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## Diploid *Triticum* species as a potential source of resistance to powdery mildew

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Diploidalne gatunki z rodzaju *Triticum* jako potencjalne źródło odporności  
na mączniaka prawdziwego

**Summary.** Powdery mildew is one of the most important fungal diseases of cereal plants. It reduces the yield and the quality of harvested grain. One of the best ways to protect crops against powdery mildew is to introduce effective resistance genes into new cultivars. In this study, diploid species of the genus *Triticum* were screened to identify potential sources of genetic resistance to powdery mildew. Among the analyzed forms, 21 genotypes were identified as fully resistant to all three powdery mildew isolates used. Four of them belong to *T. urartu*, 12 to *T. monococcum*, and 4 to *T. boeoticum*. The tests performed showed that each of the tested species can be a source of genes determining resistance to powdery mildew. The most resistant genotypes were identified among accessions from Lebanon and Syria. Genotypes from Türkiye showed the lowest level of resistance, which indicates that this area is not a region with high pathogen pressure.

**Key words:** resistance, powdery mildew, *Triticum monococcum*, *Triticum urartu*, *Triticum boeoticum*

### INTRODUCTION

Powdery mildew, induced by *Blumeria graminis* f. sp. *tritici* (Bgt) infection, is one of the most dangerous wheat diseases, and it has become a significant threat to wheat production throughout the world [Yue et al. 2023]. Appearance of this pathogen cause up to 40% grain loss, or even worse during severe epidemics [Wang et al. 2021]. Although chemical and agricultural treatments are the most popular methods of disease control, the best way

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to protect wheat from powdery mildew infection is to introduce genetic resistance into the host plants [Yue et al. 2023, Zhang et al. 2023]. Up to now, 68 formally designated powdery mildew resistance genes at 63 loci (*Pm1–Pm68*, *Pm8=Pm17*, *Pm18=Pm1c*, *Pm22=Pm1e*, *Pm23=Pm4c*, *Pm31=Pm21*) have been reported [McIntosh et al. 2020, He et al. 2021]. Most of them are race-specific, which causes the pathogen to break down the immunity very quickly due to its rapid evolution [Singh et al. 2018]. Recent studies indicate that *Pm2*, *Pm3a*, *Pm3b*, *Pm3f*, *Pm4a*, *Pm6*, *Pm8*, and *Pm17* have been overcome in the USA, while *Pm1a*, *Pm3a*, and *Pm8* have been overcome in Australia, China, and Egypt [Parks et al. 2008, Cowger et al. 2018]. Even the most effective *Pm21* gene, derived from *Dasypyrum villosum*, is also undergoing strong selection pressure and the risk of losing its resistance [He et al. 2020].

*Pm* genes can be divided into two categories. The first one is all-stage resistance (ASR) genes, and the second one is adult plant resistance (APR) genes. Most of the genes are ASR genes except for *Pm38*, *Pm39*, *Pm46*, and *Pm62*, *Pm6*, which show resistance only since the fourth leaf stage.

One of the effective ways to introduce plant resistance to powdery mildew is to introduce resistance based on the *Mlo* gene. The *Mlo* gene was first used in barley but is also found in other species. The protein encoded by this gene attenuates general defense responses in the epidermal cells of the host plant. Its proper functioning allows the pathogen to penetrate cells. Mutations in the gene result in an unstable or non-functional protein that at the macroscopic level is reflected in powdery mildew resistance [Zhang et al. 2023].

Regardless of the nature of the resistance used, it is necessary to constantly monitor the effectiveness of genes that protect against pathogen attacks and to introduce new, effective sources into crops. Pathogen populations are subject to constant changes; therefore, it is necessary to search for new sources of resistance that can be used in breeding programs [Adhikari et al. 2011, Olivera et al. 2018, Shi et al. 2020, Hinterberger et al. 2022]. The presented work aimed to determine the potential of using diploid species of the genus *Triticum* as a source of resistance to powdery mildew.

