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ORIGINAL PAPER

Diverse origins of aluminum-resistance sources in wheat

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Abstract Aluminum (Al) toxicity is a major constraint for wheat production in acidic soils. Wheat producers now routinely use Al-resistant cultivars as one cost-effective means to reduce risks associated with acidic soils. To date, diverse Al-resistant materials have been identified, but their genetic relationship has not been well characterized. A total of 57 wheat accessions, including the majority of the parents of Al-resistant accessions we identified in a previous study, were evaluated for Al resistance and analyzed with 49 simple sequence repeat (SSR) markers and 4 markers for Al-activated malate transporter (*ALMT1*). Pedigree and principle coordinate analysis (PCA) both separated Al-resistant accessions into four groups labeled according to common ancestry or geographical origin: US-Fultz, Polyssu, Mexican and Chinese. Al resistance in the four groups may have three independent origins given their distinct geographic origins and gene pools. Fultz originated in the USA as a major ancestor to soft red winter wheat, Polyssu originated in Brazil as a major source of Al resistance used in most genetic studies worldwide, and the Chinese group originated in China. Based on *ALMT1* marker haplotypes, the Al resistance in the Polyssu and Mexico groups was likely derived from Polyssu, while most Al-resistant cultivars developed in the USA most likely inherited most of Al resistance from Fultz. Fultz was released about 50 years earlier than Polyssu. Norin 10 likely played a pivotal role in passing Al-resistant gene(s) from Fultz to better adapted, semi-dwarf wheat cultivars developed in the USA. Further characterization of Al resistance in the three different sources could reveal multiple Al-resistant mechanisms in wheat.

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Introduction

Aluminum (Al) toxicity is a major constraint for crop production in the acidic soils, which occupy about 30–40% of world arable lands (von Uexküll and Mutert 1995). When soil pH is lower than 5, Al^{3+} is the dominant form of Al in soil solution. It affects plant growth by inhibiting root cell division and elongation of plant root tips, subsequently reducing nutrient and water uptake essential for plant growth (Samac and Tesfaye 2003). Direct application of lime to acidic soils may increase soil pH to reduce Al toxicity, but the cost of material and transportation has prompted producers to consider alternative solutions. Fortunately, significant genetic variation in Al resistance has been found among wheat cultivars and landraces (Aniol and Gustafson 1984; Stodart et al. 2007; Zhou et al. 2007a). Growing Al-resistant cultivars has been considered as a

cost-effective means to improve wheat production in acid soils.

To date, several sources of Al resistance have been identified in wheat. Most well known is the Al-resistant germplasm from Brazil, where acid soils are the predominant soil environment for wheat production. The spring wheat line, BH 1146 (Ponta Grossa 1//Fronteira/Mentana) from Brazil, and the winter wheat cultivar Atlas 66 (Frondoso//Redhart 3/Noll 28) from the USA, have been extensively used to study the genetic mechanisms of Al resistance (Riede and Anderson 1996; Tang et al. 2002; Ma et al. 2005). Atlas 66 and BH 1146 share a common progenitor, Polyssu, in their pedigrees, as Frondoso was a progeny of Polyssu and Ponta Grossa 1 was a selection from Polyssu. Near-isogenic lines were developed from Polyssu's derivatives and have been used for cloning the Al-activated malate transporter gene (*ALMT1*, Sasaki et al. 2004) and for gene expression profiling (Guo et al. 2006).

Both monogenic and multigenic inheritance of Al resistance have been reported in wheat (Basu et al. 1997; Somers et al. 1996; Ma et al. 2005; Raman et al. 2005; Zhou et al. 2007b; Cai et al. 2008). A major locus for Al resistance has been located on the long arm of chromosome 4D (4DL) in several wheat populations (Luo and Dvorak 1996; Riede and Anderson 1996; Rodriguez-Milla and Gustafson 2001; Raman et al. 2005; 2006; Zhou et al. 2007a; Cai et al. 2008). Markers linked to the major QTL on 4DL have been identified (Ma et al. 2005; Raman et al. 2005). Malate release has been considered as a major mechanism of Al resistance in wheat (Sasaki et al. 2004; Snowden and Gardner 1993). *ALMT1* has been isolated and associated with Al resistance in several species (Hoekenga et al. 2006; Magalhaes 2006; Sasaki et al. 2006; Fontecha et al. 2007). The gene markers for exon four (Sasaki et al. 2004), intron three (Raman et al. 2006) and the promoter region (Sasaki et al. 2006) of *ALMT1* have been developed and mapped in the 4DL QTL region (Ma et al. 2005; Raman et al. 2005). Therefore, *ALMT1* is likely the crucial contributor to the QTL for Al resistance on chromosome 4DL of wheat. By comparing allele diversity of the gene markers of *ALMT1* among 179 common wheat cultivars, Raman et al. (2008) concluded that no single marker was able to differentiate all Al-resistant cultivars from sensitive ones. Of the four markers tested, the promoter marker gave the best prediction of the Al resistance QTL among the 179 cultivars studied.

Based on co-linearity among the genomes of rice, wheat, barley, rye, and sorghum, Al resistance loci corresponding to Al resistance QTL on wheat 4DL were mapped in chromosome 3 in rice and 7RS in rye (Luo and Dvorak 1996; Riede and Anderson 1996; Devos and Gale 2000; Nguyen et al. 2002; 2003; Miftahudin et al. 2004; Matos et al. 2005; Raman et al. 2005). *ALMT1* or *ALMT1*-like genes have also been isolated from several other species (Hoekenga et al. 2006; Magalhaes 2006; Fontecha et al. 2007). More recently, a gene for Al-activated citrate secretion has been isolated and associated with Al resistance in barley and sorghum (Furukawa et al. 2007; Magalhaes et al. 2007; Wang et al. 2007). In addition, cysteine synthase was reported to play a key role in Al response in rice (Yang et al. 2007). Therefore, malate release is a major mechanism for Al resistance, but other mechanisms such as citrate release also play an important role in Al resistance in those crops (Kochian et al. 2005, Magalhaes et al. 2007).

