Quantitative Trait Loci (QTL) Associated With Leaf Development in Winter Triticale (*× Triticosecale* Wittmack) Seedlings

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Abstract

The vitality and the development in the seedling stage is crucial in winter cereal's life cycle, especially before and during winter. It has been reported that rapid seedling establishment and early growth may lead to higher crop yield. Localization of cereal genome regions is not often analysed in the seedling stage. The aim of this study was to identify winter triticale genome regions (QTL) associated with seedling leaves development. Based on 'Hewo' x 'Magnat' DH lines population genetic map composed of 3539 molecular markers assigned to 20 linkage groups with 4997.4 cM map length, in total 40 *loci* were identified by a composite interval mapping (CIM). Among them, 22 *loci* appeared in at least two experiments, were common for all analyzed traits as well as were identified on wheat chromosome 4B and on rye chromosomes 1R, 4R, 5R and 6R. Those *loci* explained up to 21.7% of phenotypic variation (*Qwsl.hm.4R.2*) and had LOD value up to 31.1 (*Qlsl.hm.5R.1*). The results of QTLs of seedling leaves development could be successively associated with QTLs of the freezing and fungal infection seedlings tolerance identified in this mapping population.

Introduction

Triticale (x *Triticosecale* Wittmack) is a man-made cereal developed by crossing wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) with a genomic constitution of $2n = 6x = 42$ (Ayalew et al. 2018; Mergoum et al. 2019). It combines valuable traits like high fertility and grain quality received from wheat with high resistance to diseases, drought, low temperature, higher tolerance to environmental stress factors and winter hardiness obtained from rye (Tyrka and Chełkowski 2004; Pronyk and Mazza 2011; Mergoum et al. 2019). All of those qualities make triticale a valuable and well-established worldwide crop which grain is used for animal food as well as human food consumption (Losert et al. 2017; Ayalew et al. 2018). Triticale is also a valuable genetic bridge for transferring eligible genes among rye and wheat genetic pool using molecular breeding techniques (Tams et al. 2004; Tyrka and Chełkowski 2004; Badea et al. 2011). QTL mapping, MAS, genomic selection and next-generation sequencing (NGS) are widely used to improve crop species, especially wheat and rye; in contrast, molecular breeding in triticale is still limited (Jannink et al. 2010; Tyrka et al. 2018; Mergoum et al. 2019). So far, a few research studies describe genetic maps of triticale and QTL analysis of mapping important traits in this cereal (Alheit et al. 2011; Tyrka et al. 2011, 2015, 2018; Wen et al. 2018).

Plant development is a process controlled by plant genetic information together with environment conditions e.g. light, temperature, water and soil (Von Arnim and Deng 1996; Hochholdinger et al. 2004; Chen et al. 2008). The cereal seedling development is controlled by metabolite and hormonal signals which participate in gene expression, growth, and metabolism (Thomas and Rodriguez, 1994). This process is very crucial in cereal life cycle because the health of seedlings affects the size and quality of the grain (Stratonovitch and Semenov 2015; Maulana et al. 2018). In winter cereals, the low temperature period during seedling development (hardening) provides to increasing crop's low-temperature and fungal pathogens tolerance (Tronsmo et al. 2001; Hudec and Bokor 2002). Also, our previous studies on triticale, especially 'Hewo' x 'Magnat' DH population showed that plant's hardening leads to increased tolerance to

Microdochium nivale infection (Gołębiowska and Wędzony, 2009; Gołębiowska et al. 2011; Szechyńska-Hebda et al. 2011, 2013; Dyda et al. 2019). The studies on the genetic control of morphological and yield-related traits in cereals have been mainly focused on adult plants of wheat, barley, maize and rice (Peng et al. 2011; Li et al. 2015; Gao et al. 2016; Kumar et al. 2016; Mora et al. 2016; Kumari et al. 2018; Cao et al. 2019; Li et al. 2020; Khahani et al. 2020; Kuang et al. 2020; Xin et al. 2020). Contrary, for triticales not many studies have been reported so far (Wajdzik et al. 2019; Neuweiler et al. 2020).

The aim of this study was to locate winter triticales genome regions (QTL) associated with morphological traits in the seedling stage: number of leaves, first and second leaf length and width, the second leaf sheath and blade length as well as fresh mass of leaves. Triticales genetic map developed and described by Tyrka et al. (2015) for 'Hewo' x 'Magnat' DH lines population enabled to identify significant QTL regions for the analysed traits.

