

The QTL Controlling Partial Resistance to *Stagonospora nodorum* Blotch Disease in Winter Triticale ‘Bogo’

Ewelina Reszka^{1,5}, Qijian Song^{2,3}, Edward Arseniuk¹, Perry B. Cregan³
and Peter P. Ueng^{4,5}

¹ Plant Breeding and Acclimatization Institute, Radzików, 05-870 Błonie, Poland.

² Department of Natural Resource Sciences & Landscape Architecture, University of Maryland, MD 20742, USA.

³ Soybean Genomics and Improvement Laboratory, U.S. Department of Agriculture, ARS, Beltsville, MD 20705, USA.

⁴ Molecular Plant Pathology Laboratory, U.S. Department of Agriculture, ARS, Beltsville, MD 20705, USA.

⁵ Corresponding authors: E. Reszka (E-mail: e.reszka@ihar.edu.pl; Tel: 48-22-7253611; Fax: 48-22-7254714) and P.P. Ueng (E-mail: uengp@ba.as.usda.gov; Tel: 301-504-6308; Fax: 301-504-5449)

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ABSTRACT

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Stagonospora nodorum blotch (SNB), is one of the most important foliar and glume diseases in triticale as well as other cereals. Due to the complexity of resistance mechanisms, it is difficult to assess the resistance of triticale cultivars by conventional inoculation methods. The application of marker-assisted selection in breeding is an important strategy to develop new highly resistant triticale cultivars. A population of double haploids derived from a cross between foliar susceptible cultivar Pinokio and resistant cultivar Bogo was evaluated based on responses measured by three SNB resistance components (incubation period, disease severity and latent period) at the 5th leaf stage (GS15). With polymorphic wheat and rye simple sequence repeats (SSRs) markers, three quantitative trait loci (QTLs) located on chromosomes 4B, 5B and 6A were identified. The QTLs on 5B and 6A were consistent with reports of SNB resistance at similar chromosomal locations in wheat.

Key words: triticale, *Stagonospora nodorum*, simple sequence repeat (SSR) markers

The fungus *Stagonospora nodorum* (Berk.) Castellani & Germano [syn. *Septoria nodorum* Berk; teleomorph: *Phaeosphaeria nodorum* (E. Müller) Hedjaroude, syn. *Leptosphaeria nodorum* Müller] is one of the most destructive foliar and glume fungal pathogens affecting wheat (*Triticum aestivum* L.) as well as other cereals and grasses^(17, 42, 45, 46). The *Stagonospora nodorum* blotch (SNB) disease results in significant yield reductions and economic losses in cereals worldwide⁽¹⁴⁾. Most wheat

cultivars are susceptible to SNB with the exception of a few that have partial resistance. Partial resistance is expressed as a delay of symptom development^(15, 26). The SNB disease response in certain wheat cultivars is organ specific. Seedling leaves have high levels of SNB resistance while in adult plants both leaves and glumes are susceptible to the disease⁽²³⁾. Partial resistance in glumes is moderately correlated with SNB resistance in leaves⁽⁷⁾.

Triticale (\times *Triticosecale* Wittmack) is a synthetic

hybrid derived from wheat and rye (*Secale cereale* L.). The commonly grown triticales are hexaploids composed of genomes from durum wheat (AABB) and rye (RR)⁽³⁰⁾. Triticale has been suggested as a source of disease resistance genes and provides a bridging species for the transfer of such genes to wheat⁽¹⁸⁾. However, the level of SNB resistance of most triticales cultivars is relatively low. This is one factor that negatively impacts the expansion of triticales production in Poland and around the world^(2, 36). As is true in the case of wheat, the SNB disease reaction in triticales is not only genotype-dependent but also highly influenced by the environment^(3, 35).

