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ORIGINAL ARTICLE

Adult-plant resistance to *Septoria tritici* blotch in hexaploid spring wheat

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Abstract

Key message **New QTL for *Septoria tritici* blotch detected in hexapoid spring wheat under field conditions across diverse environments.**

Abstract *Septoria tritici* blotch caused by the ascomycete fungus *Zymoseptoria tritici* presents a serious and consistent challenge to global wheat production. In particular the augmented use of soil management practices that leave large amounts of wheat stubble on the soil surface and global warming increases the chance of *Septoria tritici* blotch epidemics to emerge more frequently including in developing countries. Two recombinant inbred line populations developed from a cross between the susceptible Moroccan spring bread wheat variety 'NASMA' and the CIMMYT resistant lines, 'IAS20*5/H567.71' and 'RPB709.71/COC' were used to study the genetics and map adult-plant resistance to *Septoria tritici* blotch under field conditions in different environments. Resistance to *Septoria tritici* blotch in both populations was quantitative and overall, five across environment consistent resistance loci on chromosomes 1BS, 3AL, 5AL and 7AS were detected in the two populations. The QTL on chromosome 1BS and 7AS are likely to be allelic with the known *Septoria tritici* blotch genes *Stb3* and *Stb11*. All identified QTL were additive and explained between 4 and 27 % of the phenotypic variation. Epistatic interaction was not observed. Low cost KASP assays were developed as flanking markers for all five QTL that will facilitate molecular breeding. Our study represents the first mapping effort under field conditions utilizing two spring bread wheat resistant sources evaluated over multiple environments.

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Introduction

Septoria tritici blotch caused by the ascomycete fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (anamorph: *Septoria tritici*, teleomorph: *Mycosphaerella graminicola*) is among the most economically important biotic factors limiting wheat production in several wheat growing areas. *Septoria tritici* blotch can be severe in certain spring wheat environments in the developing world, particularly in North and West Asia and North Africa and parts of South America (Duveiller et al. 2007; Nazari et al. 2009). Yield losses due to *Septoria tritici* blotch in disease-conductive climates can reach 35–50 % through the reduction of photosynthetic area (Eyal et al. 1987). In Ethiopia, the largest wheat producer in Sub-Saharan Africa, 25–82 % wheat production losses due to Septoria diseases have recently been reported with increasing disease prevalence in the

major wheat growing areas (Bekele et al. 2011). In particular the augmented use of soil management practices that leave large amounts of wheat stubble and debris on the soil surface increase the chance of *Septoria tritici* blotch epidemics under favorable climatic conditions and are therefore expected to emerge more frequently in developing countries in the future. Due to global warming rising cost and environment deterioration of fungicide application and the emergence and prevalence of fungicide-resistant/insensitive strains of the causative pathogen (including to widely used strobilurins or demethylase inhibitors), resistance breeding provides one approach of controlling this disease (Siah et al. 2014; Cools et al. 2013).

The inheritance of resistance to *Septoria tritici* blotch in wheat can either be qualitative, isolate-specific, depending on major genes or quantitative, isolate-nonspecific with polygenic inheritance (Goodwin 2012). Up to now, 18 major resistance loci (*Stb1* to *Stb18*) (Chartrain et al. 2009; Goodwin 2012; Tabib Ghaffary et al. 2011, 2012) along with many quantitative genes with minor effects (Simón et al. 2012; Kelm et al. 2012; Risser et al. 2011; Kosellek et al. 2013) have been identified. Major resistance loci can be effective at seedling and adult-plant stage or just at seedling and adult-plant stage, respectively, depending on the gene (Arraiano et al. (2001); Kema and van Silfhout 1997). Given the high diversity and complex population structure within local populations of *Z. tritici* due to high gene flow within and between populations and frequent sexual reproduction, quantitative, isolate non-specific resistances at adult-plant stage is favored (Kema et al. 1996; McDonald et al.1996). Nevertheless, little is known about the inheritance of adult-plant resistance to *Septoria tritici* blotch especially under field conditions over diverse wheat growing environments. In a DH population derived from a cross between European winter wheat lines Senat and Savannah tested in three different environments Eriksen et al. (2003) observed four QTL altogether explaining 62–77 % of phenotypic variation. Risser et al. (2011) identified nine and six QTL for *Septoria tritici* blotch disease ratings over five and four environments explaining together 55 and 51 % of phenotypic variation in two European winter wheat populations, respectively. Kosellek et al. (2013) mapped five QTL altogether explaining 20 % of the phenotypic variance in the European winter wheat population Solitär × Mazurka tested in six environments. These studies suggest that field resistance to *Septoria tritici* blotch is due less to major genes than to genetic factors conferring partial resistance. In this study, we developed two bi-parental recombinant inbred line (RIL) populations of spring bread wheat to analyze the inheritance of adult-plant resistance to *Septoria tritici* blotch by inoculation in field trials in four different environments.

Materials and methods

Plant materials

A total of 200 and 193 $F_{6:7}$ RILs were developed through single seed decent from a cross between the Moroccan spring bread wheat variety 'NASMA' and the two CIMMYT wheat lines 'IAS20*5/H567.71' and 'RPB709.71/COC', respectively. The variety "NASMA" with the pedigree 'BT1149//Florence/AuroreC' was bred by the "Institute National de la Recherche Agronomique" in Morocco in 1973 (CIMMYT 2015) and has shown high susceptibility to *Septoria tritici* blotch. The CIMMYT lines IAS20*5/H567.71 and RPB709.71/COC were previously reported to have reduced *Septoria tritici* blotch severity at the CIMMYT experimental station in Toluca, State of Mexico, a location where *Septoria tritici* blotch is highly endemic (Jlibene et al. 1994). In addition, low pycnidial coverage two leafs below the flag leaf was observed in the cross IAS20*5/H567.71//CAR853/COC at four geographically different locations in Morocco inoculated with a group of 27 different isolates (Jlibene et al. 1995). Test entries were derived from F_6 lines and tested after a seed multiplication as F_7 bulks.

Inoculation procedures and disease assessment

The inoculum of *Septoria tritici* blotch was produced in the CIMMYT wheat pathology laboratory using a mixture of six aggressive strains: St1 (B1), St2 (P8), St5 (OT), St6 (KK), 64 (St 81.1) and 86 (St 133.4) with a concentration of 1×10^7 conidia/mL. These six isolates were selected from a larger Mexican collection based on their strong virulence to a set of wheat genotypes at CIMMYT. The first inoculation of the mapping population was conducted between 28 and 30 days after planting i.e. four to five leaf stage. The inoculation was continued every week with a total of three applications. The same inoculation procedure was used for a set of differential lines which were planted in the field and greenhouse to verify the avirolence-virolence profile of the isolates.

