Validation of a SNP-KASP marker for the Fusarium head blight resistance quantitative trait loci on chromosome 5AS
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Abstract: Fusarium head blight (FHB) is a devastating wheat disease with a significant economic impact. Fhb5 is an important quantitative trait loci (QTL) conferring type I resistance to FHB. In this study, we accessed the usability of a Kompetitive allele-specific polymerase chain reaction (KASP) marker for the QTL on Fhb-5AS. The KASP clustering results were compared with the linked simple sequence repeat marker, wmc705 for Qfhb-5AS. Our results indicate that the single-nucleotide polymorphic locus wsnp_Ra_c24707_34262900 (IWA7777) provides reliable information for Qfhb-5AS-based resistance and would be amenable to marker-assisted selection, introgression of the resistance loci, and pyramiding of FHB resistance in wheat cultivars.

Key words: Qfhb-5AS, SNP, KASP assay, marker-assisted selection.

Résumé : La brûlure de l’épi causée par Fusarium (FHB — « Fusarium head blight ») est une maladie dévastatrice du blé qui a d’importantes répercussions économiques. Le locus quantitatif (QTL — « quantitative trait loci ») Fhb5 joue un rôle important sur ce plan en conférant une résistance de type I à la maladie. Les auteurs ont tenté de déterminer si on pourrait recourir à un marqueur KASP (« Kompetitive Allele Specific PCR ») avec le QTL sur Fhb-5AS. Ils ont comparé les résultats des grappes KASP à ceux obtenus avec le marqueur SSR wmc705 associé à Qfhb-5AS. Les résultats indiquent que le locus du polymorphisme mononucléotidique wsnp_Ra_c24707_34262900 (IWA7777) fournit des informations fiables sur la résistance conférée par Qfhb-5AS et qu’on pourrait s’en servir pour la sélection assistée par marqueur, l’introgression des locus de résistance et le cumul de FHB chez les cultivars de blé. [Traduit par la Rédaction]

Mots-clés : Qfhb-5AS, polymorphisme mononucléotidique, technique KASP, sélection assistée par marqueur.

Introduction

Fusarium head blight (FHB), primarily caused by Fusarium graminearum Schwabe [teleomorph: Gibberella zeae (Schwein.) Petch], is a major fungal disease affecting wheat production globally. Canadian wheat production is severely affected by FHB, with the incidence and the severity of the disease expanding considerably in recent years due to changes in the wheat farming practices and climate change (McCartney et al. 2016). FHB has been a problem in eastern Canada since the 1980s, and it migrated into western Canada in the 1990s. Under favorable conditions for disease development, FHB significantly lowers wheat grain yield and quality and contaminates grains with the mycotoxin deoxynivalenol (DON) (McCartney et al. 2004). The development of FHB-resistant wheat cultivars is an effective, economic, and environmentally friendly strategy for sustainable disease management.

FHB resistance is a complex quantitative trait and is controlled by several small- to medium-effect quantitative trait loci (QTL). More than 250 QTL for FHB resistance distributed across all 21 wheat chromosomes have been identified, but only seven (Fhb1–Fhb7) have been formally designated (Steiner et al. 2017). A QTL for FHB on 5A (Qfhi.nau-5A) flanked by gwm293 and gwm415 in cultivar Wangshuibai was designated as Fhb5 (Xue et al. 2011). Fhb5 predominantly confers resistance to initial infection (type I), and to a lesser extent, also contributes resistance to the spread of infection through the rachis (type II). Fine mapping of Qfhs.ifa-5A (Buerstmayr et al. 2018) using a near-isogenic recombinant inbred line population separated Qfhs.ifa-5A (barc186–wmc805) into two...
closely linked QTL: Qfhs.ifa-5Ac (cfa2250–wmc705) and Qfhs.ifa-5AS (barc56–ldk49) (Steiner et al. 2019). The two tightly linked QTL are highly associated with anther retention and to a lesser extent plant height (Steiner et al. 2019).

