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QTL mapping of resistance to Ug99 and other stem rust pathogen races in bread wheat

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Abstract Most wheat cultivars planted worldwide are susceptible to the stem rust Ug99 race group. To prepare for the potential spread of these races into South America, we aimed to identify genomic regions responsible for resistance to Ug99 race group in germplasm adapted to South America. Two RIL populations from a cross between a stem rust susceptible parent "Baguette 13" and resistant local parents "INIA Tero" and "BR23//CEP19/PF85490" were developed. Phenotypical evaluation was completed at the seedling stage in Uruguay and under field conditions in Uruguay and Kenya. Both RIL populations were genotyped using the GBS approach. Besides *Sr24*, three other resistance loci in "INIA Tero" were detected on chromosomes 2B, 6A, and 7B. All four QTL were effective to local races, whereas only the QTL on chromosome 2B was effective against the Ug99 race group. Besides *Sr31*, "BR23//CEP19/PF85490" also carries two other stem rust resistance loci on chromosomes 2B and 6A. All three explained the resistance in Uruguay, while only the QTL on 2B was effective to Ug99 in Kenya. The physical location suggested that the QTL identified on chromosome 2B in both populations may correspond to *Sr28*, which was confirmed using specific molecular markers. Further studies are needed to determine the relationship between QTL for resistance to local races identified on chromosomes 6A and 7B and previously reported resistance genes and QTL. The results of this study are highly relevant for breeding wheat cultivars with diverse and durable resistance to stem rust.

Keywords *Triticum aestivum* · QTL mapping · Genetic resistance · *Puccinia graminis f.sp. tritici*

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Introduction

Bread wheat (*Triticum aestivum L.*) is the second most important cereal crop in the world in terms of total production (FAOSTAT 2016), and it plays an important role as food for the world's population. In South America, wheat is cultivated annually over approximately 8.6 Mha (FAOSTAT 2016). Cereal diseases are one of the major constraints for obtaining high grain yields (Germán et al. 2007). Wheat stem rust, caused by the pathogenic fungus *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. (Pgt), is considered as one of the most destructive diseases of wheat worldwide (Roelfs et al. 1992). Yield losses under severe epidemics can reach up to 100% in susceptible cultivars. Due to long-term efforts to breed for stem rust resistance, the disease was successfully controlled by genetic resistance (Singh et al. 2006) until the appearance of race TTKSK (Ug99) in Uganda in 1998 (Pretorius et al. 2000). The evolution and spread of Ug99 in several Eastern African countries represent a major threat to wheat production and food security worldwide (Singh et al. 2011a).

The use of rust-resistant wheat varieties is considered the most economical, efficient, and environmentally safe method to control wheat stem rust. To date, more than 60 stem rust resistance genes have been designated (McIntosh et al. 1995, 2014; Yu et al. 2014). Most of these genes are inherited qualitatively and provide race-specific resistance which expresses at the seedling stage (all-stage resistance, ASR) (Singh et al. 2015). In contrast to ASR, adult plant resistance (APR) is expressed at post-flowering stage and is often inherited quantitatively. Generally, a single race-specific resistance gene provides high level of resistance, whereas four to five race non-specific resistance genes are required to confer a complete or near immune response. In recent studies, several quantitative trait loci (QTL) for stem rust resistance have been identified and chromosome arms 3BS, 6BS, 5BL, and 2BL are the hotspots where many of these QTL are located (Yu et al. 2014). However, the Pgt population is dynamic, and new races of the pathogen are constantly emerging, causing high infections on cultivars that were initially resistant (Singh et al. 2015). Therefore, it is essential to develop wheat cultivars with durable and effective resistance based on combinations of effective resistance genes to decrease the probability of selection of virulent races and/or to use quantitative resistance (Singh et al. 2005, 2011b).

Until the emergence of Ug99, the gene *Sr31,* located on the 1BL/1RS translocation transferred from rye (*Secale cereal L.*) to wheat, provided protection against all known Pgt races (Jin et al. 2008). *Sr31* has been extensively deployed in CIMMYT wheat lines as a source of stem rust resistance (Jin and Singh 2006). During the last two decades, several derivatives of the Ug99 race have emerged and spread to different countries of East Africa. These derivatives rendered ineffective several of the stem rust resistance genes, including S*r38* transferred into wheat from *T. ventricosum* (Bariana and McIntosh 1993). In 2006 and 2007, variants within the Ug99 lineage with additional virulence on *Sr24* and *Sr36* were detected (Jin et al. 2008, 2009). At that point, about 80–90% of wheat cultivars around the world were susceptible to the Ug99 race group (Singh et al. 2015). By 2009, cultivars carrying the genes *Sr24* and *Sr31* conferring resistance to stem rust represented half of the total planted area in South America (Germán et al. 2009). The Ug99 race group spread to Southern and Eastern Africa (Pretorius et al. 2010) and Iran (Nazari et al. 2009) causing concern that its spread could lead to major epidemics (Singh et al. 2011a).

In the past two decades, major efforts around the world have been directed at identifying novel sources of stem rust resistance especially against the Ug99 race and its derivatives (Kaur et al. 2009; Hiebert et al. 2010; Haile et al. 2012; Singh et al. 2013; Lopez-Vera et al. 2014; Babiker et al. 2016; Li et al. 2016; Gao et al. 2017). Likewise, screening of South American wheat cultivars and lines for resistance to the Ug99 race group in Kenya identified resistant lines. Among these, the Uruguayan wheat cultivar "INIA Tero" and the Brazilian advanced line "BR23//CEP19/PF85490" were identified and used as sources of stem rust resistance for breeding. The objective of this study was to identify the genetic basis of the resistance to local stem rust races and the Ug99 race group in "INIA Tero" and "BR23//CEP19/PF85490."

Materials and methods

Development of mapping populations

Two recombinant inbred line (RIL) populations were developed from crosses of the susceptible wheat European cultivar "Baguette 13" (abbreviated B.13) (Castro et al. 2012) with two stem rust-resistant wheat cultivars.

The "INIA Tero" cultivar (abbreviated as I.Tero, cross LI107/C-CH-91-1642) was released by the Instituto Nacional de Investigación Agropecuaria (INIA), Uruguay and the Brazilian line BR23//CEP19/PF85490 (abbreviated as BR23//C/P) was developed by EMBRAPA-CNPTrigo. Both RIL populations were developed by advancing F_2 progeny to the F_5 generation by single spike descent. Seeds from each F_5 plant were harvested in bulk and advanced to produce enough seeds for phenotyping. The RIL populations INIA Tero/Baguette 13 (abbreviated as I.Tero/B.13) and BR23//CEP19/PF85490/3/Baguette 13 (abbreviated as BR23//C/P/3/B.13), included 174 and 107 lines, respectively.

