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Quantitative trait loci for Aluminum resistance in wheat cultivar Chinese Spring

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Abstract

Aluminum (Al) toxicity is one of the major constrains for wheat production in many wheat growing areas worldwide. Further understanding of inheritance of Al resistance may facilitate improvement of Al resistance of wheat cultivars (*Triticum aestivum* L.). A set of ditelosomic lines derived from the moderately Al-resistant wheat cultivar Chinese Spring was assessed for Al resistance. The root growth of ditelosomic lines DT5AL, DT7AL, DT2DS and DT4DS was significantly lower than that of euploid Chinese Spring under Al stress, suggesting that Al-resistance genes might exist on the missing chromosome arms of 5AS, 7AS, 2DL and 4DL of Chinese Spring. A population of recombinant inbred lines (RILs) from the cross Annong 8455 × Chinese Spring-Sumai 3 7A substitution line was used to determine the effects of these chromosome arms on Al resistance. A genetic linkage map consisting of 381 amplified fragment length polymorphism (AFLP) markers and 168 simple sequence repeat (SSR) markers was constructed to determine the genetic effect of the quantitative trait loci (QTLs) for Al resistance in Chinese Spring. Three QTLs, *Qalt.pser-4D*, *Qalt.pser-5A* and *Qalt.pser-2D*, were identified that enhanced root growth under Al stress, suggesting that inheritance of Al resistance in Chinese Spring is polygenic. The QTL with the largest effect was flanked by the markers of *Xcfd23* and *Xwmc331* on chromosome 4DL and most probably is multi-allelic to the major QTL identified in Atlas 66. Two additional QTLs, *Qalt.pser-5A* and *Qalt.pser-2D* on chromosome 5AS and 2DL, respectively, were also detected with marginal significance in the population. Some SSR markers identified in this study would be useful for marker-assisted pyramiding of different QTLs for Al resistance in wheat cultivars.

Abbreviations: AFLP – amplified fragment length polymorphism; Al – Aluminum; DT – ditelosomic line; QTL – quantitative trait locus; RILs – recombinant inbred lines; SSR – simple sequence repeat

Introduction

Aluminum (Al) toxicity is one of the major constrains for crop production in acid soils where low pH facilitates the release of Al^{3+} into the soil solution. This trivalent cation is toxic to the plant root system by limiting root growth and development (Kochian, 1995). Al toxicity is the primary limitation on crop production for 37.9% of farmland in Southeast Asia, 30.9% of Latin America, and approximately 20% in East Asia, Sub Saharan Africa, and North America (Wood et al., 2000). High sensitivity of wheat to Al stress directly threatens food security in many areas where food security is most tenuous.

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Furthermore, in developed countries such as the United States, extensive farming practices such as the extensive use of ammonia fertilizers and continuous removing cations from soils through harvesting grain crops are causing further acidification of agricultural soils, creating new acid soils from previously neutral ones (Jackson and Reisenauer, 1984).

There is a wide range of genetic variation in Al resistance, both within and across plant species (Delhaize and Ryan, 1995; Ryan et al., 1995; Taylor, 1988). The genetic analysis of Al resistance has been an active area of research for several decades. The genetic architecture of Al resistance in wheat (*Triticum aestivum* L.) is still equivocal, being proposed to be monogenic in some studies but polygenic in the others. Evaluation of Al resistance in populations from the crosses between cultivars contrasting in Al resistance suggested that Al resistance was attributed to the action of a single dominant gene (Delhaize et al., 1993; Riede and Anderson, 1996) that was located on chromosome 4DL (Riede and Anderson, 1996; Rodriguez-Milla and Gustafson, 2001). Studies on ditelosomic lines of the moderately Al-resistant wheat cultivar Chinese Spring indicated a polygenic control of Al resistance in wheat. When Al resistance was assayed by root re-growth after Al stress, Chinese Spring lacking of chromosome arms 5AS, 6AL, 7AS, 2DL, 3DL, or 4DL showed reduced Al resistance relative to the euploid Chinese Spring, suggesting that each of these missing chromosome arms might be involved in Al resistance (Aniol, 1990; Aniol and Gustafson, 1984; Papernik et al., 2001; Takagi et al., 1983).