Materials And Methods

Plant material and genetic linkage map

Study was performed on 89 doubled haploid (DH) lines derived from F_1 generation of a cross between two winter triticales cultivars 'Hewo' (Strzelce Plant Breeding – IHAR Group Ltd., Poland) as the female parent and 'Magnat' (DANKO Plant Breeders Ltd., Poland) as the pollen donor. The population was developed by the anther culture according to the method described by Wędzony et al. (2003). Parental cultivars differed in morphology in the seedling stage (here in), adult plant stage (Wajdzik et al. 2019), tolerance to *Microdochium nivale* infection (Gołębiowska and Wędzony 2009; Gołębiowska-Pikania et al. 2019), as well as freezing tolerance in pre-tests (unpublished data).

For this mapping population, the genetic triticales map with total 4997.4 cM length and composed of 3539 molecular markers was developed. The map consists of 20 linkage groups assigned to triticales genomes: A (7), B (7) and R (6) (Tyrka et al. 2015). Plant material was available for the experiments at the Institute of Plant Physiology Polish Academy of Science (IPP PAS) in Kraków, Poland.

Plant growth conditions and experiment design

Analysis of seedlings morphological traits were performed in two-years' time period in controlled conditions in completely randomized blocks design (CRBD). Analysis were divided in three series of independent experiments. Experiments 1 and 2 (Exp. 1, Exp. 2) were performed on cold-hardened 7-weeks old seedlings. Experiment 3 (Exp. 3) was performed on 4-weeks old unhardened seedlings. In all experiments, plants were cultivated as described in Gołębiowska and Wędzony (2009) with detailed modifications. All DH lines, together with parental cultivars, were grown in multi-pots in an isolated chamber with 67% of humidity. Three replicates were performed in a randomized complete block design in order to limit the error resulting from the marginal position of an individual genotype. Kernels were sown in 10 rows of 6 kernels/ genotype/ box. One row per each parental line was sown in variable positions in every box as a control.

In Experiments 1 (1st year) and 2 (2nd year), plants were grown in temperature 21°C/16°C, at 8h/16h (day/night) photoperiod for one week and were watered and supplemented with Hoagland and Arnon's (1938) sterile medium. Then seedlings were pre-hardened in 12°C/12°C, at 8h/16h (day/night) photoperiod for next 14 days and subsequently hardened in 4°C/4°C, at 8h/16h (day/night) photoperiod for the next 28 days. In the Experiment 3 (2nd year), plants were grown in 21°C/16°C, at 8h/16h (day/night) photoperiod for three weeks then were transferred into a greenhouse for one week and watered and supplemented with Hoagland and Arnon's (1938) sterile medium.

Seedlings phenotyping

Seedlings phenotyping was performed on 7-weeks old cold-hardened seedlings (Exp. 1 and 2) as well as 4-week old unhardened seedlings (Exp. 3). It included: (1) total number of seedling leaves (NL); (2) length of the first (LFL) and second (LSL) leaf measured from the soil surface together with leaf sheath; (3) length of second leaf sheath from the soil surface the top of the leaf (LSSL); (4) length of second leaf blade (LSLB) from the base to the top; (5) the width of the first (WFL) and second (WSL) leaf in the widest point; (6) the fresh mass of five youngest and fully developed leaves (LFM).

Statistical analysis and QTL mapping

All data were analyzed using Statistica 13.0 PL software (Statsoft, Tulsa, OK, USA). Distribution of the data was checked using histograms and analyzed with a Shapiro-Wilk test, together with skewness and kurtosis. Additionally, one-dimensional variance analysis was performed at $p < 0.05$. Linear correlation coefficients (Pearson's) were calculated for each of three experiments separately on the basis of mean value of replicates. The regression line was presented with a 95% coefficient interval.

QTL regions were identified with Windows QTLCartographer 2.5 software according to Wang et al. (2012) using composite interval mapping (CIM) with significance at the $p = 0.05$ level and 1000 permutations with LOD threshold ≥ 3.0 . The percentage of the phenotypic variation covered by each QTL was calculated with a single factor regression (R^2). Favourable alleles in each QTL region were selected on the basis of the additive effect (Add): negative additive effect referred to cv. 'Magnat'; positive additive effect referred to cv. 'Hewo'. Results of QTL analysis were visualized using CorelDRAW9 software and the label of each QTL was created from the short name of each morphological trait; Hewo × Magnat (hm), chromosome names (wheat A and B group, rye group R) and QTL number on the chromosome (1–3).

Results

The cv. 'Hewo' seedlings had an upright habit, while cv. 'Magnat' seedlings - lying, with leaves spread close to the soil surface. Additionally, the dimensions of the leaves differed between parental cultivars at the same 7-weeks old cold-hardened seedlings stage (Fig. 1S). The first leaf was on average 69 mm longer and 0.94 mm narrower in cv. 'Hewo' than in cv. 'Magnat' seedlings ($p < 0.05$). The second leaf sheath and the lamina were on average 7.9 mm and 55.5 mm longer in 'Hewo' than in 'Magnat' ($p < 0.05$) seedlings. The second leaf blade was almost 1/3 narrower in 'Hewo' than in 'Magnat' ($p < 0.05$) seedlings.

