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Introgression of the coupled *Fhb1-Sr2* to increase Fusarium head blight and stem rust resistance of elite wheat cultivars

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Research Article

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Abstract

Fusarium head blight (FHB) and stem rust (SR) threaten the sustainability of wheat production worldwide. *Fhb1* and *Sr2* confer partial durable resistance to FHB and SR, respectively. Despite resistant alleles of both genes are linked in repulsion, lines with *Fhb1-Sr2* in coupling were developed at the University of Minnesota, USA. Marker-assisted backcrossing was used to incorporate the coupled *Fhb1-Sr2* into four elite INIA-Uruguay spring wheat varieties lacking both genes and expressing different levels of FHB and SR resistance. In each case, the initial cross between the donor line and recurrent parent was backcrossed three times. Genotypes carrying *Fhb1-Sr2* were selected using the molecular marker *UMN10*. In BC_3F_3 families, retention of *Fhb1-Sr2* was further confirmed with the markers *SNP3BS-8* and *Sr2-ger9* for *Fhb1* and *Sr2*, respectively. BC_3F_3 homozygous lines contrasting at *UMN10*, *SNP3BS-8* and *Sr2-ger9* were obtained to quantify the effect of *Fhb1-Sr2* on the resistance to FHB under controlled conditions and to SR under field conditions. After 26 months period, successful introgression of *Fhb1-Sr2* into the four cultivars was achieved, representing novel wheat genetic resources. Lines homozygous for the resistant alleles of *Fhb1* were significantly more resistant to FHB as reflected by an 18% reduction of average FHB area under the disease progress curve. A significant effect of *Sr2* on SR field resistance was observed in lines derived from the most susceptible cultivar 'Génesis 2375'. The most resistant lines to both diseases are expected to be valuable genetic resources in breeding for durable resistance to FHB and SR.

Introduction

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch], is a serious threat to wheat production worldwide (Stack, 2003; McMullen *et al.*, 2012; Petronaitis *et al.*, 2021). FHB can reduce yield and affect grain quality and safety, particularly due to contamination with mycotoxins such as deoxynivalenol (DON, Desjardins, 2006). After severe epidemics, the harvested grains may not be marketable due to toxin limits imposed for commercialization. The control of FHB presents great challenges, requiring the adoption of disease management strategies that integrate appropriate cultural practices, fungicide applications and most importantly the use of cultivars with acceptable levels of genetic resistance.

The genetics of FHB resistance is complex and associated with the presence of quantitative trait loci (QTLs; van Eeuwijk *et al.*, 1995; Mesterházy *et al.*, 1999; Buerstmayr *et al.*, 2009). More than 100 QTLs for resistance to FHB have been identified. Buerstmayr *et al.* (2009) summarized 22 regions present on 16 chromosomes containing QTLs with high stability and an effect on FHB resistance. The most important source of FHB resistance worldwide is the Chinese cultivar 'Sumai 3'. Many studies of the FHB resistance of 'Sumai 3' and its derivatives have been published (Anderson *et al.*, 2001; Liu *et al.*, 2008; Niwa *et al.*, 2014; Brar *et al.*, 2019). The most important QTL in 'Sumai 3', *Fhb1*, is located on chromosome 3BS and confers a high level of type II FHB resistance (i.e. resistance to the spread of infection within the spike; Liu *et al.*, 2006). Rawat *et al.* (2016) reported a gene encoding a pore-forming toxin-like protein as responsible for the FHB resistance conferred by *Fhb1*. Conversely, two subsequent independent studies (Li *et al.*, 2019; Su *et al.*, 2019) reported on the cloning of *Fhb1* and postulated a histidine-rich calcium-binding (*TaHRC* or *His*) gene as the *Fhb1* candidate. The current knowledge of the underlying genetic basis of *Fhb1* may boost the use of technologies for accelerating genetic improvement such as gene editing (Hao *et al.*, 2020). In two mapping populations, Anderson *et al.* (2001) found that simple-sequence repeat (SSR) markers linked with *Fhb1* accounted for 24.8 and 41.6% of the variation in FHB resistance. Subsequently, other researchers further confirmed the effect of *Fhb1* on other wheat populations

(Buerstmayr *et al*., 2002; Zhou *et al*., 2002; Pumphrey *et al*., 2007). Liu *et al*. (2008) and Bernardo *et al*. (2012) developed the diagnostic *Fhb1* molecular markers *UMN10* and *SNP3BS-8*, respectively, which are useful for marker-assisted selection (MAS).

Stem rust (SR), caused by *Puccinia graminis* f. sp. *tritici* (Pgt), was historically considered the most destructive disease of wheat (Singh *et al*., 1995). SR was successfully controlled in most of the wheat cropping regions for over 30 years using resistant cultivars (Singh *et al*., 2008). However, since 1998, new virulent races of the pathogen, named the Ug99 race group were detected in Eastern Africa (Pretorius *et al*., 2000; Wanyera *et al*., 2006). Ug99 and derived races are virulent to most of the known *Sr* resistance genes. About 90% of the current wheat varieties worldwide exhibited partial to complete susceptibility to these races when tested in the annual SR assessments conducted in Kenya and Ethiopia from 2005 to 2014 (Singh *et al*., 2015). Ug99 and/or derived races have already migrated to other countries in Eastern Africa and Asia (Sharma *et al*., 2013) and their possible migration to other regions poses a major threat to wheat production worldwide.

SR can be successfully controlled by genetic resistance. More than 60 genes associated with SR resistance have been identified (McIntosh *et al*., 2017). Most of these genes are major genes conferring qualitative race-specific and all-stage resistance and have been extensively used in wheat breeding programmes. However, qualitative SR resistance has most frequently been effective for short periods due to the rapid adaptation of the pathogen to the major resistance genes through mutations, that turn avirulent into virulent phenotypes (Dawkins and Krebs, 1979). Five minor resistance genes to SR have been catalogued: *Sr2*/*Lr27*/*Yr30*/*Pbc1* (Mago *et al*., 2011), *Sr55*/*Lr67*/*Yr46*/*Pm46*/*Ltn3* (Herrera-Foessel *et al*., 2014), *Sr56* (Bansal *et al*., 2014), *Sr57*/*Lr34*/*Yr18*/*Pm38*/*Sb1* (Singh, 2012) and *Sr58*/*Lr46*/*Yr29*/*Pm39* (Singh *et al*., 2013). These genes confer quantitative non-race specific resistance at the adult plant stage. They also most often confer or are tightly linked to genes that confer resistance to other diseases such as leaf rust (*Lr* genes), yellow rust (*Yr* genes) and powdery mildew (*Pm* genes). *Sr2* has been the most widely used minor gene, usually in combination with major or other minor genes, since *Sr2* provides insufficient protection by itself (Singh *et al*., 2006). Molecular markers for *Sr2* have been developed; csSr2, *Sr2-ger9* (Mago *et al*., 2011) and *Wms533* (Röder *et al*., 1998) are the most commonly used.

The introduction of *Fhb1* and *Sr2* in commercial wheat cultivars has been a high priority in wheat breeding programmes in North America, Europe and in the International Maize and Wheat Improvement Center (CIMMYT, Anderson *et al*., 2001; Yang *et al*., 2003; Miedaner *et al*., 2006; Ellis *et al*., 2007; He *et al*., 2015). However, a major constraint for their combined introduction in advanced germplasm was that these two genes were linked in repulsion phase and in close proximity, approximately 3 cM apart (Anderson *et al*., 2001). Recently, Zhang *et al*. (2016) overcame this restriction by developing lines that contain both genes in coupling phase which represents a major breakthrough for breeding for resistance to SR and FHB.