Field trials

The two RIL populations together with their parental lines were evaluated in four different environments (location–year combinations). Field trials were conducted at CIMMYT's experimental stations in the State of Mexico, at Toluca (latitude 19°17′N, longitude 99°40′W, 2600 m above sea level), and Boximo (latitude 19°36′N, longitude 99°41′W, 2600 m above sea level) in 2010 and 2011. Both locations are characterized by monthly average

temperatures of 12–17 °C and yearly rainfalls of approximately 1000 mm which mostly occur during the wheat growing season. At both locations also natural infections with *Z. tritici* are observed. All entries were grown with two replications at each location adjacent to each other in a completely randomized design. Each entry was sown in sets of 2 m rows with 4 g kernels per row. Approximately 4 weeks after the last inoculation, disease severity was visually scored for each plot, using the double-digit scale (00–99) developed as a modification of Saari and Prescott's severity scale for assessing wheat foliar diseases (Saari and Prescott 1975). The first digit (D1) indicates disease progress in canopy height from the ground level and the second digit (D2) refers to severity measured based on diseased leaf area. Both D1 and D2 were scored on a scale of 1–9. Disease evaluation was repeated three to four times at weekly intervals. For each evaluation, percentage disease severity was estimated based on the following formula:

$$\% \text{ severity} = (\text{D1}/9) \times (\text{D2}/9) \times 100$$

The area under disease progress curve (AUDPC) was subsequently calculated using the formula: $\text{AUDPC} = \sum_{i=1}^{n} \left[\left\{\left(Y_i + Y_{(i+1)}\right)/2\right\} x \left(t_{(i+1)} - t_i\right)\right]$ where Y_i *Septoria tritici* blotch severity at time t_i, $t_{(i+1)} - t_i$ = time interval (days) between two disease scores, n = number of times when Septoria tritici blotch was recorded. In addition to the disease scores, plant height was measured in cm at maturity from the ground to the ear tip in all location-year combinations except in Boximo 2011.

DNA isolation and selective genotyping

Eight to ten seeds of each $F_{6:7}$ RIL were germinated for DNA extraction in the greenhouse. Genomic DNA was isolated from young seedling leaves according to CIMMYT laboratory protocols (http://repository.cimmyt.org/xmlui/handle/10883/3221). Selective genotyping was applied in both populations. DNA of the ten most resistant RILs based on disease assessment across environments was pooled in equal quantities to construct a resistant (R) bulk. Likewise, DNA from the ten most susceptible RILs was pooled to create a susceptible (S) bulk. The three parental lines (NASMA, IAS20*5/H567.71, and RPB709.71/COC) and four bulks were screened for polymorphism with SSR markers from diverse sources: (1) wheat microsatellite consortium (WMC) primers (Gupta et al. 2003); (2) Beltsville Agriculture Research Center (BARC) primers (Song et al. 2000), (3) Gatersleben Wheat Microsatellite (GWM) primers (Röder et al. 1998), and (4) Clermont Ferrand A- and D genome (CFA, CFD) primers (Sourdille et al. 2004; Guyomarc'h et al. 2002). Once polymorphisms were identified between the parents, the R and S bulks of each population and individuals of the two bulks then the entire RIL populations were screened for that marker. SSR marker screening also included flanking markers previously published to be associated with different *Stb* loci (Table 1). The DNA of the parents and selective genotypes was furthermore sent for SNP genotyping to the USDA-ARS Small Grain Genotyping Center, Fargo, USA (http://wheat.pw.usda.gov/GenotypingLabs) for testing the Illumina iSelect 90 K SNP Assay (Wang et al. 2014b). SNP allele clustering and genotype calling was performed with the Genome Studio software v2011.1. The default clustering algorithm implemented in Genome Studio was used to identify assays that produced three distinct clusters corresponding to the AA, AB and BB genotypes expected for bi-allelic SNPs. For poorer cluster separation manual clustering was applied. For a subset of polymorphic SNP markers between parents and selective genotypes in each population, KASPar assays (LGC Genomics, Herts, UK) were utilized to genotype the corresponding RILs.

PCR amplification of the SSR markers was conducted in a 10-ml reaction mix, which contained 1 × buffer (5X Green or Colorless GoTaq® Flexi, Promega, USA), 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.25 µM of each oligonucleotide primers, 40 ng of DNA, and 1 unit of *Taq* polymerase (GoTaq® Flexi, Promega, USA, Cat. # M8295). PCR was performed in an ABI Geneamp 9700 PCR thermocycler (Applied Biosystems, USA). The samples were denatured at 94 °C for 2 min, followed by 35 cycles consisting of 94 °C for 30 s, 55 °C (or 60 °C) for 40 s, 72 °C for 1 min, with a final extension at 72 °C for 7 min. The amplified products were separated on 12 % acrylamide gels (29:1), and silver staining was used for visualization (http://repository.cimmyt.org/xmlui/handle/10883/3221).

KASPar assays genotyping was partly conducted in-house and partly outsourced to LGC Genomics. In-house, the KASP assays were visualized with a PHERAstar FS microplate reader (BMG Labtech, Ortenberg, Germany) in a final volume of 4 µl containing 2x KASPV4.0 Mastermix (LGC Genomics) and 120 nM of each allele-specific primers and 300 nM or of common primer. The following thermal profile was used for all PCR reactions: 15 min at 94 °C, 20 cycles of 10 s at 94 °C, 5 s at 57 °C, and 10 s at 72 °C (http://repository.cimmyt.org/xmlui/handle/10883/3221).