All independent factors (plant genotype and experiment number) as well as the interaction between them had significant influence on the studied traits ($p < 0.05$). For that reason QTLs were calculated for the data of each experiment separately. The Shapiro-Wilk test as well as skewness and kurtosis results confirmed the normal distribution of values for every experiment and each trait (Table 1), what allowed to perform further QTL analysis.

Table 1

The values range of the analyzed traits: number of leaves (NL), length of first (LFL) and second (LSL) leaf, width of first (WFL) and second (WSL) leaf, second leaf sheath length (LSSL), second leaf blade length (LSLB) and fresh mass of leaves (LFM). Experimental mean value, together with standard deviation as well as the results of the Shapiro-Wilk test, skewness and kurtosis were presented.

Trait	Exp. No.	Minimum - Maximum	Mean value $\pm$ SD	Normality	Skwesness	Kurtosis
NL	1	6–9	8 ± 0.6	0.19	-0.30	0.47
Number of leaves	2	4–8	5 ± 0.8	0.15	0.43	-0.11
	3	2–3	3 ± 0.3	0.11	-0.67	0.27
LFL	1	8.52–14.4	11.3 ± 1.2	0.23	-0.07	0.31
First leaf length (cm)	2	8.7–16.5	12.6 ± 1.5	0.25	0.14	0.24
	3	9.3–16.9	13.3 ± 1.8	0.28	-0.03	-0.60
WFL	1	2.4–4.9	4.1 ± 0.4	0.16	-0.37	0.13
First leaf width (cm)	2	2.8–5.1	4.1 ± 0.3	0.12	-0.45	0.12
	3	3.4–5.1	4.3 ± 0.3	0.12	-0.05	0.26
LSL	1	16.2–35.5	25.2 ± 4.6	0.56	0.11	-0.96
Second leaf length (cm)	2	13.6–31.3	22.3 ± 3.8	0.54	0.04	-0.73
	3	12.4–28.8	21.5 ± 3.4	0.49	0.20	-0.28
WSL	1	3.3–6.7	5.7 ± 0.6	0.17	-0.15	0.96
Second leaf width (cm)	2	3.2–6.2	4.9 ± 0.6	0.14	-0.15	0.12
	3	2.4–5.8	4.6 ± 0.5	0.13	-0.70	0.60
LSSL	1	0.9–6.1	3.6 ± 0.8	0.18	-0.25	0.38
Second leaf sheath length (cm)	2	1.9–5.5	3.5 ± 0.9	0.15	-0.01	-0.82
	3	1.6–7.7	4.2 ± 1.2	0.17	0.12	0.02
LSLB	1	14.7–29.6	21.6 ± 3.9	0.48	0.15	-0.93
Second leaf blade length (cm)	2	11.7–26.4	18.8 ± 2.9	0.43	0.09	-0.45
	3	9.7–22.8	17.4 ± 2.4	0.37	-0.24	0.17
LFM	1	0.1–0.9	0.4 ± 0.2	0.14	0.05	-0.61
Fresh mass of leaves (g)	2	0.1–1.1	0.6 ± 0.2	0.09	0.21	0.35
	3	0.2–1.1	0.6 ± 0.2	0.09	0.17	0.17

QTL analyses revealed 22 *loci* identified by a composite interval mapping (CIM) with LOD value ≥ 3.0 which appeared in at least two experiments with range common for all analyzed traits (in cM). They included: 2 *loci* for the first leaf length (LFL), 3 *loci* for the first leaf width (WFL), 5 *loci* for the second leaf length (LSL), 3 *loci* for the second leaf width (WSL), 4 *loci* for the second leaf sheath length (LSSL) and 5 *loci* for second leaf blade length (LSLB) (Table 3). Additionally, 18 *loci* of QTL regions with LOD value ≥ 3.0 are presented for all analyzed traits (Table 1S): 4 *loci* for the number of leaves (NL), 3 *loci* for the first leaf length (LFL), 2 *loci* for the first leaf width (WFL), 2 *loci* for the second leaf length (LSL), 2 *loci* for the second leaf width (WSL), 1 *locus* for the second leaf blade length (LSLB) and 4 *loci* for the fresh mass of the leaves (LFM). Those 18 *loci* were specific only for one trait and appeared in one or two experiments.

