Theor Appl Genet (2008) 117:49–56
DOI 10.1007/s00122-008-0751-1

ORIGINAL PAPER

Quantitative trait loci for aluminum resistance in Chinese wheat landrace FSW

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Received: 18 December 2007 / Accepted: 14 March 2008 / Published online: 1 April 2008

Abstract Aluminum (Al) toxicity is a major constraint for wheat production in acid soils worldwide. Chinese landrace FSW demonstrates a high level of Al resistance. A population of recombinant inbred lines (RILs) was developed from a cross between FSW and an Al-sensitive Chinese line, ND35, using single seed descent, to map quantitative trait loci (QTLs) for Al resistance. Wheat reaction to Al stress was measured by net root growth (NRG) in a nutrient solution culture containing Al^{3+} and hematoxylin staining score (HSS) of root after Al stress. After 1,437 simple sequence repeats (SSRs) were screened using bulk segregant analysis, three QTLs were identified to control Al resistance in FSW. One major QTL (*Qalt.pser-4DL*) was mapped on chromosome 4DL that co-segregated with Xups4, a marker for the promoter of the Al-activated malate transporter (*ALMT1*) gene. The other two QTLs (*Qalt.pser-3BL*, *Qalt.pser-2A*) were located on chromosomes 3BL and 2A, respectively. Together, the three QTLs accounted for up to 81.9% of the phenotypic variation for HSS and 78.3% of the variation for NRG. The physical positions of flanking markers for *Qalt.pser-4DL* and *Qalt.pser-3BL* were determined by analyzing these markers in corresponding nulli-tetrasomic, ditelosomic, and 3BL deletion lines of Chinese Spring. *Qalt.pser-3BL* is a novel QTL with a major effect on Al resistance discovered in this study. The two major QTLs on 4DL and 3BL demonstrated an additive effect. The SSR markers closely linked to the QTLs have potential to be used for marker-assisted selection (MAS) to improve Al resistance of wheat cultivars in breeding programs.

Communicated by J.-L. Jannink.

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Introduction

Aluminum (Al) toxicity is a major constraint for crop production in acidic soils worldwide. When the soil pH is lower than 5, exchangeable Al^{3+} is released to soil solution and enters into root tip cells, crippling root development of plants (Hoekenga et al. 2003). Poor root development reduces nutrient and water uptake from the soil (Kochian 1995), which significantly reduces plant growth and eventually plant yield. Although irrigation or direct application of lime to acidic soils can reduce harmful Al^{3+} effects on crop performance, the associated high cost has prompted producers to consider alternative solutions (Alva et al. 1986). In wheat, significant genetic variation in Al resistance has been reported among cultivars (Garvin and Carver 2003; Kochian 1995; Matsumoto 2000; Zhou et al. 2007b). Use of Al-resistant cultivars is an economically effective approach to improve crop productivity in acidic soils.

Inheritance of Al resistance in wheat has been extensively studied. A major QTL on 4DL has been identified in wheat cultivars BH 1146, Atlas 66, and Chinese Spring (Luo and Dvorak 1996; Ma et al. 2005; Raman et al. 2005; Riede and Anderson 1996). Markers are available for screening this QTL in wheat materials (Ma et al. 2005;

Raman et al. 2005, 2006). In addition, diagnostic markers for *ALMT1* gene were reported (Ma et al. 2005; Raman et al. 2006; Sasaki et al. 2004, 2006) and also mapped on the 4DL QTL region of Atlas 66 (Ma et al. 2005).

However, some studies demonstrated that more than one gene might be involved in Al resistance of wheat. Berzonsky (1992) reported that Al resistance in Atlas 66 was determined by a complex genetic mechanism involving several genes. Near-isogenic lines containing a single Al-resistance gene from Atlas 66 show only partial Al resistance, providing indirect evidence to support this assumption (Carver et al. 1993). Further study of the near-isogenic lines suggested that at least two genetic loci might contribute to Al resistance in Atlas 66 (Tang et al. 2002). More recently, Zhou et al. (2007a) reported a minor QTL for Al resistance on chromosome 3BL of Atlas 66, in addition to the major QTL on 4DL. However, the expression of the minor QTL on 3BL was suppressed by the major QTL on 4DL. Because polymorphic markers in the QTL region of the mapping population were lacking, the flanking markers for the 3BL QTL were not identified.

