Candidate Genes Underlying QTL for Powdery Mildew Resistance in Triticale (x *Triticosecale* Wittm.)

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Abstract

Combining to tolerance to biotic and abiotic stresses is important target for modern triticale breeding. Cultivation of varieties resistant to fungal pathogens is economically and environmentally important and may lead to reducing of the use of fungicides. Molecular markers are necessary for accumulation of advantageous alleles in the best genotypes by means of marker-assisted and genomic selection approaches. In present research, QTL regions associated with the powdery mildew resistance at adult plant stage were evaluated in order to provide the effective selection tools. Testing of DH population in multiple environments under natural infestation revealed 20 QTL on wheat (4A, 3B, 4B) and rye (2R, 4R, 5R, 6R) chromosomes. Regions explained 8.1% - 29.3% of phenotypic variation depending of the trait, localization and year of the experiment. Main QTL with effect exceeding 15% were found on chromosomes 3B, 4B, 2R, 5R and 6R. QTL and candidate genes located on chromosomes 4B, 2R, 5R and 6R are so far reported for the first time as regions associated with PM resistance in the adult triticale plants. Additionally, within all QTL, 21 candidate genes associated with the PM resistance were revealed. Predicted function of protein encoded by these genes include triggering a defense system which restricts the pathogen growth, enzyme activity, regulation of hormone-activated pathways, transcriptional corepressor complex and cell wall construction. Availability of QTL, molecular markers together with candidate genes linked with the powdery mildew resistance can be validated on triticale lines and varieties and then, used in MAS to improve modern breeding.

Introduction

Triticale (x *Triticosecale* Wittm.) is a self-pollinated, man-made cereal obtained by crossing wheat (*Triticum* spp.) and rye (*Secale cereale* L.) (Ammar et al. 2004; Mergoum et al. 2019). Hexaploid triticale (2*n* = 6x = 42, AABBRR) which is currently the most commonly cultivated, combines valuable traits received from both parental species i.e. high grain quality and yield efficiency of wheat and resistance to biotic and abiotic stresses transferred from rye (Klocke et al. 2013; Blum 2014; Ayalew et al. 2018). These qualities make triticale an important crop with world acreage covering almost 4 million hectares and a grain yield over 14 million tons (FAO Stat, 2021). Triticale is mainly used as an animal food (Blount et al. 2017) but also, the potential in biofuel production has been recognized (Petersen et al. 2020) as well as an alternative in various food and beverage applications (Zhu 2018; Leonova et al. 2021). Additionally, this cereal crop is widely used as a genetic bridge to transfer important genes associated with fungal disease resistance from rye to wheat genome (Mergoum et al. 2019). Preliminary high disease-resistance of triticale transferred from rye has been recently overcome and nowadays, triticale is susceptible to various fungal diseases e.g. stem and leaf rust and *Fusarium* head blight (FHB). The most important, triticale become vulnerable to powdery mildew (Losert et al. 2017; Galiano-Carneiro et al. 2019) that may lead to significant economic losses (Arseniuk and Góral 2015; Mergoum et al. 2019).

The epidemic powdery mildew appearance has been observed in many European countries, including Poland in the last few years (Walker et al. 2011). This disease is caused by biotrophic fungus *Blumeria graminis* (DC.) Speer. The first symptoms are fluffy white-grey spots on the lower leaves that appear in late autumn or early spring. The spots grow and connect over the time cover stem and later on also ears, affect kernels development and decrease the yield (Hückelhoven 2005; Pietrusińska and Tratwal 2020). The breeding of resistant triticale varieties can be the most environmentally-friendly and economical alternative to using fungicides (Mergoum et al. 2019).

Up to date, almost 80 genes/alleles associated with powdery mildew resistance have been identified on 18 wheat chromosomes and only 8 *Pm* resistance genes have been found on rye chromosomes (Tyrka and Chelkowski 2004; Huang and Röder 2004; Yang et al. 2017; Shah et al. 2018). Recent advances in wheat and rye genomics together with access to the annotated genomes are extremely useful for triticale improvement (Chapman et al. 2015; Karbarz et al. 2020; Li et al. 2021). A number of triticale genetic maps were developed (González at al. 2005; Alheit at al. 2011; Tyrka et al. 2015; Tyrka et al. 2011, 2018; Dyda et al. 2021; Wąsek et al. 2021) and used to locate quantitative trait loci (QTL) linked with multiple agronomic traits (Niedziela et al. 2012; Ayalew et al. 2018; Sapkota et al. 2018; Wen et al. 2018; Wąsek et al. 2021) including fungal pathogen resistance (Kalih et al. 2015; Miedaner et al. 2016; Dhariwal et al. 2018; Zhao et al. 2018; Karbarz et al. 2020; Dyda et al. 2021). Molecular markers and next generation sequencing (NGS) play a major role in genetic improvement and can be widely used in marker-assisted selection (MAS) and genomic selection (GS) for reducing breeding cycle and costs of a crop production (Collard and Mackill 2008; Bhat et al. 2016; Mergoum et al. 2019).

In present study, the genetic map of 'Hewo' x 'Magnat' doubled haploids (DH) population (Tyrka et al. 2015) was used for localization of QTL regions associated with powdery mildew resistance in adult plant stage. Phenotyping data obtained under natural field conditions in multiple environments for three-years were used for identification respective regions of genome and linked the candidate genes.

