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Identifying QTLs for cold-induced resistance to *Microdochium nivale* in winter triticale

Magdalena Szechyńska-Hebda[1], Maria Wędzony[1,2]*, Mirosław Tyrka[3], Gabriela Gołębiowska[1,2], Małgorzata Chrupek[1,3], Ilona Czyczyło-Mysza[1], Ewa Dubas[1], Iwona Żur[1] and Elżbieta Golemiec[1]

[1]*Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, Krakow 30-239, Poland,* [2]*Pedagogical University of Krakow, Podchorazych 2, Krakow 30-084, Poland and* [3]*Rzeszow University of Technology, Wincentego Pola 2, 35-959 Rzeszów, Poland*

Abstract

Snow mould caused by *Microdochium nivale* (Fr.) Samuels & Hallett is the most widespread seedling disease in winter cereals. Due to the complexity of the resistance mechanisms, a poorly understood genetic background and strong interaction with winter weather conditions, it is difficult to assess the resistance of triticale cultivars via conventional inoculation methods. Genetic resistance is the most economical and environmental friendly way to control *M. nivale* infection; therefore, the objective of this study was to detect the quantitative trait loci (QTLs) associated with resistance components of winter triticale in a mapping population derived from a cross of the 'Modus' (partly resistant) and 'SaKa 3006' (sensitive) varieties. High-resolution mapping was conducted by using 1518 molecular markers (diversity arrays technology, simple sequence repeat and amplified fragment length polymorphism). Partial resistance components assessed in this study, i.e. candidate QTLs, were detected on chromosomes 1B, 2A, 3A, 3B, 5A, 5B, 6A, 6B and 7B, whereas QTLs describing overall seedling vitality in non-infected control plants were located on chromosomes 1B, 2B, 3A, 5A, 7B and 7R.

Keywords: disease resistance; *Microdochium nivale*; quantitative trait loci; seedling vitality; triticale

Introduction

Microdochium nivale (Fr.) Samuels & Hallett is the most widespread snow mould fungus. It is a pathogen-caused disease which develops under high humidity and low temperature, and results in major damage to economically important winter cereals and grasses. It is the source of seedling blight, stem rot, leaf blotch and a part of the disease complex called 'fusarium head blight'. Genetic resistance is the most economical and environmental friendly way to control *M. nivale* infection. Triticale has been suggested as a source of disease resistance genes since it is a bridging species for the transfer of such genes to wheat and rye (Kuleung *et al*., 2004). However, even resistance of the best triticale cultivars is far from satisfactory. According to Nakajima and Abe (1996), Ergon *et al.* (1998), Tronsmo *et al*. (2001), and from our own experiments (Gołębiowska and Wędzony, 2009), cold-hardening is the factor switching on cereal defence responses to *M. nivale* infection. The mechanisms of plant resistance induced by cold have not yet been recognised and the genotypes differ in their ability to obtain resistance expression (Hömmo, 1996; Tronsmo, 1994; Pulli *et al*., 1996). Thus, it is important to identify the genetic background and the markers that can be exploited to increase natural resistance via marker-assisted selection. Moreover, the most agronomically important characters in cereals, including resistance, are classified as polygenic or quantitative (Reszka *et al*., 2007; Carter *et al*., 2009). Therefore, we present the results of studies in which we tried to identify QTLs related to seedling survival and its vitality while recovering from snow mould infection in triticale.

* Corresponding author. E-mail: niwedzon@cyf-kr.edu.pl

The studies exploit the first triticale marker map (paper in preparation) and the mapping doubled haploids (DH) population being developed at SPB Institute (Germany) by Dr. Eva Bauer.

Materials and methods

The mapping population consisting of 90 DH lines was derived by the maize method (Wędzony *et al*., 1998; Wędzony, 2003) from triticale (× *Triticosecale Wittmack*) F_1 generation of 'Modus' (partially resistant) × 'SaKa3006' (sensitive) (Fig. 1).

Seedlings were planted in multipots in a randomised complete block design with three replicates. Plants grew in a chamber at 16/12°C, 10/12 h light/darkness for 2 weeks. Then they were subjected to a pre-hardening period (12°C for 7 d) and hardening (2°C for 28 d) in the same light regime. Hardened seedlings were inoculated with soil-borne mycelium of *M. nivale* (monosporal isolate no. 38z/5a/01). Then the plants were covered with moist paper and black plastic bags to imitate a snow cover and to keep high humidity in darkness at 2°C. The control plants were treated the same way, except for the infection. The covers were removed after 21 d of incubation and the seedlings were moved for 2 weeks to optimal conditions for recovery. Five traits describing seedling survival and vigour were selected for analysis (Table 1).

