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Quantitative trait locus mapping of rust resistance and agronomic traits in spring wheat

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Abstract

Marker-assisted selection requires the identification of molecular markers associated with major genes and quantitative trait loci (QTLs). In this study, we used 167 doubled haploid lines derived from two unregistered spring wheat (*Triticum aestivum* L.) parental lines that belong to the Canada Western Special Purpose wheat market class to map QTLs associated with five traits using inclusive composite interval mapping (ICIM). Using ICIM, the least-squares means phenotype data across three to four environments, and a genetic map of 2676 single-nucleotide polymorphisms (SNPs) out of the wheat 90K SNP array, we identified 10 QTLs associated with days to maturity (4A and 5B), plant lodging (4B, 5A, 5D, and 7D), grain yield (2D), leaf rust (4A), and stem rust (1A and 2B). Each QTL individually explained 6.0%–22.3% of the phenotypic variance and together accounted for 8.6%–38.2% of the total variance per trait. Two of the QTLs associated with rusts (*QLr.dms-4A* and *QSr.dms-1A*) had a minor effect (6.0%–9.0%), whereas the second QTL for stem rust (*QSr.dms-2B*) had a major effect (22.3%). Although chromosome 2B harbors multiple disease resistance QTLs, the physical location of *QSr.dms-2B* has not been reported in previous studies. Results from this study provide additional valuable information to wheat researchers; in particular, the area on chromosome 2B should be considered for future analyses.

Key words: *Triticum aestivum* L., leaf rust, stem rust, Canada Western Special Purpose wheat, 90K SNP array

Résumé

La sélection assistée par marqueur exige qu'on identifie les marqueurs moléculaires associés aux principaux gènes et locus quantitatifs (QTL). Les auteurs ont recouru à 167 lignées à haploïdie double issues de deux lignées parentales non homologuées de blé de printemps (*Triticum aestivum* L.) de la catégorie « blé de l'Ouest canadien à des fins spéciales » (CWSP) pour situer les QTL liés à cinq caractères par cartographie d'inclusion des intervalles composés (ICIM). En recourant à l'ICIM, aux données phénotypiques (moyenne des moindres carrés) dans trois ou quatre environnements et à la carte génétique de 2 676 SNP sur un ensemble de 90 000 SNP, les auteurs ont identifié dix QTL associés au nombre de jours jusqu'à la maturité (4A et 5B), à la verse (4B, 5A, 5D et 7D), au rendement grainier (2D), à la rouille de la feuille (4A) et à la rouille de la tige (1A et 2B). Chaque QTL expliquait 6,0 à 22,3 % de la variance du phénotype et, collectivement, 8,6 à 38,2 % de la variance globale pour chaque caractère. L'effet de deux des QTL associés à la rouille (*QLr.dms-4A* et *QSr.dms-1A*) n'était que secondaire (6,0 à 9,0 %), mais le deuxième QTL de la rouille de la tige *(QSr.dms-2B*) avait un effet majeur (22,3 %). Bien que le chromosome 2B porte de nombreux QTL de résistance à la maladie, l'emplacement du locus *QSr.dms-2B* n'avait encore jamais été signalé. Les résultats de cette étude procureront des informations précieuses à ceux qui poursuivent des recherches sur le blé, en particulier sur la partie du chromosome 2B, sur laquelle les analyses ultérieures devraient s'attarder. [Traduit par la Rédaction]

Mots-clés : *Triticum aestivum* L., rouille de la feuille, rouille de la tige, blé de l'Ouest canadien à des fins spéciales, jeu de 90 000 SNP

Introduction

Wheat (*Triticum aestivum* L.) is a major crop in Canada with an estimated total production of 35.2 million tonnes (Mt) in 2020 (Statistics Canada 2020) of which 19.6 Mt was exported. To support the strong demand for modern wheat varieties (cultivars) across 17 market classes (10 classes in western and 7 classes in eastern Canada), breeders in both the public and private sectors have registered 591 cultivars from 1961 to 2020, which include 336 spring wheat, 205 winter wheat, and 50 durum wheat cultivars (CFIA 2021). Most Canadian spring

and durum wheat are grown in Alberta, Saskatchewan, and Manitoba, which account for over 90% of the total wheat production in the country (https://www150.statcan.gc.ca/n1/pub/95-634-x/2017001/article/54904-eng.htm). The Canadian Food Inspection Agency (CFIA), with the recommendations from the Prairie Grain Development Committee (PGDC), is responsible for the registration of cultivars. CFIA requires that each candidate cultivar for registration be evaluated in comparison to appropriate check varieties for various traits (depending on the market class) of which maturity, plant height, lodging tolerance, grain yield, grain protein content (GPC), test weight, thousand kernel weight, and resistance to five priority diseases are mandatory across all market classes (PRCWRT 2018).