Seedling response

Greenhouse seedling tests were carried out during 2017 at INIA La Estanzuela Experimental Station (INIA LE), Uruguay. Approximately six seeds of each parent and RILs from the I.Tero/B.13 and BR23//C/P/3/B.13 populations were sown in trays of 28 lines in an incomplete block design (IBD) with two replications. Pgt race RHKTF isolate 2372, with avirulence/virulence *Sr8a*,*9e*,*11*,*24*,*31*,*36*/*5*,*6*,*7b*,*9a*,*9d*,*9 g*,*10*,*17*,*21*,*30*,*38*,*Tmp*,and *McN* (Jin et al. 2008), and race RRTTF isolate 2749, with avirulence/virulence *Sr8a*,*9e*,*24*,*31*/*5*,*6*,*7b*,*9a*,*9d*,*9 g*,*10*,*11*,*17*,*21*,*30*,*36*,*38*,*Tmp*,and *McN*, were used individually to inoculate seedlings 8–10 days after planting. Urediniospores were suspended in light mineral oil Soltrol 170. After inoculation, seedlings were incubated in a dew chamber overnight and then grown in the greenhouse with an average temperature of 20 °C. Infection types (IT) were assessed 14 days after inoculation using the 0–4 scale developed by Stakman et al. (1962), where IT 0 to 2+ were considered resistant (R) and 3 or higher were considered susceptible (S). For QTL mapping of seedling response to stem rust, IT data were converted into a quantitative scale of seedling response (avirulent phenotypes: 0 = 0,;=0, 1 = 1, 2 = 2, virulent phenotypes: 3 = 3, 4 = 4, + = +0.25, − = −0.25) according to Maccaferri et al. (2010).

Field response

Field trials were conducted to evaluate the field response of the I.Tero/B.13 and BR23//C/P/3/B.13 populations and parents against Pgt races in Uruguay and Kenya. In Uruguay, trials were carried out at (i) INIA LE, Colonia (34°20′18"S, 57°41′26"W) during the 2015 growing season (abbreviated as LE-15); (ii) INIA LE during the off-season 2016 (abbreviated as LE-16); and (iii) Universidad de la República, Facultad de Agronomía, M.A. Cassinoni Experimental Station, Paysandú, (EEMAC) (32°22′49"S, 58°03′09"W) during the 2016 growing season (abbreviated as EEMAC-16). In Kenya, trials were carried out at the Kenya Agricultural and Livestock Research Organization (KALRO) nurseries (0°20′31"S, 35°56′30″E) at Njoro, during the 2018 off-season (abbreviated as K-18).

In Uruguay field trials (LE-15, LE-16, EEMAC-16), each population was planted in an incomplete block design with two replications at each location with the respective parents as repeated checks. RILs and parents were planted as single row plots of 1.0 m length with 0.40 m spacing between rows. To facilitate uniform disease pressure within the nursery, spreader rows of the stem rust susceptible cultivar B.13 were planted perpendicular to all entries on one side of the plots, in the middle of alleys, and around the field. In addition to natural infection, trials were artificially inoculated by spraying the spreader rows two to three times with freshly collected urediniospores of the races RHKTF (isolate 2372), RRTTF (isolate 2749), and SPLKC (isolate 2047, with avirulence/virulence *Sr6*,*7b*,*9a*,*9b*,*17*,*24*,*30*,*31*,*38*/*5,8a,9d,9e,9 g,10,11,21,36,Tmp,McN*) suspended in a lightweight mineral oil Soltrol 170 (Phillips Petroleum Co., Borger, TX, USA). Two additional inoculations through injection of a suspension of urediniospores in distilled water into two tillers per plot and random tillers of the spreader row were made. In Kenya, RILs and the respective parents were planted as double-row plots of 0.7 m with 0.3 m distance between plots. The two populations were planted in one replication, whereas the respective parents were planted as repeated checks (five susceptible and six resistant checks for the I.Tero/B.13 population and four susceptible and four resistant checks for the BR23//C/P/3/B.13 population). Spreaders comprised of a mixture of stem rust susceptible cultivars "Cacuke" and "Robin," and six *Sr24*-carrying lines (GIDs: 5391050, 5,391,052, 5,391,056, 5,391,057, 5,391,059, and 5,391,061) were planted as hill plots on one side of each test plot in the middle of the 0.3-m-wide pathways. Spreaders were also planted along the borders of the experimental field in 1.0-m plots.

Inoculations were carried out by spraying a mixture of urediniospores suspended in water plus Tween 20 and needle inoculations with the same suspension as described in Njau et al. (2013). Based on differential lines and race analysis of samples from screening nurseries at the Cereal Disease Laboratory (USDA, USA), the races present in the nursery were TTKSK (Ug99), TTKST (Ug99 high on *Sr24*), TTKTT (Ug99 high on *Sr24* and *SrTmp*), TKTTF (low on *Sr11*, *Sr24*, and *Sr31*), TKPTF (low on *Sr11*, *Sr9b*, *Sr24*, and *Sr31*), and TTTTF (low on *Sr24* and *Sr31*).

Stem rust disease severity (DS) was recorded at the peak of disease development using the modified Cobb's scale (Peterson et al. 1948). Infection response (IR) was scored according to Roelfs et al. (1992) as susceptible (S), moderately susceptible (MS), moderately resistant (MR) and resistant (R). The coefficient of infection (CI) was calculated by multiplying DS and IR where coefficients were assigned to each IR as R = 0.2, MR = 0.4, MRMS = M = 0.6, MS = 0.8, and S = 1 (Stubbs and Prescott 1986). Calculated CI values were used for QTL analysis.

Days to heading were recorded at LE-15 and LE-16 field trials for all RILs and parental lines of both populations according to Zadok's scale (Zadoks et al. 1974).

Genotyping

Genomic DNA was isolated from pooled leaves of six seedlings of each F_6 RIL and parent lines, using a modified CTAB method (Dreisigacker et al. 2017).

The presence or absence of stem rust resistance alleles of *Sr24* and *Sr31* in both RIL populations along with the parents were tested using the gene-linked PCR markers *Sr24#12* (Mago et al. 2005) and *iag95* (Mago et al. 2002), respectively.

Single nucleotide polymorphism (SNP) markers were obtained using the genotyping by sequencing (GBS) approach. The library construction protocol included two enzymes, *Pst*I and *Msp*I, as described by Poland et al. (2012). The libraries were sequenced with an Illumina HiSeq2000. The SNP markers were called using TASSEL 5 GBS v2 pipeline (Glaubitz et al. 2014), and the unique sequence tags were aligned to the reference Chinese Spring wheat assembly v1.0 (International Wheat Genome Sequencing Consortium 2014). The SNP filtering parameters used were minor allele frequency (MAF) < 0.25 and missing data > 20%, and all heterozygous allele calls were excluded.

Statistical analyses

Analysis of variance (ANOVA) of field CI and seedling response were analyzed using the following linear model with the aov function in the base package in statistical R Development Core Team (2014):

$$Y_{ijk} = \mu + G_i + \beta_j + \delta_{k(j)} + \varepsilon_{i(j)k}$$

where Y_{ijk} is the stem rust CI or IT values, G is the effect of i-th genotype, β is the effect of the j-th replication or block, δ is the effect if the k-th incomplete block within j-th replication, and ε is the experimental error with N(0, σ_ε^2). Days to heading was used as a fixed effect covariate in the model to account for the possible differences in maturity between lines, but as its effect was not significant; therefore this parameter was not included in the final model. Principal coordinate analysis (PCoA) was performed with SNP information.