Table 2

Correlation coefficients between the number of leaves (NL), length of first (LFL) and second (LSL) leaf, width of first (WFL) and second (WSL) leaf, second leaf sheath length (LSSL), second leaf blade length (LSLB) and the fresh mass of leaves (LFM) in a DH 'Hewo' x 'Magnat' winter triticale mapping population evaluated in three different experiments.

	LFL	WFL	LSL	WSL	NL	LSLB	LSSL
WFL	0.428*						
LSL	0.676*	0.238***					
WSL	0.293**	0.689*	0.171**				
NL	-0.050**	0.004***	-0.062**	0.332*			
LSLB	0.672*	0.212***	0.992*	0.152**	-0.036***		
LSSL	0.637*	0.271**	0.943*	0.161**	-0.145**	0.895*	
LFM	-0.291***	-0.107**	-0.168***	-0.014***	0.048**	-0.171**	-0.145***
*, **, *** - significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.							

Table 3

The characteristics of the most significant QTL regions identified by CIM method for the first leaf length (LFL), first leaf width (WFL), second leaf length (LSL), second leaf width (WSL), second leaf sheath length (LSSL) and second leaf blade length (LSLB) in DH 'Hewo' x 'Magnat' mapping population.

QTL name	Flanking markers ^a (position in cM)	Exp. No	LOD	LOD max. position	Marker closest to the LOD peak	R ² (%) ^b	Add ^c	Favorable allele ^d
First leaf length (LFL)								
Qlfl.hm.4R.1	4345445 : <i>rPt-402563</i> (141.4–149.5)	2	4.4	142.7	4200451	15.1	-6.3	M
Qlfl.hm.6R.2	3623117 : 3045717 (18.7–39.5)	3	4.3	18.7	3623117	16.3	7.3	H
First leaf width (WFL)								
Qwfl.hm.1R.1	4349664 : 4204964 (123.6–146.3)	3	4.3	123.6	4349664	14.9	19.0	H
			5.3	136.9	<i>rPt-507790</i>	16.8	19.8	H
			4.4	143.8	3609054	15.0	18.6	H
Qwfl.hm.4R.1	3611142 : 3620564 (132.8–160.8)	1	4.0	132.8	3611142	14.8	-21.1	M
			4.2	143.9	4355123	14.9	-20.5	M
			3.5	152.9	3043032	13.2	-19.6	M
Qwfl.hm.4R.2	3620564 : 4371409 (160.8–175.8)	3	5.8	168.7	<i>rPt-509173</i>	18.5	-21.0	M
Second leaf length (LSL)								
Qlsl.hm.4B.1	4218883 : 3046835 (0.0–17.6)	2	4.0	6.3	4213221	5.1	9.1	H
			3.9	15.0	6044038	5.0	9.3	H
Qlsl.hm.5R.1	<i>rPt-506433</i> : <i>rPt-507500</i> (0.0–12.0)	1	31.1	6.8	<i>rPt-390294</i>	6.9	3.8	H

a – an identifiable region of the QTL defined by the first and last marker of the QTL region; b – R² (%) – percentage of the phenotypic variance explained by the QTL; c – Add – additive effect; d - favorable allele for each QTL: H - cv. Hewo and M - cv. Magnat.

QTL name	Flanking markers ^a (position in cM)	Exp. No	LOD	LOD max. position	Marker closest to the LOD peak	R ² (%) _b	Add _c	Favorable allele ^d
Qlsl.hm.5R.2	4205026 : 3610765 (27.6–35.6)	2	23.4	29.8	3040546	6.5	3.2	H
Qlsl.hm.5R.3	4339461 : 3610765 (22.0–35.6)	3	13.6	24.2	4343102	4.3	2.3	H
			14.0	29.8	3040546	4.3	2.3	H
Qlsl.hm.6R.3	3623117 : 3045717 (18.7–39.5)	3	3.6	35.5	3617691	8.1	1.1	H
Second leaf width (WSL)								
Qwsl.hm.4R.1	wPt-4487 : 304302 (119.8–152.9)	1	3.9	141.4	4345445	13.9	-23.2	M
			3.6	150.6	rPt-390504	12.5	-22.1	M
Qwsl.hm.4R.2	rPt-509173 : 3603973 (168.7–173.2)	3	5.2	170.9	rPt-505225	20.6	-27.5	M
			5.2	175.8	4371409	21.7	-27.8	M
Qwsl.hm.6R.1	4213428 : 4202378 (365.1–383.1)	1	3.5	365.1	4213428	12.5	-23.9	M
			4.1	379.7	4201137	14.3	-25.0	M
Second leaf sheath length (LSSL)								
Qlssl.hm.1R.1	rPt-507790 : 4215936 (135.9–151.4)	1	4.3	149.9	3603565	6.5	-25.7	M
Qlssl.hm.5R.1	rPt-506433 : rPt-507500 (0.0–12.0)	1	23.2	6.8	rPt-390294	6.2	7.6	H
Qlssl.hm.5R.2	4343102 : 3047417 (24.2–31.4)	2	22.2	29.8	3040546	5.7	7.6	H
Qlssl.hm.5R.3	4343102 : 3047417 (24.2–31.4)	3	15.7	29.8	3040546	3.9	8.1	H

a – an identifiable region of the QTL defined by the first and last marker of the QTL region; b – R² (%) – percentage of the phenotypic variance explained by the QTL; c – Add – additive effect; d - favorable allele for each QTL: H - cv. Hewo and M - cv. Magnat.