In wheat, the most extensively studied Al-resistant sources all have the Brazilian ancestor, Polyssu, in their pedigrees (Garvin and Carver 2003; de Sousa 1998). BH 1146 and Atlas 66 have been used widely in inheritance and gene expression studies (Berzonsky 1992; Guo et al. 2007; Riede and Anderson 1996) and they both can be traced back to Polyssu although Atlas 66 was developed in the USA. More recently, a Chinese wheat landrace, FSW, was found to have Al resistance similar to Atlas 66, but FSW has a different haplotype pattern for the markers derived from *ALMT1* (Zhou et al. 2007b). Inheritance of Al resistance in FSW has not been reported, and the genetic relationship between Al-resistance genes from FSW and Brazilian sources remains to be characterized.

The objectives of this study were to (1) elucidate the genetic relationship between Al-resistance QTLs from FSW and Brazilian sources, (2) identify new QTLs for Al resistance in FSW, and (3) to develop high-throughput PCR-based markers suitable for marker-assisted selection (MAS) in wheat breeding programs.

Materials and methods

Plant materials

A mapping population of 199 recombinant inbred lines (RIL) was derived from the cross between FSW and ND35 by single-seed descent. FSW is an Al-resistant landrace from China, and ND35 is an Al-susceptible wheat line from Jiangsu Academy of Agricultural Science, Nanjing, China. Chinese Spring and its nulli-tetrasomic lines N3BT3D and N4DT4B, ditelosomic lines 20″ + T″(3BL), 20″ + T″(4DL) and 20″ + T″(4DS), and seven 3BL deletion lines were used to physically map the markers linked to the QTLs conferring Al resistance on the chromosomes 3BL and 4DL.

To evaluate Al resistance of the RILs, wheat seeds were placed on moistened filter paper in a petri dish at 4°C for 24 h and then moved to room temperature (22 ± 2°C) for an additional 24 h. Three germinated seeds with similar viability were transferred onto a nylon net at the open bottom of a plastic cup. The cups were supported by a plastic cup holder floating on deionized water at 22 ± 2°C with 16 h fluorescent light daily. Two bubble rods in the water connected to an air pump provided aeration during the culture period. After 48 h of hydroponic culture, the deionized water was replaced with a nutrient solution (pH = 4.0) consisting of 4 mM $CaCl_2$, 6.5 mM KNO_3, 2.5 mM $MgCl_2{\cdot}6H_2O$, 0.4 mM NH_4NO_3, 0.1 mM $(NH_4)_2SO_4$, and 0.36 mM $AlK(SO_4)_2{\cdot}12H_2O$. Reactions of parents and RILs to Al stress were evaluated by measuring root growth during Al stress and the degree of hematoxylin staining of Al-treated root tips. The principal root of each seedling was measured after growing in the deionized water for the first 48 h. After 72 h of Al exposure, the same root was measured again. The difference between the two measurements was calculated as net root growth (NRG). After the second measurement of root length, excess Al^{3+} on the root surface was rinsed off in deionized water for 1 h, with two to three water replacements. Clean roots were then submerged in a hematoxylin solution containing 0.2% hematoxylin (w/v) and 0.02% (w/v) KIO_3 for 15 min. Following that, roots were rinsed with deionized water three to four times. The stained root tips of each stained seedling were visually scored as hematoxylin stain score (HSS) using a three-grade scale. The experiment was repeated three times with two replicates in each experiment using a randomized complete-block design. A total of 199 F_6 RILs were evaluated for Al resistance in first experiment and 170 F_7 RILs were evaluated in second and third experiments due to sterility of 29 F_6 RILs.

Marker analysis

After hematoxylin staining, wheat seedlings from the first experiment were transplanted to a greenhouse for about 2 weeks to harvest leaf tissue for DNA isolation. Leaf tissue from each line was collected in a 1.5-mL tube and dried in a freeze dryer for 2 days. Dry leaf tissue in the tube was ground by shaking the tube containing a 3.2-mm metal bead for 3 min at 30 times per second using a Mixer Mill (Retsch GmbH, Haan, Germany). DNA was extracted using the cetyltrimetryl ammonium bromide method (Saghai-Maroof et al. 1984). Bulked segregant analysis was used to screen polymorphic SSR markers associated with Al resistance. The Al-resistant bulk

consisted of five highly Al-resistant RILs, and the Al-susceptible bulk consisted of five highly Al-sensitive RILs. SSR primers screened included 552 BARC primers (Song et al. 2005), 291 WMC primers (Somers et al. 2004), 126 CFD and 56 CFA primers (Guyomarc'h et al. 2002; Sourdille et al. 2003), 236 GWM primers (Roder et al. 1998), 64 GDM primers (Pestsova et al. 2000), 22 DUP primers (Eujayl et al. 2002), and 90 KSM primers. Polymorphic markers between the bulks were further analyzed in the F_6 RIL population.