Materials And Methods

Double haploid (DH) population

The mapping population used in the experiment was composed of 92 doubled haploid (DH) lines derived from F_1 'Hewo' (used as a female parent) and 'Magnat' hybrid. 'Hewo' and 'Magnat' cultivars were registered by Strzelce Plant Breeders Ltd (Plant Breeding and Acclimatization Institute Group, Poland) and Danko Plant Breeders Ltd, respectively and differed in tolerance to *Microdochium nivale* (Gołębiowska and Wędzony 2009; Dyda et al. 2019) and *Blumeria graminis* infection (unpublished breeders data indicate that cv. 'Hewo' shows higher resistance to powdery mildew than cv. 'Magnat'). The DH population was developed by the androgenesis in the anther culture according to method described by Wędzony (2003).

Plant growth conditions and phenotyping

Triticale kernels for the first year of the experiment were reproduced in greenhouse in the Institute of Plant Physiology Polish Academy of Science in Kraków and for the second and the third year of experiment were obtained in the field of Danko Plant Breeders Ltd by the isolation of five spikes per each DH line before flowering.

Powdery mildew resistance was tested in the field conditions during the three-year period (2013–2015) in three different locations in Poland: Choryń (52°2'26"N 16°46'59"E; seasons 2013–2015), Laski (51°47'N 21°12'E; seasons 2014–2015) and Modzurów (50°9'20"N 18°7'52"E; season 2015). All 92 lines along with parental cultivars were sown in two rows (1 m long each) at the 20 x 2.5 cm spacing. During plant growth period, no chemical protection was applied. The degree of the powdery mildew infection was measured under natural field conditions using a 1–9 scale according to Ziems (2014), where 1 refers to immune (healthy-look) plant and 9 to susceptible (damaged) one. All observations were conducted in periods of heading and flowering. Depending on the different weather conditions (high temperature and drought which limited the proper plant development and led to death of some plants) field observations of the infection degree were conducted once, twice or three times in each location and year. Data obtained from three terms of observation during vegetative season 2013 in Choryń localization were used to calculate Area Under Disease Progress Curve (AUDPC) according to Shaner and Finney (1977) and Jeger and Viljanen-Rollinson (2001) while data obtained from one or two terms of observations in remaining seasons and localizations were used to establish the average value of powdery mildew infection (avPM) according to the 9-grade scale.

Statistical analysis, genetic map, QTL and candidate genes identification

The Shapiro-Wilk test was performed to calculate deviations from a normal distribution of all data together with skewness and kurtosis using Statistica version 12.0 (StatSoft, Inc. USA). Additionally, mean values from all observations were used to calculate the Pearson's correlations.

Genetic linkage map for the 'Hewo' x 'Magnat' population described in details by Tyrka et al. (2015) was 4997.4 cM long. The map consisted of 842 DArT, 2647 DArT-seq and 50 SSR markers ordered into 20 linkage groups assigned to the A (7), B (7) and R (6) genomes. The quantitative trait loci (QTL) were identified using Windows QTLCartographer version 2.5 (Wang et al. 2012). Composite interval mapping (CIM) method was used to obtain QTL regions associated with measured traits. The threshold logarithm of the odds (LOD) scores was calculated using 1000 permutations and a 1-cM walk speed. QTL with the LOD score ≥ 3.0 were accepted. The percentage of phenotypic variation was calculated using a single-factor regression (R^2) and favorable allele was selected based on additive effects (Add) at LOD peaks obtained by Windows QTLCartographer 2.5. The candidate genes were *in silico* located within all identified QTL regions according to method described by Wąsek et al. (2021).

Results

Phenotypic analysis

The powdery mildew resistance of 'Hewo' x 'Magnat' DH population and parental lines across years and locations showed a normal distribution according to the Shapiro-Wilk test which was also confirmed by skewness and kurtosis analyses (Table 1). AUDPC values for all 92 DH lines, calculated for observations conducted in Choryń location in season 2013 were in range 2046.5 to 2728.8. The average PM values recorded were in exact range of the used scale (1 to 9) and varied by location and year of the experiment (Table 1). The values for parental lines differ in each experiment and showed that cv. 'Hewo' was more immune than cv. 'Magnat' (Table 1) but the range of variation was higher for all DH lines than in parents. Furthermore, highly positive correlations were found between different powdery mildew scores measured within and among all locations (Table 2). Values for powdery mildew infection measured in Choryń within all three seasons were positively correlated. The same positive correlations were observed also within observations in Laski (Table 2).

Table 1
The ranges of powdery mildew resistance measures (as AUDPC or in 9-grade scale) for the 'Hewo' x 'Magnat' DH mapping population together with parental lines evaluated in three locations for three years. Mean values, standard deviations, the normality test using Shapiro-Wilk statistics as well as skewness and kurtosis values are provided.