Besides the QTL on 4DL of wheat, several additional QTLs were discovered from different germplasm. Berzonsky (1992) reported that the Al resistance in Atlas 66 was controlled by several genes on the D and other genomes. Ma et al. (2006) identified two additional minor QTLs for Al resistance on chromosome 5AS and 2DL of Chinese Spring. Zhou et al. (2007a, b) reported an additional QTL with a minor effect on Al resistance on chromosome 3BL of Atlas 66. More recently, Cai et al. (2008) discovered that the 3BL QTL in FSW also showed a major effect on Al resistance.

Utilizing new sources of Al resistance can improve wheat resistance to Al stress and, consequently, increase wheat yields in acidic soils without additional inputs. Based on the diversity of *ALMT1* gene markers in a world collection of wheat cultivars, Raman et al. (2008) suggested that Al resistance in modern wheat germplasm was derived from several independent sources. After screening 590 wheat accessions from the USA and several other countries, we found 88 wheat accessions with at least moderate resistance to Al toxicity (Zhou et al. 2007a). Many selected accessions with similar Al resistance to Atlas 66 were derivatives of Jagger (Sears et al. 1997). However, Jagger does not have any discernible connection with Brazilian germplasm in its pedigree. Further investigation of the origin of Al resistance in Jagger and other cultivars may provide useful information for understanding the evolution of Al-resistance genes and for identifying new sources of resistance for breeding applications. The objective of this study was to identify the genetic relationships among Al resistant sources used in the US wheat breeding programs and those with Brazilian and Chinese origins.

Materials and methods

Plant materials

A total of 55 wheat accessions (Table 1) were obtained from the USDA National Small Grains Collection at

Aberdeen, Idaho, USA, and two Chinese landraces, FSW and Chinese Spring, were obtained from Jiangsu Academy of Agricultural Sciences, Nanjing, China. Accessions represented major sources of Al resistance from 11 countries identified in wheat to date (Table 1). All accessions were parents or ancestors of Al-resistant germplasm lines identified in a previous study, except for the two Chinese Al-resistant landraces. Atlas 66 was used as a resistant control, and Century and Danby were used as susceptible controls (Zhou et al. 2007a).

Evaluation of Al resistance

Aluminum resistance was evaluated by measuring relative root elongation and hematoxylin staining of root tips after 2 days of Al stress in a nutrient solution culture (Polle et al. 1978). Wheat seeds were placed on moist paper in a Petri dish at room temperature (22–25°C) overnight and transferred to a refrigerator (4°C) for an additional 2 days. Three germinated seeds per accessions with similar viability were transplanted onto a piece of nylon net at the bottom of a plastic cup with an open bottom. The cups with germinated seeds were supported by a plastic cup holder floated on deionized water at 22°C with 16 h of fluorescent light daily. For aeration, two bubble rods in the water were connected to an air pump. After 48 h, the deionized water was replaced with a nutrient solution (pH 4.0) containing 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 $AlK(SO_4)_2{\cdot}12H_2O$. For the control treatment, de-ionized water was replaced with the same nutrient solution without $AlK(SO_4)_2{\cdot}12H_2O$.

The length of the principal root of each seedling was measured both before and after seedlings were placed in the Al-containing nutrient solution for 48 h. Root elongation during this period was called net root growth (NRG). Root elongation in the control treatment [0 mM $AlK(SO4)_2{\cdot}12H_2O$, 48 h] was called control root growth (CRG). Root-resistance index (RRI) for each line was calculated as NRG/CRG. After root length was measured, the roots were rinsed to remove excess Al^{3+} using deionized water for 1 h with two to three water replacements. Rinsed roots were submerged in a hematoxylin solution containing 0.2% hematoxylin (w/v) and 0.02% (w/v) KIO_3 for 15 min and rinsed three to four times with de-ionized water. Hematoxylin staining score (HSS) for each line was visually scored as three grades, with 1 = no stain on root tips, 2 = light stain, and 3 = heavy stain. The experiment was repeated once with three replicates (cups) of each accession per experiment using a randomized complete-block design.

Marker and data analysis

After hematoxylin staining, leaf tissue from the first experiment was harvested in a 1.5-mL tube and dried for 2 days in a freeze drier for DNA isolation (Ma et al. 2005). The tubes with dried tissue were shaken for 3 min at 30 times per sec using a Mixer Mill (Retsch GmbH, Haan, Germany) with a 3.2 mm stainless bead in each tube. SSR PCR amplification and PCR fragment analysis were conducted in a Li-Cor 4300 DNA analyzer (Ma et al. 2005). CAP, SSRs and up-stream promoter markers of the *ALMT1* gene were analyzed as described by Raman et al. (2006, 2008) and Sasaki et al. (2006).

A total of 49 pairs of SSR primers were selected to analyze 57 accessions. Primers included 17 WMC primers (Somers et al. 2004), 14 GMW primers (Roder et al. 1998), 11 BARC primers (Song et al. 2005), 5 CFD primers and one CFA primer (Guyomarc'h et al. 2002; Sourdille et al. 2003), and one GDM primer (Pestsova et al. 2000). This set of primers accounted for all 21 chromosomes, including 13 primers from genome A, 18 from genome B and 18 from genome D (Supplemental Table 1).

Individual marker alleles were scored as present (1) or absent (0) using Saga^{TM} Software (Version 3.3, Li-Cor Inc., Lincoln, NE, USA). All data were re-checked visually, and ambiguous data were eliminated. Similarities for paired accessions were calculated using the SIMQUAL module of NTSYSpc software (version 2.0, Rohlf 1998). A principal coordinate analysis (PCA) was performed using the DCENTER module of the NTSYSpc program. Polymorphic information content (PIC) was calculated according to Anderson et al. (1993), assuming homozygosity for all wheat accessions.

Results

Sensitivity of wheat accessions to Al stress

Absolute sensitivity of wheat roots to Al stress was measured by NRG, or the amount of root growth during the Al stress treatment. Mean NRG was 1.31 cm for all accessions and varied widely from 0.36 to 3.33 cm (Table 1). The duration (48 h) and intensity (0.36 mM Al^{3+}) of the Al treatment impeded root growth in some accessions but did not substantially affect root growth in others. The relative resistance index (RRI) averaged 33.4% and varied from 8 ± 6 to 90 ± 2% (Table 1). Variance analysis indicated that NRG, RRI and HSS differed significantly ($P < 0.01$) among accessions. HSS showed significant negative correlations with both NRG ($r = -0.89$, $P < 0.01$) and RRI ($r = -0.57$, $P < 0.01$).