QTLs were identified using Windows QTLCartographer 2.5 and the results were analysed using composite interval mapping (CIM). Threshold logarithm of the odds (LOD) scores were calculated based on 1000 permutations. A QTL was accepted when the LOD score was greater than 3.

Results and discussion

Genetic maps are the fundamental tools that identify the features of phenotypes that are linked to specific genetic loci, including those that influence QTLs. Over 40 maps with at least 300 markers have been published for different *Triticeae* populations; however, to date, only a few rye and one partial triticale map with more than 300 markers have been produced (Gonzalez *et al*., 2005; Lehmensiek *et al*., 2009). Therefore, reports identifying and mapping QTLs in triticale are limited (Reszka *et al*., 2007; Hura *et al*., 2009). In our research, the population of 90 DH lines derived from the F1 hybrid of triticale 'Modus' and 'SaKa3006' was mapped with 1518 markers selected from a total of 1670 wheat and rye candidate markers: simple sequence repeat, amplified fragment length polymorphism and diversity arrays technology markers. The map spans a distance covering a total of 2197 cM genomic regions with an average interval of 1.44 cM between adjacent marker loci. The highest marker density was established in rye chromosomes 4R, 5R, 6R and 7R.

Resistance to *M. nivale* showed a wide variation range within the mapping population, 23% of lines had lower survival capability than resistant Magnat, 43% lines did not differ from Magnat and 20% lines fully regenerated after infection. However, CIM approaches, performed for 14 wheat and 7 rye chromosomes, revealed that reaction to snow mould in this population is not determined by 'plant survival after infection' because the QTLs were not identified. In the case of plants regenerated after infection, two QTLs were detected on chromosomes 2A and 3B (around the *Xbarc212* and *wPt-6802* regions, respectively) at a similar position for both traits concerning the

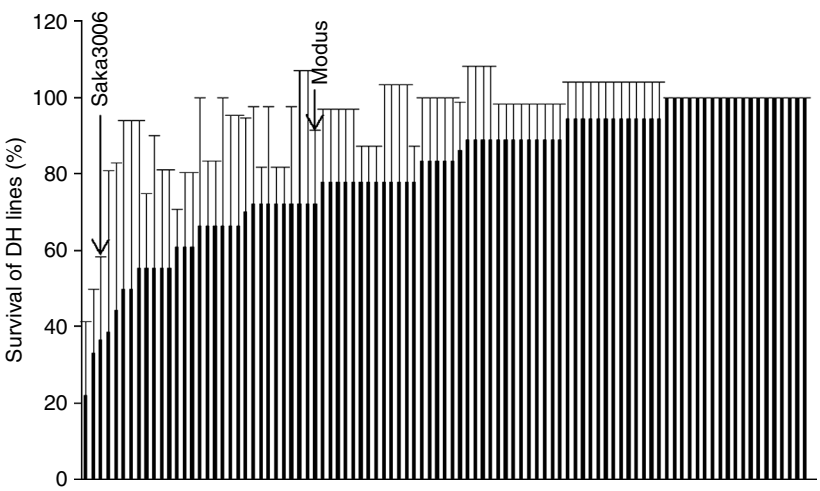

Fig. 1. Survival of doubled haploids (DH) lines selected from a triticale mapping population after infection with *M. nivale*. Parents are indicated with the arrows.

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Table 1. Results of the CIM analysis – detected main QTLs for the studied traits

Trait	QTL	Chromosome	Marker	Position QTL (cM)[a]	LOD	R^2 (%)	Add[b]
No. of regenerated leaves/planted plant – infected	1	2A	Xbarc212	5.2	3.66	11.44	0.3457
	2	3B	wPt-6802	25.9	3.61	11.29	−0.3458
	3	3B	wPt-4625	31.5	3.03	9.61	−0.3170
	4	5B	wPt-9872	32.9	3.17	10.93	0.3343
No. of regenerated leaves/ planted plant – control	1	1B	rPt-399959	87.8	7.82	27.54	0.4384
	2	7R	rPt-399750	11.3	3.85	11.61	−0.2862
Plant survival control (%)	1	2B	rPt-509666	56.7	3.42	12.57	−0.0121
No. of regenerated leaves/recovered plants – infected	1	2A	Xbarc212	5.2	6.27	20.35	0.3455
	2	3B	wPt-6802	25.9	4.58	14.22	−0.3324
No. of regenerated leaves/recovered plants – control	1	5A	Xwmc713	0	4.18	12.51	−0.2900
	2	1B	rPt-399959	83.8	7.49	25.78	0.4226
	3	7B	wPt-3723	84.1	3.36	10.01	0.2567
Height of regenerated plants – infected	1	5A	rPt-389308	7.9	6.59	21.02	0.6645
Height of regenerated plants – control	1	5A	rPt-389308	7.9	3.60	10.9	0.5601
	2	7B	wPt-9880	116.4	3.20	9.61	0.5251
Dry mass regenerated leaves – infected	1	6A	Xgwm1009	55.6	3.40	12.48	−0.0175
	2	6A	rPt-507997	64.3	5.75	20.08	−0.0255
Dry mass regenerated leaves – control	1	3A	Xcfa2193	106.9	4.54	16.47	0.0122