Molecular marker and genome mapping allow one to identify individual chromosomal region containing genetic factors that contribute to variation in a complex trait. They have been extensively used to dissect complex traits such as Al resistance in wheat and other cereal crops (Luo and Dvorak, 1996; Magalhaes et al., 2004; Miftahudin et al., 2004; Nguyen et al., 2001, 2003; Riede and Anderson, 1996; Sibov et al., 1999; Wu et al., 2000). In wheat, Riede and Anderson (1996) identified a single gene (Alt_{BH}) for Al resistance on 4DL of BH 1146 by using an RFLP marker. Further study of the same population demonstrated that Alt_{BH} was located to a 5.9-cM interval between markers Xgdm125 and Xpsr914 on 4DL (Rodriguez-Milla and Gustafson, 2001). In Chinese Spring, another gene for Al resistance, *Alt2*, was reported from the same chromosome arm by using disomic substitution lines (Luo and Dvorak, 1996).

Our objectives in this study were to evaluate the responses of ditelosomic lines of Chinese Spring to Al stress and to identify genetic loci for Al resistance in Chinese Spring by QTL mapping.

Materials and methods

Plant materials

Seeds of wheat cultivar Chinese Spring (*Triticum aestivum* L.) and its ditelosomic lines were provided by Wheat Genetics Resource Center, Kansas State University, Manhattan, KS, USA. Because loss of a chromosome arm caused sterility in some ditelosomic lines, only 31 ditelosomic lines were used for evaluation of Al resistance.

The mapping population consisted of 90 F_6 RILs derived by single-seed descent from the cross of Annong 8455/CS-SM3DS7A. CS-SM3DS7A has all chromosomes of Chinese Spring except for chromosome 7A substituted by the same chromosome of cultivar Sumai 3 (Zhou et al., 2002). Chinese Spring is a landrace from China with moderate Al resistance, whereas Annong 8455 is an Al-sensitive cultivar released from Anhui Agricultural University in China.

Al resistance of Chinese Spring and its derivatives

Ditelosomic lines and euploid of Chinese Spring, the mapping population of RILs and their parents were evaluated for Al resistance by measuring root growth of each line after Al stress. Wheat seeds were placed on moist paper in a petri dish. The seeds were incubated at 4 °C for 24 h and then moved to room temperature (22–25 °C) for another 24 h. Three germinated seeds with similar root lengths were selected and transferred onto a nylon-net at the bottom of a plastic cup with the bottom removed. Cups with germinated seeds were placed in a 30-well plastic cup holder. The cup holder floated on 7-L of deionized water in a 27-L plastic tray. Two bubble

rods in the bottom of each tray were connected to an air pump for aeration during hydroponics culture. After 48 h, the deionized water was replaced with a nutrient solution consisting of 4 m*M* $CaCl_2$, 6.5 m*M* KNO_3, 2.5 m*M* $MgCl_2{\cdot}6H_2O$, 0.1 m*M* $(NH4)_2SO_4$, and 0.4 m*M* NH_4NO_3 (Polle et al., 1978). The pH of the nutrient solution was set at 4.0, and monitored and adjusted daily.

After incubated in the nutrient solution for 24 h at 22 °C with 16 h of fluorescent light, the longest root of each seedling was measured to the nearest millimeter from the bottom of the plastic cup to the tip of the root. The seedlings were then transferred to a fresh nutrition solution, with addition of 0.36 m*M* $AlK(SO_4)\cdot 2H_2O$ at pH 4.0. After 48 h of Al stress, the same root in each seedling was measured again. The same procedure was followed for control plants, except without addition of $AlK(SO_4)\cdot 2H_2O$ in the nutrition solution. The experiment was repeated three times at different dates. Root elongation during 48 h of Al stress was referred to as stress root growth (SRG). Root elongation in the control solution (0 m*M* $AlK(SO_4)\cdot 2H_2O$) during 48 h was described as control root growth (CRG). The root-resistance index (RRI) (%) for each line in each replication was calculated as 100×SRG/CRG.