Phenotypic data analyses

Arithmetic means across replications for genotypes in each environment were used for analysis of variance (ANOVA). Estimation of variance components was performed using the *lmer* function in R (r-project.org/) with genotype and environments as random effects. Heritability (h^2) was calculated based on the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/\text{re})$ (Allard 1960), where σ_g^2, σ_{ge}^2, and σ_ε^2 are the estimates of

Table 1 Published molecular markers flanking assigned *Stb* resistance genes used for screening of two RIL populations NASMA × IAS20*5/H567.71 and NASMA × RPB709.71/COC

Marker name	Stb gene	Chr.	Germplasm Sources	Reference
barc74	*Stb1*	5BL	Bulgaria 88	Adhikari et al. 2004a
gwm335				
barc008	*Stb2*	1BS	Veranapolis	Liu et al. 2013
wmc230				
wmc406				
wmc83	*Stb3*	7AS	Israel 493	Goodwin et al. 2014
gwm111	*Stb4*	7DS	Tadinia	Adhikari et al. 2004b
gwm44	*Stb5*	7DS	Cs Synthetic 6x	Arraiano et al. 2001
gwm369	*Stb6*	3AS	Flame	Brading et al. 2002
wmc313	*Stb7*	4AL	Estanzuela Federal	McCartney et al. 2003
wmc219				
gwm146	*Stb8*	7BL	M-6 Synthetic W7984	Adhikari et al. 2003
gwm577				
wmc317	*Stb9*	6AS	Courtot	Chartrain et al. 2009
gwm848	*Stb10*	1D	Kavkaz-K4500 L.6.A.4	Chartrain et al. 2005a
barc008	*Stb11*	1BS	TE 9111	Chartrain et al. 2005b
barc137				
wmc313	*Stb12*	4AL	Kavkaz-K4500 L.6.A.4	Chartrain et al. 2005a
wmc219				
wmc396	*Stb13*	7B	Salamouni	http://wheat.pw.usda.gov//ggpages/awn/53/Textfile/Wgc.html
wmc623	*Stb14*	3B	Salamouni	
wmc500				
wmc494	*Stb16q*	3D	Cando/R143//Mexi'S'/3/Ae. Tauschii	Tabib Ghaffary et al. 2012
hbg247	*Stb17*	5A	Cando/R143//Mexi'S'/3/Ae. Tauschii	
gpw5176	*Stb18*	6DS	Balance	Tabib Ghaffary et al. 2011
gpw3087				

the genotypic variance, the genotype × environment interaction variance, and the error variance, and *e* and *r* are the numbers of environments and replications per environment, respectively. Pearson correlation coefficients among arithmetic means of traits in each environment were calculated.

Maps of linkage groups and QTL analyses

Genetic linkage groups were established with IciMapping 3.3 (Wang et al. 2014a; https://www.integratedbreeding.net/supplementary-toolbox/genetic-mapping-and-qtl/icimapping) using the 'BIN' tool to reduce the redundant markers. 'Two Opt' and 'SARF' were used for linkage criteria and algorithm, respectively, with the logarithm of odds (LOD) set at 3.0 for grouping. Inclusive composite interval mapping (ICIM) with IciMapping 3.3 was carried out to detect the positions of QTL for AUDPC in each environment, respectively. The parameters used for one dimensional ICIM were a threshold LOD = 3 to declare significant QTL, a 1 cM genome scanning step, a *P* value ≤0.01 for entering variables in the forward–backward stepwise regression of residual phenotype on marker variables.

The percentages of phenotypic variance explained (PVE) by individual QTL and the additive effect at the LOD peaks were obtained. ICIM for digenic epistatic QTL was performed using the same parameters. QTL were then confirmed additionally with QTL cartographer (Basten et al. 1994) version 2.5 using the composite interval mapping function. The threshold LOD score in cartographer was calculated using 1000 permutations.

Results

Phenotypic evaluation

There was good *Septoria tritici* blotch disease development in all environments studied. Each field trial revealed differentiation of disease ratings between the parental lines 'NASMA', 'IAS20*5/H567.71' and 'RPB709.71/

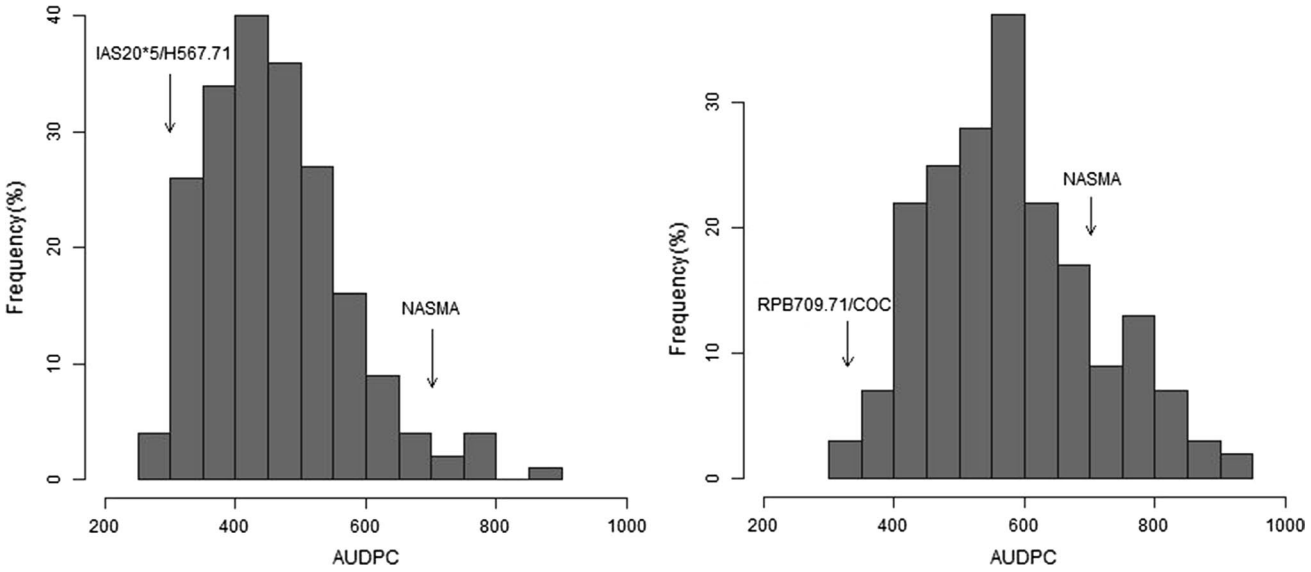

Fig. 1 Histogram of AUDPC for *Septoria tritici* blotch averaged across four environments of the NASMA × IAS20*5/H567.71 and NASMA × RPB709.71/COC RIL populations. The mean values for the parents are indicated by *arrows*

Table 2 Mean and range of AUDPC for *Septoria tritici* blotch for the susceptible parent NASMA and resistant parents IAS20*5/H567.71 RPB709.71/COC and derived RIL populations in four environments