Experiment location	Experiment season	Experiment term	Trait	Values of powdery mildew infection			Mean value ± SD	Normality	Skewness	Kurtosis
				Minimum - Maximum	Value of 1–9 scale					
				DH lines	'Hewo'	'Magnat'				
Choryń	2013	1	AUDPC	2046.5–2728.8	1	2	2482.1 ± 229.7	0.94	-0.61	-0.92
		2			3	4		0.82	-0.79	0.84
		3			3	4		0.92	-0.74	0.63
	2014	1	avPM	2–8.5	3	4	6.2 ± 1.2	0.84	0.92	0.62
	2015	1	avPM	4.5–8	3	4	6.6 ± 0.7	0.90	-0.93	0.51
		2		3–8	3	4	6.6 ± 0.8	0.82	-0.50	0.71
Laski	2014	1	avPM	4.5–9	1	2	7.1 ± 1.3	0.95	-0.41	-0.78
		2		1–8	3	5	3.7 ± 1.7	0.93	0.33	-0.60
	2015	1	avPM	1–5.5	2	6	3.1 ± 1.1	0.91	0.20	0.74
Modzurów	2015	1	avPM	4–9	5	6	6.3 ± 1	0.98	0.23	0.17

Table 2
The Pearson's correlations between mean values of powdery mildew resistance measured in 9-grade scale for 'Hewo' x 'Magnat' DH mapping population evaluated in three locations (Ch, L, M - locations Choryń, Laski and Modzurów, respectively) in three years (2013, 2014 and 2015) and different terms of observations (1, 2 and 3).

	Ch 2013_1	Ch 2013_2	Ch 2013_3	Ch 2014_1	Ch 2015_1	Ch 2015_2	L 2014_1	L 2014_2	L 2015_1
Ch 2013_2	0.489 *								
Ch 2013_3	0.315	0.708 ***							
Ch 2014_1	0.024	0.041	0.075						
Ch 2015_1	-0.057	0.655 ***	-0.887	0.787 **					
Ch 2015_2	0.235	0.101	0.0214	0.727 **	0.080				
L 2014_1	0.032	0.211	-0.019	0.874 ***	0.024	0.042	0.321 *		
L 2014_2	0.422 **	0.065	0.107	0.012	0.098	0.665 *	0.775 ***	0.409 **	
L 2015_1	-0.089	0.298 *	-0.388**	0.032	0.365 **	0.600 *	0.344 *	0.350 *	0.076
M 2015_1	-0.271	-0.347 *	0.039	0.421 *	0.789 *	0.359 **	0.387*	0.032	0.331 *

*, **, *** : Significant at P < 0.05, P < 0.01 and P < 0.001, respectively.

QTL regions associated with powdery mildew resistance

Quantitative trait loci were calculated from the mean values of the data obtained for each experiment separately. Identification of QTL regions associated with triticale resistance to powdery mildew infection was carried out based on the previously described, genetic map for 'Hewo' x 'Magnat' DH population (Tyrka et al. 2015). A total of 20 significant QTL effects were identified with Composite Interval Mapping (CIM) method. These QTL effects had overlapping positions and 5 independent QTL were assigned to 3 wheat chromosomes (4A, 3B and 4B) and 7 QTL were located on rye chromosomes 2R, 4R, 5R and 6R (Table 3, Fig. 1).

Table 3
Characteristics of the QTL regions associated with powdery mildew resistance in triticale 'Hewo' x 'Magnat' DH population located for AUDCP (Ch2013) and avPM evaluated in three locations in 2013–2015.

QTL name	Environment	Flanking markers (cM) [a]	LOD	LOD max. position (cM)	Marker closest to the LOD peak	R^2 (%) [b]	Add [c]	Fav. allele [d]
Qpm.hm.4A.1–2	L2014_1	*3613305 : 3041392* (103.2 : 118.5)	4.6	110.2	*4356897*	11.5	-0.49	M
	M2015_1	*4356897 : 4357848* (110.2 : 125.6)	3.6	119.7	*3046898*	10.9	-0.38	M
Qpm.hm.3B.1	M2015_1	*tPt-9923 : wPt-0990* (254.4 : 264.9)	3.1	254.4	*tPt-9923*	10.5	0.37	H
Qpm.hm.3B.2–5	Ch2013	*4365124 : 4366967* (331.9 : 349.4)	3.6	337.5	*tPt-4541*	12.1	81.8	H
	L2014_1	*3616572 : 4366967* (334.1 : 349.4)	4.5	337.5	*tPt-4541*	11.7	0.47	H
	L2014_2	*4362533 : tPt-4541* (327.3 : 337.5)	3.3	334.1	*3616572*	8.6	0.51	H
	Ch2015_1	*wPt-0531 : wPt-3301* (323.9 : 345.0)	6.1	337.5	*tPt-4541*	18.1	0.31	H
Qpm.hm.4B.1–3	L2014_2	*4202451 : 4353436* (21.4 : 32.3)	4.4	25.2	*4366886*	12.1	0.63	H
	L2015_1	*4366886 : 4363322* (25.2 : 28.2)	9.4	25.2	*4366886*	23.4	0.54	H
	Ch2015_2	*3044038 : 4363322* (15.0 : 28.8)	4.8	25.2	*4366886*	13.5	0.32	H
Qpm.hm.4B.4	Ch2015_1	*wms149 : wPt-7412* (46.0 : 56.2)	6.5	50.5	*wPt-8543*	19.8	0.35	H
Qpm.hm.2R.1	Ch2014_1	*rPt-390554 : 4347964* (7.8 : 17.2)	5.9	11.9	*rPt-508776*	15.9	0.48	H
Qpm.hm.4R.1	Ch2014_1	*rPt-399468 : rPt-507026* (0.0 : 11.3)	5.0	2.3	*4356439*	13.8	0.51	H
Qpm.hm.5R.1–2	L2015_1	*4205026 : 3047417* (27.6 : 31.4)	11.2	29.8	*3040546*	29.3	0.59	H
	M2015_1	*4343102 : 4341499* (24.2 : 37.0)	3.7	31.4	*3047417*	11.0	0.37	H
Qpm.hm.5R.3	Ch2014_1	*4204714 : 3046190* (222.3 : 235.3)	3.1	228.4	*4358930*	8.1	0.36	H
Qpm.hm.6R.1–2	L2014_1	*3605937 : 3045646* (35.5 : 52.8)	9.9	50.1	*3615688*	27.6	-0.75	M
	L2014_2	*rPt-399922 : 3040652* (43.4 : 60.2)	3.6	50.1	*3615688*	9.5	-0.56	M
Qpm.hm.6R.3	Ch2015_2	*4363416 : 3620326* (156.3 : 172.1)	3.2	167.9	*3605086*	8.9	-0.26	M
Qpm.hm.6R.4	Ch2013	*scm180 : 4352708* (194.7 : 209.4)	3.1	203.7	*3617138*	9.9	-75.1	M