SSR and AFLP analysis

A total of 1013 pairs of SSR primers were screened between parents, including 101 GWM primers (Röder et al., 1998), 432 BARC primers (Song et al., 2005), 374 WMC primers (Somers et al., 2004), 8 GDM primers (Pestsova et al., 2000), and 98 CFD or CFA primers (Guyomarc'h et al., 2002; Sourdille et al., 2003). The PCR reactions were performed in a DNA Engine Tetrad® Peltier thermal cycler (MJ Research, Waltham, MA, USA). A 10-μL PCR mixture contained 40 ng of template DNA, 0.1 μM of each primer, 0.2 m*M* of each dNTP, 1 × PCR buffer, 2.5 m*M* $MgCl_2$, and 0.6 units of Taq polymerase (Promega, Madison, WI, USA). Forward primer was labeled with IRDye-700 or -800 (Li-Cor, Lincoln, NE, USA). A touch-town program was used for PCR amplification, in which the reaction incubated at 95 °C for 5 min, then continued for five cycles of 45 s at 95 °C, 5 min at 68 °C with a decrease of 2 °C in subsequent cycles, and 1 min 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 45 s at 94 °C, 2 min at 50 °C, and 1 min at 72 °C with a final extension at 72 °C for 5 min.

For AFLP analysis, 300 ng template DNA was double digested with *Pst*I and *Mse*I restriction enzymes and ligated to corresponding AFLP adaptors before pre-amplification. For pre-amplification, 40 μL PCR mixture consisted of 1 × PCR buffer, 2.5 m*M* of $MgCl_2$, 0.2 m*M* of dNTP mix, 75 ng each of unlabeled *Pst*I (5′-GACTGCGTACATGCAG) and *Mse*I (5′-GATGAGTCCTGAGTAA) primers, 0.75 u of *Taq* polymerase and 10 μL of ten-fold diluted DNA. PCR was run for 25 cycles at 94 °C for 30 s, at 56 °C for 60 s, and at 72 °C for 60 s. The pre-amplified PCR product was then used as a template for further selective amplification. A 10 μL of selective PCR mixture contained 2 μL of ten-fold diluted pre-amplified DNA, 1 × PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of dNTP mix, 10 ng of unlabeled *Mse*I primer, 0.35 pmol of IR dye-labeled *Pst*I primer and 0.2 u of *Taq* polymerase. The PCR was run at 94 °C for 2 min followed by 13 cycles of 94 °C for 30 s, 65 °C for 30 s with a touchdown temperature of –0.7 °C/cycle in each following cycle, and 72 °C for 60 s followed by additional 23 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s. To detect AFLP products, *Pst*I primers were labeled with either IRDye-700 or -800 fluorescent dyes compatible with the Li-Cor DNA analyzer (Li-Cor Inc, Lincoln, NE, USA). PCR products were mixed with 5 μL of formamide loading dye from Li-Cor (Li-Cor, Lincoln, NE, USA).

The PCR products of simple sequence repeat (SSR) and AFLP were denatured for 5 min at 94 °C, and then quickly cooled on ice. To 20 mL of 6.5% Gel Matrix (Li-Cor, Lincoln, NE, USA), 75 μL of 20% ammonium persulfate and 15 μL TEMED were added right before the gel was cast with 25-cm glass plates from Li-Cor (Li-Cor, Lincoln, NE, USA). The gel was cast at least 1 h in advance and was pre-run in 1 × TBE buffer (50 mM TRIS, 50 m*M* boric acid, 1 m*M*

EDTA) for 10 min before the samples were loaded. A sample of 0.8 μL was loaded into each well of the gel. The electrophoresis condition was set at 1500 V, 40 W at 45 °C. The gel image was collected simultaneously as the electrophoresis progressed by a scanner inside the analyzer, and was visualized on a computer screen.

Data analysis

Standard analysis of variance (ANOVA) was performed to test the significant differences among ditelosomic lines and RILs by using SAS (SAS Institute Inc, Cary, NC. USA). Broad-sense heritability (h^2) was computed as $\sigma_g^2/(\sigma_g^2+\sigma_e^2)$ based on the estimates of genetic and error variances. Linkage map of the SSR and AFLP markers was constructed by using Joinmap® 3.0 (Van Ooijen and Voorrips, 2001). MapQTL® 5 was used for composite interval mapping and estimation of determination coefficients (R^2) (Van Ooijen, 2004). Threshold of the LOD value to declare a significant QTL was determined by running 1000 permutations.