Chi-square (χ^2) analyses were performed to test the goodness-of-fit of the number of observed resistant and susceptible lines to expected numbers according to models for one, two, or more resistance genes in the seedling stem rust resistance trial. The analyses were carried out in R statistical software (R Development Core Team 2014).

Genetic linkage map construction

The genetic linkage maps for both populations were constructed using QTL IciMapping software version 4.1(Meng et al. 2015). Redundant markers which were completely correlated within each population were excluded, keeping only one marker for each group of correlated markers. Linkage groups were constructed using the options of grouping, ordering, and rippling. Grouping was done considering the chromosome in which each tag was aligned with a minimum LOD of 5.0. Ordering was done by the two-opt heuristic algorithm (nnTwoOpt). Rippling was done by permutation of a window of 5 markers ($m = 5$) with the sum of adjacent recombination frequency criteria. Recombination frequencies were converted to centimorgans (cM) using the Kosambi mapping function (Kosambi 1944) to calculate genetic distances between markers. The presence and absence of alleles for the *Sr24* and *Sr31* markers were included in the map as additional markers.

QTL mapping and physical location

The QTL analyses of days to heading, stem rust seedling IT, and field CI were performed using the R/qtl package (Broman et al. 2003) of R Development Core Team (2014) for each population and each trial individually. The parameters for the composite interval mapping function (CIM) were: 50 cM window for cofactors, and QTL were considered significant if their LOD scores were higher than the threshold determined by 1000 permutation tests ($\alpha = 0.01$). For each detected QTL, the percentage of the phenotypic variance explained (R^2), and the allelic effects were estimated using a multiple QTL analysis.

For each QTL, the nucleotide sequences flanking the SNP markers (defined by a 95% Bayesian interval) were used to BLAST against the reference Chinese Spring wheat assembly v1.0 (International Wheat Genome Sequencing Consortium 2014) in the available tools online to obtain the physical coordinates of chromosomal position. The alignment with the highest percentage of identity was selected.

Molecular markers for *Sr* genes located on chromosome 2B

The parents and eight selected resistant and eight susceptible RILs from each population were later tested with specific markers for *Sr9a (GWM47,* Tsilo et al. 2007) and *Sr28* (*wPt_7004_PCR*, Rouse et al. 2012). Stem rust differential lines carrying *Sr9h* (SrWeb gene#46 selection from Webster) or *Sr28* (line AD diff hosts#24 W2691/Kota) were used as controls. Standard procedures and protocols were followed for PCR amplification and agarose gel electrophoresis of amplified products of each marker (Dreisigacker et al. 2017). The expected amplification product for the *Sr9a* marker is a 190-bp (base pairs) amplicon associated with the resistant allele and a 165-bp amplicon associated with the susceptible allele (Tsilo et al. 2007). Additionally, marker GWM47 is linked in coupling with *Sr9h* resistant allele from cultivar Webster (Hiebert et al. 2010) and linked in repulsion with *Sr9h*-resistant allele from cultivar Gabo 56 (Rouse et al. 2014). The expected amplification products for *Sr28* maker are two amplicons (166-bp and 194-bp) for all lines, where the preferential amplification of the 194-bp band is associated with the presence of the resistant allele, and either equal or preferential amplification of the 166-bp band is associated with the susceptible allele (Rouse et al. 2012).

Results

Genotypic information and construction of genetic linkage maps

Principal coordinate analysis (PCoA) of SNP marker information from both mapping populations (1262 markers for I.Tero/B.13 and 1209 for BR23//C/P/3/B.13) showed the expected distribution of individuals without transgressive segregation (data not shown).

Genetic linkage maps with 21 linkage groups were constructed for both populations, corresponding to each of the 21 wheat chromosomes. The I.Tero/B.13 linkage map had a total genetic distance of 2816 cM and an average distance between markers of 2.27 cM, (range 0.32 cM to 43.72 cM). The BR23//C/P/3/B.13 linkage map had a total genetic distance of 3236 cM and an average distance between markers of 2.72 cM (range 0.51 cM to 34.95 cM). On average, genome A was covered with 82 and 79 markers, genome B with 78 and 73, and genome D with 20 and 21 markers per chromosome in the I.Tero/B.13 and BR23//C/P/3/B.13 RIL populations, respectively.

Seedling response

The resistant parent I.Tero had an incompatible IT that ranged from 0 to;1 = (numerical IT 0 to 0.25) and 0 to 1 = (numerical IT 0 to 0.25), and the resistant parent BR23//C/P had IT 1- to 2- (numerical IT 0.75 to 1.75) and 1 = to 2 = (numerical IT 0.5 to 1.5) to stem rust races RHKTF and RRTTF, respectively, when tested at the seeding stage. The susceptible parent B.13 displayed compatible IT 4 to both races, RHKTF and RRTTF, at the seedling stage. The distribution of seedling ITs in both populations did not show transgressive segregation (Fig. 1a and b).

Inheritance of seedling resistance

Using molecular markers linked to *Sr24* and *Sr31*, it was confirmed that the parental line I.Tero carried the resistant allele of *Sr24*-linked marker *Sr24*#12, and the parental line BR23//C/P carried the resistant allele of *Sr31*-linked marker *iag95* (data not shown). The RILs of both