For SSR analysis, a 10-μL PCR mixture contained 40 ng of template DNA, 250 nM each of reverse and M13-tailed forward primers, 200 μM each of dNTPs, 1× PCR buffer, 2.5 mM $MgCl_2$, and 0.6 units of *Taq* polymerase. For PCR detection, 1 pmole fluorescence-labeled M13 primer (Li-Cor Inc., Lincoln, NE, USA) was added to each PCR reaction. A touch-down PCR program was used for PCR amplification, in which the reaction mixture was incubated at 95°C for 5 min then underwent five cycles of 1 min of denaturing temperature at 96°C, 5 min of annealing temperature at 68°C with a decrease of 2°C in each of subsequent cycles, and 1 min of extension at 72°C. For another five cycles, the annealing temperature started at 58°C for 2 min with a decrease of 2°C for each subsequent cycle. Then, PCR went through an additional 25 cycles of 1 min at 96°C, 1 min at 50°C, and 1 min at 72°C with a final extension at 72°C for 5 min. The amplified PCR fragments were separated in a Li-Cor 4300 DNA analyzer (Li-Cor Inc., Lincoln, NE, USA) using a 6.5% Gel Matrix (Li-Cor Inc., Lincoln, NE, USA). All marker data were scored by visual inspection and rechecked once to remove ambiguous data. All markers mapped on 3BL, 4DL, and 2A were reanalyzed in an ABI 3730 DNA Analyzer (Applied Biosystem, Foster City, CA, USA).

Data analysis

Analysis of variance and heritability was conducted according to Bernardo (2002) using the SAS system for windows v8 (SAS Institute, Inc., Cary, NC). Genetic linkage groups of SSR markers were constructed using JoinMap3.0 (van Ooijen and Voorrips 2001). Recombination fractions were converted into centiMorgans (cM) using the Kosambi function (Kosambi 1944). The threshold value of logarithm of odd (LOD) score was set at 3.0 to claim linkage between markers with a maximum fraction of recombination at 0.4. For QTL analysis, interval mapping was performed with MapQTL 5 (van Ooijen 2004). QTL analysis was done on the basis of line means from each individual experiment and overall line means across three experiments. Permutation tests of 1,000 times identified LOD at 3.0 as the threshold for declaring a significant QTL at $P < 0.05$ (Doerge and Churchill 1996).

Results

Responses of RILs and their parents to Al stress

The roots of FSW were longer (5.64 cm) than those of ND35 (1.47 cm) after 72 h of hydroponic culture in a nutrient solution with 0.36 mM Al^{3+}. After 3 days of Al treatment, the root tips of ND35 were fully stained by hematoxylin (grade 3), whereas those of FSW were not (grade 1). Therefore, the Al concentration used in this study was appropriate for differentiating the resistant genotypes from the susceptible genotypes by measuring either NRG or HSS.

The frequency distribution of NRG of the RILs under Al stress was continuous with the major peak toward ND35 (Fig. 1). A similar distribution was observed for HSS (data not shown). A highly significant correlation coefficient ($r = 0.85$, $P < 0.01$) was observed between NRG and HSS in the mapping population. The correlation between untreated root length and HSS or NRG was not detected in the RIL population ($r = 0.01$), therefore, HSS and NRG were independent of variation in natural root growth among RILs. Variance analysis showed that effects of RILs were significant on both HSS and NRG (Table 1). Heritibility was high for HSS (0.74) and NRG (0.78). Results suggested that Al resistance as measured by HSS and NRG is highly inheritable and more than one gene may be involved in Al resistance in the population.