Location	Year	NASMA	IAS20*5/H567.71	RPB709.71/COC	Population mean (NASMA × IAS20*5/H567.71)	Population mean (NASMA × RPB709.71/COC)	Population range (NASMA × IAS20*5/H567.71)	Population range (NASMA × RPB709.71/COC)
Boximo	2010	445.1	250.6	188.0	271.9	329.8	112.3–713.0	155.6–587.7
	2011	885.8	309.9	418.2	576.7	818.8	217.6–1330.6	388.0–1397.5
Toluca	2010	650.3	304.6	267.9	367.8	424.3	242.0–650.3	259.3–734.6
	2011	828.4	333.3	442.0	524.4	616.6	324.1–914.8	355.6–1023.5

COC' and within the two RIL populations (Fig. 1). Higher disease severities were observed in 2011 than 2010 (Table 2). The resistant parent 'RPB709.71/COC' expressed superior disease resistance compared to line 'IAS20*5/H567.71' in 2010, but the reverse was monitored in 2011. The population mean of the derived RIL population NASMA × RPB709.71/COC was higher than of the NASMA × IAS20*5/H567.71 population in each environment (Table 2). Correlations between arithmetic means of disease ratings across environments and RIL populations ranged from 0.48 to 0.79 (data not shown). Correlations between disease ratings and plant height were negative and moderately low in each population with an overall range from −0.04 to −0.27.

Analysis of variance showed that in both populations year variability had the highest contribution reflecting the difference in disease pressure over the 2 years (Table 3). Second highest contribution was the variability between lines, followed by variability between locations. The contribution of interaction components (genotype × location and year) was minor (Table 3). Population NASMA × IAS20*5/H567.71 had a considerably smaller genotypic, environment and error variance. The mean heritability was moderately high for both populations, $h^2 = 0.77$ for population NASMA × IAS20*5/H567.71 and $h^2 = 0.71$ for population NASMA × RPB709.71/COC.

QTL analyses

A total of 283 SSR markers were initially screened among the two parents and the two bulks. Thirty-eight and 35.8 % of the markers amplified polymorphism between the parental lines NASMA, IAS20*5/H567.71 and NASMA, RPB709.71/COC, respectively. Between the parental lines, the contrasting bulks and their constituent lines 65 markers in the NASMA × IAS20*5/H567.71 population and 43 in the NASMA × RPB709.71/COC population were polymorphic and were subsequently utilized to evaluate the entire RIL populations. With the Illumina iSelect SNP assay we revealed 37,386 (45.8 %) SNPs with distinct

Table 3 Estimates of variance components and heritability for AUDPC of *Septoria tritici* blotch of two RIL populations NASMA × IAS20*5/H567.71 and NASMA × RPB709.71/COC in four environments (location-year combinations)

Population	Source of variation												h^2
	Genotype		Year		Location		Genotype × year		Genotype × location		Residual		
	DF[a]	Variance components	DF	Variance components	DF	Variance components	DF	Variance components	DF	Variance components	DF	Variance components	
NASMA × IAS20*5/H567.71	202	7638***	1	25,950***	1	279***	202	200	202	0	203	8569	0.77
NASMA × RPB709.71/COC	192	9744***	1	57,970***	1	1408***	192	0	192	0	192	16,320	0.71

DF degrees of freedom, h^2 heritability

*** indicates F test significant at $P < 0.001$

clusters. A total of 6205 (16.6 %) SNPs were polymorphic among the parents and bulks in the NASMA × IAS20*5/H567.71 population and 9738 (26.1 %) SNPs in the NASMA × RPB709.71/COC population. Between the parental lines, the contrasting bulks and their constituent lines 187 markers in the NASMA × IAS20*5/H567.71 population and 199 in the NASMA × RPB709.71/COC population were polymorphic. Out of these a subset of SNPs that presented a high contrast between selective bulks and that were distributed across the occurrent chromosome regions were selected and converted to KASP assays. KASP assays for 26 and 35 SNPs were subsequently used to genotype all lines in the two populations, NASMA × IAS20*5/H567.71 and NASMA × RPB709.71/COC, respectively.

QTL were declared significant at LOD ≥3.0. In regions where such loci were also detected, QTL with LOD≥ 2.0 at $P \leq 0.1$ were taken into account. QTL analyses identified seven QTL for *Septoria tritici* blotch resistance in the NASMA × IAS20*5/H567.71 population of which two QTL were consistent across three of the four environments (Table 4). The QTL with the highest additive effect was located on chromosome 1BS spanning a 1.5 cM region and closely linked to markers *wmc230* and *Ku_c16938_25916260* (Table 4). The second QTL was mapped to a 7.6 cM centromeric region of chromosome 5AL flanked by the markers *Ex_rep_c68441_67261679* and *gwm186* (Table 4). The two QTL together explained on average 31.8 % of the phenotypic variation. Resistance effects for both QTL were derived from the resistant parent 'IAS20*5/H567.71'. Segregation distortion towards the resistant allele was observed for the first QTL. Digenic epistatic effects among the *Q*TL were not detected. The LOD scores were plotted to illustrate the QTL (Fig. 2).

In the NASMA × RPB709.71/COC population six QTL were detected by the ICIM analyses. Three QTL on chromosome 5AL, 3BL, and 7AS were consistent across three and four environments (Table 4). The highest additive effect QTL was on chromosome 3BL located within a 2.5 cM region and flanked by the SNP marker *Ex_c19778_28779907* and SSR marker *wmc291*. The QTL *QStb.cim.5AL-2* was the only QTL significant in all four environments. Flanked by the SNP markers *Ex_c621_1231444* and *Ex_c5998_10513766* at a genetic distance of 1.7 cM apart, the QTL was closely linked to *Vrn-A1*. The third QTL (*QStb.cim.7AS*) was of minor affect and mapped within a 3.3 cM region flanked by SNP markers *barc174* and *Ra_c2094_4066675*. The three QTL together explained 36.8 % of the phenotypic variance. Segregation distortion towards the susceptible allele was observed for the QTL on chromosome 5AL. Similar to the first population, digenic epistatic effects among the QTL were not observed and resistance effects of QTL were derived from the resistant parent. The LOD scores were plotted to

Table 4 Summary of resistance QTLs for *Septoria tritici* blotch consistent across at least three out of four environments