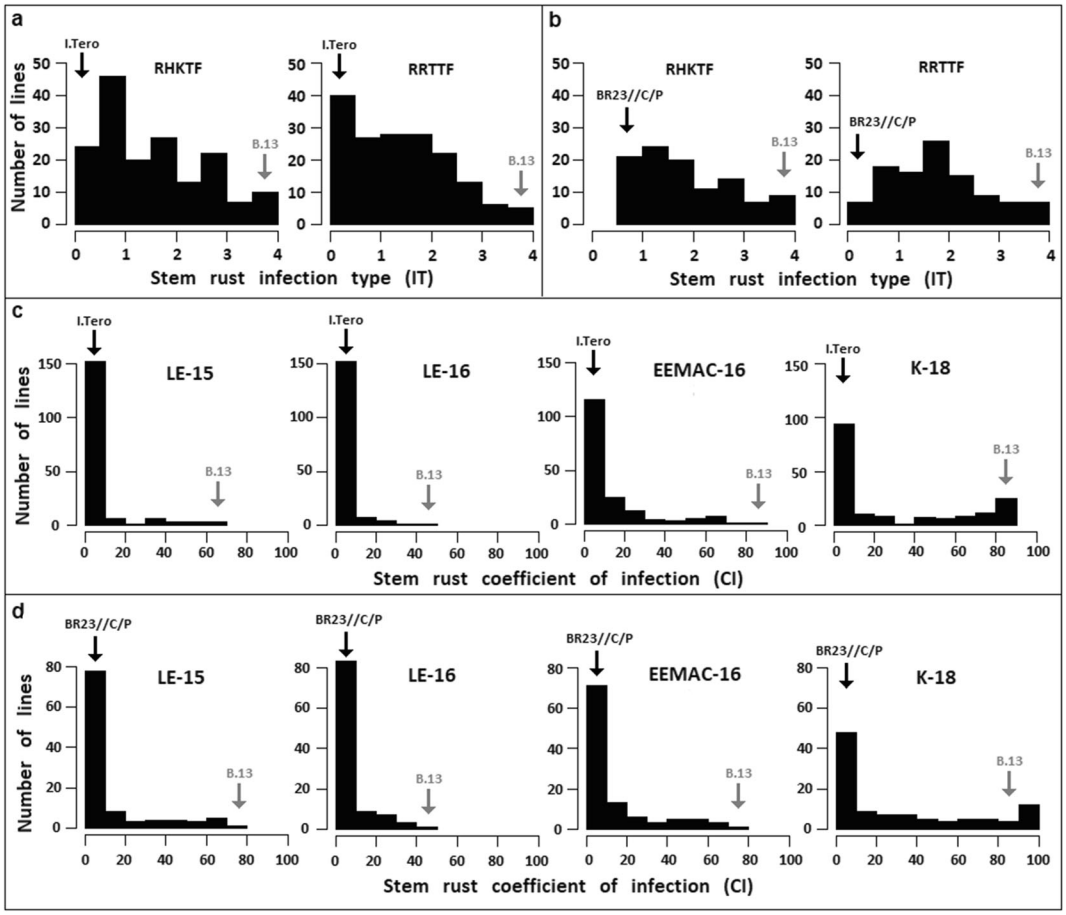

Fig. 1 Frequency distribution of stem rust (**a**) seedling infection type in INIA Tero/Baguette 13 RIL population, (**b**) seedling infection type in BR23//CEP19/PF85490///Baguette 13 RIL population, (**c**) coefficient of infection in INIA Tero/Baguette 13 RIL population, and (**d**) coefficient of infection in BR23//CEP19/PF85490/3/Baguette 13 RIL population. Coefficient of infection was determined for La Estanzuela main season 2015 (LE-15), La Estanzuela off-season 2016 (LE-16), Experimental Station M. A. Cassinoni 2016 (EEMAC-16), and Kenya off-season 2018 (K-18). Seedling infection types were determined for *Puccinia graminis* f. sp. *tritici* races RHKTF and RRTTF. Black, dark gray, and gray narrows indicate coefficient of infection or infection type of INIA Tero, BR23//CEP19/PF85490, and Baguette 13, respectively

populations were also genotyped for *Sr24* and *Sr31*, and this information was used in the estimation of the number of stem rust resistance genes effective at the seedling stage in the mapping populations. The I.Tero/B.13 population segregated for two resistance genes to the race RHKTF and three resistance genes to the race RRTTF, including *Sr24* for both races (data not shown). The BR23//C/P/3/B.13 population segregated for two resistance genes to both races, including *Sr31*.

Field resistance

In the four environments where stem rust field response was tested, the susceptible parent B.13 had uniform and relatively high disease levels, from CI > 80 recorded in K-18 to CI > 40 in LE-16 (Fig. 1c and d). Both resistant parents (I.Tero and BE23//C/P) had high levels of resistance in all environments (CI = 1 or lower). There was a significant ($P < 0.0001$) genotype effect on stem rust CI (data not shown) in all environments and for both populations. The effect of days to heading was not statistically significant as a covariate and was therefore not included in the final QTL analysis for resistance.

QTL analysis

Three QTL for heading date were detected on chromosomes 2D, 5A, and 5D in the I.Tero/B.13 population at

37.5, 78.6 and, 112.5 cM, respectively. Two QTL on chromosomes 2D and 7A were found in the BR23//C/P/3/B.13 population at 95.0 and 157.0 cM, respectively.

In the I.Tero/B.13 population, four QTL for stem rust field response were detected on chromosomes 2B, 3D, 6A, and 7B (Fig. 2a). Two QTL for seedling resistance were detected on chromosomes 3D and 6A to race RHKTF, and three QTL on chromosome 2B, 3D, and 6A were detected to race RRTTF (Fig. 2b). All QTL alleles conferring resistance were inherited from the resistant parent I.Tero. The QTL located on chromosome 2B (abbreviated QTL-2B-IT) was the most consistent across field environments, had the largest effect in Uruguay (mean $R^2 = 20.5\%$), and was the only QTL explaining the resistance in Kenya (Table 1). In seedlings, QTL-2B-IT was detected only to race RRTTF and had a low LOD and low R^2, and the lines carrying only this QTL expressed mean seedling reaction of 2.5 (approximately IT 2 + 3), with a relatively high range of IT (Fig. 3a), frequently pustules of intermediate size with prominent chlorosis. The QTL on chromosome 3D (abbreviated QTL-3D-IT) detected in all Uruguayan environments had the second largest effect. In seedlings, QTL-3D-IT was significant to both races and lines carrying only this QTL expressed a mean seedling reaction of 0.7–0.75 (approximately IT 1-) to races RHKTF and RRTTK, respectively. The QTL on chromosome 6A (abbreviated QTL-6A-IT) was also detected in all Uruguayan environments and in seedlings to both races. Lines carrying only this QTL expressed mean seedling reactions of 1.5 and 1.7 (IT 2 = to 2-) to RHKTF and RRTTF, respectively. The QTL on chromosome 7B (abbreviated as QSr.iuy-7B) was only detected in LE-15 and was not significant in the seedling stage. All the QTL detected in EEMAC-16, K-18, and LE-15 explained more phenotypic variance (61.3, 55.6 and 47.9%, respectively) than the QTL detected in LE-16 (31.6%). The QTL detected for seedling resistance to races RHKTF and RRTTF explained 59.6 and 51.1% of the phenotypic variance, respectively.

In the BR23//C/P/3/B.13 population, three QTL were detected for stem rust field response on chromosomes 1B, 2B and, 6A (Fig. 2c). Two QTL for seedling resistance were detected on chromosomes 1B and 6A to race RHKTF and two QTL on chromosome 1B and 2B were detected to race RRTTF (Fig. 2d). All QTL alleles conferring resistance were inherited from the resistant parent BR23//C/P. The QTL located on chromosome 1B (abbreviated QTL-1B-BR) was detected in field tests in LE-15 and LE-16 (Uruguay) and had the highest effect on seedling resistance to both races (Table 1). Lines carrying only this QTL expressed mean seedling