QTL name	Flanking markers ^a (position in cM)	Exp. No	LOD	LOD max. position	Marker closest to the LOD peak	R ² (%) _b	Add _c	Favorable allele ^d
Second leaf blade length (LSLB)								
Qlslb.hm.4B.1	4218883 : 3046835 (0.0–17.6)	2	5.6	0.0	4218883	8.6	8.9	H
			5.7	15.0	3044038	8.4	8.8	H
Qlslb.hm.5R.1	rPt-506433 : rPt-507500 (0.0–12.0)	1	27.6	7.9	rPt-390522	6.8	3.1	H
Qlslb.hm.5R.2	4339461 : 3610765 (22.0–35.6)	2	21.6	29.8	3040546	5.3	2.3	H
Qlslb.hm.6R.1	4213428 : 4202378 (365.1–383.1)	2	4.1	377.5	rPt-505870	5.8	7.9	H
Qlslb.hm.6R.2	3623117 : 3045715 (18.7–39.5)	3	3.9	30.9	3606053	12.2	9.2	H
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 - favorable allele for each QTL: H - cv. Hewo and M - cv. Magnat.								

Number of leaves (NL)

The number of seedling leaves ranged from 4 to 9 in cold-hardened plants (Exp. 1 and 2, Table 1) as well as from 2 to 3 in unhardened ones (Exp. 3, Table 1). For this trait, four QTL regions were identified in Experiments 1 and 2 on chromosomes 2A, 3A, 6R and 7B (Table 1S). Those regions explained from 12.6–19.3% of phenotypic variation for *Qnl.hm.7B.1 locus* (Table 1S). For same *locus* the highest LOD value (5.4) was observed (Table 1S).

First leaf length (LFL)

The first leaf length ranged from 8.5 cm (Exp. 1) to 16.9 cm (Exp. 3). The longest first leaf was observed in unhardened, 4-weeks-old seedlings (Table 1). Five QTL regions were found for this trait (Table 3, Table 1S). Two of them: *Qlfl.hm.4R.1* and *Qlfl.hm.6R.2 loci* cover common region of 4R and 6R chromosomes identified for other analyzed traits; remaining *Qlfl.hm.3A.1*, *Qlfl.hm.6R.1* and *Qlfl.hm.7B.1* were specific only for LFL trait (Table 1S). The highest LOD value (5.0) was observed for *Qlfl.hm.7B.1* (Table 1S) and the highest explained phenotypic variation (16.3%) for *Qlfl.hm.6R.2* (Table 3).

First leaf width (WFL)

The first leaf width ranged from 2.4 cm (Exp. 1) to 5.1 (Exp. 2 and 3) (Table 1). For this trait, five QTL regions were identified (Table 3, Table 1S). Three of them were considered as common for other analyzed traits (*Qwfl.hm.1R.1*, *Qwfl.hm.4R.1*, *Qwfl.hm.4R.2*, Table 3). Only two regions were specific for WFL (Table 1S). Identified *loci* referred to Exp. 1 and 3. For *Qwfl.hm.1R.1 locus*, the LOD was peaked in four different markers assigned to chromosome 1R (Table 3). The highest LOD value (5.8) and explained phenotypic variation (18.5%) was observed for *Qwfl.hm.4R.2 locus* (Table 3).

Second leaf length (LSL)

The longest second leaf was observed in Exp. 1 in cold-hardened plants (25.2 cm), while Exp. 3 mean value was 21.5 cm (Table 1). For this trait, seven QTL regions were identified in all experiments and five of them were common for other traits (Table 3, Table 1S). The most interesting LSL *loci* were found on rye chromosomes 5R and 6R for which very high LOD values (23.4, 31.3 and 35.5) for *Qlsl.hm.5R.2*, *Qlsl.hm.5R.1* and *Qlsl.hm.6R.3* were observed, respectively (Table 3). Positive allele effect of all those *loci* referred to cv. 'Hewo'. The phenotypic variation of this trait was in range from 4.4% for *Qlsl.hm.5R.3* to 8.1% for *Qlsl.hm.6R.3* (Table 3).