Table 1 Pedigree, year of release, aluminum (Al) resistance score, *ALMT1* and SSR markers, and geographic origin of 57 wheat accessions

No.[a]	Name	Pedigree	Year of release	Origin	Group[b]	HSS[c]	NRG[c]	RRI[c]	*Xups4*[d]	*Xssr3a*[e]	*Xssr3b*[f]	*ALMT1-CAP*[g]	*Xwmc 331*[h]	*Xbarc 96*[h]	*Xbarc 159*[h]
12	Fronteira	Polyssu/Alfredo Chaves 6–21	1932	Brazil	1	1	3.02	0.90	1,014	233	1	1	1	1	1
25	P.G.1	Selection from Polyssu	1924	Brazil	1	1	1.99	0.45	849	221	0	0	0	1	1
26	Frontana	Fronteira/Mentana	1940	Brazil	1	1	3.33	0.57	1,014	233	1	1	1	0	1
55	Atlas 66	Frondoso//Redhart 3/Noll 28	1948	USA	1	1.15	2.17	0.45	1,014	233	1	1	1	1	1
28	Carazinho	Colonista/Frontana	1957	Brazil	1	2	1.84	0.36	1014	233	1	1	1	1	0
29	Penjamo 62	Frontana/Kenya 58//Newthatch/3/ Norin 10/Brevo	1962	Mexico	1	2.6	0.85	0.18	439	232	1	1	0	1	1
30	Tezanos Pintos Precoz	Frontana//Thatcher/Sinvalocho	1969	Argentina	1	2.65	0.80	0.20	440	223	0	0	0	1	1
14	Mentana	Rieti/Wilhelmina//Akagomughi	1945	Italy	1	2.85	0.53	0.18	471	222	0	0	0	1	0
41	Marquis	Hard Red Calcutta/Red Fife	1913	Canada	1	3	0.39	0.23	440	227	0	0	0	1	0
23	Yecora Rojo	Ciano 67//Sonora 64/Klein Rendidor/3/II-8156	1975	Mexico	2a	1	2.73	0.84	1,014	233	1	1	1	1	1
35	Pavon F76	Vicam//Ciano sib/Siete Cerros 66/ 3/Kalyansona/Bluebird	1976	Mexico	2a	1	2.09	0.55	1,014	233	1	1	1	1	1
46	Ciano F67	Pitic 62/Chris sib//Sonora 64	1967	Mexico	2a	1	2.59	0.34	1,014	233	1	1	1	1	1
48	Sonora 64	Yaktana 54//Norin 10/Brevor/3/2* Lerma Rojo 54	1964	Mexico	2a	1	2.04	0.32	1,014	233	1	1	1	0	1
49	Sonalika	II53-388/Andes//Pitic 62 sib/3/ Lerma Rojo 64	1967	India	2a	1.15	2.65	0.47	440/ 1,014	233	1	1	1	1	1
50	Bluebird'S'	Ciano sib//Sonora 64/Klein Rendidor/3/8156	1987	Mexico	2a	1.15	2.46	0.51	440/ 1,014	233	1	1	1	1	1
47	Pitic 62	Yaktana 54//Norin 10/Brevor 26-1C	1962	Mexico	2a	1.65	1.86	0.41	471	223/227/ 233	1	1	1	1	1
43	Andes	Kentana 48/Frontana//Mayo 48	1962	Colombia	2a	1.75	1.84	0.29	440	221	1	1	1	0	0
45	Lerma Rojo 64	Yaqui 50//Norin 10/Brevor/3/ Lerma 52/4/2* Lerma Rojo	1964	Mexico	2a	2.25	1.44	0.26	471	221	0	0	0	1	1
42	Kentana 48	Kenya C9906/Mentana	1948	Mexico	2a	2.85	0.54	0.30	439	221/232	1	1	0	0	0
1	Fultz	A selection from Lancaster	1871	USA	2b	1	1.91	0.38	1,014	222	1	0	1	0	0
7	Fulhio	A selection from Fultz	1920	USA	2b	1	1.82	0.39	849	222	0	0	1	0	0
15	Seneca	Portage/Fulcaster	1950	USA	2b	1	1.82	0.58	439	238	1	1	0	1	0
27	Fulcaster	Fultz/Lancaster	1886	USA	2b	1	2.27	0.70	439	234	1	1	0	0	0
52	Jagger	KS82W418/Stephens	1994	USA	2b	1.15	2.29	0.39	440/ 1,014	234	1	1	1	0	0
10	Thorne	Portage/Fulcaster	1937	USA	2b	1.35	2.24	0.49	439	237	1	1	0	1	0
9	Pawnee	Kawvale/Tenmarq	1942	USA	2b	1.5	0.73	0.20	440	231	0	0	0	1	0