QTL for Al resistance in FSW

After 1,437 SSR markers were screened, 413 primers amplified at least one polymorphic band between FSW and ND35. Among the polymorphic markers, 116 markers were polymorphic between the two bulks, and were selected for further

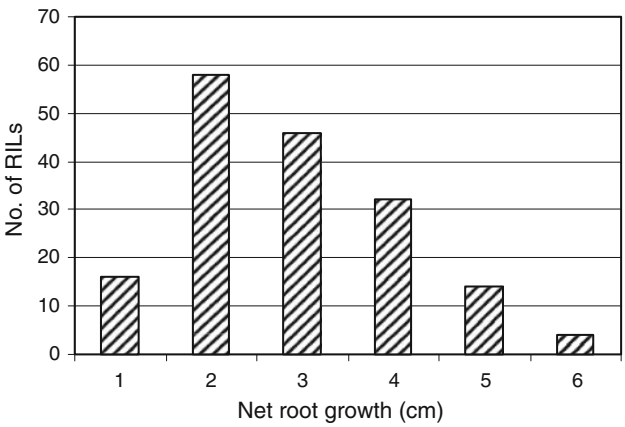

Fig. 1 Frequency distribution of net root growth (NRG) for RILs from the cross FSW/ND35 after 72 h of Al stress. *Arrows* indicate NRG for parents FSW (*right*) and ND35 (*left*)

Table 1 Variance components and heritability for net root growth (NRG) and hematoxylin stain score (HSS) in the recombinant inbred population derived from the cross FSW/ND35

Source	*df*	SS	MS	*F* value	h^2
NRG					
Environment	2	78.77	39.38	48.46**	
Block	1	0.17	0.17	0.21	
RILs	169	637.9	3.77	4.64**	0.78
RILs × block	314	261.3	0.832	1.02	
Error	467	379.58	0.812		
HSS					
Environment	2	9.76	4.88	25.33**	
Block	1	0.00	0.00	0.01	
RILs	169	514.24	3.043	15.8**	0.74
RILs × block	317	252.14	0.795	4.13**	
Error	477	91.872	0.1926		

** Significant *F* values at $P < 0.01$

analysis in 199 RILs of the population. A total of 81 markers were mapped in 14 linkage groups that spanned 257.5 cM of genetic distance. This map was used for further QTL analysis.

Interval mapping identified three QTLs, designated as *Qalt.pser-4DL, Qalt.pser-3BL and Qalt.pser-2A,* for Al resistance on chromosomes 4DL, 3BL, and 2A, respectively (Table 2). These three QTLs were all from FSW, and *Qalt.pser-4DL, Qalt.pser-3BL* showed a major effect on both NRG and HSS in the population (Fig. 2). The QTL *Qalt.pser-2A* also showed an effect on both NRG and HSS but was significant only for HSS ($P < 0.05$). *Qalt.pser-4DL*, was co-segregated with the marker Xups4 for the promoter of *ALMT1* with R^2 values of 55.7% for HSS and 46.2% for NRG. *Qalt.pser-3BL* was flanked by the markers Xbarc164 and Xbarc 344 and explained 47.0% of phenotypic variance for HSS and 41.7% of phenotypic variance for NRG (Table 2). *Qalt.pser-2A* was a minor QTL for HSS and was flanked by SSR markers Xgwm515 and Xgwm249 on chromosome 2A. This QTL only explained 9.5% of phenotypic variance for mean HSS, and 6.4% of phenotypic variance for mean NRG, which was not significant at $P < 0.05$.

Physical locations of the two major QTLs and their effects on Al resistance

Chinese Spring nulli-tetrasomic lines N3B-T3D and N4D-T4B, ditelosomic lines 20″ + T″(3BL), 20″ + T″(4DL), and 20″ + T″(4DS), and seven 3BL deletion lines were analyzed with the flanking markers of the two putative QTLs (Table 3). The SSR primer Gdm125 and STS primer Ups4 for the *ALMT1* promoter did not amplify the target band in the nulli-tetrasomic line N4D-T4B and ditelosomic line 20″ + T″(4DS), therefore, these markers were physically mapped on the long arm of chromosome 4D. Primers Gwm108, Barc164, Barc139, Gwm566, Wmc777, and Wmc078 amplified the target bands in Chinese Spring but not in nulli-tetrasomic line N3B-T3D, thus, they were mapped on chromosome 3B. Among them, Primers Wmc777 and Wmc078 amplified DNA from all seven 3BL deletion lines but not in 20″ + T″(3BL), thus, they were assigned to the short arm of chromosome 3B. Primers Gwm108, Barc164, and Barc139 amplified a band in 20″ + T″(3BL) and some of deletion lines of Chinese Spring, thus, they were mapped on 3BL. Primer Barc344