Both, FHB and SR are important diseases in wheat growing areas in South America. In Uruguay and neighbouring countries, an increased occurrence of FHB epidemics was observed in the last few decades (Pereyra and Lori, 2013). A considerable expansion in the use of wheat cultivars susceptible to SR after 2000 may have contributed to generalized SR epidemics in 2014 and 2015 in Argentina and Uruguay (Campos *et al*., 2015). *Fhb1* is present at a very low frequency in INIA-Uruguay Wheat Breeding Program's germplasm, while *Sr2* has been previously introduced to the program from germplasm which carried *Fhb1* and *Sr2* in repulsion phase.

The present study had two objectives: (1) to introduce *Fhb1* and *Sr2* in coupling phase in elite wheat germplasm from INIA-Uruguay and (2) to quantify the effect of *Fhb1* and *Sr2* in enhancing the resistance to FHB and SR, respectively, in the developed lines. A donor line with coupled *Fhb1-Sr2* was used in a backcrossing scheme with four INIA wheat cultivars lacking both genes and expressing different levels of resistance to FHB and SR. For each cross, wheat lines carrying the *Fhb1-Sr2* introgression were selected during the backcross process using the molecular marker *UMN10*. The presence of the *Fhb1-Sr2* introgression in the BC_3F_2 lines was verified with additional markers. The effect of the introduced genes on the level of resistance to both diseases was quantified in the different genetic backgrounds comparing the level of resistance of BC_3F_3 homozygous lines for resistant or susceptible alleles under greenhouse conditions for *Fhb1*, and under field conditions for *Sr2*.

Materials and methods

Plant materials and development of populations

'Sf26' (CO03W269/Bigg Red), the donor line of the coupled *Fhb1-Sr2*, was developed by the University of Minnesota Wheat Breeding Program (Zhang *et al*., 2016) and kindly provided by Dr Jim Anderson. 'Sf26' was crossed and backcrossed with four Uruguayan elite wheat cultivars 'LE 2375 – Génesis 2375' (LE2302/3/PF90099/OR1//GRANITO), 'LE 2387 – Génesis 6.87' (PF90099/OR1//GRANITO/3/BAG10), 'LE 2332 – INIA Madrugador' (E.FED//CHUANMAI/BAU) and 'LE 2331 – INIA Don Alberto' (I.TIJ/LE2229), which have different levels of resistance to FHB and SR and carry the susceptible alleles of the molecular markers *UMN10* for *Fhb1* and csSr2 for *Sr2*. According to the Uruguayan National Cultivar Evaluation Program (INIA/INASE; Castro *et al*., 2015, 2019) the resistance level to FHB type II resistance and FHB field resistance for the four cultivars were: (i) 'Génesis 2375': moderately susceptible (MS) and moderately resistant (MR), (ii) 'Génesis 6.87': MRMS and MR, (iii) 'INIA Madrugador': MS and MRMS and (iv) 'INIA Don Alberto': susceptible (S) to MS and S, respectively. The characterization of field resistance for SR were: (i) 'Génesis 2375': MRMS, (ii) 'Génesis 6.87': MS, (iii) 'INIA Madrugador': MRMS and (iv) 'INIA Don Alberto': MSMR. This characterization of resistance was based on at least 3 years of disease data from trials and specific nurseries.

'Sf26' and the four INIA elite cultivars were planted and crossed in the field in 2016 at INIA La Estanzuela (LE: latitude 34.3°S, longitude 57.7°W, elevation 70 masl). Generations F_1 to BC_3F_1 were grown in greenhouse facilities at INIA La Estanzuela at 20–30°C and light supplementation with 400 W high sodium pressure lamps between 8:00 P.M. and 6:00 A.M. Plants were grown in pots filled with 1 kg of a mixture of 1/3 of soil, 1/3 substrate (organic compost, VITATERRA) and 1/3 vermiculite (expanded vermiculite, Agrinobre). The water-soluble fertilizer Milagro (18:18:18 N–P–K plus microelements) from DM Agro Company was applied once a week, at a rate of 0.2 g/pot, starting at 15 days after planting until anthesis. The BC_3F_2 generation was grown in a growth chamber at 22°C temperature, 75–85% relative humidity and 470 W LED lightning (GCLB-8 2nd Generation, Grow Candy) in an 18 h light:6 h dark cycle, for accelerated plant development.

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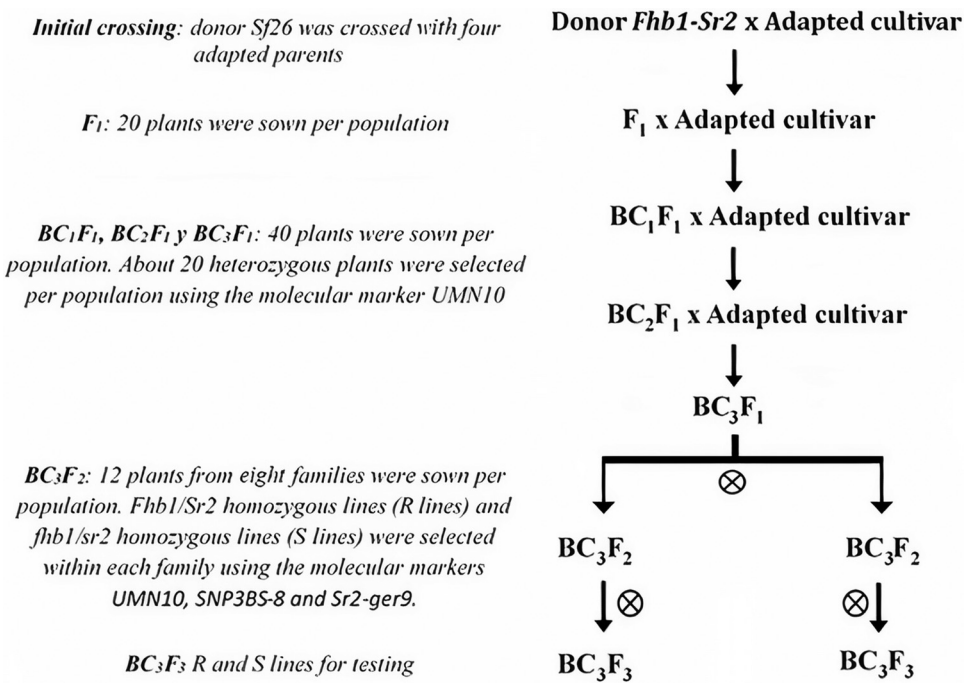

Fig. 1. Marker-assisted backcrossing protocol used for the introduction of *Fhb1-Sr2* locus in each of four INIA elite cultivars ('Génesis 2375', 'Génesis 6.87', 'INIA Madrugador' and 'INIA Don Alberto') as recurrent parents, and development of resistant (R) and susceptible (S) lines. ⊗: Selfing.

'Sf26' was used as the female parent in the initial crosses (Fig. 1). Twenty F_1 plants per population, each population derived from one of the four different crosses, were used as the female parent in the backcross with each INIA cultivar to obtain the BC_1F_1 generation. Subsequently, heterozygous *Fhb1-Sr2/fhb1-sr2* plants were selected with marker *UMN10* from 40 BC_1F_1 and BC_2F_1 plants per population, which were used as the female parents in the following crosses with the INIA cultivars. Heterozygous BC_3F_1 plants were selected among 40 plants for selfing. Twelve plants from each of eight BC_3F_2 families per population were sown, each family coming from the same heterozygous BC_3F_1 plant. The BC_3F_2 homozygous *Fhb1-Sr2* lines (R lines) and homozygous *fhb1-sr2* lines (S lines) were selfed for seed increase. Five-to-seven pairs of R and S lines were selected from each of the eight BC_3F_2 families sown per population for FHB and SR phenotyping.