Since the 1980s, DNA-based markers have become a major tool for genetic analysis. Linkage maps based on simple sequence repeat (SSR) DNA markers have been developed in many major crops, including wheat^(10, 20, 27, 28, 29, 38, 40, 41). SSR markers have become an important tool for genetic segregation analysis and for marker-assisted selection. SSR markers have been used to identify the quantitative trait loci (QTL) for SNB resistance in wheat^(1, 4, 12, 19, 37). Due to the AABBRR genome composition of triticales, it should be possible to use wheat-derived and rye-derived SSR markers for the analysis of triticales and to identify QTLs controlling partial SNB resistance in triticales. Thus, the objective of this study was to detect the QTLs in winter hexaploid triticales cultivar Bogo associated with partial resistance components of SNB development using SSR DNA markers from wheat and rye.

Thirty winter triticales cultivars were evaluated for their response to SNB disease in the field in 2000. Cultivars Pinokio and Bogo had opposite disease reactions in their plant organs (Table 1). In later field and controlled environments tests, Bogo was identified with the highest SNB resistance on leaves but the lowest on heads, while Pinokio had the opposite disease resistance characteristics with the low SNB resistance on head and high resistance on the leaves (Arseniuk, unpublished). A total of 258 doubled-haploid (DH) lines were developed from anther cultures of the F₁ generation from a cross of Bogo × Pinokio.

The DH lines and two parental cultivars were evaluated for disease reaction to *S. nodorum*. The inocula were a mixture of pycnidiospores from 15 *S. nodorum* isolates, which originated from different geographical regions of Poland (Arseniuk, unpublished data). Seedlings at the 5th leaf stage (GS15) were tested for SNB disease reaction⁽⁴⁸⁾. Four consecutive experiments were conducted in growth chambers under controlled environmental conditions. Partial SNB disease resistance in cereal leaves has been divided into three components, namely length of incubation period (INC) (number of days from inoculation

to appearance of first symptoms), disease severity (DIS) (percent of leaf area with lesions) and length of latent period (LAT) (number of days from inoculation to formation of pycnidia)⁽¹¹⁾. These three defined partial resistance components were assessed in this study. The INC was checked daily following inoculation for visible chlorotic blotch. The DIS was rated two weeks after inoculation. A 0 - 9 scale (0 = resistant, no symptoms; 1 - 9 equivalent to 10 - 90% leaf necrosis coverage) was used for evaluation⁽³³⁾. On average, 10 seedlings per replicate were evaluated for INC and DIS. The latent period (LAT) was checked every other day beginning two weeks after inoculation. Five leaves per replicate were collected to assess sporulation by the detached leaf technique^(6, 14). The phenotyping test was conducted in a randomized complete block design with four replications.

In addition, 210 of the 258 DH lines and the parental cultivars were evaluated for SNB disease reactions in the field during the 2000 - 2002 growing seasons. The experiment was designed as a randomized complete block with three replications. The plants at the boots swollen (GS45) and heading (GS50) stages of development was inoculated⁽⁴⁸⁾. DIS on leaves and heads was assessed visually with the 1 - 9 scale at weekly intervals after the appearance of the first symptoms.

A total of 323 wheat and 27 rye SSR markers were tested for PCR product length polymorphisms in the two parental triticales cultivars, Pinokio and Bogo. Of the 323 wheat SSR markers, 263 were reported by Song *et al.*⁽⁴¹⁾ and 60 by Róder *et al.*⁽²⁹⁾. The rye SSRs were described by Saal and Wricke⁽³²⁾. Genomic DNA was extracted from 258 DH lines and two parental cultivars with the cetyltrimethylammonium bromide (CTAB) method^(13, 34). Equal amounts of genomic DNA from six DH lines showing extreme levels of resistance or susceptibility to SNB partial disease resistance components were combined for bulk segregant analysis (BSA)^(9, 21). The PCR products were resolved with agarose gel electrophoresis. The SSR markers which were polymorphic in any of the three sets of bulks representing partial disease resistance components and two parental cultivars were selected for segregation analysis on the complete population of DH lines. Based upon the analysis of the DH lines a genetic linkage was constructed using JoinMap[®] 4 and QTLs were mapped using an interval mapping method (MapQTL[®] 5 softwares, Kyazma, Wageningen, Netherlands)⁽⁴³⁾.