Results

Al resistance in ditelosomic lines of Chinese Spring

The ditelosomic lines and euploid of Chinese Spring were evaluated for Al resistance by measuring the root growth of these lines under Al stress in comparison with root growth of non-Al-stressed seedlings of the same lines in three times. Plants were grown hydroponically with a full-strength of nutrient solution containing 0.36 mM $AlK(SO_4)_2$ at pH 4.0. CRG, SRG, and RRI were measured for each line after 48 h of Al treatment (Table 1). During 2 days of growth, non-Al-stressed root of Chinese Spring increased 4.33 cm, which was similar to most of the ditelosomic lines by the means over three replications. The root growth values of ditelosomic lines ranged from 2.15 to 6.03 cm, with an average of 4.29 cm, indicating significant variation in root growth among these ditelosomic lines under normal hydroponic growth conditions. When roots of the ditelosomic lines were exposed to 0.36 mM Al, root growth was significantly reduced. But the root growth reduction due to Al stress differed significantly among ditelosomic lines. The SRG values for ditelosomic lines ranged from 0.29 to 1.98 cm. DT5AL, DT7AL, DT2DS, DT4DS, DT1BS, DT7AS, and DT6DL showed significantly smaller SRG values than that of euploid Chinese Spring. Among them, reduction of SRG in lines DT5AL, DT7AL, DT2DS, DT4DS was statistically significant ($P=0.01$). The RRI was also evaluated for these lines, and a highly significant correlation coefficient was observed between SRG and RRI ($r=0.82$, $P=0.01$).

QTLs for Al resistance in Chinese Spring

Frequency distributions for root growth of the RILs and their parents under Al-stressed condition are summarized in Figure 1. The roots of two parents, CS-SM3DS7A and Annong 8455 showed differential responses to Al stress: the former had larger values for SRG and RRI, therefore is more resistant to Al. The frequency of distributions for SRG and RRI in the RIL population was basically normal distribution, indicating Al resistance in this genetic population was controlled by multiple genes. Broad sense heritability was high with 0.80 for SRG and 0.85 for RRI, suggesting that Al resistance is a highly heritable trait, and the genetic variation can be used in a wheat-breeding program for improving Al resistance.

After 1013 pairs of SSR and 112 AFLP primers were screened for polymorphism between parents, 381 AFLP markers and 168 SSR markers were selected to construct a genetic linkage map with a coverage of 2512 cM length and can be assigned to all 21 chromosomes based on previously published map information for known SSR markers in the map (Guyomarc'h et al., 2002; Pestsova et al., 2000; Röder et al., 1998; Somers et al., 2004; Sourdille et al., 2003).

Composite interval mapping was used for identification of significant QTLs for root growth under Al stress, the threshold of LOD for each linkage group was calculated by a permutation test method with 1000 permutations at different P-values to determine a significant QTL (Table 2). Three QTLs, *Qalt.pser-4D*, *Qalt.pser-5A* and *Qalt.pser-2D*, for SRG reached or

Table 1. Aluminum sensitivity of ditelosomic lines and euploid of wheat cultivar Chinese Spring as reflected by mean and standard deviation of CRG (Control root growth), SRG (Stress root growth) and RRI (Root resistance index) over three experiments

Ditelosomic line	Missing chromosome fragment	CRG (cm)[a]	SRG (cm)[a]	RRI (%)[a]
DT1AS	1AL	2.40 ± 0.34*	1.49 ± 0.17	62.04 ± 6.90
DT1AL	1AS	5.17 ± 1.25	1.43 ± 0.43	27.74 ± 5.85
DT2AS	2AL	3.50 ± 1.25	1.59 ± 0.21	45.40 ± 6.25
DT3AS	3AL	4.50 ± 0.98	1.31 ± 0.37	29.14 ± 8.34
DT3AL	3AS	5.70 ± 1.69	1.66 ± 0.47	29.04 ± 8.30
DT4AL	4AS	3.10 ± 1.05	0.93 ± 0.18	30.11 ± 6.20
DT5AL	5AS	6.03 ± 0.95	0.29 ± 0.06**	4.79 ± 0.21**
DT6AS	6AL	5.34 ± 1.37	1.17 ± 0.05	21.83 ± 0.91
DT6AL	6AS	5.70 ± 1.50	0.96 ± 0.61	16.76 ± 4.32*
DT7AS	7AL	4.03 ± 1.46	0.71 ± *0.27	17.63 ± 2.25*
DT7AL	7AS	4.40 ± 0.28	0.37 ± 0.09**	8.33 ± 0.96**
DT1BS	1BL	4.37 ± 0.39	0.49 ± 0.05*	11.20 ± 4.9*
DT1BL	1BS	3.65 ± 1.02	1.51 ± 0.41	41.40 ± 8.36
DT2BL	2BS	4.02 ± 1.67	1.10 ± 0.05	27.36 ± 1.43
DT3BS	3BL	4.15 ± 1.54	1.98 ± 0.34	47.66 ± 9.02
DT3BL	3BS	4.10 ± 0.88	0.87 ± 0.29	21.14 ± 7.22
DT4BS	4BL	3.90 ± 0.79	1.39 ± 0.19	35.61 ± 4.79
DT5BL	5BS	5.00 ± 1.56	1.35 ± 0.10	27.00 ± 2.16
DT6BS	6BL	3.73 ± 3.73	1.45 ± 0.37	38.84 ± 7.52
DT6BL	6BS	3.65 ± 0.66	1.35 ± 0.03	36.99 ± 0.79
DT7BS	7BL	5.30 ± 1.78	1.16 ± 0.33	21.91 ± 6.26
DT7BL	7BS	2.77 ± 0.86*	1.60 ± 0.21	57.83 ± 7.74
DT1DS	1DL	2.15 ± 0.54*	1.08 ± 0.11	50.13 ± 5.16