QTL	Environment	Left marker	Right marker	LOD value	PVE (%)	Additive effect
NASMA × IAS20*5/H567.71						
QStb.cim-1BS	Toluca 2010, Boximo 2010, 2011	*wmc230*	*Ku_c16938_25916260*	6.2	11.7	−40.3
QStb.cim-5AL-1	Toluca 2011, Boximo 2010, 2011	*Ex_rep_c68441_67261679*	*gwm186*	3.1	5.4	−28.8
NASMA × RPB709.71/COC						
QStb.cim-3BL	Toluca 2010, 2011, Boximo 2011	*Ex_c19778_28779907*	*wmc291*	12.2	22.7	−71.0
QStb.cim- 5AL-2	Toluca 2010,2011, Boximo 2010, 2011	*Ex_c621_1231444*	*Ex_c5998_10513766*	10.3	18.5	−61.8
QStb.cim-7AS	Toluca 2010, 2011, Boximo 2010	*barc174*	*Ra_c2094_4066675*	3.0	5.1	−24.0

PVE percent variance explained

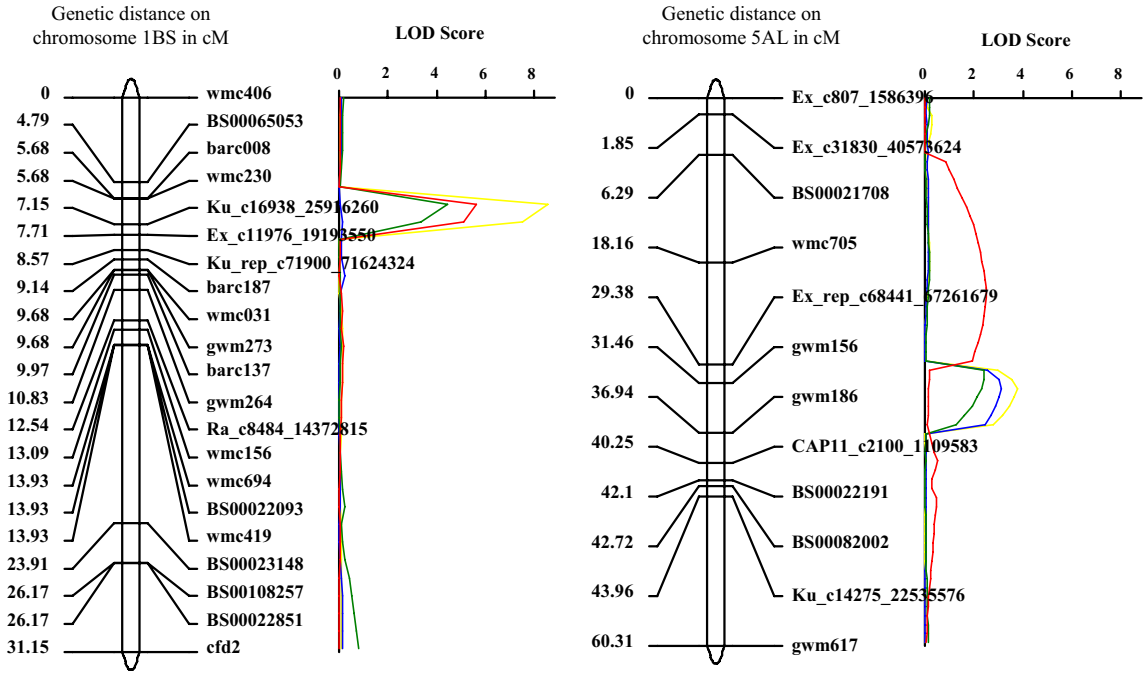

Fig. 2 LOD curves of QTL on chromosomes 1BS and 5AL in NASMA × IAS20*5/H567.71 for *Septoria tritici* blotch. *Different colors* represent different locations (color figure online)

illustrate the QTL (Fig. 3). The sequences of SSR and SNP markers linked to QTL are given in supplementary Table 1.

Individual effects of the two and three consistent QTL per population are shown in Figs. 4 and 5. The total data set was divided into four and eight groups, each carrying different haplotypes of the major QTL. In the NASMA × IAS20*5/H567.71 population, the first group carrying the susceptible allele (SS) at both loci had a mean AUDPC value of 516.7. The second and third group carried the SS allele at the 1BS locus and the resistant (RR) allele at 5AL locus or vice versa. Mean AUDPC values decreased in both groups on average 16.6 %. The fourth group carrying both RR alleles decreased AUDPC values by 23.9 %.

In the NASMA × RPB709.71/COC population, similar effects were obtained by analyzing the three consistent QTL for their phenotypic effects (Fig. 5). The maximal difference between haplotypes having all three RR and SS alleles was an AUDPC value of 218.3 (33.2 %). Between the groups including haplotypes carrying one or two RR alleles at the three loci the average difference was an AUDPC value of 82.2 (14 %). For the haplotypes carrying two RR alleles, the haplotype with resistant alleles at the