Table 2 Flanking markers, logarithm of odd (LOD) values, and coefficients of determination (R^2) of QTLs for net root growth (NRG) and hematoxylin stain score (HSS) identified in the recombinant inbred population derived from the cross FSW/ND35

Chromosome	Marker interval	Experiment	HSS			NRG	
			No. RILs	LOD	R^2	LOD	R^2
4DL	Xgdm125–Xups4	I	199	15.4	37.8	14.3	31.4
		II	170	14.3	65.2	11.5	43.3
		III	170	4.7	20.1	4.2	19.7
		Mean	170	15.9	55.7	13.7	46.2
3BL	Xbarc164–Xbarc344	I	199	8.6	28.5	9.1	29.5
		II	170	10.0	37.2	9.1	34.1
		III	170	7.7	34.2	5.8	23.3
		Mean	170	12.9	47.0	12.6	41.7
2A	Xgwm515–Xgwm296	I	199	4.9	11.9	3.7	8.6
		II	170	2.6	7.7	2.1	6.8
		III	170	1.5	4.9	1.0	3.3
		Mean	170	3.3	9.5	2.1	6.4
Total		I			67.2		70.3
		II			69.0		64.0
		III			40.3		33.7
		Mean			81.9		78.3

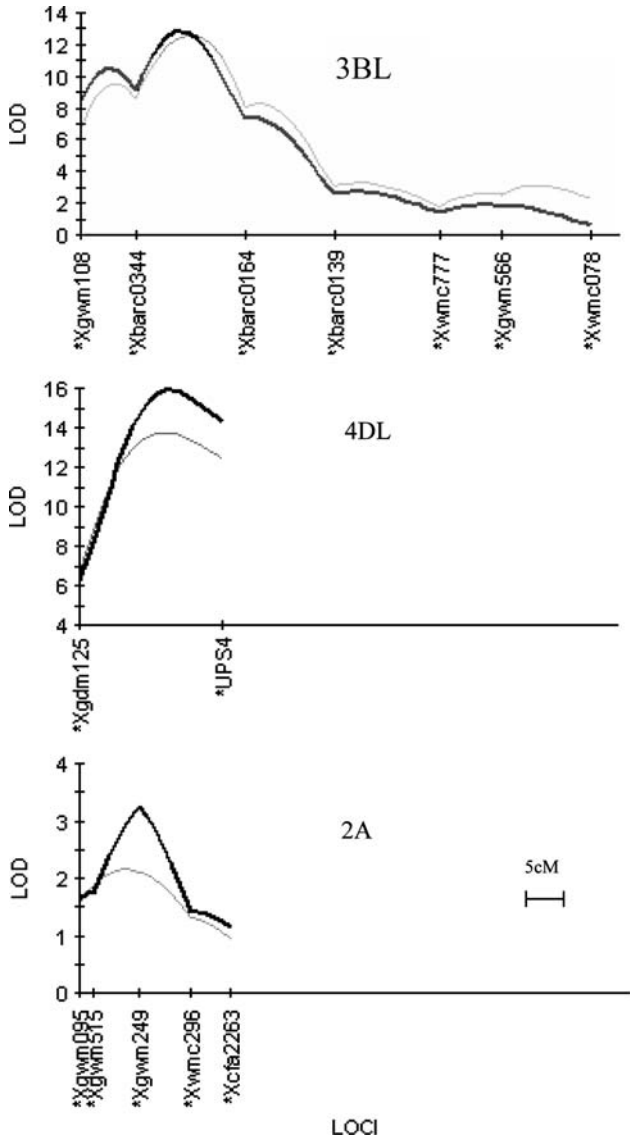

Fig. 2 Logarithm of odd (*LOD*) plot of QTLs for net root growth (*thin line*) and hematoxylin staining scores (*bold line*) on chromosome 4DL, 3BL, and 2A by using SSR markers and interval mapping (IM) based on 170 recombinant inbred lines from FSW/ND35. Phenotypic value of each RIL used for QTL mapping was derived from mean over all three experiments. Threshold for significant QTL was LOD at 3 based on 1,000-permutations