Table 1 continued

No.[a]	Name	Pedigree	Year of release	Origin	Group[b]	HSS[c]	NRG[c]	RRI[c]	*Xups4*[d]	*Xssr3a*[e]	*Xssr3b*[f]	*ALMT1-CAP*[g]	*Xwmc 331*[h]	*Xbarc 96*[h]	*Xbarc 159*[h]
16	Norin 10	Daruma/Fultz//Turkey Red	1935	Japan	2b	1.65	1.92	0.47	471	223/227	0	0	0	1	0
33	Cody	Warrior*5/Agent//Centurk 78	1986	USA	2b	1.75	1.02	0.27	1,014	222	0	0	0	1	0
54	Stephens	Nord Desprez/CI 13438	1977	USA	2b	2	1.62	0.30	440	223	0	0	0	0	0
21	Centurk	Kenya 58//Newthatch/3/Hope/2*Turkey/4/Cheyenne/5/Parker	1971	USA	2b	2.15	1.19	0.29	440	223/232	1	1	0	1	0
31	Kalyansona	Frontana//Kenya 58/Newthatch/3/Norin 10/Brevor/4/Gabo 55	1967	India	2b	2.15	1.13	0.19	439	232	1	1	0	0	0
51	Century	Payne//TAM W-101/Amigo	1986	USA	2b	2.15	0.67	0.10	471	227	1	0	0	0	0
17	Norin 10/Brevor 14	Norin 10/Brevor	1955	USA	2b	2.35	0.93	0.24	471	223/228	0	0	0	0	0
8	Redhart	A selection from Red May	1921	USA	2b	2.5	1.20	0.60	439	237	1	1	0	1	0
11	Fairfield	Purkof/Fulhio	1942	USA	2b	2.65	0.91	0.20	439	233/239	1	1	0	0	0
44	Riley	Monon sib/8/Knox/7/Kawvale/5/Fultz/Hungarian//Illinois No. 1 (W38)/3/Wabash/4/Fairfield/6/3* Trumbull//Hope/Hussar	1961	USA	2b	2.75	1.07	0.28	471	225	0	0	0	0	0
4	Wilhelmina	Squarehead/Zeeuwsche//Squarehead	1932	Netherlands	2b	2.85	0.62	0.22	471	224	0	0	0	0	0
6	Squarehead	Unknown	1920	Netherlands	2b	2.85	0.46	0.16	440	223	0	0	0	0	0
20	Siete Cerros 66	Penjamo62/Gabo55	1966	Mexico	2b	2.85	0.46	0.88	439	232	1	1	0	0	0
22	TAM W-101	Norin10/3/Nebraska60//Mediterranean/Hope/4/Bison	1971	USA	2b	2.85	0.93	0.67	471	223	0	0	0	1	0
24	Hart	Etoile de Choisy//Thorne/Clarkan/3/Pawnee/CI12454	1976	USA	2b	2.85	0.58	0.18	440	224	0	0	0	1	0
34	Collin	Agent/Tascosa//Sturdy	1986	USA	2b	2.85	0.61	0.17	471	229/223	1	0	0	0	0
36	2137	W2440/W9488A//2163	N/A[b]	USA	2b	2.85	0.49	0.10	440	221	0	0	1	1	1
37	Turkey Red	Unknown	1991	USA	2b	2.85	0.45	0.08	440	222	0	0	0	0	0
38	TAM 300	TAM 106/Collin	1993	USA	2b	2.85	0.45	0.09	440	224	0	0	0	0	0
2	Silversheaf	American Bronze//Lancaster/Longberry 91	1903	USA	2b	3	0.45	0.11	440	225	0	0	0	0	0
3	Dawson	A selection from Seneca	1893	Canada	2b	3	0.44	0.12	440	227	0	0	1	0	0
5	Triumph	Kanred/Blackhull//Quality/3/Kanred/Blackhull	1940	USA	2b	3	0.51	0.35	439/471	224/232	1	1	0	0	0

Table 1 continued

No.[a]	Name	Pedigree	Year of release	Origin	Group[b]	HSS[c]	NRG[c]	RRI[c]	*Xups4*[d]	*Xssr3a*[e]	*Xssr3b*[f]	*ALMT1-CAP*[g]	*Xwmc 331*[h]	*Xbarc 96*[h]	*Xbarc 159*[h]
13	Brevor	CI 11912/4/Oro/Turkey Red/ Florence/3/Oro//Fortyfold/ Federation	1949	USA	2b	3	0.55	0.18	440	223	0	0	0	0	0
18	Agent	Triumph/KS464708	1967	USA	2b	3	0.36	0.12	471	223	0	0	0	0	0
19	Sturdy	Sinvalocho/Wichita//Hope/ Cheyenne/3/2*Wichita/4/Seu Seun 27	1966	USA	2b	3	0.64	0.21	471	229	0	0	0	0	0
32	Colt	Agate sib (NE69441)//391-56-D8/ Kaw (Tx65A1503-1)	1983	USA	2b	3	0.56	0.24	471	225	0	0	0	0	1
40	Rieti	Landrace	1900	Italy	2b	3	0.44	0.10	–	224	0	1	0	0	0
53	Danby	Trego/Jagger sib	2006	USA	2b	3	0.69	0.15	439	232	1	1	0	0	0
56	FSW	Landrace	N/A	China	3	1	1.57	0.22	471	226	0	0	0	1	0
57	Chinese Spring	Landrace	N/A	China	3	1.25	2.06	0.49	440	221	0	0	0	1	0
39	Transfer	Chinese/*Aegilops umbellulata*	1955	USA	3	1.85	1.74	0.34	440	221	0	0	0	1	0

[a] Original entry number

[b] Group number from principal coordinate analysis in Fig. 2: *1* Polyssu group, *2a* Mexico-Fultz subgroup, *2b* US-Fultz group and *3* Chinese group

[c] Al resistance scores: *HSS* hematoxylin staining score ($LSD_{0.05}$ = 0.16, $LSD_{0.01}$ = 0.21), *NRG* net root growth in cm ($LSD_{0.05}$ = 0.18, $LSD_{0.01}$ = 0.24) and *RRI* relative root index in % ($LSD_{0.05}$ = 0.05, $LSD_{0.01}$ = 0.06). All values are means over six experiments

[d] *Xups4* (a upstream promoter marker) from *ALMT 1* (Sasaki et al. 2006) on chromosome 4DL showed four fragments in different germplasm and only 849 bp and 1,014 bp fragments appeared to be correlated with Al tolerance

[e] *Xssr3a* from *ALMT 1* on chromosome 4DL (Raman et al. 2006) showed 11 fragments in different wheat accessions and only the 233 bp fragment that was putatively associated with Al resistance. – missing data

[f] *ssr3b* of *ALMT 1* on chromosome 4DL (Raman et al. 2006) is a dominant marker and the 171 bp fragment was associated with Al resistance

[g] *CAP* marker from *ALMT1* on chromosome 4DL (Sasaki et al. 2004)

[h] *Xwmc 331 Xbarc96* and *Xbarc15*9 are SSR markers from chromosome 4DL, 2D and 6D, respectively

Genetic relationship among Al-resistant accessions

The majority of tested materials were parents of Al-resistant lines selected in a previous study (Zhou et al. 2007a), including both Al-resistant and susceptible accessions. Among the 57 accessions evaluated, 24 were considered Al resistant based on NRG (>1.5 cm) and HSS (<2). According to their pedigrees (Table 1; Fig 1), resistant accessions originated mainly from three sources: Fultz from the USA, Polyssu from Brazil, and an unknown source from China. Most of the resistant accessions, including Fulcaster, Fulhio, Thorne, Seneca, Norin 10, Norin 10/Brevor 14, Pictic 62, Sonora 64, and Jagger can be traced to Fultz, a selection from the US landrace, Lancaster (Fig. 1). Five Al-resistant accessions, including Atlas 66, P.G.1, Fronteira, Frontana and Carazino, inherited Al resistance from Polyssu (Fig. 1). Three accessions, FSW, Transfer, and Chinese Spring, carried resistance gene(s) from Chinese sources. Transfer was derived from the cross of Chinese/*Aegilops umbellulata* (Table 1). The pedigrees of Al-resistant cultivars Bluebird, Yecoro Rojo, Pavon F76, Sonalika, and Ciano F67 from Mexico can be traced back to both Fultz and Polyssu.