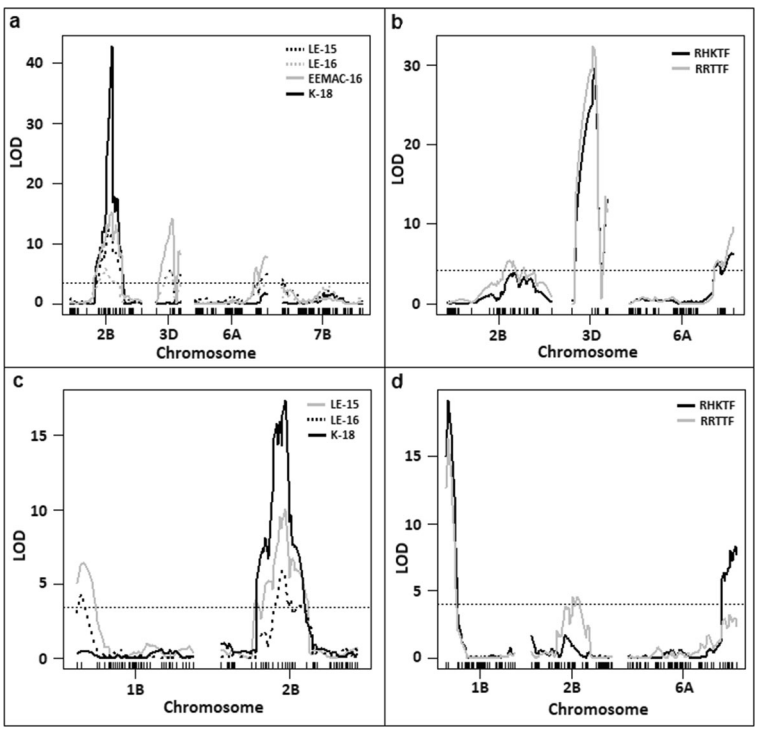

Fig. 2 QTL mapping of (**a**) field resistance in four environments (LE-15, LE-16, EEMAC-16, K-18) in the INIA Tero/Baguette 13 RIL population, (**b**) seedling resistance to races RHKTF and RRTTF in the INIA Tero/Baguette 13 RIL population, (**c**) field resistance in three environments (LE-15, LE-16, K-18) in the BR23//CEP19/PF85490/3/Baguette 13 RIL population, and (**d**) seedling resistance to races RHKTF and RRTTF) in the BR23//CEP19/PF85490/3/Baguette 13 RIL population. Threshold LOD determined by 1000 permutation test, with α = 0.01

Table 1 QTL for field resistance to stem rust in four environments and seedling resistance to Pgt races RHKTF and RRTTF detected by composite interval mapping in INIA Tero/Baguette 13 and BR23//CEP19/PF85490/3/Baguette 13 RIL populations

Environment/race	QTL	Chr[a]	Pos.(cM)[b]	LOD[c]	AE[d]	R^2 (%)[e]	Total R^2 (%)[f]
INIA Tero/Baguette 13 population: Field resistance							
LE-15	QTL-2B-IT	2B	67.8	12.5	+7.0	23.5	47.9
	QTL-3D-IT	3D	23.9	5.6	+4.1	9.2	
	QTL-6A-IT	6A	122.5	4.9	+4.5	9.0	
	QSr.iuy-7B	7B	3.7	3.7	+3.4	6.2	
LE-16	QTL-2B-IT	2B	67.6	6.4	+2.6	14.9	31.6
	QTL-3D-IT	3D	25	4.6	+2.3	10.7	
	QTL-6A-IT	6A	117.5	3.2	+1.8	6.0	
EEMAC-16	QTL-2B-IT	2B	72.5	15.8	+9.9	23.0	61.3
	QTL-3D-IT	3D	27.7	14.5	+9.2	24.4	
	QTL-6A-IT	6A	125.7	8.1	+6.9	13.9	
K-18	QTL-2B-IT	2B	72.5	42.7	+29.8	55.6	55.6
INIA Tero/Baguette 13 population: seedling resistance							
RHKTF	QTL-3D-IT	3D	27.5	29.4	+0.7	51.2	59.6
	QTL-6A-IT	6A	122.5	5.9	+0.3	8.4	
RRTTF	QTL-2B-IT	2B	75	4.9	+0.2	4.6	51.1
	QTL-3D-IT	3D	25	32.3	+0.8	36.5	
	QTL-6A-IT	6A	126	9.7	+0.3	10.0	
BR23//CEP19/PF85490/3/Baguette 13 population: field resistance							
LE-15	QTL-1B-BR	1B	5	6.4	+8.7	19.1	43.9
	QTL-2B-BR	2B	52.5	8.2	+10.6	24.8	
LE-16	QTL-1B-BR	1B	3.16	3.56	+3.3	11.0	31.9
	QTL-2B-BR	2B	55	5.9	+4.9	21.9	
K-18	QTL-2B-BR	2B	57.5	17.4	+27.5	53.9	53.9
BR23//CEP19/PF85490/3/Baguette 13 population: seedling resistance							
RHKTF	QTL-1B-BR	1B	3.2	19.4	+0.8	50.0	67.4
	QTL-6A-BR	6A	162.5	8.3	+0.7	17.4	
RRTTF	QTL-1B-BR	1B	3.2	16.7	+0.7	44.5	54.0
	QTL-2B-BR	2B	70	5.1	+0.3	9.5	

[a] Chromosome

[b] Position in centimorgans (cM)

[c] Logarithm of the odds (to the base 10)

[d] Additive effect of the QTL (+ indicates that segregated from the resistant parent and – from the susceptible parent)

[e] Phenotypic variance explained by the QTL

[f] Percentage of total variance explained by all QTL detected in each environment

reaction of 1.25 and 1.20 (approximately IT 1+) to RHKTF and RRTTF, respectively. The QTL on chromosome 2B (abbreviated QTL-2B-BR) was the most important conferring field stem rust resistance in the two LE environments and the only QTL detected in Kenya. QTL-2B-BR was also detected in the seedling stage to race RRTTF, and lines carrying only this QTL expressed a mean seedling reaction of 2.1 (approximately IT 22+) with a relatively high range of IT (Fig. 3b), frequently pustules of intermediate size with prominent chlorosis. The QTL on chromosome 6A (abbreviated QTL-6A-BR) was only significant at the seedling stage to race RHKTF, and lines carrying only this QTL expressed a mean seedling reaction of 2.00 (IT 2). The QTL detected in K-18 explained more phenotypic variance (53.9%) than the QTL detected in LE-15 and LE-16 (43.9 and 31.9%, respectively). No QTL were detected in EEMAC-16 in this RIL population. The QTL

detected for seedling resistance to races RHKTF and RRTTF and explained 67.4 and 54.0% of the phenotypic variance, respectively.

The effect of single QTL and their combination on stem rust resistance was evaluated in both RIL populations (Fig. 3). In the I.Tero/B.13 population at the adult plant stage, in LE-15 and LE-16, lines carrying only QTL-2B-IT or QTL-3D-IT had high resistance, similar to that of lines carrying combinations of two or more QTL (Fig. 3a). Lines with only QTL-6A-IT or QSr.iuy-7B expressed lower levels of resistance than the rest of the lines. At EEMAC-16, lines with only QTL-3D-IT and combinations of two or three QTL were highly resistant, whereas lines carrying only QTL-2B-IT or QTL-6A-IT expressed lower levels of resistance. In the K-18 field trial, QTL-2B-IT conferred a reduction of stem rust CI from 65 to 10. At the seedling stage, QTL-3D-IT conferred the same level of resistance as the combination of two or three QTL detected.