Efficient introgression and tracking of QTL into elite breeding lines requires linked genetic markers. Among the simple sequence repeat (SSR) markers (barc117, gwm129, gwm293, gwm304, gwm415, and wmc705) linked to Fhb-SAS, only wmc705 was distinguishable and polymorphic in Canadian germplasm (McCartney et al. 2004). The polymorphic information content of the marker wmc705 (0.83) was the highest among the tested SSRs; the others were gwm415 (0.46), barc117 (0.53), gwm129 (0.62), gwm293 (0.77), and gwm304 (0.81) (McCartney et al. 2004). McCartney et al. (2016) resubstantiated the applicability of wmc705 in marker-assisted selection (MAS), and the marker continues to be used in Canadian breeding programs (Toth et al. 2019). Screening SSR markers on large breeding populations, however, is laborious and time consuming. Single-nucleotide polymorphisms (SNPs) are prevalent genetic changes that are distributed throughout the genome and are often available with tighter linkages to the gene(s) of interest. Kompetitive allele-specific polymerase chain reaction (PCR) (KASP) is a high-throughput and breeder friendly fluorescence-based genotyping assay for SNP markers and highly desirable for MAS (Toth et al. 2019). Various studies have identified SNPs associated with FHB QTL on 5A (Ren et al. 2019). The objective of this study was to develop a KASP assay for Qfhb-5AS to replace and (or) supplement the most informative SSR marker wmc705 and to enhance breeding efficiency for FHB resistance in the Canadian germplasm.

Materials and Methods

DNA extraction and primer design

A set of 85 Canadian spring wheat genotypes from various classes of wheat quality and disease resistance was used to test usability of the KASP marker. Genomic DNA was isolated from embryos using Qiagen DNeasy 96 Plant Kit (Qiagen, Canada, Toronto, ON, Canada) with slight modifications. Using an in silico validation to assess marker specificity based on the available wheat genome assembly (IWGSC 2018), several previously identified SNPs associated with Fhb-5A QTL were converted to KASP markers. One of the KASP primer sets for the loci wsnp_Ra_c24707_34262900 (IWA7777) was usable based on known disease resistance phenotype and the previously known wmc705 SSR marker.

Results and Discussion

The KASP assays were performed using the KASP master mix (LGC Genomics, Middlesex, UK) or PACE master mix (3crbio, Essex, UK) in a 10 μL reaction volume. The reaction consists of 5 μL of 2x master mix, 0.14 μL of primer mix, and 5 μL of sample DNA at 20 ng μL⁻¹. The PCR reactions were performed in a Bio-Rad CFX-96 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the touchdown PCR program: 94 °C for 15 min, followed by 10 cycles of touchdown PCR from 65 to 57 °C with 0.8 °C decrease per cycle, then followed by 32 cycles of 94 °C for 20 s and 57 °C for 1 min. The PCR plates were read with FLUOstar Omega microplate reader (BMG Labtech Inc., Cary, NC, USA), and the reader files were imported into ClusterCaller software (LGC Genomics, Middlesex, UK) for visualization of clusters and SNP allele calling.

SSR marker (wmc705) was amplified by PCR in a 25 μL reaction volume containing 100 ng of genomic DNA, 200 μmol L⁻¹ deoxynucleoside triphosphate, 1x Qiagen PCR buffer containing 1.5 mmol L⁻¹ MgCl₂, and 0.2 μmol L⁻¹ of each primer (For: 5′-GGTTGGCTTCCTGTCCTGA-3′ and Rev: 5′-CTCTGCACCTCCCCATGCTCT-3′) (McCartney et al. 2004), and 0.125 units HotStarTaq DNA polymerase (Qiagen, Valencia, CA, USA). Thermal cycling conditions comprised an initial denaturation at 95 °C for 15 min, followed by 32 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and a final extension step of 72 °C for 10 min.