could not be physically mapped on 3BL because it amplified a non-target band in all Chinese Spring genetic stocks used in the study. Marker Xgwm108 was absent in the deletion line 3BL-3 but present in the deletion line 3BL-10, which located Xbarc108 in bin 3BL-10. Xbarc164 was absent in the deletion line 3BL-10 but present in the deletion line 3BL-6, which located Xbarc164 in the bins 3BL-6. Since Xgwm108 and Xbarc164 flanked *Qalt.pser-3BL, Qalt.pser-3BL* was located within bins DT3BL-10-6.

To analyze the effect of *Qalt.pser-4DL* and *Qalt.pser-3BL* on Al resistance, the closest markers, Xups4 on 4DL

Table 3 Physical mapping of markers linked to the Al-resistance QTL on 3BL and 4DL

Marker	CS	N3B-T3D[a]	20″ + T″(3BL)[b]	3BL-8[c]	3BL-1[c]	3BL-9[c]	3BL-3[c]	3BL-10[c]	3BL-6[c]	3BL-7[c]	N4D-T4B[a]	20″ + T″(4DS)[b]	20″ + T″(4DL)[b]	Location
Xgwm108	Y	–	Y	–	–	–	–	Y	–	Y	Y	Y	Y	3BL-10
Xbarc164	Y	–	Y	–	Y	–	–	–	Y	Y	Y	Y	Y	3BL-6
Xbarc139	Y	–	Y	Y	Y	Y	–	Y	Y	Y	Y	Y	Y	3BL
Xwmc777	Y	–	–	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	3BS
Xwmc078	Y	–	–	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	3BS
Xgdm125	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	–	–	Y	4DL
XUPS4	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	–	–	Y	4DL

Y indicates that a target band was amplified in the Chinese Spring genetic stock; "–" indicates that a target band was not amplified in the Chinese Spring genetic stock

CS Chinese Spring

[a] N3B-T3D and N4D-T4B are two nullisomic–tetrasomic lines that carry double chromosomes of 3D and 4B but are missing chromoeomes 3B and 4D, respectively

[b] 20″ + T″(3BL), 20″ + T″(4DS), and 20″ + T″(4DL) lines are ditelosomic lines that have double chromosome arms 3BL, 4DS and 4DL without 3BS, 4DL, and 4DS, respectively

[c] Different deletion lines with a missing part of chromosome fragment starting from different bins

and Xbarc0334 on 3BL, were selected to analyze the effect of MAS for the two QTLs (Fig. 3). Four possible combinations of the two QTLs are: 4DL+/3BL+, 4DL+/3BL−, 4DL−/3BL+, and 4DL−/3BL−, where 4DL+ and 3BL+ represent Al-resistance marker alleles of the QTLs from 4DL and 3BL of FSW, respectively, and 4DL− and 3BL− represent Al-sensitive marker alleles of the two QTLs from ND35. Comparisons of means among these genotypic classes indicated that a combination of the two Al-resistance marker alleles linked to *Qalt.pser-4DL* and *Qalt.pser-3BL* in a line increased NRG by about 2.5 cm and decreased HSS by 1.5 relative to the lines carrying neither resistance allele. The effects of these two resistant alleles appeared to be additive, and the presence of either Al resistance allele on 4DL or 3BL showed a significant increase in Al resistance in terms of NRG and HSS (Fig. 3).

Discussion

Net root growth of Al-stressed seedlings has been used to measure plant resistance to Al toxicity in several studies (Parker and Pedler 1998; Taylor and Foy 1985). Hematoxylin staining score has been used widely to evaluate Al resistance by estimating the content of Al^{3+} accumulation in the cells of tip roots in several crops (Anas 2000; Cancado et al. 1999; Delhaize et al. 1993). In this study, both NRG and HSS were used to measure Al resistance of parents and the RIL mapping population. Two parents showed a great contrast in NRG and HSS. Significant variation in HSS and NRG was observed among RILs and heritability was high for both measurements. The correlation between natural root growth rate and HSS or NRG was not detected. Therefore, the phenotypic data from both measurements were not associated with variation in root growth rate and appropriate for QTL analysis. In addition, both measurements of Al resistance were significantly correlated ($r = 0.85$, $P < 0.01$) and the QTLs for both traits were identified on the same three chromosome regions. *Qalt.pser-2A* showed a significant effect on mean HSS, but not on mean NRG. However, *it* showed a significant effect on NRG at $P < 0.05$ in the first experiment and a significant effect on NRG at $P < 0.10$ in the second experiment and mean HSS from three experiments. Therefore, the QTL on 2A appeared to be real but has a minor effect on Al resistance and be more vulnerable to non-genetic variation. Results suggested that both HSS and NRG were highly inheritable and most likely controlled by the same QTLs. Because the HSS method is simpler, less prone to environmental variation, and less labor-intensive than direct root length measurement, it is a more practical method for large-scale screening of Al-resistant wheat materials in breeding programs.