#### MATERIALS AND METHODS

The objects of this study were 29 accessions belonging to three different diploid species of the genus *Triticum*. *T. urartu* was represented by 19 accessions (Tab. 1), *T. monococcum* subsp. *monococcum* by 14 accessions (Tab. 2) and *T. boeoticum* by 6 (Tab. 3). Tested genotypes originated from different parts of the world. All genotypes were received from the National Small Grains Collection (USDA ARS GRIN).

The resistance of the analyzed genotypes was determined by host-pathogen tests [Hsam et al. 1997] based on three *B. graminis* f. sp. *tritici* isolates collected from different wheat fields in Poland. As a susceptible control, three wheat cultivars were used: Błyskawica, Syntia, and Diplom. These cultivars were susceptible to powdery mildew in field experiments in our previous study (unpublished). Kernels of the tested genotypes were seeded into plug trays filled with universal substrate and germinated. After ten days leaf fragments of analyzed genotypes were placed on 4-well culture plates with benzimidazole agar (6 g of agar per 1 l of water and 35 mg · l<sup>-1</sup> of benzimidazole). The control susceptible genotypes were put into every dish. Plates with leaf segments were inoculated in an

Table 1. Genotypes belonging to *Triticum urartu* and their resistance to wheat powdery mildew

No.	Country of origin	Gene Bank No.	Degree of plant infection on the Mains scale			Plant response classification		
			isolate 1	isolate 2	isolate 3	isolate 1	isolate 2	isolate 3
1	Iran	PI 428316	3	4	1	S	S	R
2	Syria	PI 662278	0	0	1	R	R	R
3	Lebanon	PI 662261	4	4	2	S	S	I
4	Iran	PI 662238	3	3	1	S	S	R
5	Syria	PI 487271	4	3	3	S	S	S
6	Türkiye	PI 428231	4	3	2	S	S	I
7	Jordan	PI 662264	4	3	3	S	S	S
8	Lebanon	PI 428327	0	1	0	R	R	R
9	Syria	PI 662281	4	4	3	S	S	S
10	Jordan	PI 662265	3	0	0	S	R	R
11	Lebanon	PI 428336	1	1	2	R	R	I
12	Lebanon	PI 428323	0	0	0	R	R	R
13	Syria	PI 662282	1	1	1	R	R	R
14	Türkiye	PI 428256	2	1	2	I	R	I
15	Iran	PI 662263	4	2	4	S	I	S
16	Iraq	PI 662262	3	2	2	S	I	I
17	Armenia	PI 428258	4	4	4	S	S	S
18	Türkiye	PI 428230	3	3	4	S	S	S
19	Syria	PI 487268	2	3	2	I	S	I

inoculation tower by placing about 500–700 *B. graminis* f. sp. *tritici* spores per 1 cm<sup>2</sup>. Then the dishes were incubated in a growing chamber at about 17°C and illuminance of approximately 4 kLx.

Infection on the leaves was rated ten days after inoculation according to a 0–4 modified scale [Mains 1934]. Reactions to the isolates were grouped into three classes: R (resistant) – from 0 to 20% leaf area affected relative to susceptible cultivars, I (intermediate)

Table 2. Genotypes belonging to *Triticum monococcum* and their resistance to wheat powdery mildew

No.	Country of origin	Gene Bank No.	Degree of plant infection on the Mains scale			Plant response classification		
			isolate 1	isolate 2	isolate 3	isolate 1	isolate 2	isolate 3
1	Austria	PI 355547	0	0	0	R	R	R
2	Austria	PI 355546	1	0	0	R	R	R
3	Türkiye	PI 341413	0	1	1	R	R	R
4	Germany	PI 277138	0	0	0	R	R	R
5	Germany	PI 352475	0	0	0	R	R	R
6	Türkiye	PI 560728	1	3	1	R	S	R
7	Germany	PI 355543	0	0	0	R	R	R
8	Austria	PI 355548	0	0	0	R	R	R
9	Albania	PI 277130	1	1	0	R	R	R
10	Germany	PI 355524	0	1	0	R	R	R
11	Türkiye	PI 573524	1	0	1	R	R	R
12	Türkiye	PI 554596	0	1	1	R	R	R
13	Switzerland	PI 355550	0	0	0	R	R	R
14	Balkans	PI 355540	0	0	1	R	R	R