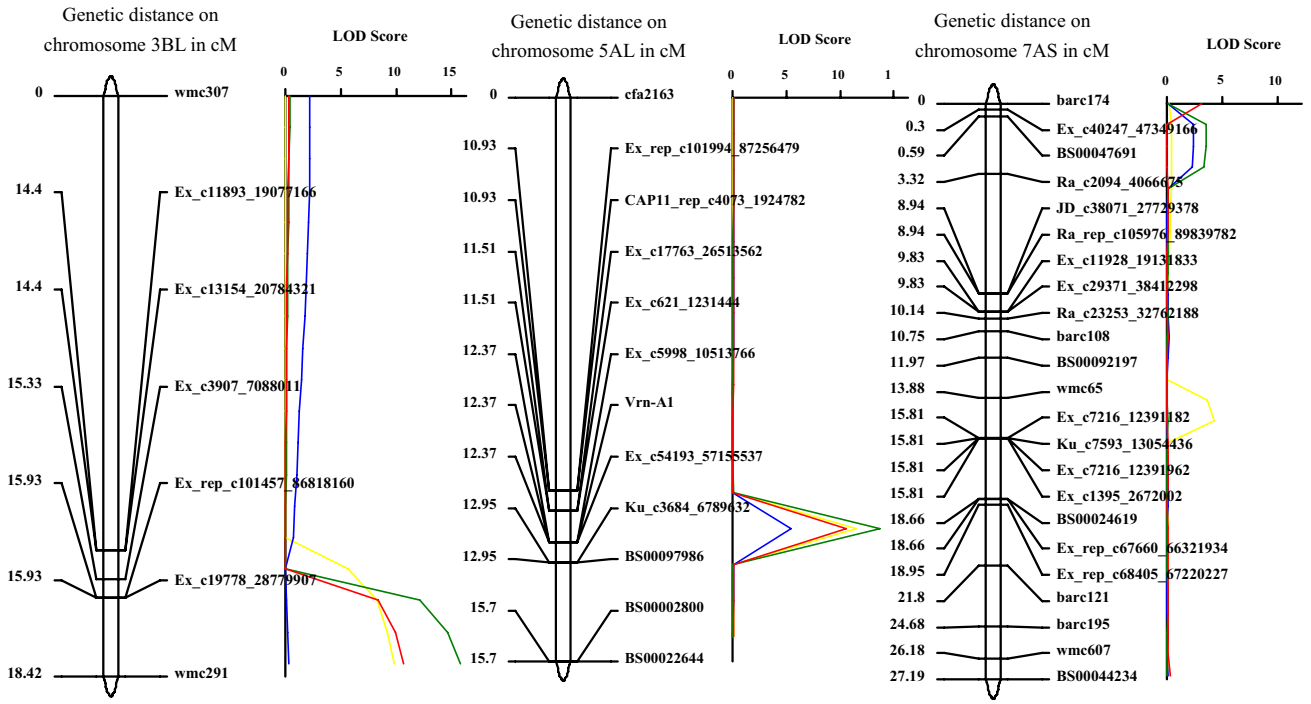

Fig. 3 LOD curves of QTL on chromosomes 3BS, 5AL and 7AS in NASMA × RPB709.71/COC for *Septoria tritici* blotch. *Different colors* represent different locations (color figure online)

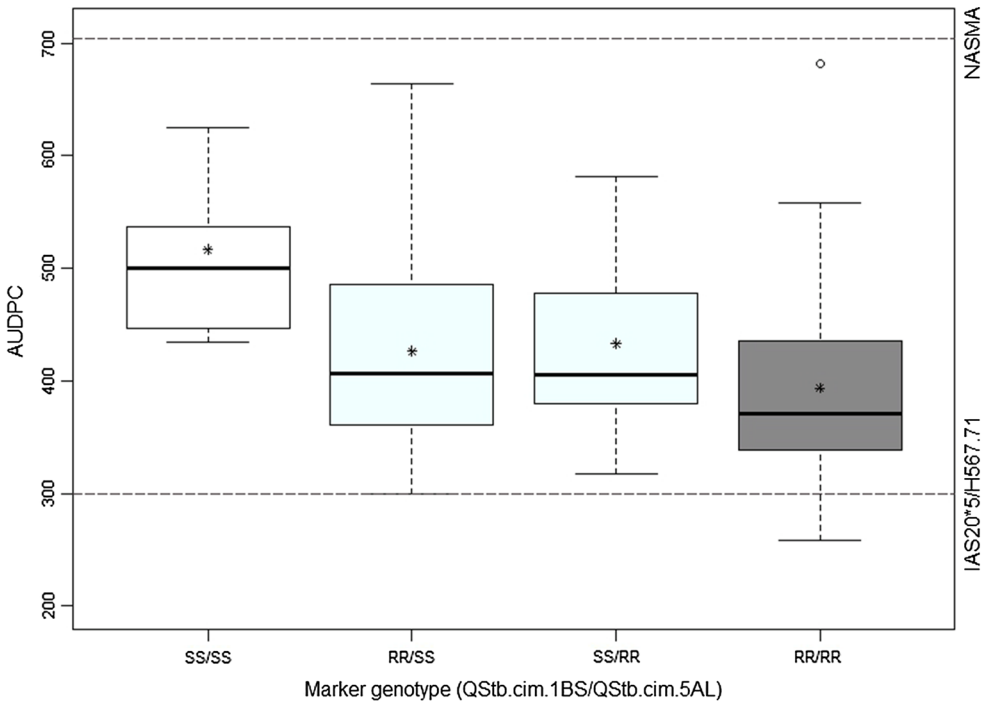

Fig. 4 *Boxplots* of RILs of the NASMA × IAS20*5/H567.71 population grouped by the genotypes of the flanking markers of two across environment consistent QTL associated with *Septoria tritici* blotch resistance. The *marker code* indicates whether the allele originates from the susceptible (SS) parent 'NASMA' or the resistant (RR) parent IAS20*5/H567.71', *represents the mean

3BL and 7AS QTL, revealed higher average AUDPC values were in comparison to the residual two haplotypes. Even so no significant digenic epistatic effects were estimated in the ICIM analyses, interaction plots (Supplementary Fig. 1–4) indicate increased interaction between the 3BL and 7AS QTL in individual environments. The observed variation within the resistant subpopulations gives the possibility for further phenotypic selection in both wheat populations.