The 57 accessions also were analyzed with 49 selected SSR primers covering 21 wheat chromosomes (Supplemental Table 1). A total of 441 SSR alleles were scored, and an average of 8.8 alleles per marker was detected, ranging from 1 to 19 per SSR primer pair. Polymorphism information content (PIC) was high for the selected set of primers, and had an average of 0.72.

Principle coordinate analysis (PCA) classified the 57 accessions into four major groups (Fig. 2). Resistant accessions were distributed in all groups. The first principle coordinate (PC1) separated the Polyssu group from the Chinese group and US-Fultz group from the Mexican group. The second principle coordinate (PC2) separated the Mexican group from the Polyssu and the US-Fultz group from the Chinese group (Fig. 2). Most Mexican accessions have a pedigree of both Polyssu and Fultz. Fultz-related Al-resistant accessions were widely scattered on the PC1 axis, reflecting that Al-resistant Fultz and its derivatives pervade numerous US and CIMMYT wheat breeding programs.

Variation in *ALMT1* and markers associated with Al resistance

The 57 accessions also were analyzed with four markers developed from the *ALMT1* gene (Table 1) and compared with the marker pattern of Polyssu. Among 24 resistant or moderately resistant accessions, 9 accessions showed the same haplotype as Polyssu for the 4 *ALMT1* markers; 2 accessions each showed 3, 2 and 1 markers as Polyssu, respectively; and 3 Chinese resistant accessions, Norin 10 and Stephens showed completely different haplotype from Polyssu for the 4 *ALMT1* markers. Ten susceptible accessions showed 2 markers and 2 susceptible accessions showed

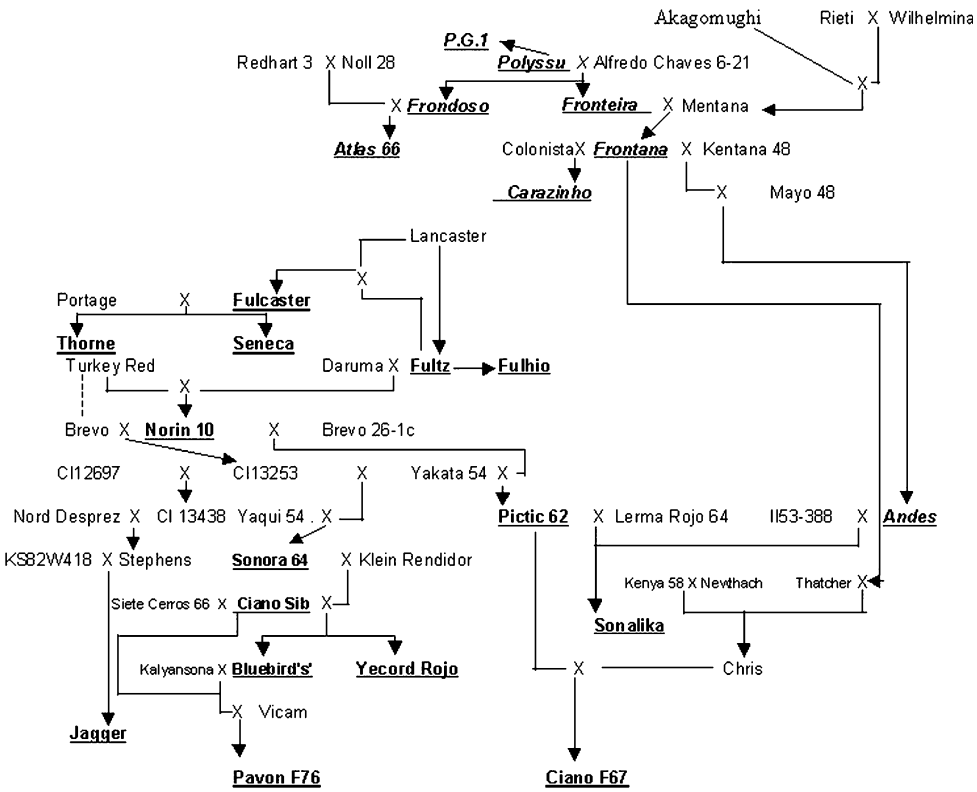

Fig. 1 Pedigrees of the Al-resistant wheat accessions used in this study. *Solid arrow* indicates the accession was derived directly from the cross or directly from the cultivar by pure-line selection; "*X*" indicates crossing between two parents; *dashed arrow* indicates the accession was made after several rounds of crosses involving several different parents. Al-resistant accessions with Fultz pedigree are indicated by *underlined bold characters*; Accessions with Al resistance from Polyssu are indicated by *underlined italic characters*; Al-resistant accessions with Fultz and Polyssu pedigree are indicated by *bold characters*