Marker-assisted selection

The presence of *Fhb1-Sr2* was determined using the closely diagnostic sequence-tagged site marker *UMN10* developed for *Fhb1* (Liu *et al.*, 2008). DNA extractions from the plant material were performed at the Biotechnology Laboratory of INIA La Estanzuela using the CTAB 2% method (Doyle, 1987). Polymerase chain reaction (PCR) was carried out with a volume of 10 µl per reaction, consisting of 3.0 µl of DNA (30 ng/µl), 1 µl of 10× PCR buffer, 0.6 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (2.0 mM), 0.1 µl of primer F (10 µM), 0.1 µl of primer R (10 µM), 0.05 µl of Taq enzyme (5 U/µl) and 4.15 µl of Milli-Q water. The amplification protocol of the *UMN10* marker was performed as indicated by Liu and Anderson (2003). The detection of the *UMN10* marker on the DNA samples was performed with the 3730xl 96-Capillary Genetic Analyzer (Applied Biosystems) at Yale University. DNA from cultivar 'Sumai 3' and the donor line 'Sf26' were used as positive controls for the *Fhb1* resistance allele. As described in Liu *et al.* (2008), the *Fhb1* allele had a band size of 240 bp, while the susceptibility allele *fhb1* had a band size of 237 bp.

In BC_3F_2, the presence of *Fhb1* and *Sr2* was further confirmed by assessing with the Kompetitive Allele-Specific PCR Genotyping System (KASP™) of *UMN10* and *SNP3BS-8* markers for *Fhb1* (Bernardo *et al.*, 2012) and the *Sr2-ger9* marker for *Sr2* (Mago *et al.*, 2011) at the Wheat Molecular Breeding Laboratory of CIMMYT, El Batán. Polymorphic marker primer sequences, type of marker and annealing temperatures used, are listed in Table 1. The PCR protocol and the visualization of the genotypic data for KASP were performed as indicated in Dreisigacker *et al.* (2016).

Evaluation of FHB resistance

Type II FHB resistance was determined under greenhouse and growth chamber conditions at INIA La Estanzuela between October 2018 and February 2019. Plants were grown in a greenhouse until anthesis as previously described for the backcrossing protocol. Two experiments were carried out with a month's-interval (sowing dates 24 October and 20 November). Each experiment had a complete block design with 10 blocks. Each experimental unit consisted of one pot with two plants. A total of 46 treatments included: five BC_3F_3 homozygous *Fhb1-Sr2* R lines, five BC_3F_3 homozygous *fhb1-sr2* S lines per population, each pair of lines derived from the same BC_3F_1 family and six checks ('Sumai 3', 'Sf26' and the INIA adapted parents).

Plants were inoculated at anthesis (Z6.5, Zadoks *et al.*, 1974) with a mix of 14 *F. graminearum* (*sensu stricto*) monosporic isolates collected from different cultivars, locations and years, representing the *F. graminearum*, 15ADON chemotype of the current population diversity in Uruguay. Inoculum concentration was adjusted to 2×10^5 conidia/ml and 0.025 ml of Tween 20 was added per ml of the suspension. Ten microlitres of the inoculum suspension was placed into each of the two central, alternate spikelets of the main spike of each plant with an Eppendorf pipette. Plants were maintained at room temperature for 1 h and then placed in a dew chamber for 72 h (20–22°C, 100% relative humidity). Plants were then transferred to a growth chamber (24°C, 75–85% relative humidity and 12 h photoperiod).

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Table 1. Characteristics of the molecular markers used to detect the presence/absence of *Fhb1-Sr2*

Marker	Primer	Sequence (5′–3′)	T_A (°C)	Reference
UMN10[a]	Allele R (VIC-tail)	GAATTACTCATTTTTAGATTTGTCTACATACG	50	Liu *et al.* (2008)
	Allele S (FAM-tail)	GAATTACTCATTTTTAGATTTGTCTACATACA		
	Common	GAAGTTCATGCCACGCATATGCTAGTA		
SNP3BS-8[a]	Allele R (VIC-tail)	CACATGCATTTGCAAGGTTGTTATCG	54	Bernardo *et al.* (2012)
	Allele S (FAM-tail)	CACATGCATTTGCAAGGTTGTTATCC		
	Common	CAAAGCAGCCTTAGGTCAATAGTTTGAAA		
Sr2-ger9[a]	Allele R (VIC-tail)	GTGCGAGACATCCAACACTCAT	63–55[b]	Mago *et al.* (2011)
	Allele S (FAM-tail)	GAATTACTCATTTTTAGATTTGTCTACATACA		
	Common	CTCAAATGGTCGAGCACAAGCTCTA		
UMN10[c]	Forward	CGTGGTTCCACGTCTTCTTA	60	Liu *et al.* (2008)
	Reverse	TGAAGTTCATGCCACGCATA		

T_A, annealing temperature.
[a]KASP marker.
[b]Touchdown over 63–55°C for 60 s 10 cycles (dropping 0.8°C per cycle).
[c]SSR marker.

Disease severity (DS) (percentage of symptomatic spikelets per spike) was evaluated at 7, 14 and 21 days after inoculation (dpi). Area under the disease progress curve (AUDPC) was calculated and used to estimate type II FHB resistance:

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{Y_i + Y_i + 1}{2}\right)(t_i + 1 - t_i)$$

where n is the total number of observations, y_i is the severity of observation ith and t_i is the time for observation ith. A Box–Cox transformation was used to correct for normal distribution of the residuals of FHB AUDPC (FHB N_AUDPC). The effect of the *Fhb1* introduction in reducing FHB disease was estimated as:

$$Fhb1 \text{ effect} = \frac{\text{FHB N\_AUDPC}_{\text{S Lines}} - \text{FHB N\_AUDPC}_{\text{R Lines}}}{\text{FHB N\_AUDPC}_{\text{S Lines}}} \times 100$$

were *Fhb1* effect is the percentage of FHB N_AUDPC reduction caused by *Fhb1* on R lines compared to S lines.

Evaluation of SR resistance

SR resistance was evaluated at La Estanzuela Experimental Station in a field experiment with an incomplete block design with three replications sown on 15 July 2019. Each experimental unit consisted of a single 1 m row. Treatments included five to seven pairs of BC_3F_3 R and S lines from each population (each pair of lines derived from the same BC_3F_1 family), and six checks ('Baguette 11', 'Baguette 601' and the four INIA parents). A spreader row of SR susceptible cultivars ('Onix', 'Baguette 11', 'Baguette 13' and 'Baguette 601') was planted perpendicular to the plots to promote a homogeneous infection. Fertilization and weed control were performed as required. The trial was artificially inoculated four times, starting on stage Z2.2 (Zadoks *et al.*, 1974). The first and the last inoculations (August 28 and October 28) were performed spraying a suspension of urediniospores on distilled water with two drops of Tween 20 on the plots and the spreader row. The second and third inoculations (September 11 and October 7) were performed injecting the same spore suspension into three tillers per plot and on random tillers of the spreader row. The inoculum was a mix of spores collected in the field during winter 2018 plus four Pgt isolates representing four different races of SR collected in previous years: isolates 2048, 2372, 2749 and 2931 corresponding to races SPLKC, RHKTF, RRTTF and QFCSC (Jin *et al.*, 2008), respectively. The Pgt races were increased on the susceptible cultivar 'Little Club' under greenhouse conditions and maintained at INIA Rust Laboratory.