Second leaf width (WSL)

The mean value of second leaf width was similar in Exp. 2 and 3 (4.9 cm and 4.6 cm, accordingly) and higher in Exp. 1 (5.7 cm, Table 1). Five *loci* were identified but only three were considered as common for all other traits; they were found on rye chromosomes 4R and 6R (Table 3, Table 1S). *Loci* specified for WSL were identified on wheat chromosome 1A as well as rye chromosome 3R (Table 1S). For all *loci*, negative allele effect referred to cv. 'Magnat'. The highest phenotypic variation was observed for *Qwsl.hm.4R.2* (21.7%) and the highest LOD value for *Qwsl.hm.1A.1* and *Qwsl.hm.4R.2 loci* (Table 3, Tab. S1).

Second leaf sheath length (LSSL)

The longest second leaf sheath (4.2. cm) was observed in unhardened plants in Exp. 3 (Table 1); for plants in Exp. 1 and 2 the length was similar (3.6 and 3.5, accordingly, Table 1). For this trait, four QTL regions were identified on rye chromosomes 1R and 5R and all of those regions were common for other analyzed traits (Table 3). The most interesting *loci* were found on chromosome 5R with the highest LOD value (23.2) for *Qlssl.hm.5R.1* (Table 3). The phenotypic variation for this trait explained 6.5% for *Qlssl.hm.1R.1 locus*. Positive allele effect of *loci* identified on chromosome 5R referred to cv. 'Hewo' whereas negative allele effect on of *locus* on 1R referred to cv. 'Magnat'.

Second leaf blade length (LSSB)

The longest second leaf blade was observed in Exp. 1 (21.6 cm, Table 1) comparing to blades in Exp. 2 and 3 (18.8 and 17.4 cm, Table 1). Six QTL regions were identified for this trait and only one *locus* *Qlslb.hm.5R.3* was specific (Table 1S). All *loci* for this trait were found on chromosomes 4B, 5R and 6R (Table 3, Table 1S). The most interesting *loci* were *Qlslb.hm.5R.1* and *Qlslb.hm.5R.2* for which LOD value

was very high (27.6 and 21.5, respectively, Table 3). Positive allele effect of all those *loci* referred to cv. 'Hewo'. The highest explained phenotypic variation (12.2%) was found for *Qlsb.hm.6R.2* (Table 3).

Comparison of common QTL regions

22 out of total 40 *loci* identified in at least two experiments were common for all analyzed traits (Table 3). Those *loci* were identified on wheat chromosome 4B and on rye chromosomes 1R, 4R, 5R and 6R (Table 3).

On chromosome 4B, two *loci* were found for LSL and LSLB in Exp. 2 - *Qisl.hm.4B.1* and *Qlsb.hm.4B.1*, respectively (Table 3). Between those traits high correlation (0.992) was also observed (Table 2). Those QTL regions were in the same position on this chromosome, from 0.0 cM to 17.6 cM. In both regions, the CIM peak showed two markers and one of them, *3044038* was common for both QTL regions. The maximum LOD position was 5.6 and 5.7 for *Qlsb.hm.4B.1* (Table 3). Positive allele effect of those *loci* referred to cv. 'Hewo'.

On chromosome 1R, two *loci* for WFL and LSSL were identified in Exp. 1 and 3 - *Qwfl.hm.1R.1* and *Qlssl.hm.1R.1* between 123.6 cM and 151.4 cM (Table 3). For *Qwfl.hm.1R.1* LOD peak revealed four markers with maximum LOD value 5.3 and it explained 16.8% of phenotypic variation (Table 3).

On chromosome 4R, five QTL regions were found for WFL, WSL and LFL in all experiments - *Qwfl.hm.4R.2* and *Qwsl.hm.4R.2* between 160.8 cM and 175.8 cM as well as *Qlfl.hm.4R.1*, *Qwfl.hm.4R.1* and *Qwsl.hm.4R.1* between 119.8 cM and 160.8 cM (Table 3). The correlation between WFL and WSL had high (0.689), and between WFL and LFL medium (0.428) value (Table 2). The highest LOD value was observed for *Qlfl.hm.4R.1* (4.4) and *Qwfl.hm.4R.2* (5.8). Negative allele effect of those *loci* referred to cv. 'Magnat' (Table 3). All of those *loci* explained 12.5% – 21.7% of phenotypic variation. For *Qwfl.hm.4R.1* and *Qwsl.hm.4R.1* maximum LOD peak was for two markers located near to each other on chromosome 4R (Table 3).