Table 3. Genotypes belonging to *Triticum boeoticum* and their resistance to wheat powdery mildew

No.	Country of origin	Gene Bank No.	Degree of plant infection on the Mains scale			Plant response classification		
			isolate 1	isolate 2	isolate 3	isolate 1	isolate 2	isolate 3
1	Türkiye	PI 427984	4	4	4	S	S	S
2	Türkiye	PI 427491	1	0	1	R	R	R
3	Türkiye	PI 538723	2	2	1	I	I	R
4	Türkiye	PI 427621	1	0	0	R	R	R
5	Türkiye	PI 427620	1	1	1	R	R	R
6	Iraq	PI 427741	0	0	0	R	R	R

– from 20 to 50% affected, and S (susceptible) – greater than 50% affected. The result of infection scored as 0 or 1 point classified the genotypes as resistant (R). The intermediate (I) reaction consists of genotypes infection which was assessed as 2. If the infection was

determined as the 3 or 4 points, the genotype was qualified as susceptible. To confirm the response of the tested accessions to the used *B. graminis* f. sp. *tritici* isolates, all tests were performed in three replications.

## RESULTS

Among the 19 genotypes belonging to *T. urartu*, 5 were completely resistant to the pathogen isolates used in the tests. The response of one genotype was marked as 0 for all isolates, the remaining ones were marked as 0 or 1 and the response was classified as resistant. These genotypes originated from Syria and Lebanon. Two further genotypes (originating from Lebanon and Türkiye) showed a resistant or moderate response to the isolates used. The remaining *T. urartu* genotypes showed a moderate or sensitive response to *B. graminis* f. sp. *tritici*.

Genotypes belonging to *T. monococcum* subsp. *monococcum* was characterized by a high level of resistance to powdery mildew. Among the 14 tested genotypes, 13 were completely resistant to the isolates used. One genotype was resistant to 2 isolates, and the third isolate was susceptible. Among the tested genotypes of this species, no ones were identified that showed a susceptible response to all isolates. Resistant genotypes originated from different parts of the world.

*T. boeoticum* genotypes were also characterized by high resistance to *B. graminis* f. sp. *tritici*. Among the 6 tested genotypes, 4 were completely resistant to the pathogen isolates used in the tests. One from Iraq scores 0 for all isolates. The rest of the genotypes came from Türkiye. Three were classified as resistant but scored 1 or 0 in host-pathogen tests. One genotype was resistant to one isolate and showed an intermediate response to the other 2. One genotype was susceptible to all pathogen isolates.

## DISCUSSION

Crop wild relatives are species that carry many beneficial traits for plant breeding. They are especially valuable because they may have traits that were lost during domestication and breeding selection, as well as novel adaptive alleles that can enhance crop diversity and productivity [Dempewolf et al. 2017]. Wild *Triticum* species are increasingly being used in wheat breeding programs around the world as donors of genes that confer resistance to biotic and abiotic stresses, as well as genes that contribute to the improvement of grain quality [Colmer et al. 2006, Nevo and Chen 2010, Bakala et al. 2021, Pour-Aboughadareh et al. 2021].