SR DS and infection response (IR) were recorded on stems and leaf sheaths on November 19 and November 28 (approximately Z.7 and Z.8, Zadoks *et al.*, 1974). DS was evaluated using the modified Cobb Scale (Peterson *et al.*, 1948) and IR was assessed as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) as described by Roelfs *et al.* (1992). The coefficient of infection (CI) was calculated as the DS multiplied by a coefficient corresponding to IR (R: 0.2, MR: 0.4, I = MRMS: 0.6, MS: 0.8, S: 1.0) as described by Stubbs *et al.* (1986). The SR AUDPC was calculated using the CI according to Campbell and Madden (1990). The effect of the *Sr2* introduction in reducing SR disease was estimated as:

$$Sr2 \text{ effect} = \frac{\text{SR AUDPC}_{\text{S Lines}} - \text{SR AUDPC}_{\text{R Lines}}}{\text{SR AUDPC}_{\text{S Lines}}} \times 100$$

were *Sr2* effect is the percentage of SR AUDPC reduction caused by *Sr2* on R lines compared to S lines.

Statistical analysis

FHB AUDPC residuals did not follow a normal distribution according to a Shapiro–Wilk test for normality (Shapiro and Wilk, 1965, P-value <0.01). After Box–Cox transformation (Box and Cox, 1964), the result from the normality test indicated that there was no evidence against the assumption of normality for the normalized FHB AUDPC (N_AUDPC, P-value of 0.12) and this variable was used for the statistical analysis.

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The model used to analyse FHB N_AUDPC data was:

$$y_{ijklm} = \mu + T_i + R_{i(j)} + M_k + \mathrm{TM}_{ik} + P_l + \mathrm{MP}_{kl} + F_{m(l)} + \varepsilon_{ijklm}$$

where y: FHB N_AUDPC, μ: general mean, T: trial main effect $l = \{1–2\}$, R: repetition or block effect $m = \{1–10\}$ nested in T $l = \{1–2\}$, M: *Fhb1* marker main effect $i = \{1$: presence, 2: absence$\}$, TM: *Fhb1* marker effect by trial effect interaction, P: population main effect $k = \{1–4\}$, MP: *Fhb1* marker effect by population effect interaction, F: family main effect $j = \{1–5\}$ nested in P $k = \{1–4\}$ and ε: experimental error with normal distribution $N(0, \sigma^2\varepsilon)$.

The model used to analyse the SR AUDPC was:

$$y_{ijklm} = \mu + R_m + B_{l(m)} + M_i + P_k + \mathrm{MP}_{ik} + F_{j(k)} + \varepsilon_{ijklm}$$

where y: SR AUDPC, μ: general mean, R: repetition $m = \{1–3\}$, B: incomplete block effect as random $l = \{1–8\}$ nested in $\underline{R}$ $m = \{1–3\}$, M: *Sr2* marker main effect $i = \{1$: presence, 2: absence$\}$, P: population main effect $k = \{1–3\}$, MP: *Sr2* marker effect by population effect interaction, F: family main effect $j = \{1–7\}$ nested in P $k = \{1–3\}$ and ε: experimental error. Random terms, B and ε, follow a normal distribution with $N(0, \sigma^2 b)$ and $N(0, \sigma^2\varepsilon)$, respectively.

First, the proposed analysis of variance (ANOVA) models were used to estimate the effect of *Fhb1-Sr2* presence/absence on the level of resistance to FHB and SR of BC_3F_3 homozygous lines and to estimate if there was an interaction between the *Fhb1-Sr2* introgression and the genetic backgrounds of the different populations. Second, multiple comparisons of means were performed (Tukey–Kramer range test; Tukey, 1949) to compare the level of resistance in the different introgressed lines, the parental INIA cultivars, the *Fhb1-Sr2* donor line and the checks. The ANOVA and Tukey–Kramer range test assumptions were tested and there were no violations of these. The significance threshold level was set as $\alpha \leq 0.05$ for all the analyses performed. Statistical analyses were performed using R statistical software with the stats package (R Core Team, 2016).

Results

Development of Fhb1-Sr2 *lines*

Seven generations were required to obtain homozygous BC_3F_3 R lines with *Fhb1-Sr2* and S lines with *fhb1-sr2*. The overall protocol took 26 months with an average duration of each generation of 3.7 months (3.2 generations per year).

Sixty-nine BC_3F_1 heterozygous individuals were obtained: 18, 19, 16 and 16 for the populations derived from 'Génesis 2375', 'Génesis 6.87', 'INIA Madrugador' and 'INIA Don Alberto', respectively. After selfing, eight BC_3F_2 families were selected from population 'Sf26'/'Génesis 2375' and seven from each of the other populations (Table 2), each family coming from the same heterozygous BC_3F_1 plant. The segregation ratio estimated in the BC_3F_2 progeny with the marker *UMN10* approximated 1:2:1 for homozygote resistance, heterozygotes and homozygote susceptible plants as expected for the segregation of one gene. Five-to-seven pairs of R (*Fhb1-Sr2*) and S (*fhb1-sr2*) BC_3F_3 lines per population were selected for FHB and SR phenotyping.

FHB type II resistance

FHB severity is described here to illustrate the level of infection obtained in the experiments. Average FHB severity of all BC_3F_3 lines and checks at 7, 14 and 21 dpi were 18.3, 38.8 and 45.9%, respectively. The FHB resistant check 'Sumai 3' and the 'Sf26' donor line had a mean severity of 16.6 and 46.2% at 21 dpi, respectively, while the average severity of the recurrent parents ranged from 38.9 to 70.6% at the same time of evaluation.

The *Fhb1* marker and population effects were highly significant (P-value <0.001, online Supplementary Table S1), while other sources of variation had no significant effect on FHB N_AUDPC.

The mean FHB N_AUDPC of R lines was 60.3, significantly lower than the FHB N_AUDPC of the S lines of 73.9 (Fig. 2a); therefore, *Fhb1* determined an overall average reduction of the FHB N_AUDPC of 18.4%. The corresponding FHB severity at 21 dpi was 35.6% for the R lines and 51.1% for the S lines.

Mean FHB N_AUDPC values of the populations derived from 'Génesis 2375' and 'Génesis 6.87' were significantly lower than the ones of the populations derived from 'INIA Madrugador' and 'INIA Don Alberto' (Fig. 2b). The population derived from 'INIA Don Alberto' had significantly higher N_AUDPC values than the population derived from 'INIA Madrugador' (Fig. 2b).

Although the *Fhb1* × Population interaction effect on FHB N_AUDPC was not significant at the threshold level of significance fixed in this study, the P-value of this interaction was low (0.09203). A trend was observed of higher FHB N_AUDPC reductions on R lines *vs* S lines in populations derived from 'Génesis 6.87' (22.7%) and 'INIA Madrugador' (21.7%), compared to the population derived from 'Génesis 2375' (13.4%, Fig. 3). R lines derived from 'INIA Don Alberto' had intermediate reduction of FHB N_AUDPC (17.0%) than S lines. In all cases, the FHB N_AUDPC of the S lines was similar to that of the recurrent parents.