Lines from BR23//C/P/3/B.13 population carrying only QTL-3D-BR had a high level of resistance at the adult plant stage in LE-15 and LE-16 trials, similar to that expressed by lines carrying the combination of both QTL detected, while lines carrying only QTL-2B-BR had lower levels of resistance (Fig. 3b). In K-18 trial, QTL-2B-BR determined a reduction of stem rust CI from 60 to 15. At the seedling stage, QTL-1B-BR conferred the same level of resistance as the combination of the two QTL detected for each Pgt race.

Physical location of stem rust resistance QTL

The physical location of the stem rust resistance loci detected on chromosome 2B, determined from the physical position of the associated SNP markers, spanned over large interval of chromosome 2B (QTL-2B-IT: 627.2 Mb and QTL-2B-BR: 145 Mb), and had a partial overlap (Table 2). QTL-6A-IT and QTL-6A-BR were mapped to the distal region of the chromosome 6A, with physical overlap between them (QTL-6A-IT interval was completely within QTL-6A-BR interval). QSr.iuy-7B was located in a relatively short interval of 10.6 cM on chromosome 7B.

Identification of the QTL on chromosome 2B resistant to the Ug99 race group

Two *Sr* genes (*Sr9* and *Sr28*) were previously reported in the QTL-2B region. Therefore, it was hypothesized that one of them could be the same as the QTL-2B identified in this study. The resistant parents of both mapping populations and the stem rust positive control for *Sr28* amplified the PCR band associated with the susceptible *Sr9h* allele *(*from Webster, Hiebert et al. 2010) when tested with *Sr9a* marker *GWM47,* while the positive control for *Sr9h* (*SrWeb* gene#46) amplified the PCR band associated with the resistant allele (Fig. 4a). The resistant parents and the positive control for *Sr28* (W2691/Kota) amplified the PCR band associated with the resistant *Sr28* allele when tested with *Sr28* marker *wPT_7004_PCR*, while the positive control for *Sr9h* amplified the PCR band associated with the susceptible allele. The *wPT_7004_PCR* marker was polymorphic and segregated according to the field phenotypic response in all RILs tested from both populations (Fig. 4b).

Discussion

QTL mapping of stem rust response in four different field environments and seedling tests indicated the presence of at least four stem rust-resistant QTL in the I.Tero/B.13 population and three in the Br23//C/P/3/B.13 population. Although maturity is often associated with the level of disease (Simón et al. 2004; Rosas et al. 2017), in this study days to heading was not associated with stem rust resistance. The QTL detected for heading date were different from the QTL for stem rust resistance. This indicates that the resistance found in this study is not due to the escape mechanism involving heading date.

The I.Tero/B.13 population of 174 RILs allowed more precise detection of QTL since generally no lines without detected QTL were resistant. The smaller population size of 107 lines of the Br23//C/P///B.13 population resulted in less variation in stem rust resistance explained by the detected QTL. Probably in this population, there is additional resistance undetected by the QTL analysis since the phenotypic values of some RILs without any of the detected QTL still had intermediate levels of resistance.

QTL-1B-BR detected on chromosome 1B in the BR23//C/P/3/B.13 RIL population explained a high proportion of the stem rust resistance in seedling and field trials in Uruguay. This QTL corresponds to *Sr31*, as the peak of the QTL co-localized with the *Sr31* specific marker *iag95* (Mago et al. 2002). As expected,

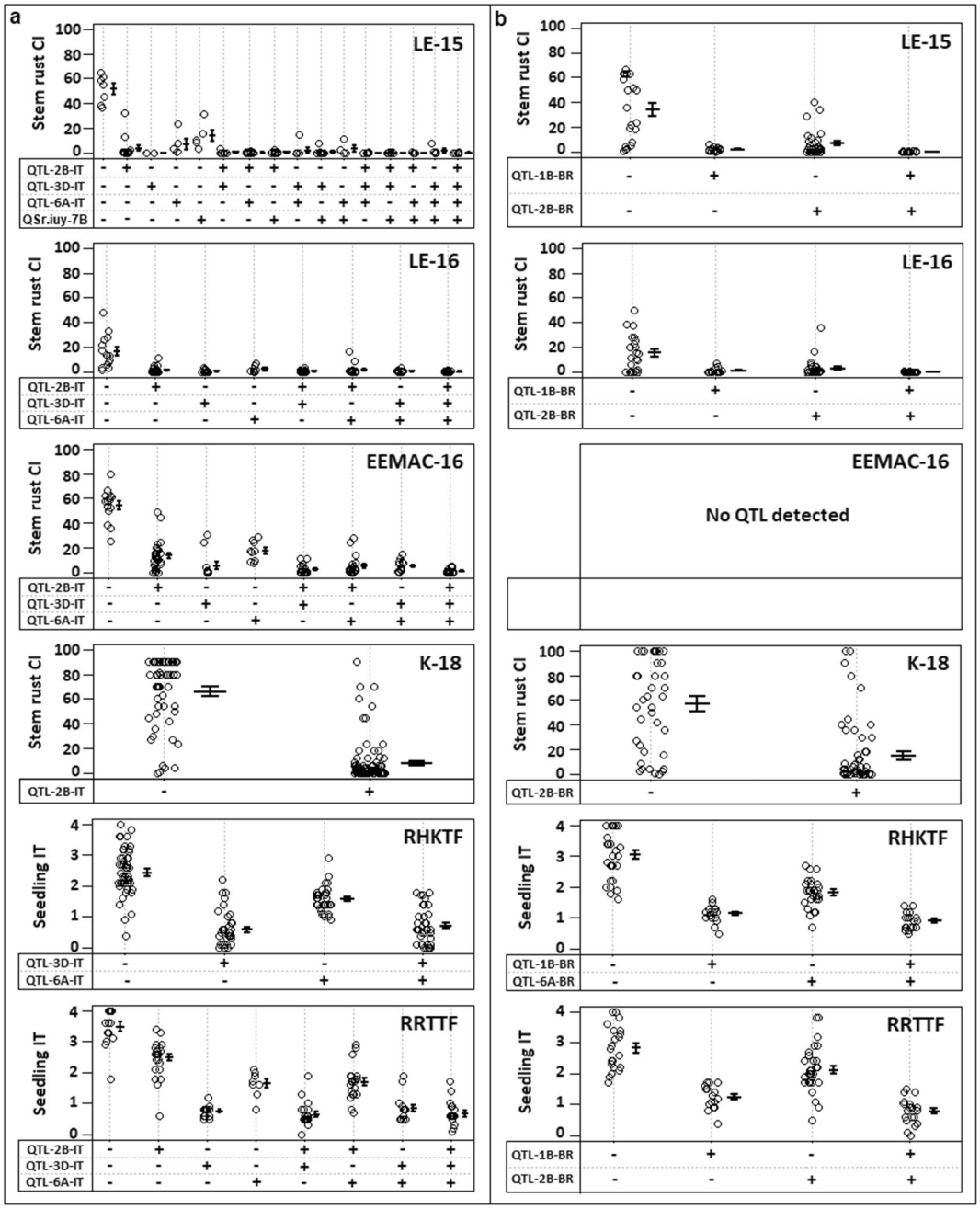

Fig. 3 Phenotypic effect of single QTL and the effect of combining more QTL for field and seedling stem rust resistance in the (**a**) INIA Tero/Baguette 13 and (**b**) BR23//CEP19/PF85490/3/Baguette 13 RIL populations. Stem rust CI: stem rust coefficient of infection. Seedling IT: seedling infection type. Coefficient of infection was determined for La Estanzuela main season 2015 (LE-15), La Estanzuela off-season 2016 (LE-16), Experimental Station M. A. Cassinoni 2016 (EEMAC-16), and Kenya off-season 2018 (K-18). Seedling infection types were determined for *Puccinia graminis* f. sp. *tritici* races RHKTF and RRTTF. Each point corresponds to the mean phenotypic value of one RIL. +: line carrying the resistant allele of the QTL. -: line carrying the susceptible allele of the QTL