a – an identifiable region of the QTL defined by the first and last marker of the QTL region; b – R^2 (%) – percentage of the phenotypic variance explained by the QTL; c – Add – additive effect; d –source of favourable allele for each QTL: H - cv. 'Hewo' and M - cv. 'Magnat'.

Four QTL located on chromosomes 4A, 3B, 4B, and 5R showed significant effects across environments and are the most important PM resistance loci in 'Hewo' x 'Magnat' population. Additionally, three of these loci (on chromosomes 3B, 4B, and 5R) with resistance alleles contributed by 'Hewo' explained 18.1–23.4% of variation in selected environments and can be classified as the main effective loci. The QTL located on chromosome 4A was derived from 'Magnat' and explained up to 11.5% of variation (Table 3, Fig. 1).

The second important group of QTL were those with main effects in selected environments. Three QTL: *Qpm.hm.4B.4*, *Qpm.hm.2R.1* and *Qpm.hm.6R.1–2* from this group explained from 15.9–27.6% of variation in reaction to PM. Favorable alleles for loci on 4B and 2R chromosomes were contributed by 'Hewo' and for locus on 6R by 'Magnat'. Remaining 5 QTL *Qpm.hm.3B.1, Qpm.hm.4R.1, Qpm.hm.5R.3, Qpm.hm.6R.3* and *Qpm.hm.6R.4* were significant only in selected environments and explained below 14% of variation (Table 3, Fig. 1).

Candidate genes for triticale PM resistance

Within all identified QTL regions, powdery mildew candidate resistance genes were *in silico* located in physical regions corresponding to AUDCP and avPM (Table 3, Table 4). Those 21 genes encoded: (1) disease resistance protein RGA3 (LOC119294473) transcript variant X4, (2) pirin-like protein (LOC119287429), (3) serine/threonine-protein kinase PBL25 (LOC119346574), (4) ras-related protein RABH1b-like (LOC119291733), (5) NAC domain-containing protein 22-like (LOC119294086), (6) acetyltransferase At3g50280-like (LOC109780458), (7) chloroplastic protein FAF-like (LOC119306943), (8) SKP1-like protein 4, (9) ATP-dependent Clp protease ClpB family protein, (10) protein SMAX1-LIKE 3-like (LOC119281963), (11) xyloglucan endo-transglucosylase/hydrolase protein 31-like (LOC119304359), (12) E3 ubiquitin-protein ligase RGLG3-like (LOC119278774), (13) F-box family protein-like, (14) replication factor C subunit 5, (15) G-type lectin S-receptor-like serine/threonine-protein kinase SD2-5 (LOC119314736), (16) heavy metal-associated isoprenylated plant protein 3-like (LOC119321558) as well as five uncharacterized proteins LOC119309372, LOC119294058, LOC119294131, LOC119307103 and LOC109783890 (Table 4). In addition to the listed genes, one of the QTL (*Qpm.hm.4R.1–2*) was identified in the powdery mildew region for *Triticum timopheevii* subsp. *timopheevii* isolate *QPm.tut-4A* (Table 4).

Table 4

Powdery mildew candidate resistance genes localized in physical regions corresponding to QTL regions for AUDCP and avPM in triticale 'Hewo' x