The choice of wild species for identifying valuable genes should be based on their potential to transfer genes to cultivated forms through crossbreeding. Based on the emergence of crossbreeding barriers and the possibility of gene transfer, wild species related to cultivated wheat have been categorized into four gene pools [Harlan and de Wet 1971]. *T. boeoticum*, *T. monococcum* subsp. *monococcum* and *T. urartu* (the A-genome donor for hexaploid wheat) have been identified as the primary gene pool ancestors of cultivated wheat species. Because of their homology with the A subgenome of cultivated wheat, genes can be transferred through direct hybridization and gene introgression

[Feldman 2015, Gao et al. 2017]. These species were selected for our study to determine which diploid species have a higher potential for increasing powdery mildew resistance. *T. monococcum* exhibits high genetic variability, making it a significant gene pool for other species. It has been reported as a source of valuable genes, such as disease resistance genes, including leaf rust resistance genes [Badaeva et al. 2015]. *T. boeoticum* (syn. *T. monococcum* subsp. *aegilopoides*) has been reported as drought stress resistant [Ahmadi et al. 2018], as a donor of resistance to biotic stresses like stripe rust [Chhuneja et al. 2008]. *Triticum urartu* is a non-domesticated species and serves as the A genome donor of wheat. This makes it a valuable genetic resource for improving wheat cultivars, as stated in studies by Dvorak et al. [1993], Wang et al. [2017], and Brunazzi et al. [2018]. *T. urartu* and *T. monococcum* have been identified as good sources of resistance against fungal diseases, particularly stem rust, as observed by Rouse and Jin [2011]. Moreover, Hovhannisyan et al. [2011] discovered that both species are equipped with many of the genes that provide resistance against leaf rust and powdery mildew (*Pm1*, *Pm2*, *Pm3*, *Lr10*, *Lr47*, *Lr25*, and *Lr28*) [Fedak et al. 2007, Fedak 2015]. A powdery mildew resistance gene, *Pm60*, has been introduced from *T. urartu*. *Pm60* encodes a protein with a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) domain [Zou et al. 2018]. Diploid wild wheat species are also a source of genes for resistance to various biotic stresses, including stripe rust [Chen et al. 2021, Wu et al. 2022], stem rust [Fedak 2015], Fusarium head blight [Fedak et al. 2007, Fedak 2015], Karnal bunt resistance gene [Vasu et al. 2000], Hessian fly resistance genes, mosaic virus resistance gene [Fedak 2015] as well as biotic stresses like cold resistance [Miller et al. 2006, Knox et al. 2008].

Using a group of the same genes in cultivated forms may break their resistance [He et al. 2020]. Constantly searching for new effective sources will expand the spectrum of genes used and increase the effectiveness of resistant breeding [Fedak et al. 2007]. When it comes to disease resistance, wild forms related to cultivars usually have some level of resistance, but not all species have a high potential for resistance against a particular pathogen. Our study aimed to identify which tested species showed the most potential for effective sources of resistance to powdery mildew. Despite existing literature suggesting that certain species could be a source of resistance to powdery mildew, our findings indicate that *T. monococcum* and *T. boeoticum* have greater potential than *T. urartu*. Therefore, searching for sources of powdery mildew resistance in these species is more likely to lead to the identification of effective genes. Rouse and Jin [2011], identifying genotypes resistant to stem rust, showed that the most resistant genotypes of *T. urartu* came from Lebanon. They indicated this region as a priority for searching for new, effective genes for resistance to stem rust. The conducted research showed that the *T. urartu* genotypes originating from Lebanon and Syria were characterized by the highest level of resistance, which confirms that this region should also be a priority when looking for genes for resistance to powdery mildew. In the case of *T. monococcum* and *T. boeoticum* genotypes, no relationship was observed between the level of resistance and the geographical origin of the accessions. Resistant genotypes came from European countries as well as from the Middle East. However, all genotypes with a moderate or susceptible response came from Türkiye, which may indicate that this region is not a region where powdery mildew occurs with a high frequency, and which forced the plants to adapt to emerging diseases.

## CONCLUSIONS

1. Diploid wheat species are a valuable source of resistance to powdery mildew.
2. Identified sources of resistance should be defined in terms of what resistance genes they contain.
3. Identified resistance genes can be introduced into cultivated wheat during new cultivars development.

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