Of 323 wheat and 27 rye SSR markers, a total of 121 wheat (38%) and 5 rye (19%) markers amplified polymorphic fragments between the parental cultivars, Pinokio and Bogo. In the bulked segregation analysis, 22 SSR markers were polymorphic in at least one set of three bulks. However, none of the 22 SSR markers distinguished

Table 1. Response of winter triticale cultivars to *Stagonospora nodorum* blotch (SNB) disease under field conditions in the year of 2000

Cultivars	Plant height (cm)	Heading (days)	Disease severity (1 — 9; resistant — susceptible)	
			Leaves	Heads
Bogo	92.5	145.0	3.1	3.7
Piano	102.5	143.0	3.1	1.8
Felo	107.5	147.5	3.2	1.8
Hewo	107.5	145.0	3.3	2.2
Alzo	105.0	145.0	3.4	2.2
Eldorado	97.5	145.5	3.4	1.5
Fidelio	95.0	146.0	3.4	1.5
Ugo	115.0	141.5	3.4	1.0
Vero	112.5	142.0	3.4	1.7
Lamberto	95.0	143.0	3.5	0.8
Prego	97.5	146.0	3.5	2.2
Anwo	97.5	145.5	3.6	2.0
Marko	100.0	143.0	3.6	2.2
Moniko	112.5	144.0	3.6	2.5
Presto	112.5	139.0	3.6	2.0
Tewo	95.0	145.0	3.6	1.5
Tornado	102.5	145.0	3.6	2.2
Almo	97.5	145.0	3.7	1.2
Disco	107.5	143.0	3.7	1.5
Lasko	102.5	145.0	3.7	1.7
Kitaro	100.0	139.0	3.7	2.0
Krakowiak	97.5	145.0	3.7	2.0
Dagro	110.0	142.5	3.8	1.7
Moreno	97.5	145.0	3.9	1.8
Nemo	100.0	144.0	3.9	1.7
Prado	97.5	144.0	3.9	1.0
Salvo	102.5	145.0	3.9	0.7
Mundo	105.0	138.0	4.0	1.0
Pinokio	92.5	150.5	4.2	2.2
Malno	110.0	143.0	4.5	1.5
Minimum	92.5	138.0	3.1	0.7
Maximum	115.0	150.5	4.5	3.7
LSD _{0.05}	-	-	0.3	0.5
CV%			12.61	23.5

Matrix of correlation coefficients

	Height	Heading	LeavesE
Heading	-0,491	1	
Leaves	-0,023	-0,034	1
Heads	-0,123	0,234	-0,339

$P_{28;0.01} = 0.463$; $P_{28;0.05} = 0.361$.

all six individual resistant *versus* susceptible DH lines. With logarithm of odds (LOD) scores higher than 2.0, a total of nine (Xbarc77, Xbarc84, Xbarc249, Xbarc229, Xbarc344, Xbarc164, Xbarc132, Xbarc203 and Xbarc251), five (Xbarc193, Xgwm149, Xbarc106, Xgwm495 and Xgwm513) and eight (Xbarc107, Xbarc171, Xbarc195, Xbarc104, Xbarc206, Xgwm570,

Xgwm169 and Xgwm617) SSR markers associated with SNB resistance were assigned to chromosomes 3B, 4B and 6A, respectively. Due to their distorted segregation from the expected 1:1 ratio, Xbarc249 ($\chi^2 = 12.16$), Xbarc251 ($\chi^2 = 11.57$), Xbarc104 ($\chi^2 = 59.60$), Xgwm169 ($\chi^2 = 24.14$), and Xgwm617 ($\chi^2 = 17.24$) were excluded from the QTL analysis.