QTL name	Candidate gene	Confidence	Position	Sequence ID	Predicted encoded protein
Qpm.hm.4A.1–2	TraesCS4B01G005100	High	Chr4B:3732446..3736051 (+ strand)	XM_037572659.1	*Triticum dicoccoides* putat disease resistance protein (LOC119294473), transcrip X4
	TraesCS4A03G0842300	High	Chr4A:619916253..619918166 (+ strand)	XM_037566969.1	*Triticum dicoccoides* pirin-protein (LOC119287429
	TraesCS5D02G514300	High	Chr5D:538043454..538045150 (+ strand)	-	-
Qpm.hm.3B.1	REPEATMASKER_CLARIrepeatwheat_809751_809827_Repeat_region_0504	-	-	-	-
Qpm.hm.3B.2–5	TraesCS3B03G1526900	High	Chr3B:851661563..851663075 (+ strand)	XM_037615869.1	*Triticum dicoccoides* proba serine/threonine-protein ki PBL25 (LOC119346574)
	TraesCS7D03G0881800LC	Low	Chr7D:486775607..486777382 (+ strand)	XR_005150494.1	*Triticum dicoccoides* uncharacterized (LOC1193
	STRG_Root.48180.1	High	Chr3B:791636985..791644366 (+ strand)	-	ncRNA
Qpm.hm.4B.1–3	TraesCS4B03G0831100	High	Chr4B:610300212..610301837 (- strand)	XM_037572332.1	*Triticum dicoccoides* uncharacterized (LOC1192
	TraesCS4B02G336700	High	Chr4B:629225922..629227213 (+ strand)	XM_037572381.1	*Triticum dicoccoides* uncharacterized (LOC1192
	TraesCS4B03G0801600	High	Chr4B:595350531..595352759 (+ strand)	XM_037570544.1	*Triticum dicoccoides* ras-re protein RABH1b-like (LOC119291733)
	TraesCS4B03G0851200	High	Chr4B:618435867..618437309 (- strand)	XM_037572350.1	*Triticum dicoccoides* NAC containing protein 22-like (LOC119294086)
Qpm.hm.2R.1	TraesCS2D03G0065000	High	Chr2D:13686882..13688977 (- strand)	XM_020339042.2	*Aegilops tauschii* subsp. *s* uncharacterized acetyltran At3g50280-like (LOC10978
Qpm.hm.4R.1	TraesCS6B02G056700	High	Chr6B:37233673..37240056 (- strand)	MG672525.1	*Triticum timopheevii* subsp *timopheevii* isolate *QPm.t* powdery mildew resistanc genomic sequence

QTL name	Candidate gene	Confidence	Position	Sequence ID	Predicted encoded protein
Qpm.hm.5R.1–2	TraesCS5B03G0004200	High	Chr5B:3371571..3373018 (- strand)	XM_037583016.1	*Triticum dicoccoides* prote chloroplastic (LOC119306
	SECCE5Rv1G0297180	High	Chr5R:210878..213334 (- strand)	XM_037583199.1	SKP1-like protein 4 *Triticu dicoccoides* uncharacteriz (LOC119307103)
	SECCE5Rv1G0373660	Low	Chr5R:860445705..860446439 (+ strand)	XM_037562349.1	ATP-dependent Clp protea family protein *Triticum dicoccoides* prote LIKE 3-like (LOC11928196
Qpm.hm.5R.3	SECCE5Rv1G0374850	High	Chr5R:865109932..865111083 (- strand)	XM_037581542.1	*Triticum dicoccoides* xylog endotransglucosylase/hyc protein 31-like (LOC11930
Qpm.hm.6R.1–2	SECCE6Rv1G0434050	High	Chr6R:763555284..763559328 (- strand)	XM_037560124.1	*Triticum dicoccoides* E3 ub protein ligase RGLG3-like (LOC119278774)
	SECCE6Rv1G0433820	High	Chr6R:761783609..761786302 (- strand)	XM_020342468.2	*Wollemia nobilis* Ref_Wollemi_Transcript_1 transcribed RNA *Aegilops* subsp. *strangulata* unchar LOC109783890
	TraesCS2D01G493500LC	Low	Chr2D:490572417..490592747 (+ strand)	MG560141.1	Pseudogene *Triticum aestivum* gamma omega gliadin-B
	SECCE6Rv1G0421170	Low	Chr6R:682135049..682135441 (+ strand)	-	F-box family protein-like
	SECCE6Rv1G0421210	Low	Chr6R:682397077..682397586 (+ strand)	-	Replication factor C subun

QTL name	Candidate gene	Confidence	Position	Sequence ID	Predicted encoded protein
Qpm.hm.6R.3	SECCE6Rv1G0424130	High	Chr6R:699387464..699389878 (- strand)	XM_037589430.1	*Triticum dicoccoides* G-typ receptor-like serine/threoni kinase SD2-5 (LOC119314
Qpm.hm.6R.4	SECCE6Rv1G0423020	High	Chr6R:692904600..692906280 (+ strand)	XM_037595188.1	*Triticum dicoccoides* heav associated isoprenylated p protein 3-like (LOC119321

The predicted function of encoded proteins include triggering a defense system which restricts the pathogen growth, enzyme activity (peptidase inhibitor, transferase and protein kinase), regulation of hormone-activated pathways (auxin signaling mediation, negative regulation of abscisic acid-activated signaling pathway, upstream mediation of jasmonate signaling in response to various stimuli like coronatine-mediated pathogen susceptibility and triggered programmed cell death), transcriptional corepressor complex, cell wall construction (accumulation of hemicelluloses, xyloglucan metabolic process) as well as the elongation of primed DNA templates (Table 4).

Only eight of all above candidate genes located on chromosomes 4A and 6R, encoding disease resistance protein RGA3, pirin-like protein, ubiquitin-protein ligase RGLG3-like, uncharacterized LOC109783890, F-box family protein-like, replication factor C subunit 5, G-type lectin S-receptor-like serine/threonine-protein kinase SD2-5 and heavy metal-associated isoprenylated plant protein 3-like were located in QTL with the additive effect from cv. 'Magnat'; the remaining 10 genes had additive effect coming from cv. 'Hewo' (Table 3, Table 4).