Stem, leaf, and stripe rusts caused by *Puccinia graminis* f. sp. *tritici* (Pgt), *P. triticina* f. sp. *tritici* (Ptr), and *P. striiformis* f. sp. *tritici* (Pst), respectively, are three of the five priority wheat diseases in western Canada. They are responsible for major losses in grain yield and quality. Leaf rust is the most common rust disease of wheat in western Canada annually, and its severity fluctuates every year (McCallum et al. 2021). Stripe rust has been detected in western Canada every year since 2000 with areas reporting serious epidemics in 2005, 2006, and 2011 (McCallum et al. 2007; Randhawa et al. 2012). Multiple stem rust epidemics have been reported in Canada in the early 1900s and from 1953 to 1955, which caused a loss of hundreds of millions of dollars (Peturson 1958). The severity of rusts can be reduced through agronomic management practices, the application of foliar fungicides, and the development of resistant cultivars (Wegulo 2012). The development of resistant cultivars is a more economical and environmentally friendly approach to controlling rusts. However, breeding for disease resistance is often challenging due to (*i*) the need to pyramid different sources of resistance to all three rust diseases, and (*ii*) the qualitative and quantitative inheritance of resistance to the rust diseases (Pinto da Silva et al. 2018) complicates the selection process. Qualitative resistance is controlled by a single gene with a major effect, which is effective against a subset of races. These major genes confer vertical resistance and tend to be expressed from seedling to adult plant stages, but tend to lose their effectiveness over time due to changes in pathogen populations. On the other hand, quantitative resistance is a partial level of resistance controlled by genes with incomplete resistance, which are more durable but can require the introgression of multiple genes or quantitative trait loci (QTLs). Quantitative resistances are expressed at later growth stages and provide adult plant resistance (Pilet-Nayel et al. 2017; Pinto da Silva et al. 2018; Rollar et al. 2021). Currently, a total of 61 stem rust, 80 leaf rust, and 83 stripe rust resistance genes have been identified in bread wheat, durum wheat, and their relatives (McIntosh et al. 2020). Most of these resistance genes are race-specific (qualitative), but a few are known to confer partial (quantitative) resistance at the adult stage, such as *Lr34*, *Lr46*, and *Lr67*. These genes are part of the complexes *Lr34/Yr18/Sr5/Pm38, Lr46/Yr29/Sr58/Pm39*, and *Lr67/Yr46/Sr55/Pm46* that confer resistance to leaf rust/stripe rust/stem rust/powdery mildew (Pinto da Silva et al. 2018). For stem rust, it was reported that on average a combination of four to five minor genes reduced stem rust severity to negligible levels at maturity (Singh et al. 2011). Little is known about nonspecific stem rust resistance genes beyond the above complexes. Most cultivars rely on combination of *Sr2* and other unknown slow rusting resistance genes for durable resistance to stem rust in Canada, the USA, and Australia (Singh et al. 2011).

Improved cultivars can be developed using multiple conventional breeding methods and marker-assisted selection (MAS). MAS is an indirect selection method that requires mapping genes and major effect QTLs associated with target traits, which involves developing (assembling) appropriate populations followed by coarse mapping, fine mapping, validation, and the development of high-throughput, reproducible, and breeder-friendly molecular markers (Collard et al. 2005; Schaid et al. 2018; Platten et al. 2019; Jaganathan et al. 2020). There have been continuous efforts to map and characterize genes and QTLs associated with target traits of interest using diverse linkage-based analysis (LA). As reviewed by different authors (Collard et al. 2005; Semagn et al. 2010; Gupta et al. 2019), the LA method includes simple interval mapping, composite interval mapping, inclusive composite interval mapping (ICIM), and multiple interval mapping (Kao et al. 1999; Li et al. 2010; Akond et al. 2019), which all depend on well-defined biparental populations, such as F_x-derived families, backcross, near-isogenic lines (NILs), doubled haploids (DHs), and recombinant inbred lines (RILs). Such types of mapping populations are often developed by crossing two parents with contrasting phenotypic trait(s) of interest. Although LA is the most widely used method since the early 1990s, it has four major drawbacks: (1) the time and cost of developing the mapping populations, (2) the low resolution of the method due to a limited number of recombination events, (3) the use of old populations developed five or more years before the mapping studies, and (4) the biparental populations capture only alleles originated from their parents.

Our group at the University of Alberta conducted multiple studies to map genes and QTLs in RILs derived primarily from the Canada Western Red Spring (CWRS) class on agronomic traits (Semagn et al. 2021*a*, 2021*b*) and reaction to diseases (Perez-Lara et al. 2017; Zou et al. 2017; Bemister et al. 2019). However, we have not conducted any mapping study in biparental populations derived from the Canada Western Special Purpose (CWSP) wheat market class, which forms the basis in the present study. Advanced breeding lines and cultivars in the CWSP class produce high grain yield with high starch content but low GPC, and are considered desirable for ethanol production and animal feed (Canadian Grain Commission 2021). The objective of the present study was to map QTLs associated with leaf and stem rust resistance and major agronomic traits using ICIM.

Materials and methods

Phenotyping

This study was conducted on 167 DH lines derived from a cross between "HYAYT12-10" and "GP146" using the wheat–maize hybridization method (Sadasivaiah et al. 1999). Both

parents are unregistered lines that belong to the CWSP class. "HYAYT12-10" is an advanced breeding line from the University of Alberta breeding program, which was derived from a cross between "Hidhab" and "AC Andrew". "Hidhab" was extracted from "HD1220/3∗Kal/Nac CM40454" and characterized by a relatively good level of resistance to leaf, stem, and stripe rusts, an average grain yield with a relatively high GPC, strong gluten, late maturing, and well adapted to drought (Aissaoui and Fenni 2021). "AC Andrew" is a DH cultivar developed by Lethbridge Research Center from "Dirkwin"/"SC8021V2"//"Treasure"/"Blanca" and characterized by higher grain yield, high lodging tolerance, resistance to the prevalent races of stripe rust, stem rust, and powdery mildew, and moderately resistant to leaf rust (Sadasivaiah et al. 2004). "GP146" is a high grain yielder with a soft white grain developed from a cross between "Bhishaj" (Randhawa et al. 2011) of Agriculture and Agri-Food Canada (AAFC) and a synthetic line "SKAUZ"/"PASTOR"/3/"CROC_1"/"AE.SQUARROSA(224)"//"OPATA" from the International Maize and Wheat Improvement Center (CIMMYT). The DH population was originally chosen to explore QTL for stripe rust resistance, based on observations made at a contra-season nursery in New Zealand in the winter of 2015. However, stripe rust nurseries using the prevailing races in western Canada showed insufficient variation in resistance/susceptibility for further study.