SR resistance

SR CI on the second date of evaluation is described here to illustrate the level of infection obtained in the experiments. The susceptible check 'Baguette 601' had the highest SR DS with an average CI of 77.3 (range from 72 to 80), while the check 'Baguette 11' had a mean SR disease CI of 56,0. Due to very low infection (SR disease CI average of 0.7 and maximum CI 3 and 7, respectively), 'INIA Don Alberto' and its derived lines were removed from the statistical analysis.

The effects of *Sr2* marker, population, family and *Sr2* marker × Population interaction on SR AUDPC were highly significant (P-value <0.001, online Supplementary Table S2).

The effect of family nested in population (F) was significant. Multiple comparisons of means (Tukey–Kramer range test) showed significant differences in mean SR AUDPC between families 8 and 6 derived from 'Génesis 2375' (17.0 and 50.4 respectively) and families 3 and 4 derived from 'Génesis 6.87' (4.4 and 40.6, respectively). The interaction between *Sr2* marker and population (MP) on SR AUDPC was highly significant.

The susceptible check 'Baguette 601' had significantly higher SR AUDPC than the rest of the treatments (Fig. 4). The R lines (*Fhb1-Sr2*) derived from 'Génesis 2375' had significantly lower SR AUDPC than the S lines (*fhb1-sr2*) (54.5% reduction). The reduction of SR AUDPC caused by the presence of *Sr2* of 18.9 and 17.2% observed in R lines compared to S lines from 'Sf26'/'Génesis 6.87', and 'Sf26'/'INIA Madrugador', respectively, was not significant. The average SR AUDPC of the S lines did not differ significantly from the AUDPC of their respective recurrent parents.

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Table 2. Number of selected BC_3F_2 families and homozygous and heterozygous plants per population

		Number of plants		
Population	Number of families BC_3F_2	*Fhb1-Fhb1* *Sr2-Sr2*	*Fhb1-fhb1* *Sr2-sr2*	*fhb1-fhb1* *sr2-sr2*
Sf26/G.2375	8	24	47	25
Sf26/G.6.87	7	17	53	25
Sf26/I.Mad	7	17	51	28
Sf26/I.DAl	7	23	49	22
Total	29	81	200	100

G.2375, 'Génesis 2375'; G.6.87, 'Génesis 6.87'; I.Mad, 'INIA Madrugador'; I.DAl, 'INIA Don Alberto'.

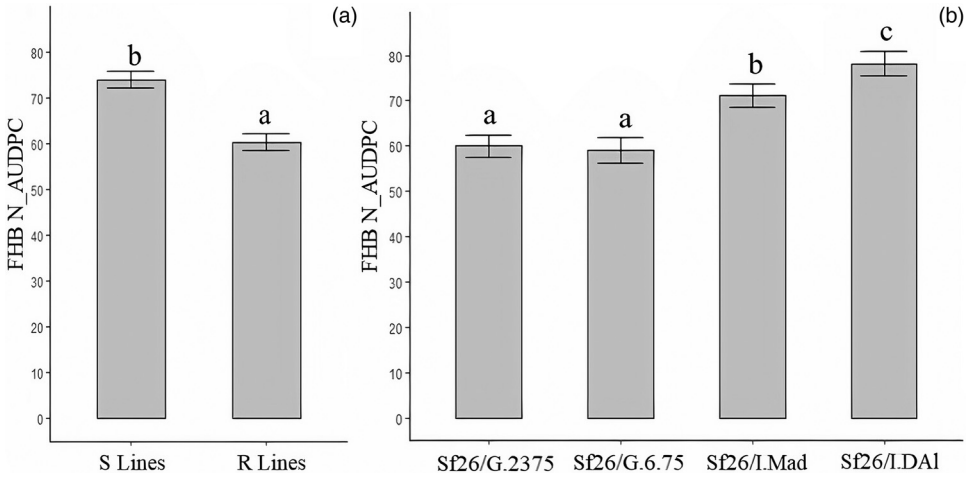

Fig. 2. Comparison of FHB normalized area under the disease progress curve (FHB N_AUDPC) means between R (*Fhb1-Sr2*) and S (*fhb1-sr2*) lines (a) considering all populations, and (b) between the four populations. N_AUDPC values with different letters are significantly different based on a Tukey–Kramer Range test (*P*-value ≤0.05). The error bars represent the 95% confidence interval around the estimate.

Discussion

The coupled resistance genes *Fhb1* and *Sr2* were successfully backcrossed into four Uruguayan wheat cultivars and their effects in enhancing the resistance to FHB and SR were quantified. *Fhb1* increased the FHB resistance in all genetic backgrounds, but *Sr2* had a statistically significant effect on SR only in the genetic background of the most susceptible recurrent parent.

The marker-assisted backcrossing (MABC) protocol developed in this work was efficient, allowing the rapid selection of lines homozygous for *Fhb1* at the seedling stage in 26 months, while in the case of INIA's wheat breeding traditional protocol with one generation per year it would have taken 7 years. MABC can be performed in the seedling stage while under the traditional schemes of phenotypic selection, screening must be done in adult plants. MAS also allowed the selection of the minor gene *Sr2*, which is often challenging because its partial resistance phenotype may be masked by the presence of major effective resistance genes. This protocol is a relevant technological contribution for Uruguay, since it was the first MAS used to enhance resistance to FHB in this country. Our results confirm that the use of the single marker *UMN10* during the backcrossing process was sufficient for the selection of plants with the *Fhb1-Sr2* introgression, as expected due to the strong linkage between these two genes. Subsequently, the presence of *Sr2* in the developed lines was further confirmed using the *csSr2* marker at the end of the MABC protocol. Based on these results, we could confirm that the functional marker *UMN10* is a reliable marker for the selection of the coupled *Fhb1-Sr2* genes.

A significant effect of *Fhb1* in reducing FHB was found in the lines derived from all crosses. The lines with *Fhb1* had an average reduction of the disease of 18.4% compared to lines carrying *fhb1*. He *et al.* (2020) introgressed the coupled *Fhb1-Sr2* genes in elite CIMMYT wheat lines. Twenty-five derived lines were tested in 32 countries and 10 showed good FHB resistance with less than 30% FHB field severity. The donor lines carrying the coupled genes used in their study came from a different origin than our donor line. Other authors estimated reductions of 24.8 and 41.6% in two different populations (Anderson *et al.*, 2001), and 15.4% (Waldron *et al.*, 1999) in the FHB infection attributed to *Fhb1* under greenhouse conditions. Pumphrey *et al.* (2007) studied the effect of *Fhb1* in 19 pairs of near isogenic lines developed from breeding lines of the University of Minnesota and reported that *Fhb1* increased 31% type II FHB resistance under greenhouse conditions. While the average FHB reduction attributed to *Fhb1* in our study was in the same range of values obtained in other studies, several factors should be taken into account to compare these results. Most of the reports estimated *Fhb1* QTL effect in