Discussion

Molecular breeding applies molecular tools and techniques, such as quantitative trait loci (QTL) mapping, marker-assisted selection (MAS) and genomic selection (GS) for crops improvement (Mergoum et al. 2019). The next-generation sequencing (NGS) technologies including DArTseq found prominent role in GS (Xu et al. 2012). Quantitative trait loci analysis based on genetic maps saturated with DArTseq markers may lead to development or optimization of selection tools and to localization of candidate genes potentially associated with agronomically important traits. Understanding of PM resistance factors may contribute to better control of biotic stresses that is important direction in modern breeding, and lead to sustainable food supply (Talas e al. 2016; Karbarz et al. 2020).

Up to date, numerous genetic maps are available for many crop species including cereals: wheat (Somers et al. 2004; Mantovani et al. 2008; Peleg et al. 2008; Avni et al. 2014; Cui et al. 2017; Xu et al. 2020), rye (Korzun et al. 2001; Milczarski et al. 2011; Gawroński et al. 2016), barley (Hearrden 2007; Zhou et al. 2015) and triticale (González et al. 2005; Alheit et al. 2011; Tyrka et al. 2011, 2015, 2018; Karbarz et al. 2020; Dyda et al. 2021; Wąsek et al. 2021). Also, numerous QTL regions associated with resistance to most common fungal diseases were reported (Saintenac et al. 2018; Odilbekov et al. 2019; Lin et al. 2020; Liu et al. 2020). However, number of reports describing QTL regions and associated with them, candidate genes of resistance to *Blumeria graminis* in triticale is very limited (Karbarz et al. 2020; Dyda et al. 2021). Therefore, analysis of PM resistance in 'Hewo' x 'Magnat' triticale population can result in an unique set of QTL and candidate genes associated with powdery mildew resistance and respective molecular markers useful for molecular breeding.

Powdery mildew caused by fungal pathogen *Blumeria graminis* (DC.) Speer is the most serious disease limiting cereal production in many regions of the world (Zhang et al. 2016). It affects host-plant's photosynthesis of nutrient organs (stems, leaves and spikes) which may lead to decrease of grain quality and yield (Gao et al. 2018). In appropriate conditions and in short time, conidia develop a tube which elongates to appresorium, produces the haustoria to penetrate the surface of a host plant (Bruggmann et al. 2005; Wang et al. 2012). Fast spread of this pathogen can significantly affect a whole field (Gao et al. 2018) that is why, breeding and cultivation of resistant triticale varieties of triticale is needed (Mergoum et al. 2019).

Based on observation on the triticale 'Hewo' x 'Magnat' DH population resistance to powdery mildew infection under natural conditions, 12 QTL regions were identified associated with this trait on three wheat (4A, 3B and 4B) and four rye (2R, 4R, 5R and 6R) chromosomes (Table 3, Fig. 1). Three main QTL (on 3B, 4B and 5R), stable across selected environments were associated with the area under disease progress curve (AUDPC) or the average value of powdery mildew infection (avPM) and explained from 18–29% of variation.

On wheat chromosome 4A, locus *Qpm.hm.4A.1–2* explained about 11% of phenotypic variation was identified (Table 3). Up to date only one QTL *Qpm.gz.4A.1*, explaining up to 13.7% of phenotypic variation has been reported as locus associated with PM resistance in triticale (Dyda et al. 2021). Chantret et al. (2001) and Mingeot et al. (2012) described QTL associated with powdery mildew resistance in wheat located on 4A chromosome which may correspond to *Pm16* resistance gene or *Rp1*-like protein coding gene that determines leaf stripe and rust resistance (Reader and Miller 1991; Marone et al. 2012, 2013).

Also, a cluster of resistance QTL on 4A chromosome for both powdery mildew and leaf rust resistance was described by Li et al. (2014) that is why this chromosome can be considered as important source of PM resistance in triticale.

Two QTL for AUDPC or avPM traits were found on chromosome 3B (Table 3). *Qpm.hm.3B.2–5* was stable in Laski and Choryń locations and explained 8.6% – 18.1% of phenotypic variation. *Qpm.hm.3B.2–5* shared the common region between 331.9 cM and 337.5 cM on chromosome 3B and the DArT marker *tPt-4541* was closest to the peak (Table 3). QTL on 3B chromosome were also previously described in wheat as loci significantly linked with adult plant resistance (APR) for powdery mildew (Chen et al. 2009; Asad et al. 2013, 2014; Jia et al. 2018). These QTL were in a close distance to previously described *Pm13* (Donini et al. 1995) and *Pm41* genes (Li et al. 2009) and play a significant role in PM resistance. Furthermore, our previous studies also revealed loci on chromosome 3B significantly linked with APR in triticale evaluated in natural field conditions (Dyda et al. 2021). Physical mapping of those QTL confirmed that *Qpm.hm.3B.2–5* is located in the same physical position as in above research, which indicates that loci on 3B chromosome is strongly linked with PM resistance. Two QTL with main effects were found on chromosome 4B including *Qpm.hm.4B.1–3* stable across environments (Table 3). The phenotypic variation for this QTL was as in range between 12.1 % and 23.4%, and the DArTseq marker *4366886* was appointed as marker closest to the LOD peak (Table 3). Powdery resistance locus on chromosome 4B flanked by RFLP markers *Xpsr593b* and *Xpsr1112* was described (Keller et al. 1999) as *Pm7* resistance gene (Friebe et al. 1994). QTL between SSR markers *Xgwm375* and *Xgwm251* explaining 5.9% of phenotypic variation was also identified (Liang et al. 2006). Locus *QPm.heau-4BL* with high and significant effects common for two analyzed wheat RIL mapping populations was also reported (Ren et al. 2017) and compared with other loci described so far (Börner et al. 2002; Asad et al. 2013). But physical mapping confirmed that loci *Qpm.hm.4B.1–3* are located in a different physical position than previously described loci so it and can be reported as an unique and a new source of powdery mildew resistance.