Table 1. Continued.

Ditelosomic line	Missing chromosome fragment	CRG (cm)[a]	SRG (cm)[a]	RRI (%)[a]
DT1DL	1DS	3.35 ± 1.29	1.17 ± 0.26	34.83 ± 7.78
DT2DS	2DL	5.90 ± 0.78	0.34 ± 0.16**	5.78 ± 1.80**
DT4DS	4DL	4.00 ± 0.84	0.38 ± 0.04**	9.58 ± 1.10**
DT4DL	4DS	5.23 ± 1.34	1.23 ± 0.24	23.46 ± 4.69
DT5DL	5DS	3.90 ± 0.93	1.48 ± 0.19	38.03 ± 4.95
DT6DS	6DL	4.50 ± 1.57	0.96 ± 0.20	21.36 ± 4.59
DT6DL	6DS	4.65 ± 0.98	0.84 ± 0.26*	18.16 ± 5.70*
DT7DL	7DS	4.78 ± 1.45	1.04 ± 0.30	21.73 ± 6.34
Chinese Spring	0	4.33 ± 1.03	1.22 ± 0.40	28.21 ± 7.84

* and ** indicate significant difference between ditelosomic line and euploid of Chinese Spring at $p = 0.05$ and $p = 0.01$ level, respectively, in a LSD test.

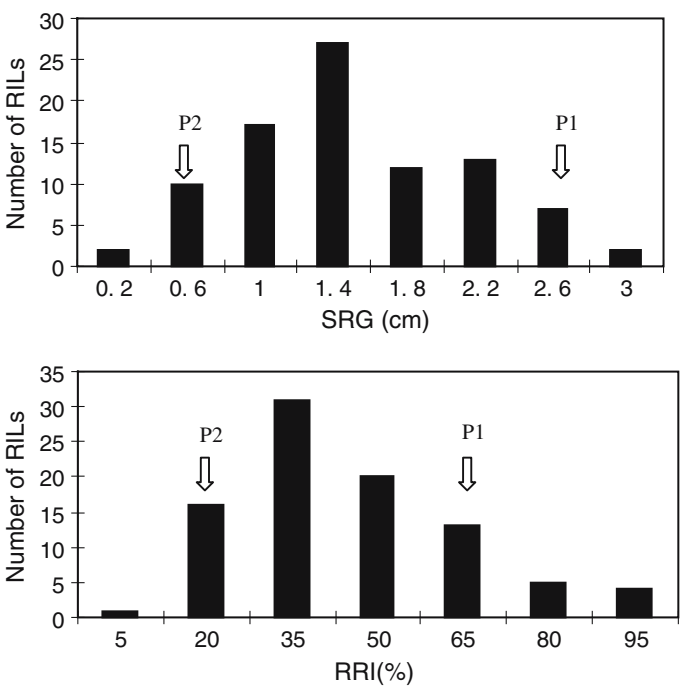

Figure 1. Frequency distributions of stress root growth (top) and root resistant index (bottom) of RILs. The values were the means over three experiments. P1 and P2 represent Al resistant parent and sensitive parent, respectively.