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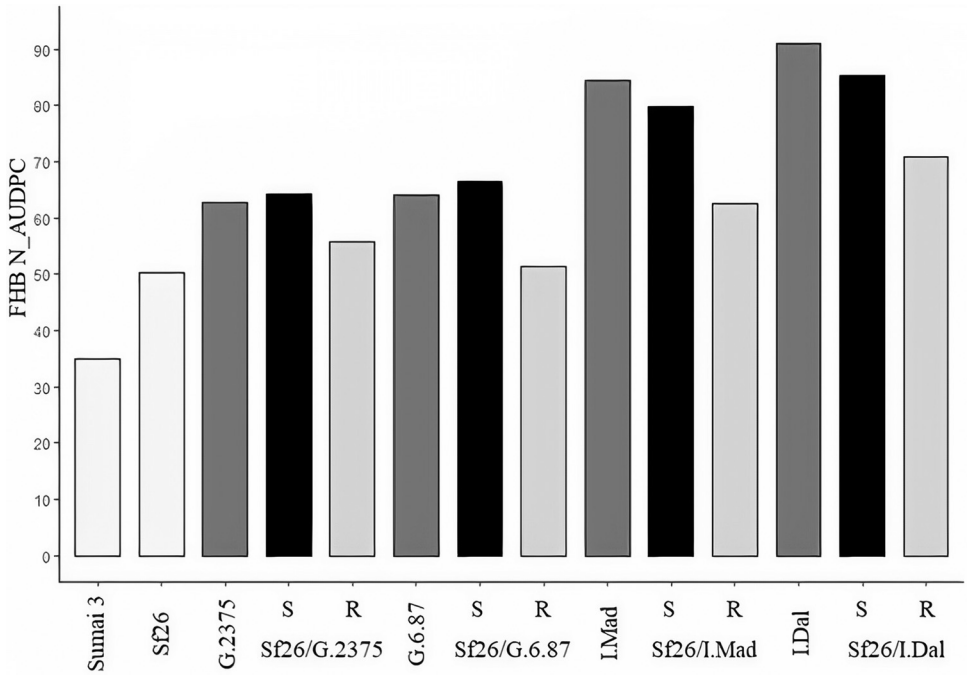

Fig. 3. Mean FHB normalized area under the disease progress curve (FHB N_AUDPC) of the resistant check ('Sumai 3'), the donor parent ('Sf26'), the recurrent parents (G.2375: 'Génesis 2375', G.6.87: 'Génesis 6.87', I.Mad: 'INIA Madrugador', I.DAl: 'INIA Don Alberto') and the R (*Fhb1-Sr2*) and S lines (*fhb1-sr2*) from each population. The percentage of FHB N_AUDPC reduction of R lines compared to S lines is shown above the R lines bars.

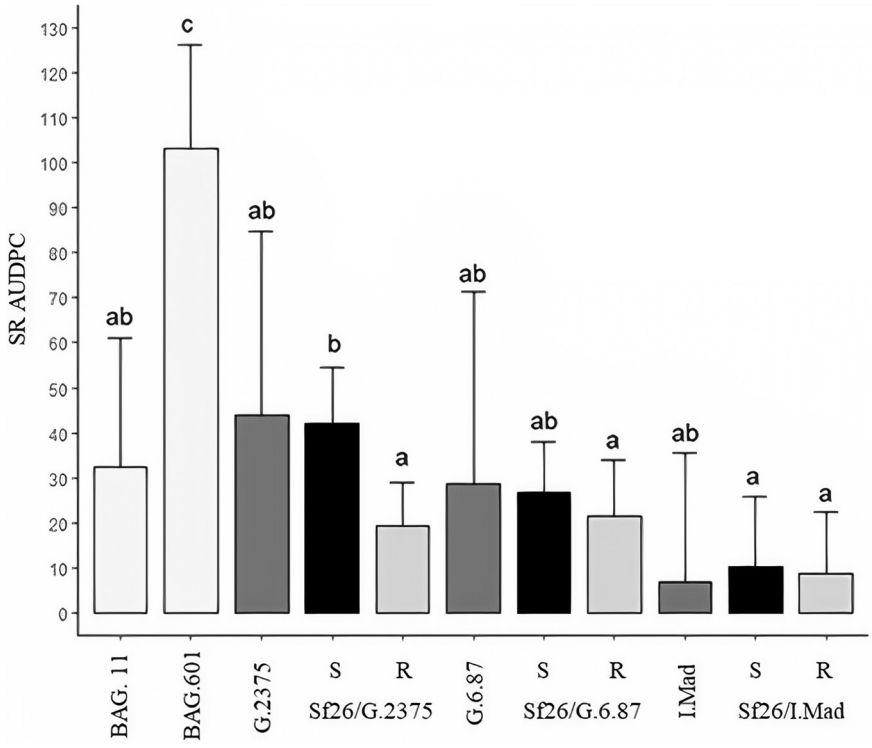

Fig. 4. Mean SR AUDPC of checks (BAG.11: 'Baguette 11', BAG.601: 'Baguette 601'), recurrent parents (G.2375: 'Génesis 2375', G.6.87: 'Génesis 6.87' and I.Mad: 'INIA Madrugador'), and R and S lines from each population. AUDPC values with different letters are significantly different (Tukey–Kramer Range, *P*-value ≤0.05). The black lines represent the 95% upper confidence interval around the estimate.

mapping populations, i.e. Zhang *et al.* (2012), derived in general from parents with highly distant genetic background, which might affect the *Fhb1* QTL effect. Also, the QTL effects can be influenced by experimental conditions, growth environment and inoculation method being the most relevant (Pumphrey *et al.*, 2007). Jin *et al.* (2013) and Salameh *et al.* (2011) estimated an *Fhb1* effect of 25.1 and 35%, respectively, in breeding materials under field conditions comparing genotypes with and without *Fhb1*.

R lines derived from 'Génesis 2375' and 'Génesis 6.87' were more resistant than lines derived from 'INIA Don Alberto' and those derived from 'INIA Madrugador' had intermediate

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resistance. The resistant lines from the different crosses followed the same pattern as the resistance of their respective recurrent parents, indicating that at least part of the background resistance was recovered, as expected for three backcrosses.

Even when only type II resistance was evaluated in our study, the FHB infection of the parental cultivars under greenhouse conditions (Fig. 2b) followed the same trend of the resistance characterized in field studies: 'Génesis 2375' and 'Génesis 6.87' were the most resistant, followed by 'INIA Madrugador', and 'INIA Don Alberto' was the most susceptible cultivar. This confirms the high contribution of type II resistance to FHB field resistance.

Sr2 interacted with the genetic components associated to SR resistance in the genetic background of the cultivars used since the *Sr2* marker × Population interaction was significant (online Supplementary Table S2). A significant difference in SR AUDPC among R and S lines was only found in the population derived from the most susceptible cultivar to SR tested, 'Génesis 2375' (Fig. 4). The introduction of *Sr2* into the 'Génesis 2375' genetic background resulted in a high reduction of disease under field conditions, of 54.5% on R lines (*Sr2*) related to S (*sr2*) lines. The relatively low infection on 'INIA Don Alberto' and 'INIA Madrugador' populations indicates that the races used were avirulent on these recurrent parents. This may explain the lack of significance of the effect of *Sr2* in those populations. The relevance of the incorporation of *Sr2* in resistant backgrounds will be its capacity to reduce the SR infection in the case that virulent races to the recurrent parents become prevalent in the pathogen population.

The disagreement between the low field infection of 'INIA Madrugador' and 'INIA Don Alberto' with the expected field performance was most probably due to the avirulence/virulence patterns of the races present in the field experiment. The information of the resistance provided by the National Cultivar Evaluation Program (see *Plant materials and development of populations* section) was most probably obtained under epidemics caused by different races. The characterization of field resistance is based on at least 3 years of disease data. However, the maximum susceptibility is kept even when in a certain period the level of infection is lower, since the potential for disease development on the cultivar is intended to be expressed by the characterization.