Identification of QTL and genes associated with powdery mildew resistance in rye is poorly described so far, comparing to wheat. So far, only eight *Pm* resistance genes were characterized and reported on rye chromosomes (Tyrka and Chelkowski 2004; Huang and Röder 2004). But due to close relationship with common wheat, rye has been extensively used as a valuable source of genes, adaptation to environment, yield improvement and mostly, for various wheat diseases (Schlegel 2016; An et al. 2019). Moreover, for triticale R homologous group, number of QTL regions and candidate genes associated with PM resistance is limited (Karbarz et al. 2020; Dyda et al. 2021). In present study, total of seven QTL regions were identified on chromosomes 2R, 4R, 5R and 6R (Table 3, Fig. 1).

Locus *Qpm.hm.2R.1* was found for avPM evaluated in 2014 in Choryń location and explained 15.9% of phenotypic variation (Table 3). It has been reported that *Pm7* gene has been derived from 2RL of Rosen rye (Rahmatov et al. 2016) but no QTL regions on rye chromosome 2R associated with powdery mildew resistance has been described so far.

Locus *Qpm.hm.4R.1* was also found for avPM measured in 2014 in Choryń explaining 13.8% of phenotypic variance. Lind (1982) and Heun and Friebe (1990) reported that chromosome 4R did not condition resistance against powdery mildew isolates tested on wheat-rye addition lines. But afterwards, An et al. (2013) confirmed stable wheat-rye T4BL·4RL and T7AS·4RS translocation line WR41 which exhibited a high level of resistance to PM. Also, Fu et al. (2014) described that lines of 'Mianyang 11'×'Kustro' rye carry powdery mildew resistance at the adult stage and located a resistant gene on chromosome 4RL of Kustro rye. Only few QTL regions have been reported so far on 4R chromosome. Karbarz et al. (2020) identified locus *QPm-4R* in triticale resistance based on AUDPC method. Also, our previous analysis revealed total of six loci for both, AUDCP and avPM methods explaining up to 15.2% of phenotypic variation (Dyda et al. 2021). These QTL can be considered as a significant PM resistance regions as their physical position correspond to physical position of *Qpm.hm.4R.1* described in this paper together with identified gene coded protein associated with race non-specificity and incomplete resistance (Table 4).

Two QTL were identified on 5R chromosome, one minor QTL for avPM in 2014 for Choryń location and one major *Qpm.hm.5R.1–2* for Laski and Modzurów locations in 2015 explaining up to 29.3% of phenotypic variation (Table 3). Additionally, *Qpm.hm.5R.1–2* shared a common region on 5R chromosome between 27.3 cM and 31.4 cM (Table 3). Up to date few reports show presence of QTL regions on 5R chromosome associated with biomass yield (Busemeyer et al. 2013) and plant height (carrying a dwarfing gene *Ddw1*; Alheitet al. 2011). In contrast, only one describe QTL, *Qpm.gz.5R.1* linked to PM resistance in triticale (Dyda et al. 2021). It has been also confirmed that 5RL might be associated with that resistance as includes *Pm4* resistance gene (Schlegel et al. 1998). Therefore, loci on chromosome 5R presented in this study is located in a different physical position comparing to *Qpm.gz.5R.1* so it can be reported as a new source of powdery mildew resistance, strongly related with that trait.

Three QTL on 6R were significant in selected environments only and two of them contributed minor effects. Main locus, *Qpm.hm.6R.1–2* was associated with avPM measured in Laski location and explained 27.6% of phenotypic variation (Table 3). Only one report describes identification of QTL linked with powdery mildew resistance on 6R chromosome (Dyda et al. 2021). Locus *Qpm.gz.6R.2* identified for AUDCP method explaining 11.1% of phenotypic variation was found together with gene encoded cyclin-dependent kinase A-2-like protein linked to those regions (Dyda et al. 2021) however we suggest *Qpm.hm.6R.1–2* as a new source of resistance, located in a different physical position than *Qpm.gz.6R.2*. For confirmation of importance of 6R chromosome, the *Pm20* resistance gene has been identified and derived from 6RL of Prolific rye (Zhuang 2003; An et al. 2015).