The DH lines, the two parents, and four CWSP checks ("AAC Awesome", "Pasteur", "AC Andrew", and "Sadash") were planted in hill plots in disease nurseries using a randomized complete block design with two replications. Hills were seeded with 1 g of seed at a spacing of 20–30 cm between hills. Reaction to both leaf and stem rust was evaluated in 2016, 2017, and 2018 in Morden, Manitoba, Canada. Leaf rust was also evaluated for 2 years in 2016 and 2017 at the University of Alberta South Campus Research Station, Edmonton, Alberta. At each location, urediniospores of both rusts were collected from infected plants in nurseries in mid-August of the previous year and frozen in −80 °C in 1.5 mL vials until needed for inoculation in June of next year. Urediniospores were recovered from −80 °C on the day of inoculation, allowed to acclimate for a few minutes, heat shocked in a 42 °C water bath, and suspended in 2 L of Soltrol 170. To create homogeneous disease epidemics within each trial, plants were inoculated at the five- to six-leaf stage (Zadok's 15–16) in the early evening using a low-volume sprayer. The nursery was inoculated a second time about 3–5 days later and for a third time after another 3–5 days. Visual disease assessment was done using a scale of 1 (no visible sign or symptom = resistant) to 9 (leaf area totally covered with spores = highly susceptible) on each hill plot basis. The disease severity was rated when the susceptible checks ("AC Barrie" and "Park") had many pustules, the moderate check ("Peace") had fewer pustules, and the resistant check ("Carberry") appeared uninfected. Reaction to stem rust was also scored in the same way as the leaf rust except that "Hoffman HRF" was used as the susceptible check, "Columbus" as an intermediate check, and "Glenn" as the resistant check. "HYAYT12-10" showed resistant and moderately resistant reactions to rusts, while "GP146" showed intermediate to moderate susceptibility to rusts.

The DH population, parents, and the four CWSP checks were also evaluated for agronomic traits in conventionally managed fields both at the University of Alberta South Campus Research Station, Edmonton, AB and at the Lethbridge Research and Development Centre, Lethbridge, AB in both 2016 and 2017. All agronomic trials were conducted using a randomized incomplete block design, with two replications. Plots were 3.0 × 1.0 m^2, with six rows of 19 cm apart, and seeded in mid-May of every year at a rate of 300 seeds m^{-2}. Weeds were controlled using registered herbicides following local recommendations and label directions. The 4-year crop rotation in conventional land was a rotation of two-row barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.), field pea (*Pisum sativum* L.), and wheat. Each entry was evaluated for plant lodging, days to maturity, and grain yield. Lodging score was recorded on a plot basis at the time of harvest on a 1-to-9 scale, with 1 and 9 representing no lodging and completely lodged, respectively. Days to maturity from the time of seeding were scored when more than 50% of the peduncles in a plot turned yellow. Plots were individually harvested with a small plot Wintersteiger Nursery Elite combine. Seed was collected into cotton bags and dried for 4 days using an industrial dryer at 80–90 °F for 4–5 days after harvest. Each bag of grain was cleaned with a Pfeuffer four-sieve seed cleaner. Yield per plot was recorded in kg and converted to t ha^{-1}.

DNA extraction and genotyping

Seedlings from the two parents and the DH lines were raised in a growth chamber until the three- to four-leaf stage. Genomic deoxyribonucleic acid (DNA) was extracted from seedlings collected at three- to four-leaf stage using a modified cetyl trimethylammonium bromide method (Doyle and Doyle 1987). DNA quality was checked by running an aliquot on 0.8% agarose gel stained with SYBR® Safe. The DNA concentration was normalized to approximately 100 ng μL^{-1} after being assessed with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). Fifty microliters of each DNA sample were sent to the National Research Council (NRC) in Saskatoon, Saskatchewan. The samples were genotyped by the NRC with the Illumina 90K Infinium Wheat Array (Wang et al. 2014).

Data analysis: phenotype and genotype

For each trait, F statistics and least-squares means were computed across all environments using a mixed linear model in R, software v3.5.2. Parameters were estimated by the restricted maximum likelihood method with the nlme package using the lme function. A mixed-effects analysis of variance approach was used to estimate least-squares means for each entry. The observed variable was explained with the linear model:

$$\Upsilon_{tjqk} = \mu_t + G_{jt} + E_{tj} + (G \times E)_{tjk} + B_{tjqk} + \varepsilon_{jqkt}$$

in which Υ_{jqkt} is the response observed in trait t of entry j in block q in environment k, μ_t is the overall mean effect of trait t, G_{jt} is the effect of the genotype of j on trait t, E_{tj} is the effect of environment on trait t in entry j, $(G \times E)_{tjk}$ is the interaction effect of genotype and environment on trait t in entry j in en-

vironment k, B_{tjqk} is the blocking effect on trait t of entry j in block q in environment k, and ε_{jqkt} is the residual error. Genotypes (G) were considered fixed, while environments (E), replications, blocks (B) within replications, and $G \times E$ interactions were considered as random effects. Broad-sense heritability was computed using multi-environment trial analysis with R for Windows (MetaR) v.6.04 (Alvarado et al. 2020). Test for normality on the least-squares means, box plots, frequency distribution plots, and Pearson correlation coefficient plots from the phenotype data were generated using the nlme package in RStudio Version 1.1.4 (R Core Team 2020; RStudio Team 2020).

The 90K genotype data were filtered as described in a previous study (Xiang et al. 2021). First, we removed all single-nucleotide polymorphisms (SNPs) that were monomorphic between the two parents, missing or heterozygous in both parents, and those with greater than 20% missing data in the DH lines. This initial stage of filtering resulted in 4799 SNPs for linkage analysis. We performed linkage analysis using JoinMap v4.0 (Van Ooijen 2006) and further excluded all markers that showed segregation distortion at $p < 0.01$, and those that were either unlinked or formed a linkage group (LG) with <5 markers using a minimum logarithm of odds (LOD) score of 3, a recombination frequency of 0.35, and Kosambi mapping function (Kosambi 1943). We finally retained 2676 SNPs for map construction using MapDisto for Windows v2.1.7.10 (Heffelfinger et al. 2017). For each SNP, we obtained the International Wheat Genome Sequence Consortium (IWGSC) RefSeq v2.0 information from https://urgi.versailles.inrae.fr as described in our previous study (Semagn et al. 2021*a*). The SNP genotype data and physical information were then sorted using chromosome name and physical position in ascending order (this is a step required to obtain the correct marker order). We then created a temporary new SNP ID that consisted of "Chr" as a prefix for chromosome, followed by 01 to 21 to represent each chromosome, and the physical positions in bp (e.g., Chr01-29183813 to represent the first SNP on chromosome 1A that mapped at 29 183 813 bp). In cases where two or more SNPs on the same chromosome had the same physical position, we added 1 bp to avoid duplicates and make each position unique to serve as SNP ID. We then loaded the SNP data with the new SNP ID into MapDisto and constructed linkage map using "Extract LG's from loci" option. The latter option generates linkage maps based on the predefined LGs using the physical positions for locus ordering and converting the positions into cM.