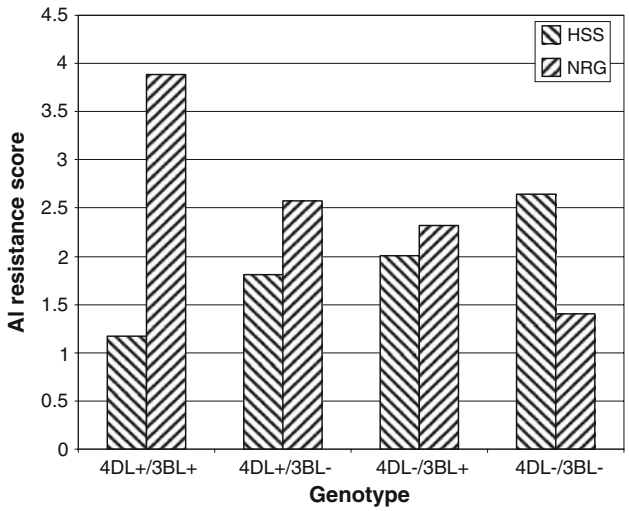

Fig. 3 Additive effect of 4DL and 3BL QTLs on Al resistance in RIL population from the cross FSW/ND35. 4DL+ and 3BL+ represent Al-resistance marker alleles of the QTLs from 4DL and 3BL of FSW, respectively, and 4DL− and 3BL− represent Al-sensitive marker alleles of the two QTLs from ND35, respectively. HSS and NRG represent hematoxylin staining score and net root growth (cm), respectively

Previous studies demonstrated that Al resistance was controlled by a single gene, *Alt1,* or a QTL on chromosome 4DL (Ma et al. 2005; Riede and Anderson 1996). Although *Alt1* in BH 1146 was reported to control complete Al resistance (Riede and Anderson 1996) and the 4DL QTL in Atlas 66 was reported to control partial Al resistance (Ma et al. 2005), they were most likely the same gene/QTL because they were located in the same chromosome region and shared a common ancestor, Polyssu, from Brazil. Malate release from root tips to prevent A^{3+} entering cells of the root tips has been considered the major mechanism for Al resistance in these cultivars (Sasaki et al. 2004). Locating the *ALMT1* gene within the 4DL QTL region of Atlas 66 provided further evidence to support that idea (Ma et al. 2005; Sasaki et al. 2004). The *ALMT1* gene explained the large portion of phenotypic variation for Al resistance in the Atlas 66 mapping population (Ma et al. 2005). In another study, a gene (*Alt2*) from Chinese Spring, a Chinese landrace unrelated to Brazilian Al-resistant sources, also was reported on the same location of 4DL (Luo and Dvorak 1996). These early studies indicated oligogenic control of Al resistance in these cultivars. However, other genes also have been associated with Al resistance in Atlas 66 (Basu et al. 1997; Pellet et al. 1996, 1997). Berzonsky (1992) proposed that genes in genomes A and/or B in addition to a dominant gene in the D genome also might be involved in Al resistance of Atlas 66. Tang et al. (2002) suggested that at least two QTLs might be involved in Al resistance of Atlas 66. Pellet et al. (1996, 1997) suggested the possibility of phosphate release from the root apex as another mechanism conferring Al resistance besides malate

release. More recent QTL mapping studies demonstrated that two or three QTLs might be involved in Al resistance in wheat (Ma et al. 2006; Zhou et al. 2007a). The current study identified three QTLs for Al resistance and provided further evidence to demonstrate that more than one QTL is involved in Al resistance in wheat.