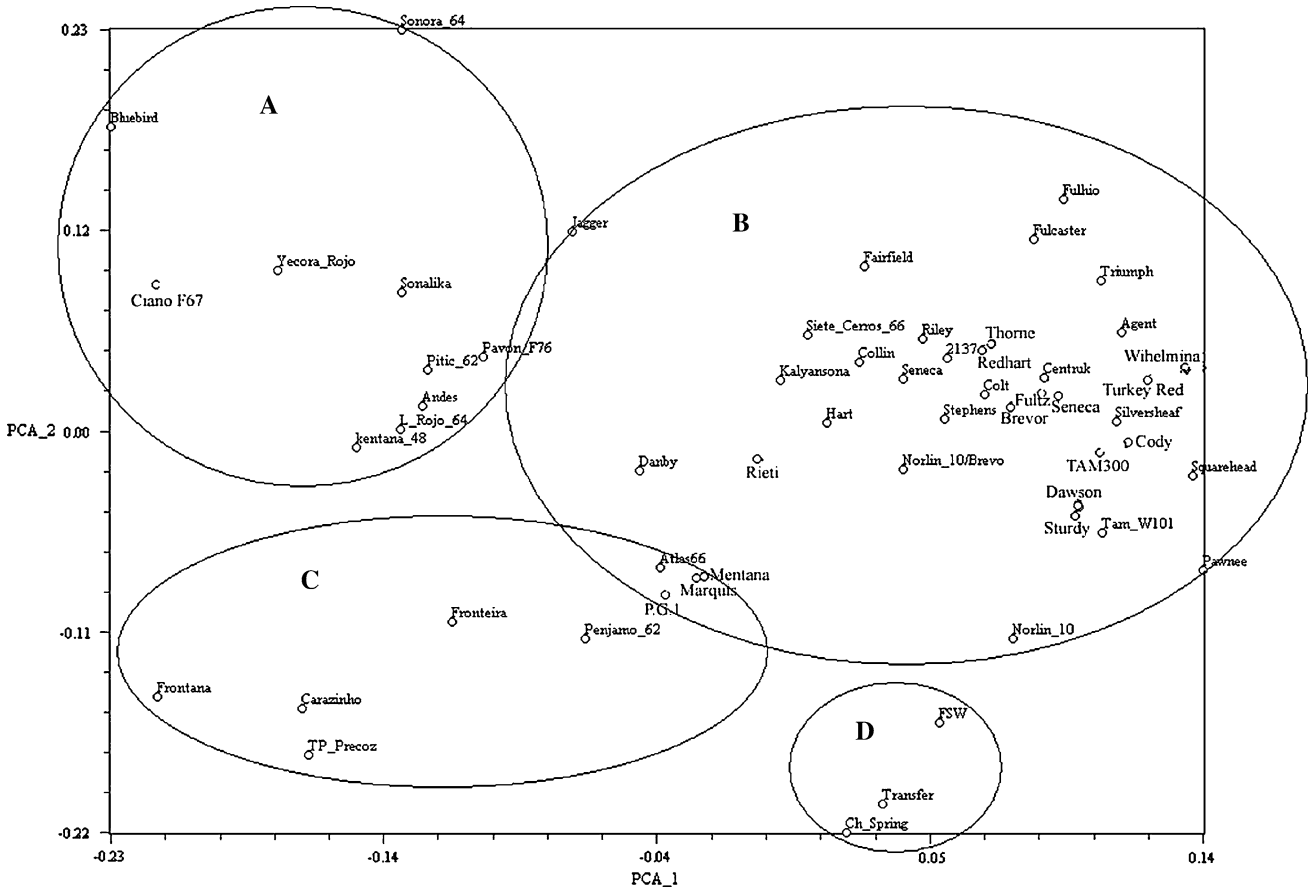

Fig. 2 Principal coordinate map for the first and second coordinates (PCA1, PCA2) estimated from 441 SSR markers alleles and *ALMT1* marker alleles by means of the genetic similarity matrix for 57 accessions. A = Mexican group, B = US-Fultz group, C = Polyssu group, D = Chinese group

1 marker as seen in Polyssu. For the *ALTM1*-CAP, 26 accessions showed the *ALMT1-1* marker as seen in Polyssu. Although a significant correlation between the CAP marker and RRG was observed (Table 2), some highly resistant accessions such as FSW, Fultz and Fulhio showed *ALMT1-2* sequence that associated with Al susceptibility while some highly susceptible accessions such as Danby, Rieti and Triumph showed *ALMT1-1* as seen in Polyssu. *Xssr3a* and *Xssr3b* were from intron 3 region of *ALMT1*. SSR3a amplified 11 fragments from 221 bp (Chinese Spring) to 238 bp (Seneca), but only the 233-bp fragment showed significant association with Al resistance (Tables 1, 2). The fragments of 224 and 225 bp appeared to be associated with Al susceptibility. SSR3b is a dominant marker. The 171-bp fragment was in 28 accessions and significantly correlated with Al resistance. Primer set 4 upstream of the *ALMT1* coding region (Ups4) amplified 5 fragments (439, 440, 471, 849 and 1,014 bp). Among them, 849 and 1,014 bp fragments correlated with Al resistance while the 440 and 471 bp fragments were correlated with Al susceptibility (Table 1). Correlation coefficients between the 4 *ALMT1* markers and 3 Al tolerance scores were all significant across all accessions, however, highly significant correlations were only observed among accessions from both the Polyssu and Mexico-Fultz subgroup, except that between HSS and *Xups4* for US-Fultz group (Table 2).

Correlation analysis was also conducted between Al phenotypic data and the other SSRs. The results revealed three additional markers that showed significant associations with Al resistance (Table 2). Marker *Xwmc331* from chromosome 4DL showed 6 fragments in 57 accessions and the 151-bp fragment was significantly associated with Al resistance. In addition, *Xbarc96* and *Xbac159* also correlated with Al resistance in the 57 accessions, but *Xbarc96* appeared to correlate with Al resistance in the Chinese and US-Fultz groups while *Xbac159* appeared to correlate with Al resistance in Polyssu and Mexico-Fultz group (Table 2).

Discussion

Aluminum toxicity is a major constraint to wheat production in acidic soils across the world. Identification of potentially novel sources of Al resistance will provide genetically

Table 2 Correlation coefficients between markers and Al resistance

Traits	*Xups4*[a]	*Xssr3a*[b]	*Xssr3b*[c]	*ALMT1-CAP*[d]	*Xwmc331*[e]	*Xbarc 96*[e]	*Xbarc 159*[e]
US-Fultz group							
HSS	−0.52**	0.00	−0.28	−0.16	−0.28	−0.33*	0.20
NRG	0.44**	0.00	0.36*	0.30	0.26	0.26	−0.20
RRI	0.12	0.00	0.41*	0.44**	−0.03	0.27	−0.15
Polyssu and Mexico-Fultz groups							
HSS	−0.86**	−0.64**	−0.50*	−0.50*	−0.78**	0.00	−0.68**
NRG	0.82**	0.69**	0.58*	0.58*	0.83**	−0.04	0.60**
RRI	0.66**	0.53*	0.42	0.42	0.58*	0.13	0.46
All accessions							
HSS	−0.69**	−0.50**	−0.40**	−0.35**	−0.55**	−0.37**	−0.40**
NRG	0.71**	0.63**	0.48**	0.48**	0.63**	0.34**	0.49**
RRI	0.43**	0.40**	0.45**	0.47**	0.32*	0.33*	0.29*