QTL mapping

ICIM was performed on the least-squares means of each trait, and both the genetic map in cM and the physical map in kilobase pair (kb) using QTL IciMapping version 4.2.53 (Li et al. 2007; Meng et al. 2015) with the following parameters: mean replacement for missing phenotypic data, a minimum LOD score of 3.0, and an additive model to determine the effect of individual QTL. The walking distance was set to 1 cM for genetic maps and 2 kb for physical maps. In cases where two or more QTLs were detected for the same trait with an overlapping confidence interval or common flanking markers, only one of them was retained. QTLs that explained <10%, 10%–20%, and >20% of the phenotypic variation were arbitrarily classified into minor, moderate, and major effects, respectively. QTL names were assigned by following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), which consisted of trait acronym, lab designation (dms = Dean Michael Spaner), and chromosome number. MapChart v2.1 (Voorrips 2002) was used to construct genetic maps and QTL graphs.

Results

Phenotypic and genetic variation

"HYAYT12-10" matured about 3 days later, yielded 740 kg ha^{-1} more grain, and was more tolerant to lodging, and resistant to both leaf and stem rust than "GP146" (Table 1). The 167 DH lines required 105–112 days to mature, varied in lodging score from 1 to 5, and yielded 4.6–6.8 t ha^{-1} grain. The average leaf and stem rust scores in the DH lines varied from 1.2 to 8.8 with an overall average score of 3.5 for leaf rust and 3.8 for stem rust. Of the 167 DH lines, only 10 DH lines produced more grain yield, 11 lines were more resistant to leaf rust, and 15 lines were more resistant to stem rust than the high-yielding and rust-resistant parent "HYAYT12-10" (6.3 Mg ha^{-1}, with scores of 1.5 for both leaf and stem rust). The genotype effect was significant ($p < 0.05$) in the model for all traits. Broad-sense heritability was moderate to high, which varied from 0.41 for maturity to 0.78 for leaf rust. The phenotypic distribution of least-squares means averaged across all environments was normal ($p < 0.05$) for both maturity and grain yield but skewed for lodging score, leaf rust, and stem rust (Fig. 1) with most of the DH lines showing moderate scores in all three traits. Statistically significant ($p < 0.05$) correlations were observed only between maturity and lodging (−0.33) and between leaf and stem rust (0.88) (Fig. 2).

Inclusive composite interval mapping

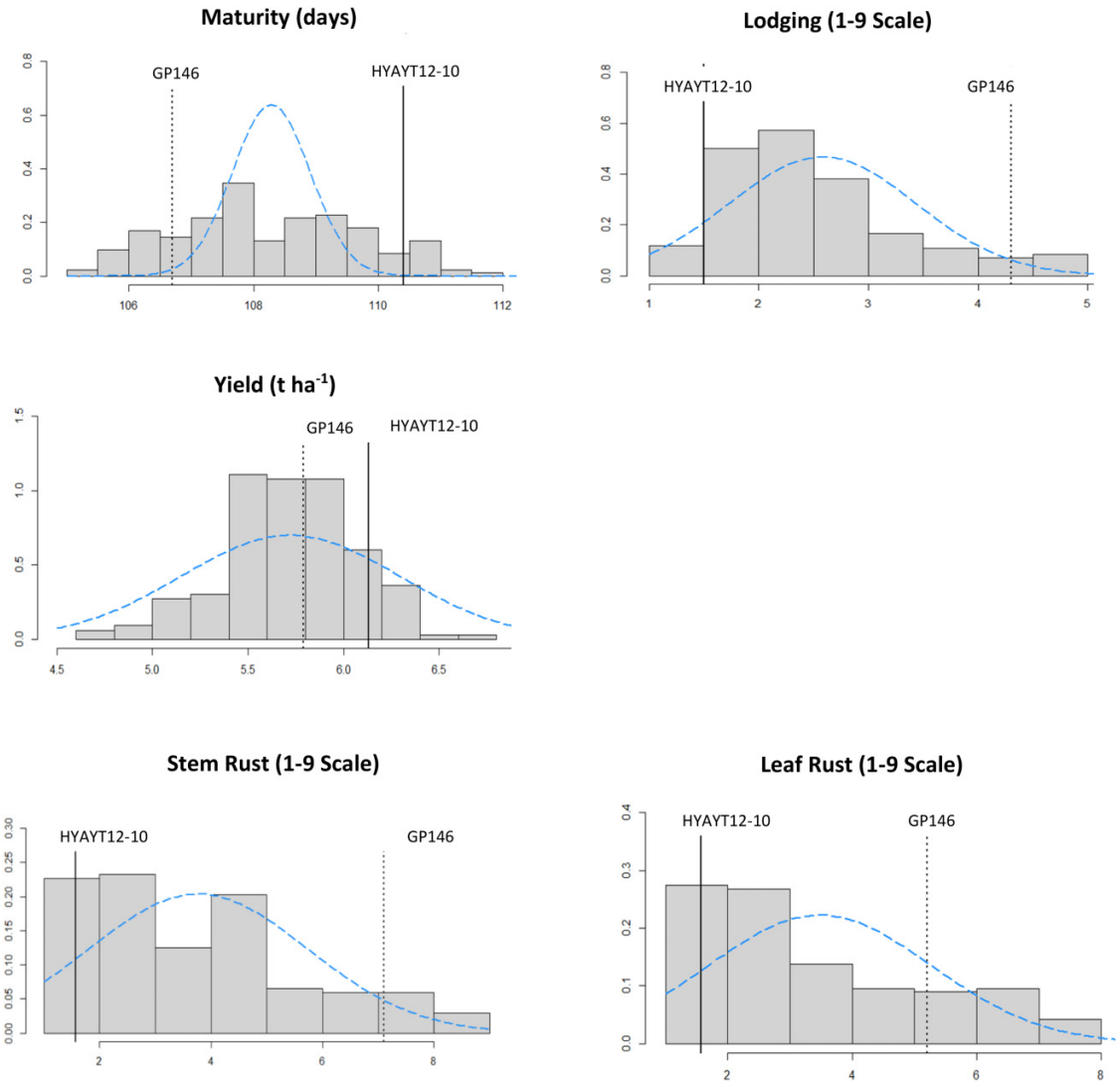
Of the wheat 90K iSelect array used for genotyping the DH population, we integrated 2676 SNPs in the final genetic maps (Table 2). The number of mapped markers per chromosome varied from 35 on chromosome 6D to 379 on 3B with an overall average of 127 SNPs per chromosome. ICIM identified a total of 10 QTLs (Table 3 and Fig. 3) associated with maturity (2), lodging (4), grain yield (1), leaf rust (1), and stem rust (2). The two QTLs for maturity were mapped at 524 cM on chromosome 4A (*QMat.dms-4A*) and at 2171 cM on 5B (*QMat.dms-5B*), which explained 10.8% and 12.0% of the phenotypic variance, respectively. The four QTLs associated with plant lodging were mapped at 1517 cM on chromosomes 4B (*QLdg.dms-4B*), at 538 cM on 5A (*QLdg.dms-5A*), at 568 cM on 5D (*QLdg.dms-5D*), and at 1102 cM on 7D (*QLdg.dms-7D*). Each QTL for lodging explained from 7.7% to 12.2% and together accounted for 38.2% of the phenotypic variance. The single QTL associated with grain yield was mapped at 1221 cM on chromosome 2D (*QYld.dms-2D*) that explained 8.6% of the phenotypic variance. DH lines that were homozygous for the HYAT12-10 alleles at the two flanking markers of *QYld.dms-2D* yielded on average 250 kg ha^{-1} more grain than those with the "GP146" alleles.