R^2 (%), % of phenotypic variance explained by the QTL; Add, additive effects of QTLs expressed in the trait unit.
[a] Position of QTL peak from the first marker in cMorgans. [b] A positive value means that the allele from the Modus increases the value of the trait.

number of regenerated leaves (traits 1 and 4, Table 1), with LOD from 3.03 to 6.27. Other QTLs for trait 1 were discovered on chromosome 3B (near *wPt-4625*) and 5B (near *wPt-9872*), which explained 9.61 and 10.93% of the phenotypic variation. The QTLs on chromosomes 3B and 5B were consistent with reports of resistance to *Stagonospora nodorum* blotch disease in triticale and wheat (Reszka *et al.*, 2007). The nearby region between the loci *Xgwm533.1* and *Xgwm493* on chromosome 3B was shown to harbour a QTL related to *Fusarium* head blight resistance in wheat cultivars Ning894037 and Wangshuibai (Jia *et al.*, 2005; Shen *et al.*, 2006). Carter *et al.* (2009) mapped the putative QTLs on 3B wheat chromosome, which is significantly associated with resistance to stripe rust. These coincidences suggest the possibility that resistance to certain fungal diseases may be controlled by the same resistance loci in wheat and triticale.

QTL distribution differed for the control plants. One QTL for traits that related the number of leaves was mapped on chromosome 1B with R^2 values of 27.5 (trait 2, Table 1) and 25.8% (trait 5, Table 1). The presence of one QTL on chromosome 7R (near *rPt-399750)* for trait 2 and two QTLs on chromosomes 5A and 7B for trait 5 were also identified.

QTLs controlling variation in 'height of regenerated plant' (traits 6 and 7), each showing significant LOD values (about 3 for control and 6.6 for infected plants), were mapped on wheat chromosome 5A. Another QTL for the trait 'height of regenerated plant-control', related rather to seedling morphology than disease resistance, was located on chromosome 7B. Unlike what was reported in rye (Börner *et al.*, 2000), we detected one QTL for height on chromosome 5R, which confirmed that the results obtained depend on the population. Zhang *et al.* (2008) constructed a linkage map for the DH lines of the Huapei3/Yumai57 population and they detected four additive QTLs and five pairs of epistatic effects for plant height on chromosomes 3A, 4B, 4D, 5A, 6A, 7B and 7D. However, within the population of Hanxuan10/Lumai14, three additive QTLs significantly affecting plant height were found on chromosomes 1B, 4D and 5B, and three pairs of epistatic QTLs were found on chromosomes 1B–1B, 2A–2D, 2D–5B (Wang *et al.*, 2010). Candidate QTLs for other assessed components that could be related to resistance in this study were detected on chromosomes 1B, 2A, 2B, 3A, 6A, 6B and 7B (Table 1).

In conclusion, the results were consistent with the observation that plant resistance to *M. nivale* is polygenic in nature. Principal component analysis showed that 95.7% of the variation can be explained by the interaction of five independent factors. Finally, although the triticale are hexaploids composed of genomes from durum

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wheat (AABB) and rye (RR), it seems that seedling vitality is mainly controlled by the wheat genome.

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References

Börner V, Korzun AV, Voylokov AJ, Worland W and Weber E (2000) Genetic mapping of quantitative trait loci in rye (*Secale cereale* L.). *Euphytica* 116: 203–209.

Carter AH, Chen XM, Garland-Campbell K and Kidwell KK (2009) Identifying QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. *Theoretical and Applied Genetics* 119: 1119–1128.

Ergon A, Klemsdal S and Tronsmo AM (1998) Interactions between cold hardening and *Microdochium nivale* infection on expression of pathogenesis-related genes in winter wheat. *Physiological and Molecular Plant Pathology* 53: 301–310.