Table 2. Effects of putative QTLs for SRG and RRI detected in the RIL population derived from the cross CS-SM3DS7A/Annong 8455 using composite interval mapping

Chr.	Flanking marker	SRG								RRI								
		1st exp.		2nd exp.		3rd exp.		Average[a]			1st exp.		2nd exp.		3rd exp.		Average[a]		
		LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	Add.	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	Add.
4D	Xcfd23/Xwmc331	2.67***	13.4	3.02***	18.8	2.24**	12	2.97***	18.4	0.29	2.46	17.5	2.72***	18.3	2.38**	15.6	2.58***	13.0	6.29
5A	XmCTGA.pACT233/XmCACG.pGTG138	1.8*	9.2	1.84*	10	1.93*	10.7	1.85*	10.5	0.20	1.71	8.8	1.95*	11.2	1.86*	10.4	1.80*	8.7	4.88
2D	XmACGC.pAG231/XmCTCG.pAGG142	2.1*	11.9	1.95*	11	1.86*	9.8	2.1*	12.8	0.27	2.2	12.9	1.94*	10.6	1.84*	10.8	1.92*	11.4	5.29

[a]Average of additive effects, LOD and R^2 values were calculated based on the mean of phenotypic values for each RIL over three experiments.
*, **, or *** indicate statistical significance of LOD values at $p = 0.1$, 0.05, or 0.01 critical value in a permutation test, respectively.

exceeded the LOD threshold for declaring the presence of a QTL, which explained 18.4%, 10.5%, and 12.8% of phenotypic variance, respectively. Among them, the *Qalt.pser-4D* with the largest effect on Al resistance is located on chromosome 4DL and is flanked by the markers of *Xcfd23* and *Xwmc331* (Figure 2). The QTL for RRI was also identified in the same region, and explained 13.0% of phenotypic variance of RRI. The *Qalt.pser-5A* and *Qalt.pser-2D*, located on chromosome 5A and 2D, were flanked by AFLP markers *XmCTGA.pACT233* and *XmCACG.pGTG138*, and *XmACGC.pAG231* and *XmCTCG.pAGG142*, respectively. The LOD values for the two QTLs were lower than that of *Qalt.pser-4D*, and were only marginally significant for both SRG and RRI. However, since these QTLs were detected in the three separate experiments performed (except for QTL on 5A for RRI in the first experiment), they suggested that genes on 5A and 2D do condition a percentage of the measured Al resistance in this population. For all three QTLs on chromosome 4D, 2D and 5A, the alleles conferring Al resistance was originated from Chinese Spring based on the comparison of alleles among CS-SM3DS7A, Chinese Spring, Annong 8455 and Sumai 3 (Figure 3).

Discussion

In wheat breeding programs, wheat plants are usually evaluated for Al resistance in acidic soils under field conditions. However, inconsistent phytotoxicity among plots may significantly increase environmental error and decrease accuracy of phenotypic data. In addition, non-stressed treatments are usually applied in a different field with normal soil pH, which may not provide a valid control for proper comparison. An alternative method for evaluating Al resistance is based on the use of a nutrient solution containing a toxic level of Al. Therefore, it has been widely used in genetic studies (Baier et al., 1995; Polle et al., 1978; Samac and Tesfaye, 2003). With this method, root growth rate during Al stress was measured to determine Al resistance of ditelosomic lines of Chinese Spring and RILs from the cross of Chinese Spring-Sumai 3 chromosome 7A substitution line and Annong 8455 in this study.