Table 1. Summary of descriptive statistics of observed maturity, lodging, grain yield, leaf rust, and stem rust of parents and the "HYAYT12-10/GP146" doubled haploid (DH) population over combined environments.

	Parents			DH lines		F statistics		
Trait	"HYAYT12-10"	"GP146"	Difference[a]	Range	Mean ± Std	*F*-value	*p*-Value	Broad-sense heritability
Maturity (days)	110.2	106.8	−3.43	104.8–111.6	108.3 ± 1.50	3.20	<0.0001	0.41
Lodging (1–9)	1.52	4.84	3.32	1.17–5.01	2.59 ± 0.87	9.30	<0.0001	0.64
Yield (tha)	6.26	5.52	−0.74	4.62–6.79	5.72 ± 0.36	2.50	<0.0001	0.47
Leaf rust (1–9)	1.50	5.10	3.60	1.20–7.80	3.48 ± 1.80	23.7	<0.0001	0.78
Stem rust (1–9)	1.50	7.00	5.50	1.20–8.80	3.79 ± 1.98	20.1	<0.0001	0.68

[a]Difference = "GP146" − "HYAYT12-10".

Fig. 1. Frequency distributions of least-squares means of 167 doubled haploid (DH) lines derived from "HYAYT12-10"/"GP146".



The single QTL detected for leaf rust was mapped at 3127 cM on chromosome 4A (*QLr.dms-4A*) and accounted for 9.0% of the phenotypic variance. DH lines that were homozygous for the "HYAT12-10" alleles at the two flanking markers of *QLr.dms-4A* had on average lower leaf rust values by 1.6 points on the 1–9 scale than those with the GP146 alleles. The two QTLs associated with stem rust were mapped at 1305 cM on chromosomes 1A (*QSr.dms-1A*) and at 3143 cM on 2B (*QSr.dms-2B*), which accounted for 6.0% and 22.3% of the phenotypic variance, respectively. DH lines that were homozygous for the "HYAT12-10" alleles at the two flanking markers for *QSr.dms-1A* and *QSr.dms-2B* had on average 1.7 and 1.9 lower stem rust scores than those with the "GP146" alleles. Overall, all QTLs detected in the present study explained from 8.6% to 38.2% of the total phenotypic variance per trait, so most of the variation in all five traits remained unexplained.

Fig. 2. Scatter plots showing Pearson correlation coefficients for lodging vs. maturity and leaf rust vs. stem rust in a doubled haploid population derived from "HYAYT12-10"/"GP146" based on least-squares means computed from all environments.

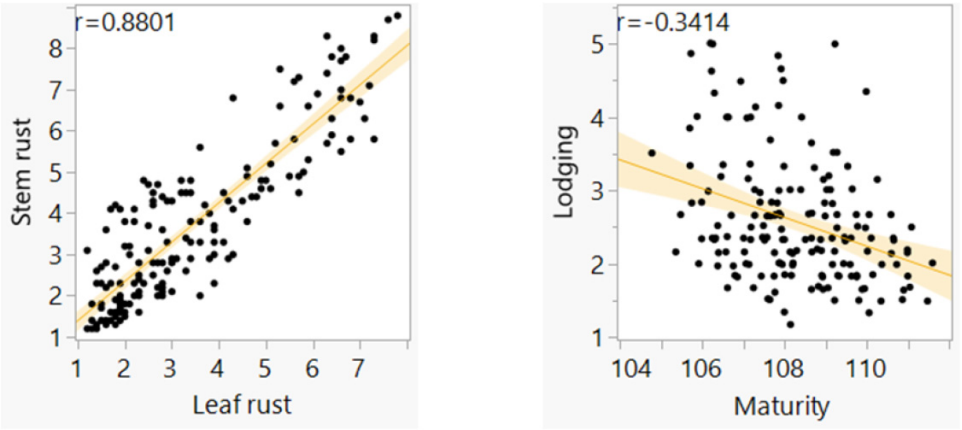


Table 2. Summary of 2767 single-nucleotide polymorphism (SNP) markers incorporated in the final linkage maps.