On rye chromosome 5R, eight QTL regions were detected in two different position (Table 3). In first 0.0–12.0 cM region, three *loci*: *Qisl.hm.5R.1*, *Qlssl.hm.5R.1* and *Qlsb.hm.5R.1* were found for LSL, LSSL and LSLB in Exp. 1. For those regions very high LOD values was observed, with maximum (31.1) value observed for *Qisl.hm.5R.1* (Table 3). The same *rPt-390294* peak marker was detected for *Qisl.hm.5R.1* and *Qlssl.hm.5R.1* (Table 3). The high correlation between LSL, LSSL and LSLB was found (Table 2). Second region identified on chromosome 5R was located between 22.0 cM and 35.6 cM. It contained five QTLs for LSL, LSSL and LSLB identified in Exp. 2 and Exp. 3 (Table 3). The LOD peak in the same marker *3040546* was observed for *loci* *Qisl.hm.5R.2*, *Qisl.hm.5R.3*, *Qlssl.hm.5R.2*, *Qlssl.hm.5R.3* and *Qlsb.hm.5R.2* (Table 3). Those *loci* were also characterized by high LOD value, with the maximum 23.4 value for *Qisl.hm.5R.2*. Positive allele effect of all those *loci* referred to cv. 'Hewo'.

Two regions of QTL common for more than one trait were found on chromosome 6R. First, containing three *loci* – *Qlfl.hm.6R.2*, *Qisl.hm.6R.3* and *Qlsb.hm.6R.2* for LFL, LSL and LSLB in Exp. 3 was located between 18.7 cM and 39.4 cM (Table 3). High correlation between those traits was also observed

(Table 2). LOD value was the highest for *Qlfl.hm.6R.2*. Positive allele effect of all those *loci* referred to cv. 'Hewo' (Table 3). In second region, two *loci* - *Qwsl.hm.6R.1* and *Qlslb.hm.6R.1* for WSL and LSLB were found in Exp. 1 and Exp. 2 on chromosome 6R, between 365.1 cM and 383.1 cM and with LOD value 4.1 (Table 3).

Discussion

The vitality and development in the seedling stage is very important in plant's life cycle, especially for winter cereals. It has been reported that rapid seedling establishment and early growth are important traits for improving yield (Von Arnim and Deng 1996, Aparicio et al. 2002). Localization of QTL regions in the cereal seedling stage is not often reported yet. In our study, analysed traits were selected on the basis of their potential role in seedlings ability to overwinter as well as potential use as an indicator of tillering and future yield early evaluation. According to our knowledge, this is the first research which describes localization of triticale genomic regions associated with leaf development in the seedling stage.

The morphology of winter cereals seedling can be correlated with tolerance to freezing and fungal infection. Number of leaves, leaf length and width are important in spreading fungal pathogens and as a main assimilating organ, largely determine the size of the future yield (Nalborczyk et al. 1995; Aparicio et al. 2002; Kobayashi et al. 2003). During 2-year experiments on unhardened and cold-hardened triticale seedlings, we localized 40 *loci* for all analysed traits on 15 chromosomes from all homologous groups: A (6 *loci*), B (7 *loci*), R (27 *loci*) (Table 3, Tab. S1, Fig. 1). Among those 40 *loci*, we selected 22 *loci* on chromosomes 4B, 1R, 4R, 5R and 6R which were common for all analyzed traits (Table 3, Fig. 1).

On wheat chromosome 4B two *loci* *Qlsl.hm.4B.1* and *Qlslb.hm.4B.1* for second leaf length and second leaf blade length were identified, respectively. Both *loci* were on the same position on this chromosome (Table 3, Fig. 1). Kruse et al. (2017) also identified QTL region on chromosome 4B associated with wheat freezing tolerance on position 8.8 cM – 18.9 cM which was very similar to the position of *loci* found in our research (0.0 cM – 17.6 cM, Table 3, Fig. 1). Yuan et al. (2017) analyzed morphological traits in the seedling and adult plant stage using RIL wheat population described several QTL clusters on 4B chromosome associated with plant height and biomass. Similarly, Zhang et al. (2008) reported wheat QTL regions on chromosome 4B, associated with plant height in DH Huapei 3/Yumai 57 population. Additionally, Li et al. (2016) identified *locus* *QLr.stars-4BL1* associated with seedling leaf rust resistance.