Gołębiowska G and Wędzony M (2009) Cold-hardening of winter triticale (× *Triticosecale* Wittm.) results in increased resistance to pink snow mould *Microdochium nivale* (Fr., Samuels & Hallett) and genotype-dependent chlorophyll fluorescence modulations. *Acta Physiologiae Plantarum* 31: 12–19.

Gonzalez JM, Muniz LM and Jouve N (2005) Mapping of QTLs for androgenic response based on a molecular genetic map of (*Tritocosecale* Wittmack. *Genome* 48: 999–1009.

Hömmo L (1996) Effect of hardening and dehardening on snow mould (*Microdochium nivale*) resistance. *Nord Jordbruksforskning* 78: 89–92.

Hura T, Hura K and Grzesiak S (2009) Physiological and biochemical parameters for identification of QTLs controlling the winter triticale drought tolerance at the seedling stage. *Plant Physiology and Biochemistry* 47: 210–214.

Jia G, Chen P, Qin G, Bai G, Wang X, Wang S, Zhou B, Zhang S and Liu D (2005) QTLs for *Fusarium* head blight response in a wheat DH population of Wangshuibai/Alondra's'. *Euphytica* 146: 183–191.

Kuleung C, Baenziger PS and Dweikat I (2004) Transferability of SSR markers among wheat, rye, and triticale. *Theoretical and Applied Genetics* 108: 1147–1150.

Lehmensiek A, Bovill W, Wenzl P, Langridge P and Appels R (2009) Genetic mapping in the *Triticeae*. *Genetics and Genomics of the* Triticeae, vol. 7. Heidelberg/London/New York: Springer, Dordrecht, pp. 201–235.

Nakajima T and Abe J (1996) Environmental factors affecting expression of resistance to pink snow mould caused by *Microdochium nivale* in winter wheat. *Canadian Journal of Botany* 74: 1783–1788.

Pulli S, Hjortesholm K, Larsen A, Gudleifsson B, Larsson S, Kristiansson B, Hömmo LM, Tronsmo AM, Ruuth P and Kristensson K (1996) *Development and Evaluation of Laboratory Testing Methods for Winter Hardiness Breeding*, vol. 32. SLU, Alnarp: Nordic Gene Bank, pp. 1–68.

Reszka E, Song Q, Arseniuk E, Cregan PB and Ueng PP (2007) The QTL controlling partial resistance to *Stagonospora nodorum* blotch disease in winter triticale Bogo. *Plant Pathology Bulletin* 16: 161–167.

Shen X, Francki MG and Ohm HW (2006) A resistance-like gene identified by EST mapping and its association with a QTL controlling *Fusarium* head light infection on wheat chromosome 3BS. *Genome* 49: 631–635.

Tronsmo AM (1994) Effect of different cold hardening regimes on resistance to freezing on snow mould infection in timothy varieties of different origin. In: Doerffling K, Brettschneider B, Tantau H and Pithan K (eds) *Crop Adaptation to Cool Climates*. Brussels: European Commission (ECSP-EEC-EAEC), pp. 83–89.

Tronsmo AM, Hsiang T, Okuyama H and Nakajima T (2001) Low temperature diseases caused by *Microdochium nivale*. In: Iriki N, Gaudet DA, Tronsmo AM, Matsumoto N, Yoshida M and Nishimune A (eds) *Low Temperature Plant Microbe Interactions Under Snow*. Sapporo: Hokkaido National Agricultural Experimental Station, pp. 75–86.

Wang Z, Wu X, Ren Q, Chang X, Li R and Jing R (2010) QTL mapping for developmental behavior of plant height in wheat (*Triticum aestivum* L.). *Euphytica* 174: 447–458.

Wędzony M (2003) Protocol for doubled haploid production in hexaploid *Triticale* (× *Triticosecale* Wittm.) by crossing it with maize. In: Maluszynski M, Kasha KJ, Forster BP and Szarejko I (eds) *Doubled Haploid Production in Crop Plants – A Manual*. ISBN 1-4020-1544-5. Dordrecht/Boston/London: Kluwer Academic Publishers, pp. 129–134.

Wędzony M, Marcińska I, Ponitka A, Ślusarkiewicz-Jarzina A and Wozna J (1998) Production of doubled haploids in triticale (× *Triticosecale* Wittm.) by means of crosses with maize (*Zea mays* L.) using Picloram and Dicamba. *Plant Breeding* 117: 211–215.

Zhang K, Tian J, Zhao L and Wang S (2008) Mapping QTLs with epistatic effects and QTL environment interactions for plant height using a doubled haploid population in cultivated wheat. *Journal of Genetics and Genomics* 35: 119–127.