Chromosome	No. of SNPs	Map length (cM)	Map length (Mb)
1A	75	2231.2	598.1
1B	142	3795.4	700.5
1D	49	1797.3	497.1
2A	164	5345.1	786.2
2B	109	4517.8	808.1
2D	54	2051.6	621.6
3A	122	3748.7	745.3
3B	379	3253.5	851.9
3D	56	2698.4	613.5
4A	169	4775.2	748.0
4B	156	2701.8	665.6
4D	39	2118.3	508.1
5A	148	2920.0	708.1
5B	332	2923.3	713.3
5D	61	1991.6	568.7
6A	244	3420.0	622.5
6B	56	3016.5	727.6
6D	35	1909.3	493.7
7A	145	3830.7	744.2
7B	96	3112.3	763.3
7D	45	2084.6	640.4
Total	2676	64 242.5	14 125.7

Discussion

The present study employed 167 DH lines derived from "HYAYT12-10"/"GP 146", which are unregistered lines belonging to the CWSP class. We uncovered a total of 10 QTLs, of which 7 were associated with agronomic traits and 3 with leaf and stem rusts. The development of early maturing wheat cultivars is always a priority in the northern areas of the world (including the Canadian prairies) where frosts can damage crops due to a short growing season (Semagn et al. 2021*b*). Our study identified two moderate effect QTLs for maturity at 29.2–29.8 Mb on chromosome 4A (*QMat.dms-4A*) from "HYAYT12-10" and at 581.5–583.5 Mb on 5B (*QMat.dms-5B*) from "GP146" (Table 3). QTLs for maturity have been reported across several wheat chromosomes, including 4A (McCartney et al. 2005*b*; Kamran et al. 2013; Perez-Lara et al. 2016; Semagn et al. 2021*b*) and 5B (Kamran et al. 2013; Semagn et al. 2021*b*). One of the minor effect maturity QTLs reported on chromosome 4A (*QMat.dms-4A.1*) by Perez-Lara et al. (2016) was flanked by *CAP12_rep_c4000_432* and *Ra_c7973_1185* SNPs, which are physically located at 24.6 and 37.0 Mb, respectively. The physical confidence interval of that QTL overlaps with the *QMat.dms-4A* identified in the present study.

Using the IWGSC RefSeq v2.0 physical map, Semagn et al. (2021*b*) recently investigated four spring wheat populations and reported the physical positions of eight QTLs for heading, flowering, and maturity on chromosomes 5B, which individually accounted for 1.8%–19.3% of the phenotypic variance. One of those QTLs was coincidentally associated with heading (*QHd.dms-5B.3*), flowering (*QFlt.dms-5B.2*), and maturity (*QMat.dms-5B.2*), which was mapped at 574.5–577.0 Mb. *Vrn-B1* (gene ID: *TraesCS5B02G396600*) is one of the major genes affecting vernalization response and flowering time in wheat (Santra et al. 2009), and is physically located between 573.8 and 577.0 Mb, based on the IWGSC RefSeq v1.0 and IWGSC RefSeq v2.0 maps, respectively. The maturity QTL detected in the present study was, therefore, about 6.5 Mb away from the *Vrn-B1* gene and far from all QTLs reported for the three earliness traits in previous studies.

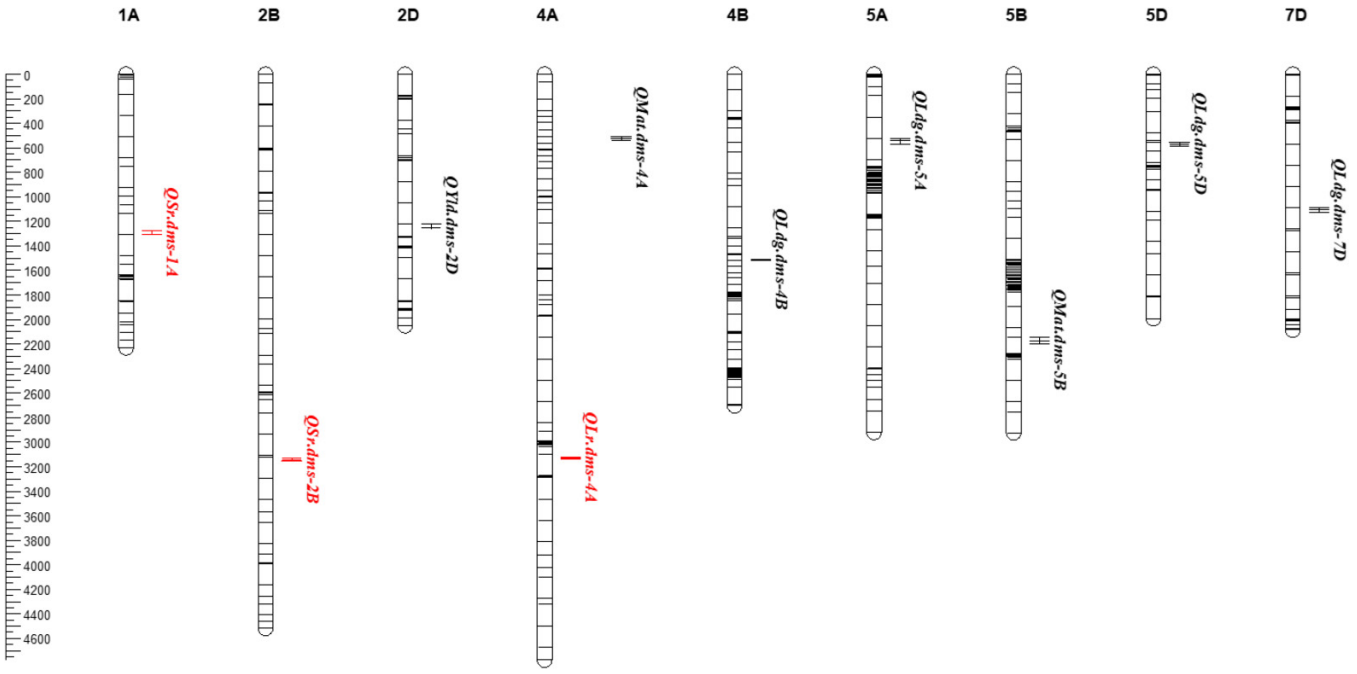
Plant lodging is another important trait in wheat breeding that directly affects grain yield. The introduction of the *Reduced height* (*Rht*) dwarfing or semi-dwarfing genes (Peng et al. 1999), such as *Rht-B1* and *Rht-D1*, has made a significant impact in modern wheat cultivars. This study uncovered four QTLs for lodging tolerance on chromosomes 4B (*QLdg.dms-4B*), 5A (*QLdg.dms-5A*), 5D (*QLdg.dms-5D*), and 7D (*QLdg.dms-7D*). Recently, Semagn et al. (2021*a*) reported the physical positions of 20 QTLs for lodging and 14 QTLs for plant height in four RIL populations, which individually accounted for 1.5%–19.4% and 1.8%–49.1% of the phenotypic variance, respectively. However, none of the QTLs reported in the pre-