Three putative QTLs significantly associated with partial resistance components were mapped on chromosomes 3B, 4B and 6A (Fig. 1). The results were consistent with the observation that partial SNB resistance in wheat is polygenic in nature^(8, 24, 25, 47). A major QTL was detected on chromosome 4B, which was strongly associated with the three SNB resistance components. The QTL on chromosome 4B explained 19%, 30% and 14% of phenotypic variation for INC, DIS and LAT, respectively. The QTL on chromosome 3B was related to two partial resistance components (DIS and LAT), and explained 10%, and 14% of the phenotypic variation for DIS and LAT, respectively. The putative QTL on chromosome 6A explained 6% of the phenotypic variation for INC but was not associated with the other two partial resistance components, DIS and LAT (Fig. 1). QTLs associated with SNB partial resistance on wheat chromosomes 3B and 6A have been confirmed in several wheat cultivars^(4, 12).

It appears that the QTL on the chromosome 3B (*Xbarc132* and *Xbarc344*) in triticale cultivar Bogo was not well defined, mainly due to the lack of polymorphic SSR markers in the region (Fig 1). This QTL was reported in two SNB partially resistant wheat cultivars Arina and Liwilla and was located at the distal end of chromosome 3B between *Xbcd907* and *XbarcC147* loci^(12, 37, 41). Recently, the nearby region between the loci *Xgwm533.1* and *Xgwm493* on chromosome 3B was shown to harbor a QTL related to partial *Fusarium* head blight (FHB) resistance in two wheat cultivars Ning894037 and Wangshuibai^(16, 39). The QTL in the region between *Xgwm251* and *Xgwm538* was reported to be responsible for SNB resistance on chromosome 4B in Swiss wheat cultivar Arina⁽³⁷⁾. The region was different from what was detected in triticale cultivar Bogo (Fig. 1). The QTL region (*Xgwm368*, *Xgwm513* and *Xgwm149*) for SNB resistance in cultivar Bogo was also the region controlling FHB resistance in