Information of significant and unique QTL regions associated with powdery mildew resistance together with candidate genes coded proteins taking part in triticale defense against fungal pathogen can be an important tool used in modern breeding programs. Physical comparison of loci and sequences of flanking markers available in the literature showed that four QTL regions identified in present study are unique. Loci *Qpm.hm.4B.1–3* were situated outside the *QPm.heau-4BL* region reported by Ren et al. (2017). Similarly, loci *Qpm.hm.5R.1–2* and *Qpm.hm.6R.1–2* are located in different physical regions comparing to *Qpm.gz.5R.1* and *Qpm.gz.6R.2*, respectively described in our previous studies (Dyda et al. 2021). Additionally, locus *Qpm.hm.2R.1* classified as the main effect was reported for the first time. All of these newly reported sources of PM resistance, after careful validation in available triticale varieties and lines can be used in marker-assisted selection (MAS) and assist molecular breeding programs.

Declarations

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Author Contribution statement

MD - writing the manuscript, QTL and statistical analysis and performing the experiments; MT - genetic map construction and writing the manuscript; GG – statistical and *in silico* candidate genes analysis, physical QTL comparison and writing the manuscript; MR - planning the experiments, obtaining the funding, text consulting; MW - planning and supervision of the experiments, obtaining the funding and text consulting.

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Conflict of interest

The authors declare that the research was con-ducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

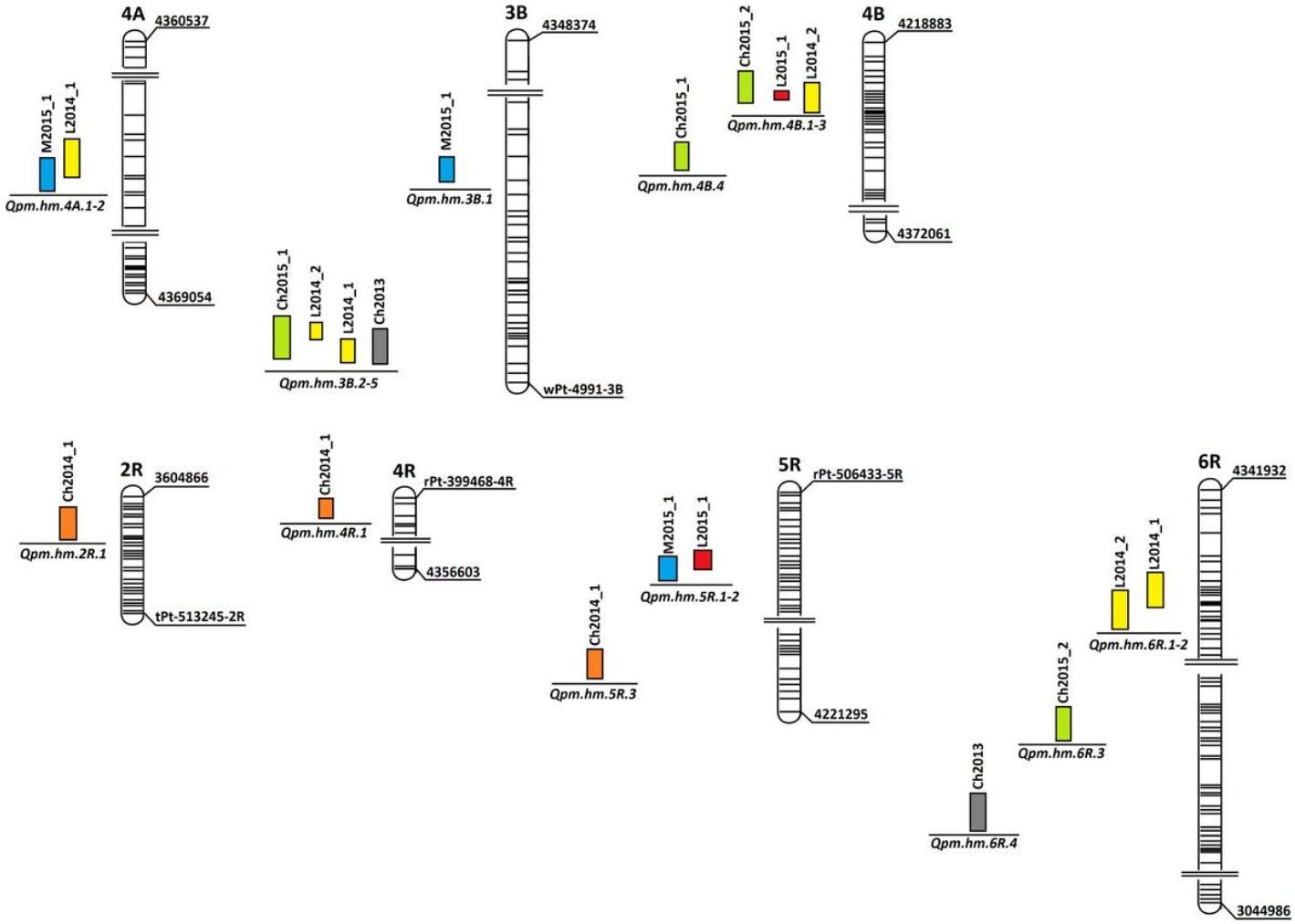

Figure 1

Interval map (cM) for 4A, 3B, 4B, 2R, 4R, 5R and 6R chromosomes of 'Hewo' x 'Magnat' DH population with QTL regions identified by CIM method for AUDCP and avPM evaluated in three locations in 2013-2015. Experiment name for AUDCP (Ch2013) and avPM (L2014_1, L2014_2, Ch2015_1, Ch2015_2, L2015_1, M2015_1) are provided above each QTL as well as common QTL name below.