Few studies estimate the contribution of *Sr2* to SR resistance. He *et al.* (2020) reported an SR field severity range of 20–80% and moderately susceptible-susceptible (MSS) to mixed (M) SR responses for lines with the coupled *Fhb1-Sr2*, while the susceptible (S) check showed 100% severity and susceptible response. Zhang *et al.* (2016) developed 59 lines carrying *Sr2* derived from two different crosses, which had a reduction of SR field severity ranging from 26 to 53% in relation to their susceptible parent. The effect of *Sr2* determined in the 'Sf26/Génesis 2375' population was consistent with the effect measured by Zhang *et al.* (2016).

The four cultivars introgressed with the coupled genes have been commercially competitive. Particularly, 'Génesis 2375' and 'Génesis 6.87' combined desirable agronomic characteristics (high and stable grain yield, acceptable bread-baking quality) with adequate multiple disease resistance when released. Having introduced the coupled genes into these elite INIA cultivars is promising due to the derived lines with higher resistance to FHB and partial resistance to SR. Particularly, three lines derived from the 'Sf26'/'Génesis 2375', 'Sf26'/'Génesis 6.87', and 'Sf26'/'INIA Madrugador' crosses (RC3F2-7.11, RC3F2-12.1 and RC3F2-22.10, respectively) are good candidates to be used for the development of commercial varieties in Uruguay.

This work is an important contribution to the knowledge about the effectiveness of the genetic resistance conferred by *Fhb1* and *Sr2* into commercial germplasm. Both genes have made important contributions to wheat breeding programmes worldwide. Breeding lines with both genes in coupling are a relevant contribution to our and other breeding programmes, allowing increased levels and durability of the genetic resistance to FHB and SR.

Conclusions

A marker assisted backcross protocol for the introduction of the coupled *Fhb1-Sr2* using the molecular marker *UMN10* was validated. In addition, we confirmed the positive effect of the coupled *Fhb1-Sr2* on type II resistance to FHB (greenhouse) and in the field resistance to SR. Adapted, homozygous lines carrying *Fhb1-Sr2* developed in this work will contribute to increase the resistance to FHB and SR in the wheat breeding program in Uruguay and elsewhere. Promising results for FHB resistance from greenhouse trials require further confirmation in field experiments, where levels of FHB disease and mycotoxin accumulation should be recorded in replicated trials.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262122000107

Data. *Terms of availability of the developed germplasm to the scientific and plant breeding community*: the lines described in this paper are stored at the Seeds and Plant Genetic Resources Unit of INIA-Germplasm Bank located in INIA La Estanzuela, Uruguay and can be provided upon request.

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Conflict of interest. The authors declare no conflicts of interest.

References

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB and Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics* **102**, 1164–1168.

Bansal U, Bariana H, Wong D, Randhawa M, Wicker T, Hayden M and Keller B (2014) Molecular mapping of an adult plant stem rust resistance gene *Sr*56 in winter wheat cultivar 'Arina'. *Theoretical and Applied Genetics* **127**, 1441–1448.

Bernardo AN, Ma H, Zhang D and Bai G (2012) Single nucleotide polymorphism in wheat chromosome region harboring *Fhb*1 for Fusarium head blight resistance. *Molecular Breeding* **29**, 477–488.

Box GE and Cox DR (1964) An analysis of transformations. *Journal of the Royal Statistical Society: Series B (Methodological)* **26**, 211–243.

Brar GS, Pozniak CJ, Kutcher HR and Hucl PJ (2019) Evaluation of Fusarium head blight resistance genes *Fhb*1, *Fhb*2, and *Fhb*5 introgressed into elite Canadian hard red spring wheats: effect on agronomic and end-use quality traits and implications for breeding. *Molecular Breeding* **39**, 44.

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M and Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). *Theoretical and Applied Genetics* **104**, 84–91.

Buerstmayr H, Ban T and Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant Breeding* **128**, 1–26.

Campbell CL and Madden LV (1990) *Introduction to Plant Disease Epidemiology*. New York: John Wiley and Sons.

Campos P, Castro M, Pereyra S, Quincke M, Milisich H, Gieco L, López J and Germán SE (2015) Re-emergence of stem rust at epidemic levels in Argentina and Uruguay in (2014). In McIntosh RA (ed.), *Proceedings 2015 Technical Workshop Borlaug Global Rust Initiative*. Sydney, Australia. Available at https://www.cabdirect.org/cabdirect/FullTextPDF/2017/20173038268.pdf (Accessed 10 January 2018).

Castro M, Germán SE and Pereyra SA (2015) Caracterización sanitaria de cultivares de trigo y cebada. Available at http://www.inia.uy/Documentos/Privados/INIA%20La%20Estanzuela/caracterizaci%C3%B3n_sanitaria_cultivares_trigo_cebada.pdf (Accessed 12 January 2018).

Castro M, Germán SE and Pereyra SA (2019) Caracterización sanitaria de cultivares de trigo y cebada. Available at http://www.inia.uy/Documentos/P%C3%BAblicos/INIA%20La%20Estanzuela/Actividades%202019/caracterizaci%C3%B3n%20sanitaria%202018%2023%20abril.pdf (Accessed 10 March 2019).

Dawkins R and Krebs JR (1979) Arms races between and within species. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **205**, 489–511.

Desjardins AE (2006) Fusarium mycotoxins. *Chemistry, Genetics, and Biology*, Vol. **531**. St. Paul, MN: American Phytopathological Society (APS) press.

Doyle JJ (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**, 11–15.

Dreisigacker S, Sehgal D, Reyes Jaimez AE, Luna Garrido B, Muñoz Zavala S, Núñez Ríos C, Mollins J and Mall S (eds) (2016) *CIMMYT Wheat Molecular Genetics: Laboratory Protocols and Applications to Wheat Breeding*. Mexico, DF: CIMMYT, pp. 14–37.

Ellis MH, Bonnett DG and Rebetzke GJ (2007) A 192 bp allele at the Xgwm261 locus is not always associated with the *Rht*8 dwarfing gene in wheat (*Triticum aestivum* L.). *Euphytica* **157**, 209–214.

Hao Y, Rasheed A, Zhu Z, Wulff BB and He Z (2020) Harnessing wheat Fhb1 for Fusarium resistance. *Trends in Plant Science* **25**, 1–3.

He X, Bonnett D, Singh PK, Hyles J, Spielmeyer W and Dreisigacker S (2015) Advanced wheat breeding lines combining *Fhb1* and *Sr2* in different genetic backgrounds. *Proceedings of the 9th International Wheat Conference*, Sydney, Australia, pp. 20–25.

He X, Brar GS, Bonnett D, Dreisigacker S, Hyles J, Speilmeyer W, Bhavani S, Singh RP and Singh PK (2020) Disease resistance evaluation of elite CIMMYT wheat lines containing the coupled *Fhb*1 and *Sr*2 genes. *Plant Disease* **104**, 2369–2376.

Herrera-Foessel SA, Singh RP, Lillemo M, Huerta-Espino J, Bhavani S, Singh S, Lan C, Calvo-Salazar V and Lagudah ES (2014) *Lr*67/*Yr*46 confers adult plant resistance to stem rust and powdery mildew in wheat. *Theoretical and Applied Genetics* **127**, 781–789.

Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R and Fetch T Jr (2008) Detection of virulence to resistance gene *Sr*24 within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease* **92**, 923–926.

Jin F, Zhang D, Bockus W, Baenziger P S, Carver B and Bai G (2013) Fusarium head blight resistance in US winter wheat cultivars and elite breeding lines. *Crop Science* **53**, 2006–2013.