Genotyping assays

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Results and Discussion

The cultivar Sumai-3 and its derivatives carrying Fhb1 and (or) Fhb5 have been used as major sources of resistance in Canadian wheat breeding programs. Resistance to FHB increases with the introgression of multiple FHB resistance alleles and significantly affect FHB index, Fusarium-damaged kernels, and DON (McCartney et al. 2016). Cultivars like AAC Cardale (Fox et al. 2013), AAC Connery (R. Cuthbert et al. unpublished data), and AAC Hodge (S. Kumar et al. unpublished data) with both resistant alleles (Fhb1 and Fhb5-AS) from Sumai-3 derivatives have moderate resistance (MR) rating to FHB and low levels of DON. Phenotypic selection of FHB usually is challenging and needs to be repeated in multiple years and locations, which makes DNA markers ideal for MAS to improve selection accuracy in breeding. Probing the presence of Fhb5-AS QTL using the SSR marker wmc705 amplifies smaller size amplicon(s) that pose challenges in scoring on regular gel-based electrophoresis. Moreover, the SSRs are difficult to scale up for high throughput screening of breeding populations.

The QTL identified on Fhb-5AS for which Sumai-3 alleles decreased FHB severity and DON content, were prioritized for the development of SNP-KASP assays. Several SNPs of the Qfhb-5AS were, therefore, selected
Table 1. Genotyping results and comparison of SNP-KASP and SSR markers on the set of wheat cultivars/line used for marker assay.

| Genotype       | Synonym     | Pedigree                  | FHB ratinga | Qfhb-5AS | IWA7777 | wmc705 |
|----------------|-------------|---------------------------|-------------|---------|---------|--------|
| AAC Tenacious  | HY615       | HY665/BW346               | R           | +       | +       |        |
| Sumai-3        | SU-49; Sobaku-3 | Fun/Taiwan-Xiaomai     | R           | +       | +       |        |
| Alsen          | BW316       | ND674//ND2710/ND688       | R-MR        | +       | +       |        |
| Cardale        | BW429       | McKenzie/Alsens          | MR          | +       | +       |        |
| AAC Cameron    | BW485       | D1125/Alsens/BW345/BW370/99B60-J26 | I   | +       | +       |        |
| AAC Connery    | PT245       | Somerset/BW865           | MR          | +       | +       |        |
| Faller         | ND805       | ND2857/ND2814            | I           | +       | +       |        |
| AAC LeRoy      | BW1049      | BW431/BW874              | MR          | +       | +       |        |
| FHB37          | 93FHB37     | HY611/Ning-8331          | R-MR        | +       | +       |        |
| Neepawa        | BW02        | Thatcher*7/Neepawa/*6/Kenya-Farmer/3/Thatcher*2//Frontana/Thatcher | I | −       | −       | −       |
| Columbus       | BW37        | Neepawa*6/RL-4137        | MS-S        | −       | −       | −       |
| Harvest        | BW259       | AC Domain*2//ND640        | S           | −       | −       | −       |
| Unity          | BW362       | McKenzie*3/BW174*2/Clark  | I           | −       | −       | −       |
| AC Barrie      | BW661       | Neepawa/Columbus//BW90    | I           | −       | −       | −       |
| AC Cadillac    | BW689       | Pacific*3/BW55            | I           | −       | −       | −       |
| Stettler       | BW867       | Prodigy/Superb           | MS          | −       | −       | −       |
| Carberry       | BW874       | Alsen/Superb             | MR          | −       | −       | −       |
| AAC Brandon    | BW932       | Superb/CDC Osler//ND744  | MR          | −       | −       | −       |
| Marquis        | Ottawa-15   | Hard Red Calcutta//Red-Fife | MS-S  | −       | −       | −       |
| Peace          | PT416       | BW165/R14660             | S           | −       | −       | −       |
| AAC Redwater   | PT457       | AC-Intrepid//Harvest//Mckenzie | I   | −       | −       | −       |
| Selkirk        | RL2769      | Mccuruchy//Exchange//3*Redman | MS-S | −       | −       | −       |
| Thatcher       | Songhuaijiang-1 | Marquis//fumillo//Marquis/| MS-S        | −       | −       | −       |
| CDC Plentiful  | PFS80       | BW282/CDC Go             | MR          | −       | −       | −       |
| AAC Warman     | BW1025      | BB07*A*637//Kane (BW342)  | MR          | −       | −       | −       |
| AAC Magnet     | BW1045      | BW388/BW430              | MR          | −       | −       | −       |
| Pasteur        | GP032       | Cadenza//Palermo//KS-91-WGRC-11 | I  | −       | −       | −       |
| Fielder        | HY908       | Yakntana-54-A*4//Norin-10//Brevor/3*Yaqui-50/4//Norin-10//Brevor//Baart/ONAS | MS-S | −       | −       | −       |