In a previous study, FSW was found to have similar Al resistance to Atlas 66 (Zhou et al. 2007b) but carry different alleles of marker loci for the *ALMT1* gene (Sasaki et al. 2004, 2006; Zhou et al. 2007b). This study confirmed previous reports and the results from the current study also coincided with the same observation in some Japanese germplasm lines (Sasaki et al. 2006). Results suggested that both *ALMT1-CAP* and promoter markers may not be diagnostic for expression of the malate release gene in FSW because the QTL with the largest effect on Al resistance was still mapped on the 4DL of FSW at the same location as the major QTL in Atlas 66 and Chinese Spring (Ma et al. 2005, 2006). It is likely that *Qalt.pser-4DL* is a conserved QTL for Al resistance across different sources of Al resistance. The 4DL QTL from different sources are likely allelic to each other despite of their different origins. It is possible that FSW and Chinese Spring have similar geographic origins and the same allele of *Qalt.pser-4DL*. The Al-resistance QTL allele in Atlas 66 appears to be different from that of Chinese origin and has a larger effect on Al resistance than that from Chinese sources, such as Chinese Spring and FSW.

In addition to *Qalt.pser-4DL*, two additional QTLs were identified in this study. *Qalt.pser-3BL* with a major effect on Al resistance was mapped on the bins 3BL10-6 of the long arm of chromosome 3B based on physical mapping of the flanking markers, Xbarc164 and Xgwm108, for the QTL. Although Xbarc344, the closed marker to the QTL, could not be physically mapped in Chinese Spring, it was mapped between Xgwm108 and Xbarc164 on 3BL in a previous study (Song et al. 2005) and the current study. Thus, Xbarc344 also should be on bins 3BL10-6. In Atlas 66, a QTL linked to marker Xbarc164 was reported previously (Zhou et al. 2007a). However, the effect of the QTL ($R^2 = 11\%$ for HSS and $R^2 = 8.6\%$ for NRG) was much smaller than that identified in the current study ($R^2 = 47.0\%$ for HSS and $R^2 = 41.7\%$ for NRG). Also, expression of the 3BL QTL in Atlas 66 was inhibited by the 4DL QTL in the Atlas 66 population, whereas expression of *Qalt.pser-3BL* in FSW appeared to be independent of *Qalt.pser-4DL* and exhibited an additive effect with *Qalt.pser-4DL*. Based on linked marker location, the QTLs on the 3BL of Atlas 66 and FSW were more likely the same QTL, but different alleles. Therefore, *Qalt.pser-3BL* also may be the second conserved QTL for Al resistance. This study is the first to identify the major effect of *Qalt.pser-3BL* on Al resistance. Because the resistance mechanism of *Qalt.pser-3BL* remains unknown, further investigation of this QTL may lead to discovery of different Al-resistance mechanisms in wheat. The flanking markers for *Qalt.pser-3BL* identified in this study will be very useful for isolating near-isogenic lines for further characterization and map-based cloning of the QTL, and also will be suitable for marker-assisted selection for the QTL in breeding programs.

Qalt.pser-2A identified in this study was also a new QTL that had never been reported previously. This QTL accounted for 9.5 and 6.4% of the phenotypic variation for HSS and NRG, respectively. Its effects on increasing NRG or decreasing HSS were much smaller than *Qalt.pser-4DL* and *Qalt.pser-3BL*. *Qalt.pser-2A* was only marginally significant for NRG. Therefore, it might not be a stable QTL, and its effect needs to be further validated before it can be used for marker assisted selection.

In summary, three QTLs were identified for Al resistance from FSW. They were mapped on chromosomes 3BL, 4DL, and 2A and together accounted for 81.9% of phenotypic variation for HSS and 78.3% of variation in NRG in FSW/ND35 population. *Qalt.pser-4DL* and *Qalt.pser-3BL* demonstrated a major additive effect on both HSS and NRG. *Qalt.pser-3BL* was a new major QTL identified in this study, and markers linked to the QTL can be used for marker assisted pyramiding of QTLs from different sources of Al resistance in breeding programs.

Acknowledgments This project is partly funded by the National Research Initiative of USDA's Cooperative State Research, Education and Extension Service, CAP grant number 2006-55606-16629. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. This is contribution No. 08-172-J from the Kansas Agricultural Experiment Station, Manhattan, Kansas, USA.

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