In present research, most of QTLs common for all analysed traits were located on rye chromosomes 1R, 4R, 5R and 6R (Table 3, Fig. 1). *Loci* *Qwfl.hm.1R.1* and *Qlssl.hm.1R.1* were localized on chromosome 1R for the first leaf width of and the second leaf sheath length in cold-hardened plants. Miedaner et al. (2012) localized several QTL regions on chromosome 1R related with important agronomic traits on rye like plant height, grain yield and 1000-kernel weight. Furthermore, Masojć et al. (2007) and Milczarski et al. (2017) revealed significant QTL region on long arm of 1R chromosome associated with preharvest sprouting resistance in rye. On chromosome 4R we found five *loci* for the first leaf length and width as well as the second leaf width both in hardened and unhardened plants (Table 3, Fig. 1). *Loci*

Qwfl.hm.4R.2 and *Qwsl.hm.4R.2* were located in similar position on chromosome 4R in unhardened plants; additionally *loci Qlfl.hm.4R.1*, *Qwfl.hm.4R.1* and *Qwsl.hm.4R.1* were located in similar position in hardened plants (Table 3, Fig. 1). On this chromosome Miedaner et al. (2012) localized QTLs for plant height and 1000-kernel weight in rye. In triticale, Dyda et al. (2019) localized on 4R chromosome QTL regions of leaf damage and F_v/F_m chlorophyll fluorescence parameter both in hardened and unhardened plants submitted to *Microdochium nivale* infection. In our study, eight QTL regions were detected on chromosome 5R for second leaf length, the second leaf sheath length and the second leaf blade length both in hardened and unhardened plants (Table 3, Fig. 1). All of those *loci* were characterized with very high LOD value, especially for *Qlsl.hm.5R.1* (Table 3). Chromosome 5R was also described as a chromosome associated with rye preharvest sprouting resistance (Masojć et al. 2007, 2009, Myśków et al. 2010, Milczarski et al. 2017). In our previous research on winter triticale genome regions associated with adult plants traits we detected nine QTL regions on chromosome 5R (Wajdzik et al. 2019). Three of them, *Qstl.hm.5R.1*, *Qph.hm.5R.1* and *Qtkw.hm.5R.1* for the straw length, plant height and thousand kernel weight, respectively, were detected in similar position to all QTL regions described in this paper (Table 3, Fig. 1). Additionally, the same marker *rPt-390522* was indicated as the nearest to the QTL peaks both in Wajdzik et al. (2019) and present study. It might be a significant correlation between genomic regions detected for the second leaf length and second leaf blade length in the seedling stage and the straw length and plant height in the adult stage. On chromosome 5R Miedaner et al. (2012) localized *loci* for plant height and 1000-kernel weight in rye which was also confirmed by Miedaner et al. (2018) who identified QTL regions on chromosome 5R for plant height in two growth stages. Myśków et al. (2018) identified on chromosome 5R *loci* associated with leaf rolling as the response of water deficit in a similar position to *loci* identified in our study. Furthermore, Dyda et al. (2019) identified *loci* associated with F_v/F_m in similar position to *loci* described in this paper in cold-hardened triticale inoculated with *M. nivale*. On chromosome 6R we detected *loci* of the first and second leaf length, the second leaf width and the second leaf blade length (Table 3, Fig. 1). Three *loci* – *Qlfl.hm.6R.2*, *Qlsl.hm.6R.3* and *Qlslb.hm.6R.2* which explained up to 16.3% of phenotypic variation, were in the same cM position and were found in Exp. 3 in unhardened seedlings (Table 3, Fig. 1). In similar position, Miedaner et al. (2012) identified *loci* associated with plant height. On the same chromosome, *loci* for preharvest sprouting were also identified (Masojć et al. 2007, 2009, Milczarski et al. 2017). Dyda et al. (2019) on 6R chromosome identified *loci* for F_v/F_m after triticale seedlings infection with three different *M. nivale* strains. Additionally, *loci* for leaf rolling in rye (Myśków et al. 2018) and yield components (Börner et al. 2000) were identified also on 6R chromosome.

In conclusion, the most significant *loci* identified in this research were located on chromosomes 4B, 1R, 4R, 5R and 6R. All those regions had a high LOD value for *Qlsl.hm.5R.1* and high phenotypic variation for *Qwsl.hm.4R.2*. Up to date this is the first paper describing QTL regions associated with leaf development in winter triticale seedling after and without cold-hardening process. Based on our results, identified *loci* can be correlated in future with seedling freezing and fungal infection tolerance.

Declarations

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Contributions: KW plant material, QTL and statistical analysis, contributing author; MD QTL and statistical analysis, contributing author; GG plant material, statistical analysis and contributing author; MW experiment design, obtaining funding, methods and text consulting.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest

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Figure 1

The interval map (cM) for chromosomes 4B, 1R, 4R, 5R and 6R of the DH 'Hewo' x 'Magnat' lines mapping population of winter triticale (xTriticosecale), with QTLs identified by CIM method for seedling traits: the first leaf length (LFL) and width (WFL), the second leaf length (LSL) and width (WSL), the second leaf sheath length (LSSL) and the second leaf blade length (LSLB). Additionally, black lines show marker closest to the LOD peak identified on each QTL.

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