Table 2 Physical location of the QTL detected in INIA Tero/Baguette 13 and BR23//CEP19/PF85490/3/Baguette 13 RIL populations mapped against the reference genome of the International Wheat Genome Sequencing Consortium

QTL	Flanking Markers	Linkage position (cM)	Interval (cM)	Physic position (Mb)	Interval (Mb)
QTL-2B-IT	*snp*_2B_142867158 - *snp*_2B_767554814	69.9 to 76.1	6.2	142.9 to 770.0	627.2
QTL-6A-IT	*snp*_6A_2869043 - *snp*_6A_9321817	114.8 to 125.7	11.0	2.8 to 9.3	6.5
QSr.iuy-7B	*snp*_7B_113572123 - *snp*_7B_309747559	0 to 10.6	10.6	720.8 to 749.2	28.4
QTL-2B-BR	*snp*_2B_71304355 - *snp*_2B_216469337	51.0 to 59.3	8.3	71.3 to 216.5	145.2
QTL-6A-BR	*snp*_2B_41105268 - *snp*_2B_794129180	152.9 to 165.1	12.2	2.4 to 12.3	9.9

this QTL was not detected in Kenya because the pathogen overcame this resistance (Pretorius et al. 2000). *Sr31* was probably inherited from Alondra, since this cultivar in the pedigree of BR23//C/P was reported to carry the *Sr31* gene (Singh and Rajaran 1991).

QTL-3D-IT with the second largest effect in all Uruguayan environments and seedling tests in the I.Tero/B.13 population corresponds to *Sr24* since the marker *Sr24#12* (Mago et al. 2005) is present in I.Tero and was the marker linked to the detected QTL. Gene *Sr24* is also effective in Uruguay and ineffective in Kenya (Jin et al. 2008). The pedigree information of I.Tero is limited, so it was not possible to identify the donor source of *Sr24.* Gene *Sr24* is common in Uruguayan varieties (Germán et al. 2007).

QTL-2B-IT and QTL-2B-BR detected in the I.Tero/B.13 and BR23//C/P/3/B.13 RIL population, respectively, had the largest effect in the adult plant stage, were consistently detected across all Uruguayan environments, and were the only two explaining the resistance to the Ug99 race group. Both were physically located in overlapping intervals of chromosome 2B and could correspond to the same resistance gene since lines carrying only these QTL had similar field and seedling responses. Chromosome 2B has been reported as a hotspot of stem rust resistance loci since 11 designated *Sr* genes and 15 QTL have been located on this chromosome (Jin et al. 2007; Yu et al. 2014; Bajgain et al. 2015). *Sr9h, Sr28*, *Sr32*, *Sr36*, *Sr39*, *Sr40*, and *Sr47* are effective to the original Ug99 race TTKSK. QTL-2B-IT and QTL-2B-BR were most probably located on 2BL, since flanking markers were located in the interval between 172.9 and 770 Mb for QTL-2B-IT and in interval between 71.3 and 216.5 Mb for QTL-2B-BR of the IWGSC reference genome, which corresponds to the long arm of the chromosome. *Sr32* and *Sr39* are located

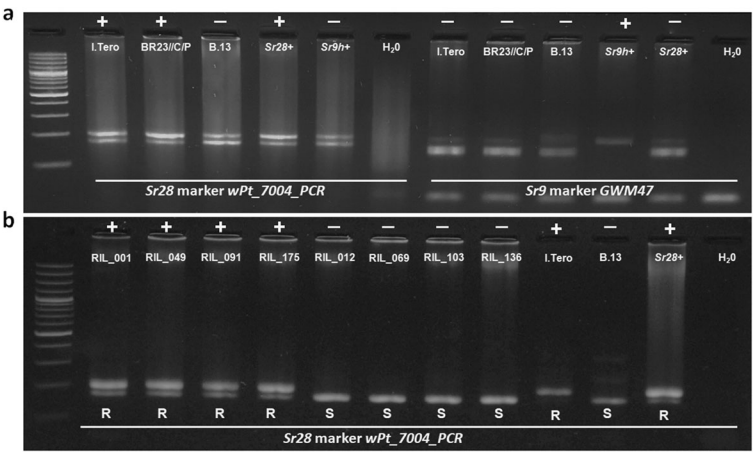

Fig. 4 PCR amplification results of (**a**) molecular marker *wPt_7004_PCR* and *GWM47* for the three parents ("INIA Tero," BR23//CEP19/PF85490, and "Baguette 13") and *Sr28*- and *Sr9h*-positive controls and (**b**) validation of molecular marker *wPt_7004_PCR* for four resistant and four susceptible RILs of the INIA Tero/Baguette 13 mapping population. I.Tero: "INIA Tero," BR23//C/P: BR23//CEP19/PF85490, B.13: "Baguette 13," *Sr28+:* stem rust positive control for *Sr28, Sr9h+*: stem rust positive control for *Sr9h*, +: resistant genotype, −: susceptible genotype, R: line with resistant phenotype carrying the resistant allele of the QTL detected on chromosome 2B, S: line with susceptible phenotype carrying the susceptible allele of the QTL detected on chromosome 2B