*, ** Significant at 0.05 and 0.01 levels, respectively

[a] Correlation coefficients between 849 bp and 1014 bp fragments of *Xups4* (a upstream promoter marker) of *ALMT 1* (Sasaki et al. 2006) on chromosome 4DL and Al tolerance scores

[b] Correlation coefficients between the 233 bp fragment of *Xssr3a* of *ALMT 1* on chromosome 4DL (Raman et al. 2006) and Al resistance scores

[c] Correlation coefficients between 171 bp fragment of *Xssr3b* of *ALMT 1* on chromosome 4DL (Raman et al. 2006) and Al resistance scores

[d] *CAP* marker of *ALMT1* on chromosome 4DL (Sasaki et al. 2004)

[e] *Xwmc 331*, *Xbarc96* and *Xbarc159* are SSR markers from chromosome 4DL, 2D and 6D, respectively

diverse germplasm for further improving Al resistance in wheat. Significant genetic variation in Al resistance has been found among wheat cultivars and landraces (Aniol and Gustafson 1984; Zhou et al. 2007a; Stodart et al. 2007). However, to date, Al resistance has been extensively investigated in a limited gene pool, usually from the Brazilian source, such as ET8 and Atlas 66 (Kochian et al. 2005; Samac and Tesfaye 2003; Tang et al. 2002). However, Atlas 66 and other Al-resistant lines with Brazilian origin have many undesired agronomic traits and are difficult to use in contemporary breeding programs in the USA. To identify new sources of Al resistance, we have screened 590 wheat accessions for Al resistance, including elite wheat breeding lines from the USA and other American countries, landraces and some commercial cultivars from East Asia, and synthetic wheat lines from CIMMYT (Zhou et al. 2007a). From those, 88 wheat accessions were identified with a high or moderate level of Al resistance. These accessions included some popular wheat cultivars currently grown in the southern Great Plains of the USA, such as Jagger and Endurance, which both have good Al resistance and adaptation to US wheat production. Pedigree analysis could not identify any lineage of Brazilian origin in these cultivars. Therefore, parental lines for most of these Al-resistant accessions were collected for the current study to determine possible sources of Al resistance in the newly identified Al-resistant accessions.

Pedigrees of the collected parental materials separated the Al-resistant accessions into four groups according to principal ancestor or geographical origin (Table 1; Fig. 1): US-Fultz group, Polyssu group, Mexican group, and Chinese group. Genetic relatedness could not be established between the groups on the basis of pedigree information except for Mexican group that can be linked to both US-Fultz and Polyssu groups. Furthermore, Al-resistance genes in these wheat accessions might have originated independently from three different geographic areas: Fultz from the USA, Polyssu from Brazil, and FSW from China.

To investigate the genetic relationships among accessions, genotypes of the 57 accessions were determined using 49 SSR primers from 21 wheat chromosomes. On the basis of 441 scored SSR alleles and 4 ALMT1 markers, PCA divided the wheat accessions into 4 major groups that corresponded to the groups indicated by pedigree analysis (Fig. 2). The Polyssu group consisted of nine accessions with five Al-resistant accessions, Frontana, Carazinho, P.G.1, Fronteira, and Atlas 66. These accessions were derived from Polyssu by either direct selection or from cross with Polyssu's derivatives. An Al-resistance gene from Polyssu has been well studied. *ALMT1* was cloned (Sasaki et al. 2004) and mapped on the long arm of chromosome 4D (Ma et al. 2005). This QTL on 4DL contributes a high level of Al resistance in Brazilian Al-resistant germplasm (Ma et al. 2005; Zhou et al. 2007b). Several gene markers were developed for the gene (Sasaki et al. 2004, 2006; Raman et al. 2005, 2006). In this study, all the Al-resistant accessions from this group have the

same haplotype of *ALMT1* markers as in Polyssu, except P.G.1 that has only one common marker (*Xups4*) with Polyssu. Therefore, the Al resistance in these accessions was most likely inherited from Polyssu.

In the Chinese group, FSW and Chinese Spring are Chinese landraces, and Transfer was derived from a wide cross between a wheat accession called Chinese and *Aegilops umbellulata*. Therefore, they were grouped together. Chinese (PI 46797) was collected from England, but it is most likely the same accession as Chinese Spring currently used in the USA (Sears and Miller 1985). Genotyping data confirmed that Chinese and Chinese Spring were the same. In this study, all Chinese accessions showed completely different haplotype of four *ALMT1* markers from that of Atlas 66. Sasaki et al. (2006) also demonstrated that the promoter sequence of the *ALMT1* gene in Japanese wheat was different from those in cultivars with Brazilian origin. Al-resistance genes in Chinese and Japanese accessions may have originated independently from those of Brazilian accessions, which is consistent with a more recent report that Al resistance in modern wheat germplasm is derived from several independent sources (Raman et al. 2008). Further investigation of Al resistance in Asian germplasm may identify new genes for Al resistance.

The pedigrees of Al-resistant wheat accessions in US-Fultz groups can be traced back to Fultz (Fig. 1), which can be divided into two groups: Mexican and USA (Fig. 2). The US-Fultz group contained 35 accessions that are mainly US cultivars, with 7 exceptions, Norin 10 from Japan, 2 accessions from the Netherlands, and 1 each from Mexico, Italy, Canada, and India. Eight accessions showed Al resistance and were developed in the USA, except Norin 10. These Al-resistant cultivars were either derived by selection from Fultz (Fulhio) or from crosses with Fultz or its derivatives as a parent.