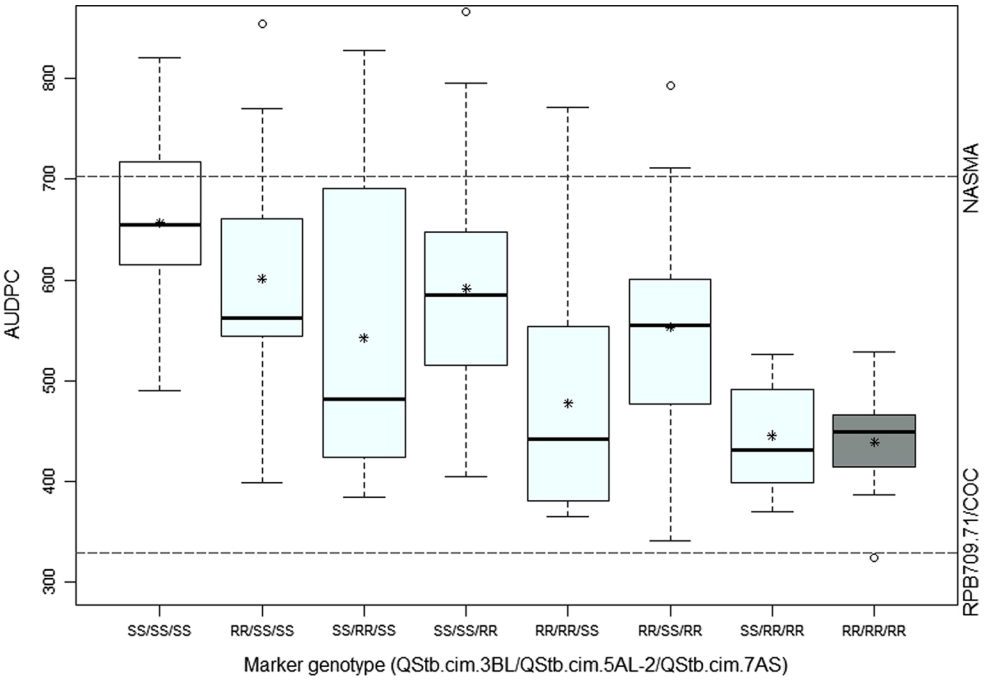

Fig. 5 *Boxplots* of RILs of the NASMA × RPB709.71/COC population grouped by the genotypes of the flanking markers of three consistent QTL associated with *Septoria tritici* blotch resistance. The marker code indicates whether the allele originates from the susceptible (SS) parent 'NASMA' or the resistant (RR) parent 'RPB709.71/COC', * represents the mean

Discussion

Due to high pathogenic variability and frequent sexual reproduction, *Z. tritici* remains a steadfast biotic stress pathogen, requiring multiple management strategies to mitigate the threat of *Septoria tritici* blotch and protect wheat production. Future disease management strategies must focus on augmented evaluations of genotype by environment by management systems; deepen the understanding of host-pathogen interaction and the improvement of varietal resistance (O'Driscoll et al. 2014). For the latter, up to date, only a few studies have deliberately evaluated adult-plant resistance under challenging field conditions over diverse wheat growing environments for the development of robust resistant phenotypes that remain durable. QTL analyses using bi-parental population assessed for *Septoria tritici* blotch resistance under field conditions were so far solely carried out using resistant European winter wheat sources. To our knowledge, the present study is the first QTL analyses targeting adult-plant resistance in multiple location-year trials using resistant spring bread wheat sources.

This study identified overall five across environment consistent QTL for *Septoria tritici* blotch resistance in the CIMMYT wheat lines IAS20*5/H567.71 and RPB709.71/COC. The QTL with the highest additive effect in the NASMA × IAS20*5/H567.71 population was observed on chromosome 1BS closely linked to markers *wmc230* and *Ku_c16938_25916260.* Segregation distortion towards the resistant allele was observed for this first QTL. As our mapping population consisted of F_7 RILs, it's reasonable to assume that QTL detected are preliminary additive effect. The studies of Wang et al. (2005) and Xu (2008) showed that, although ignoring segregation distortion in QTL analysis could result in slight power loss, the loss is negligible for QTL with additive effect, why we kept this data in our analyses. Liu et al. (2013) recently re-located the gene *Stb2* from line Veranapolis to the same chromosome location, with *wmc406* and *barc008* as flanking markers. The marker *barc008* co-segregated with *wmc230* in our study. However, *Stb2* was mapped 5 cM distal to *barc008*, and was fully effective in adult plant stage, while our QTL was mapped proximal to *barc008* and was partially effective in adult plant stage and explained only up to 13 % of the phenotypic variance (supplementary Fig. 5). The gene *Stb11,* derived from the Portuguese line TE 9111 was furthermore mapped to wheat chromosome 1BS (Chartrain et al. 2005b). The most closely linked locus to *Stb11* was once more *barc008.* Assessments of identity by state of observed marker alleles using the closest linked markers of this QTL showed that the critical band of IAS20*5/H567.71 was equal to TE 9111 for *barc008* with 275 bp (Chartrain et al. 2005b) but not for any of the other markers. *Stb11* is treated as an isolate-specific QTL due to a continuous distribution of disease scores in progeny plants after inoculation of the pathogen and was mapped as a seedling resistance gene specific to isolate IPO90012 from Mexico. Whether this isolate has similar or very different specifies to the cluster of six isolates used in our study in not known. Furthermore, in the study of Jlibene et al. (1995) line IAS20*5/H567.71 was tested as parent in the cross IAS20*5/H567.71//CAR853/COC at four geographically different locations in Morocco and inoculated with a group of 27 different isolates. Only

isolate St_{27} from Essaouira (southern Morocco) was virulent on this line and therefore suggests a rather non-isolate specific resistance. The effect of *Stb11* on resistance to *Septoria tritici* blotch under field conditions in also not yet known. Our field and greenhouse seedling tests revealed that the lines Veranapolis and TE9111 in the differential set knon to carry *Stb2* and *Stb11* respectively were resistant under Mexican field conditions in Toluca and Boximo using the same Mexican isolates (data not shown). The phenotype of both lines did therefore not help to determine if *Stb2* or *Stb11* is present in our population but confirmed the positive effect of the QTL region. An additional *Septoria tritici* blotch seedling resistance gene on 1BS was reported by Tabib Ghaffary et al. (2011) in the French cultivar 'Apache'. The most closely linked marker for this gene was DArT locus *wPt-2019*, also closely linked to *barc008* according to comparisons with shared markers in the Conan x Reeder reference map in the GrainGenes 2.0 database. A *Stb11*-like QTL named *StbWW* was reported by Raman et al. (2009) and derived from Australian wheat breeding lines 'WW1842', 'WW2451' and 'WW2449'. The gene was mapped in the same chromosome region flanked by markers *wmc230* and *wms273* (supplementary Fig. 5). This gene partially protected adult wheat plants from *Septoria tritici* blotch attack and explained up to 38 % of the phenotypic variance under greenhouse condition. Similar to our study one of the three mapping populations also showed segregation distortion towards the resistant allele. Finally, a QTL significant at seedling and adult-plant stage on 1BS was reported by Goudemand et al. (2013) using QTL meta-analyses and association mapping, but unfortunately linkage maps did not include to *Stb* genes linked microsatellite loci which would have facilitated comparative analyses. The diverse evidence from comparable genetic maps and gene action suggest that the identified QTL in our study is equal to *StbWW* and possibly allelic to *Stb11*, while validation via conventional crossing is still required. The resistance line IAS20*5/H567.71 has mainly South American cultivars in its pedigree; hence identity by decent due to the same origin of the diverse published resistance source is unlikely. The CIMMYT International Septoria Observation Nurseries (ISEPTON) has recently been characterized with linked to *Stb* genes linked SSRs including *Stb11* which was observed at very low frequency. This QTL is therefore a good candidate to be introgressed into CIMMYT wheat.