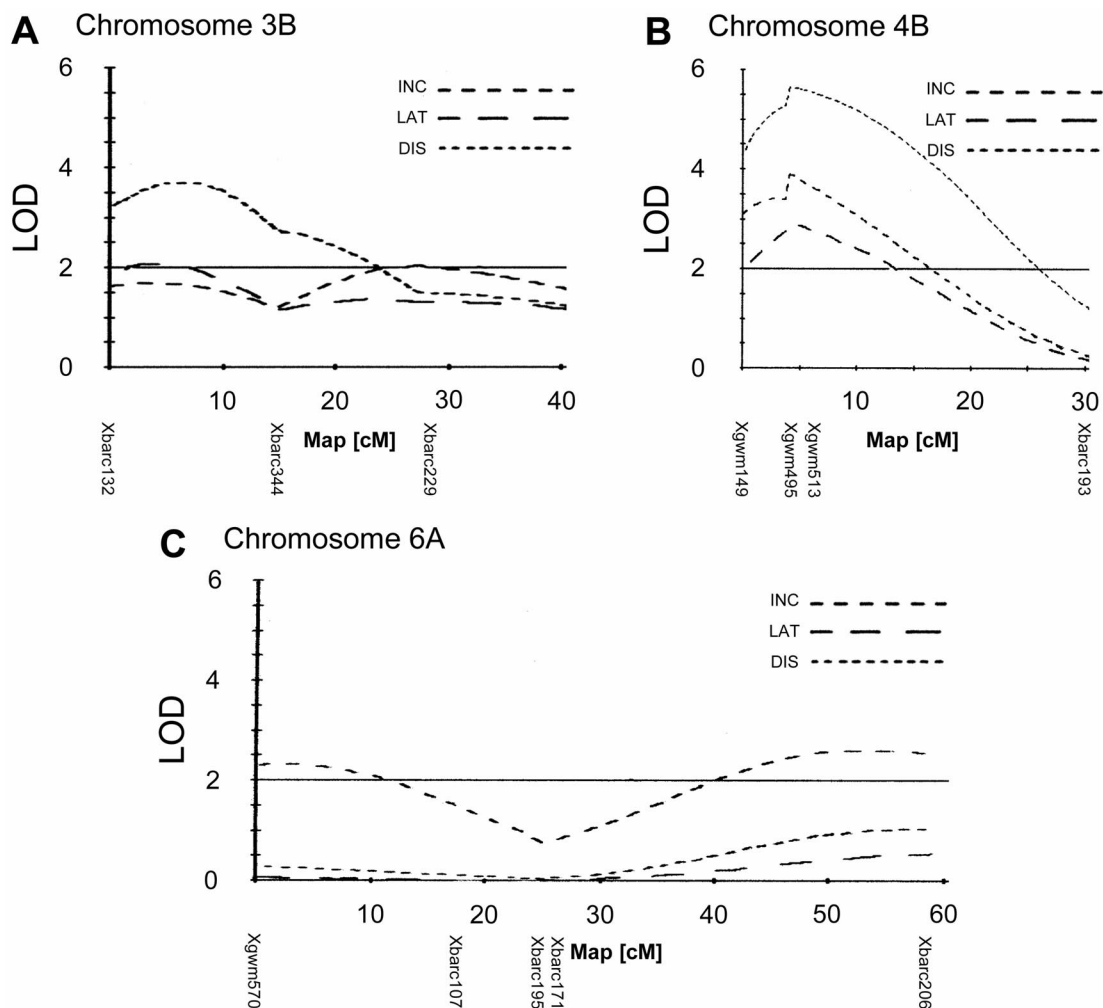


Fig. 1. Logarithm of odds (LOD) scores based on interval mapping of quantitative trait loci (QTLs) for long incubation period (INC), low disease severity (DIS) and long latent period (LAT) on linkage groups corresponding to chromosome 3B (A), 4B (B) and 6A (C).

Chinese wheat cultivar Wangshuibai as reported by Jia *et al.*⁽¹⁶⁾. This coincidence suggests the possibility that partial resistance to certain cereal fungal foliar diseases may be controlled by the same resistance loci in wheat. The presence of a QTL for SNB resistance around the *Xgwm570/Xmwg934* region on chromosome 6A was reported in wheat cultivar Alba⁽⁴⁾. However, unlike the QTL in wheat, the QTL on chromosome 6A in triticale only showed a minor effect on incubation period (INC).

In this study, no QTL for SNB resistance was derived from rye (R) genome due to the lack of SSR markers from rye. Markers derived from restriction fragment length polymorphisms (RFLP) and amplified fragment length polymorphisms (AFLP) have been used to saturate rye genetic maps in recent years^(5, 22, 32, 44). It is possible to use those types of polymorphic markers to define the QTL from the rye genome in the future.

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摘 要

Reszka, E.^{1,5}, Song, Q.^{2,3}, Arseniuk, E.¹, Cregan, P. B.³, and 翁溥^{4,5} 2007. QTL 控制冬小麥 'Bogo' 對小麥葉枯病的部份抗性. *植病會刊* 16:161-167. (¹ 波蘭植物育種與環境研究所; ² 美國馬里蘭大學自然資源科學與造園學系; ³ 美國農部大豆基因體改進研究室; ⁴ 美國農部分子病理研究室; ⁵ 聯絡作者: E. Reszka, E-mail: e.reszka@ihar.edu.pl; Fax: 48-22-7254714); P.P. Ueng, E-mail: uengp@ba.as.usda.gov; Fax: 301-504-5449)

小麥葉枯病是冬小麥及其他穀類作物葉片與穎片的重要病害之一，由於抗病機制的複雜度，以傳統接種方法難以評估冬小麥品種的抗病性。育種時應用標記輔助篩選法開發新的高抗病性品種是一項重要策略。由感病品種 Pinokio 與抗病品種 Bogo 雜交獲得的雙單倍體族群，在第五位葉階段 (GS15)。測試其靜置期、發病度及潛伏期三個葉枯病抗性反應指標加以評估。並以多型性的小麥和裸麥單一重複序列 (SSRs) 標記以及位於 4B、5B 及 6A 染色體的三個數量性狀基因座 (QTLs) 進行鑑定。結果發現位於 5B 和 6A 的數量性狀基因座 (QTLs) 與已報導的小麥葉枯病抗病基因的染色體位置一致。

關鍵詞：冬小麥、葉枯病菌、單一重複序列 (SSRs) 標記