It was a general belief that Al-resistance genes in cultivars from the USA were mainly derived from Brazilian sources such as Polyssu (Garvin and Carver 2003). This study provided contradictory evidence that Fultz was the possible source of Al resistance for most Al-resistant cultivars developed in the USA. Fultz was selected by an American farmer-breeder, Abraham Fultz, in 1862 from the cultivar, Lancaster, in Pennsylvania, USA, whereas Polyssu was identified by the Brazilian breeder Polyssu in 1914 from a wheat field in southern Brazil. Fultz was released about 50 years earlier than Polyssu. Al resistance in some early US cultivars, such as Thorne and Fulcaster, was derived mainly from crosses with Fultz. Some contemporary Al-resistant cultivars, such as Jagger and Endurance from the USA inherited Al resistance from Fultz through Norin 10. Norin 10 was a Japanese cultivar that was reported to be Al resistant and had a high level of *ALMT1* expression (Sasaki et al. 2006). It was derived from a cross among American cultivars, Turkey Red and Fultz, and the Japanese landrace, Daruma. In our study, we observed that Norin 10 from the USDA Small Grains Collection was heterogeneous for Al resistance. Though its reputation as a donor of *Rht1* is undisputed, Norin 10 appears to have played another major role as a conduit for the serendipitous transfer of Al resistance from Fultz to modern semi-dwarf wheat cultivars in many US and Mexican breeding programs (Fig. 1). Because Norin 10 contains a Japanese landrace in its pedigree, and Japanese and Chinese landraces are closely related (Yu et al. 2006), it is not surprising that Norin 10 was located between the Chinese and US-Fultz groups in the PCA map. The appearance of Siete Ceros 66, a Mexican cultivar, in the US-Fultz subgroup may be due to the major contribution from one of its parents, Norin 10/Brevor. Several entries from the Netherlands, India, Canada and Italy were grouped in the USA subgroups perhaps because only a few entries were from these countries and they had genetic compositions similar to these entries from the USA.

The haplotypes for Al-resistance markers on 4DL showed different patterns between US-Fultz and Polyssu derived cultivars. For these Fultz derived Al-resistant accessions from the USA, no accessions had the same haplotype of the four *ALMT1* markers as that in Polyssu, and most accessions had only one or two markers that were the same as Polyssu in comparison with all four markers in most Polyssu derived cultivars. Gene expression experiment indicated that the US cultivars Jagger and Endurance had a high level of expression of *ALMT1* as that in Atlas 66, a Polyssu's derivative (data not shown). A high correlation was observed between Al resistance and *Xups4* and *SSRb* markers of *ALMT1* in two breeding populations with Endurance as the source of Al resistance. Therefore, the *ALMT1* on 4DL is also responsible for Al resistance in the Fultz group but with different sequence from that of Polyssu.

The Mexican group consisted of ten cultivars from Mexico. Seven of them were Al resistant. Although these resistant cultivars can all be traced back to Norin 10 (Figs. 1, 2), most of them also have a pedigree of Polyssu. *ALMT1* marker data indicated that most of resistant accessions shared the same haplotype as Polyssu. Therefore, the Al resistance in these Mexican accessions was mainly inherited from Polyssu, but we cannot rule out the possibility that Fultz was the possible source of Al resistance for some of the Mexican Al-resistant cultivars.

Jagger, in the Fultz group, demonstrated a high level of Al resistance and has been used as a parent in many hard winter wheat cultivars in the southern Great Plains of the USA. The Al resistance in Jagger is highly heritable and most likely inherited from Fultz, not Polyssu, based on pedigree information. However, we found that only one of its parents (Stephens) showed moderate

resistance to moderate susceptibility. This could be due to modifying gene(s) that increase susceptibility of the 4DL resistance gene in Stephens while different modifier gene(s) may enhance the resistance of 4DL gene of Jagger. Another possible explanation is parental heterogeneity at the Al-resistant locus. An Al-resistant parent could have been selected in the cross to develop Jagger. Heterogeneity for Al resistance is very common for many wheat cultivars or landraces because they were not originally selected for Al resistance during cultivar development. Therefore, when selecting a resistant cultivar for crossing, the resistance should be validated for individual plants.

Among nine accessions that have the same haplotype of the four *ALMT1* markers as in Polyssu, eight can be traced to Polyssu except Sonora 64. Bluebird's, Yecora Rojo and Pavon F76 have Ciano Sib in their pedigree, while Ciano Sib was derived from cross between Pictic 62 and Chris that has Frontana in its pedigree (Fig. 1). Most US-Fultz-related resistant accessions have two *ALMT1* markers of Polyssu with one accession each having one and three *ALMT1* markers of Polyssu. The four *ALMT1* markers in three Chinese accessions are all different from those in Polyssu. These results also provide evidence to support that these sources of resistance have different origins, and different sequences of the *ALMT1* gene. Therefore, none of the four *ALMT1* markers are perfect markers for Al resistance across all sources of germplasm. However, all the four markers showed a high association with Al resistance, thus different markers can be used for marker-assisted selection when different sources of resistance are used. In general, *Xups4* appears to be the best marker for 4DL Al resistance because 849 and 1,014 bp fragments showed the highest frequency in the population and *SSR3a* is the second.

In addition to 4 *ALMT1* markers, three additional SSR markers from the D genome were identified to be associated with Al resistance, including *Xwmc331* from 4DL (Ma et al. 2005, 2006), two SSR markers on chromosomes 2D (*Xbarc159*) and 6D (*Xbarc96*). *Xwmc331* marker appears to have very high frequency in the Polyssu and Mexican groups and is a good marker for marker-assisted selection Polyssu-derived Al resistance (Ma et al. 2005). The two other SSR markers on 2D and 6D have not been previously reported to be associated with Al tolerance in wheat. *Xbarc96* significantly correlated with Al resistance in the Chinese and US-Fultz groups and *Xbac159* correlated with Al resistance in Polyssu and Mexico-Fultz group (Table 2). The association between the markers and Al resistance may be due to differences in either structure of the population studied or sources of Al resistance. Thus, the two markers need to be validated before they can be used in marker-assisted selection.

This study demonstrated that Al-resistance genes in wheat might originate independently from three countries: USA, Brazil, and China. Al resistance in most US commercial hard winter cultivars was likely derived from Fultz, mainly through Norin 10, not from the Brazilian landrace Polyssu. Fultz and Polyssu likely originated independently although the *ALMT1* is more likely the critical gene for Al resistance in all three sources. The sequences of *ALMT1* are different among different sources of germplasm. Genetics of Al resistance in Fultz or its derivatives is still unknown and needs further investigation. Al resistance in some Chinese accessions has been reported, but further investigation of those sources may reveal additional multiple Al-resistant mechanisms in wheat.

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