Note: “+” designates the presence and “−” designates the absence of the positive and (or) favorable allele. SNP, single nucleotide polymorphism; KASP, Kompetitive allele-specific polymerase chain reaction; SSR, simple sequence repeat; DON, deoxynivalenol; FHB, Fusarium head blight.

aFHB ratings: R, resistant; R-MR, resistant to moderately resistant; MR, moderately resistant; I, intermediate resistant; MS, moderately susceptible; MS-S, moderately susceptible to susceptible; S, susceptible. The ISD (Incidence Severity and DON) based rating for FHB is calculated by using the formula (0.2 × mean incidence + 0.2 × mean severity + 0.6 × mean DON).

for KASP marker development. To validate the specificity of KASP markers, it was used in conjunction with wmc705 to screen a set of 85 spring wheat genotypes (Supplementary Table S1). The SNP wsnp_Ra_c24707_34262900 (TCAP code: IWA7777, TaRefSeqv1.0_5A46232497; C/T) was converted into a robust KASP assay and used to genotype Canadian germplasm. Our findings suggest that the favorable allele for IWA7777 ("GG") is usable for the Qfhb-5AS QTL which comes from the highly Fusarium-resistant cultivar Sumai-3. The results of IWA7777 KASP marker assay aligned with the results of the SSR marker wmc705 in our assay. Of the 28 cultivars genotyped initially, 8 carried the Sumai-3 allele of the IWA7777 KASP marker, including the line FHB37 (Table 1; Fig. 1b). FHB37 (HY611/Ning 8331) has the same Qfhb-5AS allele as Ning 8331 (the male parent of FHB37) and is derived from Sumai-3 (Zhu et al. 2019). Our analysis with IWA7777 KASP marker confirms the presence of Qfhb-5AS allele in FHB37. The discrepancy between SSR wmc705 and IWA7777 KASP marker results on FHB37 for Qfhb-5AS allele could be due to the high sequence variability of the SSR wmc705 binding sites in the centromeric region. This may explain FHB37 being negative for SSR (McCartney et al. 2004) and positive for KASP, as well as moderate FHB resistance (rated MR). The validity of the IWA7777 KASP marker is further substantiated by the

1Supplementary data are available with the article through the journal Web site at https://doi.org/10.1139/CJPS-2020-0099.
discovery of the Fhb-5AS QTL (Qfhb.cau-5AS) at the same SNP loci as the IWA7777 (Ren et al. 2019).

To further substantiate our results that the IWA7777 KASP is a better marker than the SSR wmc705 using the reference genome sequence of wheat, a Blast search placed IWA7777 at the position 46.2 Mbp, close to Qfhs.ifa-5AS (70.7–119.9 Mbp) against the IWGSC RefSeq v1.0 (Fig. 1a). Steiner et al. (2019) placed wmc705 at position 290.0 Mbp on the long arm of 5A, close to the centromere. Based on this, the SSR wmc705 is further away from the Qfhs.ifa-5AS. The sequence similarity BLAST search did not return a 100% match for the position of wmc705, making it hard to predict the accurate position of the SSR marker wmc705. Centromeric regions generally have lower levels of recombination leading to discrepancy between genetic and physical distances, as observed in 5A chromosome by Xue et al. (2011). Thus, we demonstrate that IWA7777 is a better marker to assay the Fhb-5AS QTL in Canadian germplasm and should be a good replacement for wmc705 as well as amenable to high throughput screening in wheat breeding programs.

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