-T/G	0,0096	T	G	TGCAGGG[**T>G**]CGTACACAGCCAGCGTCCGCTTGGAGCCTGCGTGGCGCGGCGCCGTCGTAGGAAGGATGGT
5H	511856641	3665720-51-G/A	0,0001	G	A	TGCAGGCCAAGAAACGGCGAATTCAGTCCCCTGCCGGCGCCGCCGAAGAGG[**G>A**]AAAACAGAGCACTGCAC
6H	574992201	7243457-40-T/C	0,0000	T	C	TGCAGGTCGGCGAGCAGGGCCTGGACCGCGGCGGCGTCGG[**T>C**]GGCCCG
7H	6256505	3913074-18-G/C	0,0000	G	C	TGCAGAATCAGAGAATTT[**G>C**]ATGGGAGCAAAGCAAACCG
7H	1438903138	3919123-42-C/G	0,0096	G	C	TGCAGGAGTCAGCATACGTGATGGCTGCATACTATCGCTTCG[**C>C**]TCTCGTCATCGGTCTTTAGGGGGAAA

This results correspond to the previous study, and confirm that chromosome 6H has been pinpointed as a hotspot for *Ptt* resistance loci in numerous QTL mapping studies [2,7,15-17,19,30,31]. Most of these investigations have highlighted an extensive section of chromosome 6H associated with resistance to NFNB. Unlike this particular study, many of these research efforts have employed smaller mapping populations [30]. It's worth noting that smaller mapping populations lack the statistical robustness needed to precisely define QTL boundaries with high confidence. Therefore, the regions ((,2.00cM, 22 Mb)) on chromosome 6H and ((,1.00 cM, 0.11 Mb)) on chromosome 3H are considered high-confidence regions of interest for further investigations, including validation, fine mapping, and eventual cloning [23,25]. A BLAST search unveiled multiple predicted genes in these regions, some of which have well-established annotations related to disease resistance functions. Notably, the region on chromosome 3H contains two predicted genes from the NBS-LRR gene family.

Authors describes, that based on conducted study it worth mentioning that the region on 3H seems to be particularly relevant to 6-row germplasm, as it was identified in the 6-row panel, while the region on 6H appears to be more specialized for 2-row germplasm. Importantly, these markers can be employed in marker-assisted selection processes without the risk of significant linkage drag, as they target relatively small regions. The initial step in this direction would involve validating these markers in other populations. Plant material used in recent study was used to determine resistance to powdery mildew, barley brown rust and stem rust as the same at stages HA and MW.

4. Conclusions

Gene banks play a dual role, actively facilitating the preservation of plant genetic resources while simultaneously serving as invaluable repositories for accessing new genetic alleles This study enhances our understanding of the genomic regions linked to barley's APR (Adult Plant Resistance) against NFNB (net form of net blotch). It reaffirms the efficiency of GWAS (Genome-Wide Association Studies) with DArT (Diversity Arrays Technology) data in identifying markers associated with these traits. This opens up the

possibility of establishing a gene bank platform that includes comprehensive trait descriptions, making it well-suited for utilization in breeding programs and research. Furthermore, the study confirms the presence of closely related markers on chromosomes 1H and 6H at heading stage and at heading stage additional markers on chromosomes 1H and 7H. Lastly, the inclusion of evaluated landraces and old cultivars brings added value, as these resources can play a pivotal role in preserving agrobiodiversity through a range of diverse strategies.

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