Table 3. Summary of quantitative trait loci (QTLs) associated with five traits in the "HYAYT12-10"/"GP146" DH mapping population based on least-squares means computed from all environments.

Trait	QTL	Chr	Position (cM)	Left position (cM)	Right position (cM)	Left position (Mb)	Right position (Mb)	Left marker	Right marker	LOD	PVE (%)	Additive effect	Parental origin
Maturity	*QMat.dms-4A*	4A	524.0	508.5	536.5	29.2	29.8	Kukri_c13639_1326	BS00065607_51	6.2	10.8	0.7	P1
Maturity	*QMat.dms-5B*	5B	2171.0	2143.5	2195.5	581.5	583.5	wsnp_Ex_c621_1230852	Excalibur_c9391_1016	3.1	12.0	−0.8	P2
Lodging	*QLdg.dms-4B*	4B	1517.0	1508.5	1519.5	505.3	512.8	wsnp_Ex_c4358_7854194	GENE-2331_126	9.7	12.2	−0.4	P1
Lodging	*QLdg.dms-5A*	5A	538.0	523.5	569.5	111.8	238.5	Excalibur_rep_c69159_392	Tdurum_contig67350_494	3.4	7.7	0.3	P2
Lodging	*QLdg.dms-5D*	5D	568.0	555.5	584.5	401.7	407.6	Tdurum_contig68472_115	Kukri_rep_c79943_189	4.2	9.6	−0.3	P1
Lodging	*QLdg.dms-7D*	7D	1102.0	1086.5	1130.5	372.1	391.5	Ra_c6845_1501	wsnp_cd454041D_Ta_2_1	3.7	8.7	−0.3	P1
Yield	*QYld.dms-2D*	2D	1221.0	1218.5	1256.5	422.7	457.6	BS00090129_51	Excalibur_c24307_739	3.9	8.6	0.1	P1
Leaf rust	*QLr.dms-4A*	4A	3127.0	3118.5	3139.5	646.4	648.4	Ra_c63534_581	RAC875_c6939_1042	12.1	9.0	−1.4	P1
Stem rust	*QSr.dms-1A*	1A	1305.0	1277.5	1307.5	536.8	543.6	Excalibur_rep_c103592_955	RAC875_rep_c69334_132	5.6	6.0	−0.7	P1
Stem rust	*QSr.dms-2B*	2B	3143.0	3132.5	3155.5	694.9	695.2	BobWhite_c3871_428	BS00065914_51	9.5	22.3	−1.3	P1

Note: Chr, chromosome; cM, centi-Morgan; Mb, megabase pair; LOD, logarithm of odds; PVE, phenotypic variation explained; P1, "HYAYT12-10"; P2, "GP146".

Fig. 3. Genetic linkage maps of nine common wheat (*Triticum aestivum* L.) chromosomes showing the positions of 10 QTLs based on 167 DH lines genotyped with 2676 SNPs. Map position (cM) is shown on the left, with each horizontal line on the chromosome representing a marker. QTLs are shown on the right side of each chromosome, with bars indicating their confidence interval between two flanking markers. QTLs associated with agronomic traits and rusts resistance are in black and red fonts, respectively.



vious study were close to the QTLs identified for lodging in the present study. For example, *QLdg.dms-4B* identified in the present study was located at 505.3–512.8 Mb and had a moderate effect (accounting for 12.2% of the phenotypic variance), with the favorable allele originating from "HYAYT12-10". Multiple similar QTLs associated either with lodging or plant height on 4B have been reported (McCartney et al. 2005*b*; Verma et al. 2005; Hassan et al. 2019) using genetic positions, but none were close to *QLdg.dms-4B*. *Rht-B1* (*TraesCS4B02G043100*) is one of the genes located on the short arm of chromosome 4B that has been widely used in wheat breeding not only to reduce plant height and increase lodging tolerance but also to increase yield components and the number of productive tillers (Kertesz et al. 1991; Lanning et al. 2012; Sherman et al. 2014; Jobson et al. 2019). The exact physical position of the *Rht-B1* gene differs depending on the version of the reference sequence and varied from 30.8 Mb (based on IWGS RefSeq v1.0) to 33.6 Mb (based on IWGS RefSeq v2.1), which are far from the QTL detected in the present study. QTLs for lodging tolerance have also been reported on chromosome 5A in different studies (Keller et al. 1999; Marza et al. 2006), but their positions were reported using genetic maps in cM, which makes direct comparisons among independent studies unreliable. Song et al. (2021) reported a minor QTL for stem diameter on chromosome 5A between *RAC875_c9617_373* and *RAC875_c9617_395* that maps at 663.9 Mb, which is far from the QTL identified in the present study.