Li G, Zhou J, Jia H, Gao, Fan M, Luo Y, Zhao P, Xue S, Li N, Yuan Y, Ma S, Kong Z, Jia L, An X, Jiang G, Liu W, Cao W, Zhang R, Fan J, Xu X, Liu Y, Kong Q, Zheng S, Wang Y, Qin B, Cao S, Ding Y, Shi J, Yan H, Wang X, Ran C and Ma Z (2019) Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nature Genetics* **51**, 1106–1112. doi: https://doi.org/10.1038/s41588-019-0426-7

Liu S and Anderson JA (2003) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. *Crop Science* **43**, 760–766.

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS and Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. *Functional and Integrative Genomics* **6**, 83–89.

Liu S, Pumphrey M, Gill B, Trick H, Zhang J, Dolezel J, Chalhoub B and Anderson J (2008) Toward positional cloning of Fhb1, a major QTL for Fusarium head blight resistance in wheat. *Cereal Research Communications* **36**(Supplement 6), 195–201.

Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J and Spielmeyer W (2011) An accurate DNA marker assay for stem rust resistance gene Sr2 in wheat. *Theoretical and Applied Genetics* **122**, 735–744.

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R and Xia XC (2017) Catalogue of gene symbols for wheat: 2017. *Annual Wheat Newsletter* **53**, 1–20.

McMullen MP, Bergstrom GC, De Wolf E, Dill-Macky R, Hershman DE, Shaner G and Van Sanford DA (2012) A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Disease* **96**, 1712–1728.

Mesterházy Á, Bartók T, Mirocha CG and Komoroczy R (1999) Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. *Plant Breeding* **118**, 97–110.

Miedaner T, Wilde F, Steiner B, Buerstmayr H, Korzun V and Ebmeyer E (2006) Stacking quantitative trait loci (QTL) for Fusarium head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. *Theoretical and Applied Genetics* **112**, 562–569.

Niwa S, Kubo K, Lewis J, Kikuchi R, Alagu M and Ban T (2014) Variations for Fusarium head blight resistance associated with genomic diversity in different sources of the resistant wheat cultivar 'Sumai 3'. *Breeding Science* **64**, 90–96.

Pereyra S and Lori GA (2013) Crop residues and their management in the epidemiology of Fusarium head blight. In Alconada MT and Chulze S (eds), *Fusarium Head Blight in Latin America*. Dordrecht: Springer, pp. 143–156.

Peterson RF, Campbell AB and Hannah AE (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research* **26**, 496–500.

Petronaitis T, Simpfendorfer S and Hüberli D (2021) Importance of *Fusarium* spp. in wheat to food security: a global perspective. In Scott P, Strange R, Korsten L and Gullino M (eds), *Plant Diseases and Food Security in the 21st Century*. Switzerland: Springer, pp. 127–159.

Pretorius ZA, Singh RP, Wagoire WW and Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr*31 in *Puccinia graminis*. f. sp. *tritici* in Uganda. *Plant Disease* **84**, 203–203.

Pumphrey MO, Bernardo R and Anderson JA (2007) Validating the Fhb1 QTL for Fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. *Crop Science* **47**, 200–206.

Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick H, Bockus W, Akhunov E, Anderson J and Gill BS (2016) Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature Genetics* **48**, 1576–1580.

R Core Team (2016) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org/ (Accessed 4 January 2019).

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P and Ganal MW (1998) A microsatellite map of wheat. *Genetics* **149**, 2007–2023.

Roelfs AP, Singh RP and Saari EE (1992) *Las royas del trigo: Conceptos y métodos para el manejo de esas enfermedades*. México: Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), p. 81.

Salameh A, Buerstmayr M, Steiner B, Neumayer A, Lemmens M and Buerstmayr H (2011) Effects of introgression of two QTL for Fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on Fusarium head blight resistance, yield and quality traits. *Molecular Breeding* **28**, 485–494.

Shapiro S and MB Wilk (1965) An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591–611.

Sharma RK, Singh PK, Joshi AK, Bhardwaj SC, Bains NS and Singh S (2013) Protecting South Asian wheat production from stem rust (Ug99) epidemic. *Journal of Phytopathology* **161**, 299–307.

Singh RP (2012) Pros and cons of utilizing major, race-specific resistance genes versus partial resistance in breeding rust resistant wheat. *Proceedings, Borlaug Global Rust Initiative, 2012 Technical Workshop*, September 1–4, Beijing, China: Oral presentations, pp. 57–65. Borlaug Global Rust Initiative.

https://doi.org/10.1017/S1479262122000107 Published online by Cambridge University Press

Singh RP, Ma H and Rajaram S (1995) Genetic analysis of resistance to scab in spring wheat cultivar Frontana. *Plant Disease* **79**, 238–240.

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P and Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **1**, 1–13.

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel S and Ward RW (2008) Will stem rust destroy the world's wheat crop? *Advances in Agronomy* **98**, 271–309.

Singh S, Singh RP, Bhavani S, Huerta-Espino J and Eugenio LVE (2013) QTL mapping of slow-rusting, adult plant resistance to race Ug99 of stem rust fungus in PBW343/Muu RIL population. *Theoretical and Applied Genetics* **126**, 1367–1375.

Singh RP, Hodson DP, Jin Y, Lagudah ES, Ayliffe MA, Bhavani S and Basnet BR (2015) Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. *Phytopathology* **105**, 872–884.

Stack R (2003) History of Fusarium head blight with emphasis on North America. In Leonard KJ and Bushnell WR (eds), *Fusarium Head Blight of Wheat and Barley*. St. Paul, MN: American Phytopathological Society Press, pp. 1–34.

Stubbs RW, Prescott JM, Saari EE and Dubin HJ (1986) *Manual de Metodología Sobre las Enfermedades e los Cereales*. México: Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT).

Su Z, Bernardo A, Tian B, Chen H, Wang S, Ma H, Cai S, Liu D, Zhang D, Li T, Trick H, Amand P, Yu J, Zhang Z and Bai G (2019) A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. *Nature Genetics* **51**, 1099–1105.

Tukey J (1949) Comparing individual means in the analysis of variance. *Biometrics* **5**, 99–114.

van Eeuwijk FV, Mesterházy A, Kling CI, Ruckenbauer P, Saur L, Buerstmayr H, Lemmens M, Keizer LCP, Maurin N and Snijders CHA (1995) Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. *Theoretical and Applied Genetics* **90**, 221–228.

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW and Frohberg RC (1999) RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science* **39**, 805–811.

Wanyera R, Kinyua MG, Jin Y and Singh RP (2006) The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr*31 in wheat in Eastern Africa. *Plant Disease* **90**, 113–113.

Yang ZP, Gilbert J, Somers DJ, Fedak G, Procunier JD and McKenzie IH (2003) Marker assisted selection of Fusarium head blight resistance genes in two doubled haploid populations of wheat. *Molecular Breeding* **12**, 309–317.

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421.

Zhang H, Van der Lee T, Waalwijk C, Chen W, Xu J, Xu J, Zhang Y and Feng J (2012) Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS One* **7**, e31722.

Zhang X, Rouse MN, Nava IC, Jin Y and Anderson JA (2016) Development and verification of wheat germplasm containing both *Sr*2 and *Fhb*1. *Molecular Breeding* **36**, 85.

Zhou W, Kolb FL, Bai G, Shaner G and Domier LL (2002) Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. *Genome* **45**, 719–727.

https://doi.org/10.1017/S1479262122000107 Published online by Cambridge University Press