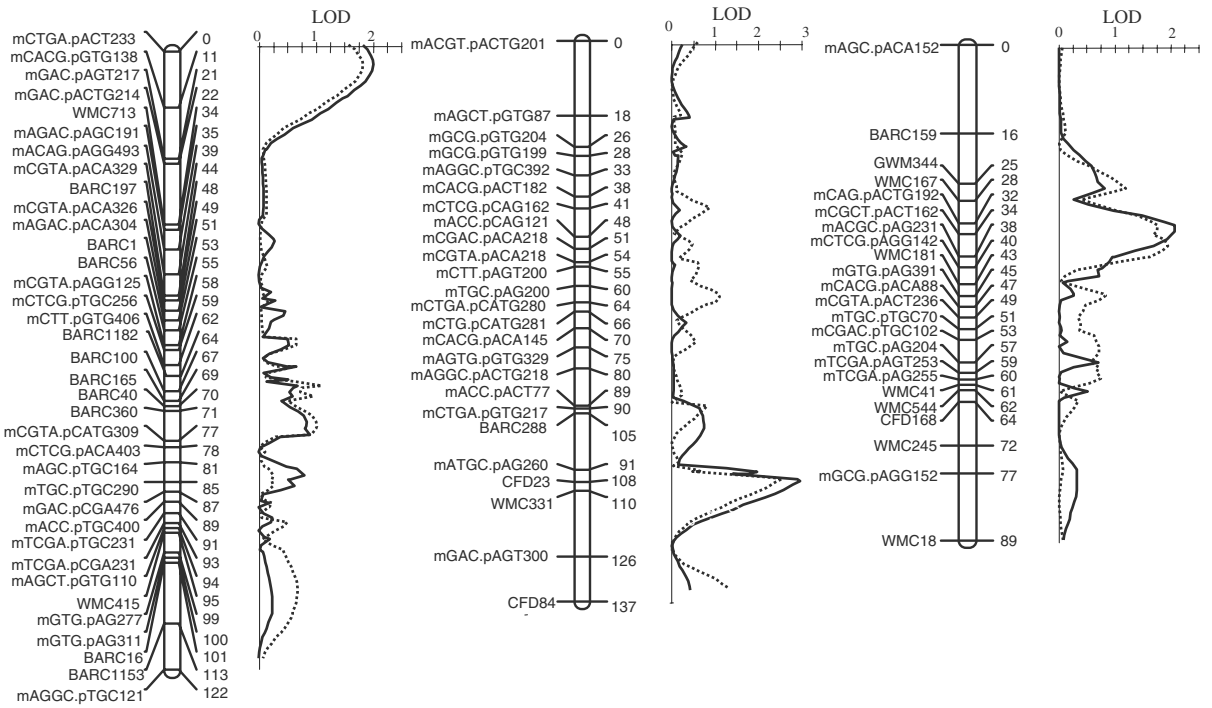

Figure 2. The QTLs on chromosome 2D (left), 4D (middle) and 5A (right) derived by composite interval mapping based on the average of three experiments. The distance between markers is given in Kosambi centiMorgans. —— SRG, ... RRI.

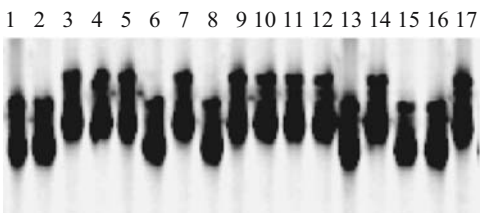

Figure 3. An electrophoresis pattern of PCR products amplified by SSR primer WMC 331 on a Li-Cor 4200 DNA analyzer. DNA samples from left to right are: 13 individuals of the RILs (1–13), cultivars Annong 8455 (14), CS-SM3DS7A (15), Chinese Spring (16) and Sumai 3 (17).

As a result, three QTLs for Al resistance were identified in the population.

Studies on Al resistance using a set of aneuploid lines of Chinese Spring indicated that genes controlling Al resistance were located on a number of different chromosome arms. Aniol and Gustafson (1984) located the genes for Al resistance on six chromosomes (6AL, 7AS, 2DL, 3DL, 4DL and 4BL) by testing 25 ditelosomic lines of Chinese Spring. Further work indicated that three factors controlling Al resistance in Chinese Spring might be on chromosome 5AS, 2DL and 4DL when plants were tested with different Al concentrations (Aniol, 1990). Papernik et al. (2001) confirmed that three chromosome arms, 4DL, 5AS and 7AS, possessed gene(s) for Al resistance when 27 ditelosomic lines of Chinese Spring were evaluated for root re-growth in the presence of Al. Ditelosomic line DT2DS was not tested in that experiment due to lack of enough seeds. In this experiment, 31 ditelosomic lines of Chinese Spring were tested. Among them, lines DT3AS, DT6AS, DT2BL, DT3BS and DT1DS have not been tested previously. Lines DT4DS, DT5AL, DT2DS and DT7AL showed a significant decrease in root re-growth after Al stress in this experiment, indicating that the genes conditioning Al resistance in Chinese Spring might be on these missing arms of these lines. This result confirmed the previous reports on Al resistance of the ditelosomic lines of Chinese Spring (Aniol 1990; Aniol and Gustafson 1984; Papernik et al. 2001). In addition, ditelosomic lines DT1BS, DT7AS and DT6DL also

reduced root growth in the presence of Al, but they were not statistically significant.