The second identified QTL *QStb.cim.5AL-1* in the NASMA × IAS20*5/H567.71 population explained only up to 9 % of the phenotypic variance (data not shown). Tabib Ghaffary et al. (2012) assigned the gene symbol *Stb17* to a QTL identified on chromosome 5AL. *Stb17* derived from durum wheat, was expressed in adult plant stage and explained 32 % of the observed phenotypic variance. The *Stb17* closely linked marker *hbg247* was mapped in our population to chromosome 5BL and was not linked with the disease ratings. In addition, *QStb.cim.5AL-1* was mapped more than 20 cM proximal of *wms617,* a second marker closely linked to *Stb17*. Thus, an additional source for *Septoria tritici* blotch resistance has been detected here.

The highest additive effect QTL in the NASMA × RPB709.71/COC population mapped to chromosome 3BL explaining 23 % of the phenotypic variance. A Meta-QTL on 3BL was published in Goudeman et al. (2013). A large effect QTL on chromosome 3B (*QStb. Isa_fb-3B*) was also observed by Risser et al. (2011) and a QTL (*QStb.risø-3B*) with smaller effect by Erikson et al. 2003. Unfortunately for all three studies, linkage maps did not allow a comparative analysis. Therefore this QTL represents either a confirmation of the reported QTL or a novel gene for STB resistance.

A second QTL on chromosome 5AL was detected in the NASMA × RPB709.71/COC population. The QTL was tightly linked to the *Vrn-A1* gene. Previous mapping studies revealed that *Septoria tritici* blotch was negatively related with earliness and plant height (Risser et al. 2011, Goudemand et al. 2013). Unfortunately, heading dates were not recorded in our field trails but breeders' notations ranging from 1 (early) to 3 (late) were available for both locations (Boximo and Toluca) in 2011. Late RILs were less prone to *Septoria tritici* blotch resistance than early RILs and correlations between the breeders' notations and disease scores were -0.43 and -0.49 for Toluca and Boximo 2011, respectively. The means of the disease scorings for 2011 were subsequently adjusted for the breeder' notation, including the notations into an ANOVA model. QTL analyses was repeated with the adjusted means but revealed the same results, which suggest that *QStb.cim.5AL-2* is either closely linked or corresponds to *Vrn-A1*. The correlation with plant height in both populations was negative, but low to moderate and no effect on resistance was observed.

The third QTL on chromosome 7AS in the NASMA × RPB709.71/COC population was of minor effect. Common relative map positions of our 7AS linkage group to previously published maps for SSRs markers *barc174*, *barc108*, *wmc65* and *barc121* were found with differences only in map distances (supplementary Fig. 6). Goodwin et al. (2014) recently re-located the gene *Stb3* to chromosome 7AS completely linked with SSR marker *wmc83*. In the NASMA × RPB709.71/COC population however, *wmc83* and nearly located markers *barc222* and *cfa2028* were monomorph with the QTL being mapped proximal not distal to *barc174*. However, since genetic distance can vary in different populations and markers are relative close, allelism between QTL *QStb.cim-7AS* and *Stb3* cannot be ruled out. In addition, the line Israel 493 in the differential set and known to carry *Stb3* showed resistance in our greenhouse seedling test (data not shown).

Unfortunately, not enough seed of the line was available to be included into the field trails, why field reactions are unknown.

The host resistance to *Septoria tritici* blotch is reported to be both qualitative and quantitative. Although gene-for-gene interactions exist between certain qualitative genes and the corresponding pathogen isolates, the resistance conferred by each gene is weak and cannot provide sufficient protection to wheat as those in rusts and powdery mildew (Goodwin 2012). Like in other diseases, breakdown of *Septoria tritici* blotch resistance genes has been observed, e.g. resistance of the wheat cultivar 'Gene' was defeated only 5 years after its release, implying its resistance nature of 'race-specific' (Cowger et al. 2000). Thus it is therefore recommended to pyramid both qualitative and quantitative resistance genes in breeding materials to achieve more durable resistance (Raman and Milgate 2012).

In this study we identified previously known and new QTL for adult-plant resistance against *Septoria tritici* blotch under field conditions in diverse environments and against a cluster of Mexican virulent isolates which are useful to accelerate the additional assembling of more resistant quantitative genes to *Septoria tritici* blotch. Further fine mapping and the characterization of the two RIL populations beyond the Mexican environments is required and aimed for, to evaluate the stability of the QTL and test their usefulness in archiving long-term durable control of the disease.

Overall five across environment consistent QTL for *Septoria tritici* blotch resistance were observed in the two RIL populations. QTL were additive and showed no major epistatic effects. However, the variance explained by the QTL varied largely from 4 to 27 % in the individual environments (data not shown) demonstrating the complex inheritance of *Septoria tritici* resistance. Quantitative genetic theory points out that the best ways of using molecular markers in selection largely depend on the genetic architecture of the trait (Bernardo 2008). Larger effect QTL are commonly used in marker-assisted selection (MAS). Despite the success in detecting larger effect QTL involved in quantitative disease resistance and with additive behavior, a deficit of commercially relevant *Septoria tritici* blotch resistant wheat germplasm so far remains and the underlying complex genetic architecture therefore challenges if MAS is the best approach for the development of durable resistant phenotypes. As an alternative concept, genomic selection has been proposed (Meuwissen et al. 2001). In genomic selection, effects are estimated for all genomic region using large populations genotyped and high-density marker panels. First experimental results applying genomic selection on *Septoria tritici* blotch resistance in wheat suggested that the approach is promising to improve breeding for quantitative disease resistances (Miedaner et al. 2013). Recently, Bernardo (2014) suggested major genes could be fitted in genomic selection prediction models as fixed effects when only a few major genes are present and each gene accounts for $\geq$10 % of the genetic variance. Thus a combined approach including high-density marker panels and major quantitative genes such as found here could be proposed. Low cost KASP assays were developed as flanking markers for all five QTL. These assays will facilitate MAS or their integration into large scale genome-wide marker data used in genomic selection approaches.

Author Contribution statement SD, P.K.S, R.J.: designed research; S.D., P.K.S., X.W., B.A.M.C., C.N.R.: Performed research; S.D.: Analyzed data; S.D.: wrote the manuscript.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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