on 2BS (Dundas et al. 2007; Mago et al. 2009); therefore these genes can be excluded as the QTL detected here. *Sr47* is an important resistance gene in tetraploid wheat (Faris et al. 1999) and was only available on a large *Ae. speltoides* chromatin introgression until 2012 (Faris et al. 1999; Klindworth et al. 2012), so it is also unlikely to be the gene detected in this study. Marker *wmc344* linked to *Sr40* (Wu et al. 2009) is located within the overlapping interval for both QTL detected in this study on chromosome 2B, while marker *wmc332* linked to *Sr28* (Rouse et al. 2012) and markers *wmc332* and *wmc175* linked to *Sr9h* (Hiebert et al. 2010) are located within the QTL-2B-IT interval. However, *Sr40* has not been used in breeding for resistance due to its linkage with undesirable traits (Singh et al. 2015) and it is not likely to be the one detected in this study. Therefore, QTL-2B-IT and/or QTL-2B-BR could be either *Sr28*, *Sr9h*, a new allele of *Sr9,* a QTL previously reported in the same region (Kaur et al. 2009; Hiebert et al. 2010; Yu et al. 2014; Bajgain et al. 2015), or a new *Sr* gene. Specific molecular markers for *Sr9a* (*GWM47;* Tsilo et al. 2007) and *Sr28* (*wPt_7004_PCR*, Rouse et al. 2012) were tested in the parents, positive controls, and selected susceptible and resistant RILs of each population. Gene *Sr9h* was discarded as marker *GWM47* amplified a 165 pb band in the resistant parents, corresponding to the susceptible *Sr9a* allele reported by Tsilo et al. (2007). Hiebert et al. (2010) obtained the same results for *Sr9h* in Webster background, as was the positive control used in this study. Rouse et al. (2014) reported that *GWM47* was linked in repulsion with *Sr9h* in Gabo 56 background. The fact that *GWM47* amplified in I.Tero and BR23//C/P further confirms that *Sr9h* is not present in the resistant parents. PCR results confirmed that the QTL detected on chromosome 2B in both resistant parents may correspond to *Sr28* gene, since the same PCR band amplified in the resistant parents and the resistant RILs as in the *Sr28* positive control. Gene *Sr28* is a valuable source of resistance for breeding programs, because it is not located on introgressed chromatin from alien species with possible linkage drag (Rouse et al. 2014) and is still effective to all races of the Ug99 group detected to date. QTL-2B-IT and QTL-2B-BR have similar seedling resistant phenotype (IT 2 to 3), higher than the typical *Sr28* IT of 0; to 2 = mentioned by McIntosh et al. (1995). However, these differences could be attributed to a bias in scoring towards a higher IT, since the reaction was similar to the illustrations provided by McIntosh et al. (1995). The relatively high variation of ITs of lines with only these QTL could be explained by experimental error and effect of genetic background. Gene *Sr28* is present in Kota (McIntosh 1978) which is in the pedigree of BR23//C/P.

QTL-6A-IT explained a high proportion of the field and seedling stem rust resistance to both races tested in Uruguay in the I.Tero/B.13 population, while QTL-6A-BR explained a high proportion of the seedling resistance to RHKTF race in Uruguay in the BR23//C/P/3/B.13 population. These *loci* were not detected in Kenya, indicating that these resistance genes are ineffective to the Ug99 race group. Both QTL were physically located on the same small interval of chromosome 6A. Four *Sr* genes (*Sr8*, *Sr13*, *Sr26*, and *Sr52*) and eight QTL (reviewed by Yu et al. 2014) have been located on chromosome 6A. *Sr13, Sr26*, and, *Sr52* are probably absent in I.Tero and BR23//C/P because these genes are effective to the Ug99 race group. Additionally, until recently *Sr26* was not used in commercial varieties due to its yield penalty (Dundas et al. 2007), and *Sr52* was translocated from *Dasypyrum villosum* (L.) lately (Qi et al. 2011). *Sr8* is located on the short arm of chromosome 6A (McIntosh 1972) and its allele *Sr8a* is effective to local RHKTF and RRTTF races. Mapping of the flanking markers of QTL-6A-IT and QTL-6A-BR indicated these are physically located in the distal chromosome arm 6AS, where *Sr8* was reported. Additionally, marker *wsnp_Ra_c3996_7334169* (reported to be 2.2 cM from *Sr8a*, Hibert et al. 2017) was also physically located within this interval. Therefore, these QTL probably corresponds to *Sr8a* as the two QTL and *Sr8a* are effective for local RHKTF and RRTTF races and have similar IT seedling reaction (McIntosh et al. 1995). Although QTL-6A-BR was not detected when the RRTTF race was used, this could be attributed to its intermediate seedling response. Also, *Sr8a* is present in the Brazilian cultivar Frontana (McIntosh et al. 1995), which is an ancestor of BR23//C/P. However, it is not possible to rule out the possibility that other *Sr8* alleles are present in the resistant parents since the phenotypic data of lines with these alleles is not available. If the QTL detected on chromosome 6A do not correspond to *Sr8a*, they could be a new *Sr* gene effective to Uruguayan races used in this study but ineffective to the Ug99 race group. Alternatively, these QTL could be one of the QTL on 6A summarized by Yu et al. (2014) effective to Ug99 if this was confirmed to be ineffective to other races present in K-18.

QSr.iuy-7B was only detected at the adult plant stage in LE-15, unlike *Sr17* which was previously reported on chromosome 7B at the seedling stage (McIntosh et al. 1967). Therefore, it is not likely that I.Tero has *Sr17*. QSr.iuy-7B is located between 720.8 and 749.2 Mb of chromosome 7B of the IWGSC reference genome and is likely an adult plant stem rust QTL. QSr.iuy-7B could also correspond to a new QTL or to some of the QTL summarized by Yu et al. (2014) on chromosome 7B if these were ineffective to races present in K-18.

Results from QTL mapping and inheritance based on phenotypic analysis of seedling resistance were mostly consistent for both populations. In the I.Tero/B.13 population, the QTL mapping and the goodness-of-fit tests indicated the presence of the same number of genes: two genes for race RHKTF (QTL-3D-IT and QTL-6A-IT) and three genes for race RRTTF (QTL-2B-IT, QTL-3D-IT, and QTL-6A-IT). Lines carrying only QTL-2B-IT had an intermediate IT to both races. For QTL-2B-IT the difference in LOD score value between both races (RHKTF and RRTTF) was low but close to LOD threshold, probably leading to its significant detection only to race RRTTF. In addition, environmental variations within and between tests that were carried out 3 weeks apart might explain why this gene with low phenotypic effect was detected for one race and not for the other. No information of seedling response to the Ug99 race group is available, therefore we do not know if these QTL correspond to an all-stage or adult plant resistance effective in Kenya.

In the BR23//C/P/3/B.13 population, the inheritance of seedling resistance and QTL mapping determined the presence of two genes to races RHKTF and RRTTF. QTL-1B-BR was detected with both races, whereas QTL-6A-BR was detected only to race RHKTF and QTL-2B-BR only to RRTTF. Despite the difference of significance of QTL-2B-BR and QTL-6A-BR, the seedling reactions of lines carrying only one of these QTL were similar to both races. The difference in the detection of these QTL could be due to environmental conditions, experimental error, or their intermediate resistant reaction.

Conclusions

This study highlights the importance of analyzing the genetic basis of resistance in breeding germplasm. Both I.Tero and BR23//C/P have been used as sources of stem rust resistance in Uruguay. Since both genotypes share the same resistance gene effective to Ug99 race group, it is extremely important to avoid the possible redundant use that can lead to low genetic diversity and increases risks of pathogen adaptation when race-specific genes are used. To achieve durable stem rust resistance to both local Pgt races and the Ug99 race group, it is necessary to combine *Sr28* with other effective major resistance genes and/or adult plant minor resistance genes.

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Author contribution SG, MQ, AC, and LG designed the hypothesis and experiments. SG created the populations. SBaraibar, RG, and SG conducted disease phenotyping in Uruguay and MR and SBhavani in Kenya. SBaraibar, BL, and PS conducted statistical analysis. SBaraibar conducted laboratory analysis. SBaraibar and SG wrote the paper. All authors contributed to the discussion of the results and edited the paper.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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