To determine individual QTL effects of all these chromosome arms on Al resistance, genome mapping with molecular markers has been used to determine the number and location of these QTLs in this study. Three regions on three different chromosomes showed effects on root growth under Al stress. The *Qalt.pser-4D*, *Qalt.pser-5A*, and *Qalt.pser-2D* were located on chromosome 4DL, 5AS, and 2DL, respectively. The QTL mapping result is in agreement with the result from evaluation of ditelosomic lines.

The QTL most strongly associated with Al resistance was on chromosome 4DL, and flanked by markers *Xcfd23* and *Xwmc331*, the allele comparison showed that the Al resistance was from Chinese Spring. The QTL on chromosome 4DL was also identified in several previous reports. Riede and Anderson (1996) identified an RFLP marker (*Xbcd1230*) tightly linked to the Alt_{BH} gene on chromosome 4DL in wheat BH1146. Further studies confined Alt_{BH} to a 5.9-cM interval between markers *Xgdm125* and *Xpsr914* (Rodriguez-Milla and Gustafson, 2001). We also mapped a locus on the same region (flanked by markers *Xwmc331* and *Xgdm125*) of Atlas 66 by using RILs from the cross of Atlas 66/Century (Ma et al., 2005). Because both BH1146 and Atlas 66 shared a common progenitor Polyssu, a Brazilian wheat with a high level of Al resistance (Foy et al., 1965), Al resistance in BH 1146 and Atlas 66 may be the same locus. Using disomic substitution lines with the D genome of Chinese Spring individually substituted by their homoeologues from *Triticum turgidum* L. cv. Langdon, Luo and Dvorak (1996) identified the *Alt2* gene for Al resistance (flanked by RFLP markers *Xps914* and *Xpsr1051*), also on chromosome 4DL of Chinese Spring. Further study indicated that Alt_{BH} and *Alt2* might be in the same chromosome region (Rodriguez-Milla and Gustafson, 2001). The result in this study further supports that a locus on 4DL of Chinese Spring is most likely located on the same region as in Atlas 66 and BH 1146, because the QTL from the Atlas 66 and BH 1146 links to the SSR markers *Xgdm125* and *Xwmc331*, and *Xwmc331* is also the closest marker to *Qalt.pser-4D* in Chinese Spring. However, they are most likely different alleles, as the marker *Xwmc331* shows different sizes of the band between Atlas 66 and Chinese Spring. In addition, *Qalt.pser-CS4D* in Chinese Spring has a much smaller effect than that in Atlas 66 and also expresses as a different allele of the *ALMT1* gene, which encodes Al-induced malate transporter (Sasaki et al., 2004). This 4DL locus has been reported as a conserved genomic region for Al resistance across different species such as rye (*Secale cereale* L.) and barley (*Hordeum vugare* L.) (Miftahudin et al. 2002; Tang et al. 2000).

In addition to the 4DL locus, genes for Al resistance in other wheat chromosome regions have never been confirmed by molecular marker mapping (Kochian et al., 2004; Samac and Tesfaye, 2003). In this study, two minor QTLs were identified to associate with Al resistance in Chinese Spring. The *Qalt.pser-CS5A* was located on chromosome 5AS, which coincides with the results from evaluation of ditelosomic lines in this study and by Aniol (1990) and Papernik et al. (2001). Another putative QTL, *Qalt.pser-2D*, was detected on chromosome 2DL, with a marginally significant LOD value. The chromosome 2DL was also identified as a chromosome arm associated with Al resistance in the present and previous studies on ditelosomic lines of Chinese Spring for Al resistance (Aniol, 1990; Aniol and Gustafson, 1984). Thus chromosome arm 5AS and 2DL of Chinese Spring most likely contain minor QTLs for Al resistance.

DT7AL also showed a highly significant reduction in root growth, but the QTL was not significant from QTL mapping. The results could be due to that 7A chromosome of the Al resistant parent was substituted by the same chromosome of Sumai 3 in this experiment, so effect of chromosome 7A of Chinese Spring on Al resistance could not be determined by molecular mapping in the study, though ditelosomic line DT7AL had significantly slower root regrowth than that for Chinese Spring under Al stress.

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ditelosomic lines. This paper reports the results of research only. 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 U.S. Department of Agriculture. This is contribution No. 05-247-5 from the Kansas Agricultural Experiment Station, Manhattan, KS, USA.

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