QYld.dms-2D was the only QTL we found for grain yield that was located at 422.7–457.6 Mb on chromosome 2D. Grain yield is a complex trait affected by multiple agronomic and yield-related traits, environments, and $G \times E$ interactions, and QTL × QTL interactions (epistasis) (Wu et al. 2012; Xing et al. 2014). Chromosome 2D harbors multiple QTLs associated with spike number and agronomic traits (Zhang et al. 2015; Perez-Lara et al. 2016; Deng et al. 2019; Ma et al. 2020) as well as the photoperiodism response *Ppd-D1*gene. However, none of the previously reported QTLs are located within the same physical interval of *QYld.dms-2D* identified in the present study.

We uncovered a minor effect QTL associated with leaf rust at 646.4–648.4 Mb on chromosome 4A (*QLr.dms-4A*), another minor effect QTL for stem rust at 536.8–543.6 Mb on 1A (*QSr.dms-1A*), and a major effect QTL for stem rust at 694.9–695.2 Mb on 2B (*QSr.dms-2B*) (Table 3). The leaf rust QTL on chromosome 4A (*QLr.dms-4A*) originated from "HYAYT12-10" and was located between *Ra_c63534_581* and *RAC875_c6939_1042* at 646.4 and 648.4 Mb, respectively. Bemister et al. (2019) reported a minor effect leaf rust QTL on 4A at 602.7 Mb, which is 43.7 Mb far from the position of our QTL. Kertho et al. (2015) reported three QTLs on chromosome 4A for seedling leaf rust resistance at 93.5, 151.3, and 198.8 cM. The closest QTL to *QLr.dms-4A* identified in the present study was flanked by marker *IWA7859* at 198.84 cM, which is physically located at 115.7 Mb; the two QTLs are over 530 Mb distant. The other QTL we detected for stem rust was mapped on chromosome 1A between *Excalibur_rep_c103592_955* at 536.8 Mb and *RAC875_rep_c69334_132* at 543.6 Mb. Other studies have reported genes (e.g., *Sr1RS*)

and QTLs associated with stem rust on 1A (Kumar et al. 2020; Leonova et al. 2020; Megerssa et al. 2020), but direct comparisons across studies were difficult due to the lack of physical information for most flanking markers.

Chromosome 2B harbors multiple genes, including *SrWeb*, *Sr28*, *Sr32*, *Sr39*, *Sr36*, *Sr40*, *Sr47* (Yu et al. 2014), *Sr9h*, and *Sr16* (McCartney et al. 2005*a*; Vanegas et al. 2008; Zurn et al. 2018; Kosgey et al. 2021). It also harbors several QTLs for stem rust resistance (Prins et al. 2011; Kosgey et al. 2021; Sharma et al. 2021), but those previously reported genes and QTLs are not within the physical confidence interval of the *QSr.dms-2B* identified in the present study. For example, Kosgey et al. (2021) found a moderate effect stem rust QTL on 2B between markers *BS00038820_51* and *Tdurum_contig54704_176*, which are located at 72.5 and 658.6 Mb, respectively. Sharma et al. (2021) reported a major effect QTL that accounted for 33.3% of the phenotypic variation for stem rust on chromosome 2B between *IWB7072* and *IWB2380* and another moderate effect QTL (16.2%) between *IWB71742* and *IWB73196*, which are located at 97.1 and 746.7 Mb, respectively.

We found moderate to high broad-sense heritability (0.41–0.78) and expected to uncover QTLs that account for most of the phenotypic variance of each trait. However, we were only able to account for <40% of the phenotypic variance of every trait. Thus, most of the phenotypic variations remained unexplained by the identified QTL, which agrees with several previous studies conducted in different Canadian spring wheat populations (Asif et al. 2015; Chen et al. 2015, 2020; Perez-Lara et al. 2016). Some of the factors that affect the probability of detecting QTL and the proportion of variance explained by each QTL include marker density, mapping population type and size, trait heritability, the number of environments, and $G \times E$ interactions (Semagn et al. 2010). DH populations are easy and quick to develop, which makes them attractive for QTL mapping in various species, but they have poor resolution due to limited recombination. They have only gone through one round of recombination as compared with multiple rounds of recombination in RIL populations (Yan et al. 2017; Alqudah et al. 2020). Other possible factors include population size, marker density, and trait heritability. Our mapping population size of 167 DH lines fell within 100–200 progenies that are widely used in QTL mapping studies (Utz, et al. 2000; Stange et al. 2013), but it had low power for detecting more numbers of QTL. For example, both leaf rust and stem rust showed highly significant positive correlations, but we did not find common QTLs associated with both diseases at LOD 3.0. At LOD 2.5, however, the 646.4–648.4 Mb on chromosome 4A was detected in both leaf and stem rusts. The failure in detecting the common QTL for stem rust on 4A at LOD 3.0 is likely due to the relatively small population that reduced its power.

Conclusion

The present study uncovered a total of 10 QTLs linked to three agronomic traits and two rusts that individually explained from 6.0% to 22.3% of the phenotypic variance and together accounted for 8.6%–38.2% of each trait. One of the QTLs on chromosome 2B (*QSr.dms-2B*) was a novel major effect QTL, which explained 22.3% of the phenotypic variance for stem rust. Follow-up studies are needed to validate and fine-map the major effect QTL *QSr.dms-2B.2* for stem rust. We found a highly significant positive correlation between stem and leaf rusts, but we did not detect a coincidental QTL for these two diseases at LOD 3.0, which is likely due to the relatively small population size that reduces the power of QTL detection.

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Data availability

Data are available upon request from the corresponding author.

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Author contributions

HR and DS conceptualized the project, acquired funding, supervised the project, and edited the paper. IC generated the data, carried out data analysis, and drafted the manuscript. K. Semagn helped with the data and QTL analysis and edited the manuscript. BM provided rust spores, generated leaf rust data in Morden, and edited the manuscript. MI revised the manuscript. K. Strenzke helped in generating agronomic and rust data in Edmonton. MV recorded rust data in Lethbridge. RD edited the manuscript.

Competing interests

The authors declare no competing interests.

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