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Comparative Mapping of a Major Aluminum Tolerance Gene in Sorghum and Other Species in the Poaceae

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ABSTRACT

In several crop species within the Triticeae tribe of the grass family Poaceae, single major aluminum (Al) tolerance genes have been identified that effectively mitigate Al toxicity, a major abiotic constraint to crop production on acidic soils. However, the trait is quantitatively inherited in species within other tribes, and the possible ancestral relationships between major Al tolerance genes and QTL in the grasses remain unresolved. To help establish these relationships, we conducted a molecular genetic analysis of Al tolerance in sorghum and integrated our findings with those from previous studies performed in crop species belonging to different grass tribes. A single locus, Alt_{SB}, was found to control Al tolerance in two highly Al tolerant sorghum cultivars. Significant macrosynteny between sorghum and the Triticeae was observed for molecular markers closely linked to putatively orthologous Al tolerance loci present in the group 4 chromosomes of wheat, barley, and rye. However, Alt_{SB} was not located within the homeologous region of sorghum but rather mapped near the end of sorghum chromosome 3. Thus, Alt_{SB} not only is the first major Al tolerance gene mapped in a grass species that does not belong to the Triticeae, but also appears to be different from the major Al tolerance locus in the Triticeae. Intertribe map comparisons suggest that a major Al tolerance QTL on rice chromosome 1 is likely to be orthologous to Alt_{SB}, whereas another rice QTL on chromosome 3 is likely to correspond to the Triticeae group 4 Al tolerance locus. Therefore, this study demonstrates a clear evolutionary link between genes and QTL encoding the same trait in distantly related species within a single plant family.

GENETIC variation for tolerance to aluminum (Al) toxicity, a major limiting factor for plant growth on acidic soils, is well documented (Duncan 1988; Pandey *et al.* 1994; Carver and Ownby 1995). However, the extent to which Al tolerance in different plant species derives from the action of orthologous or paralogous genes *vs.* that of distinctly different genes or gene ensembles has yet to be resolved.

For members of the grass tribe Triticeae including wheat, barley, and rye, comparative map data suggest that parallel mutations at a single orthologous locus on the group 4 chromosomes underlie Al tolerance (Garvin and Carver 2003). As such, Al tolerance in these crops can be readily evaluated by simple Mendelian analysis. In contrast, natural genetic variation for Al tolerance in other domesticated members of the Poaceae in different tribes, such as rice (Wu *et al.* 2000; Nguyen *et al.* 2001, 2002, 2003) and maize (Magnavaca *et al.* 1987; Ninamango-Cárdenas *et al.* 2003) appears to be quantitative in nature. While intratribe conservation of Al tolerance genes seems likely, the absence of known major Al tolerance genes outside of the Triticeae suggests that Al tolerance in these other tribes may derive from genes wholly different from those found within the Triticeae. Nevertheless, there is compelling evidence that a number of agriculturally important traits may be controlled by orthologous loci in different grass species (Lin *et al.* 1995; Paterson *et al.* 1995; Pereira and Lee 1995; Hu *et al.* 2003). It is therefore desirable to develop a comprehensive model for Al tolerance gene evolutionary relationships in the Poaceae, to answer basic biological questions regarding the evolution of this trait, and to understand what opportunities may exist to use biotechnology to improve Al tolerance by pyramiding unique Al tolerance genes from different species.

In this study, we investigated the inheritance of Al tolerance in sorghum and determined the chromosome location of the major Al tolerance gene that was detected.

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The hypothesis that different Al tolerance genes exist outside the Triticeae was tested through synteny-based analysis of the genome locations of Al tolerance genes and quantitative trait loci (QTL) in sorghum, rice, and the Triticeae. Our results demonstrate that two apparently distinct major Al tolerance genes in the Triticeae and Andropogoneae are likely to be orthologous to two major QTL in rice, a member of the Oryzeae. Thus, this study establishes a framework for understanding the genetic complexity of Al tolerance across highly diverse domesticated members of the Poaceae.

MATERIALS AND METHODS

Plant materials: The Al tolerant sorghum inbred lines SC283 and SC566-14 (*Sorghum bicolor* ssp. *bicolor*) used in this research were collected from distinct regions within Africa and also differ distinctly in their classification. SC283 belongs to the *guineae* race (Harlan and de Wet 1972), was classified by Murty *et al.* (1967) as a *conspicuum* working group, and was collected in Tanzania, whereas SC566 (*caudatum* race) was classified as a *caudatum* working group and was collected in Nigeria. SC283 and SC566 were each crossed with BR007, an Al-sensitive line from the Embrapa Maize and Sorghum breeding program. F_1 plants derived from each cross were self-pollinated and two independent F_2 populations were generated for Al tolerance studies ($n = 49$ for F_2:BR007 × SC283; $n = 135$ for F_2:BR007 × SC566). F_2 individuals from the BR007 × SC283 population were also transplanted to pots with soil in a greenhouse and self-pollinated to obtain $F_{2:3}$ families.

Hydroponic analysis of Al tolerance: Al-induced inhibition of seminal root growth in nutrient solution was used to quantify Al tolerance, using the basal nutrient solution described in Magnavaca *et al.* (1987). Seeds were surface sterilized in 0.1% NaOCl for 8 min, rinsed eight times with 50 ml of 18 MΩ H_2O, and allowed to germinate in petri dishes covered with two layers of moist filter paper for 3 days at 26° in the dark. The seminal roots from the seedlings were then inserted through the mesh bottoms of polyethylene cups, covered with black beads, and placed into holes in the lids of polyethylene containers filled with 8 liters of nutrient solution under continuous aeration (48 seedlings/container). The experiments were carried out in a growth chamber with 26° day and 23° night temperatures, a light intensity of 550 μmol photons m^{-2} sec^{-1} and a 12-hr photoperiod.

A dose response analysis at 0, 60, 110, 148, and 222 μM Al was performed with SC283, SC566, and BR007 to define the level of Al to be used in genetic studies. These concentrations correspond to free Al^{+3} activities of {0}, {11}, {20}, {27}, and {39} μM Al^{+3} (braces indicate Al^{+3} activity), respectively, as estimated with the speciation software program GEOCHEM-PC (Parker *et al.* 1995). Treatment with {27} μM Al^{+3} for 5 days elicited the largest growth differences between the Al-tolerant and Al-sensitive parents and was thus used for phenotypic evaluations of Al tolerance in the progeny (data not shown).

For genetic studies of Al tolerance, four seedlings of the relevant tolerant parent and four of the sensitive parent were planted together with 40 F_2 progeny in each container containing nutrient solution lacking Al, and the plants were given a 24-hr acclimation period. Subsequently, the initial length of each seedling's root growing in control solution (*ilc*) was measured and final lengths in control solution (*flc*) for the same roots were recorded 24 hr later. The solution was then replaced by a nutrient solution containing {27} μM Al^{+3}, and final root lengths under Al treatment (*flAl*) were obtained after 5 days of exposure to Al. Intrinsic root growth rates were assessed for each individual using the root growth data obtained during the 24-hr growth period in control solution. Accordingly, a control root growth rate was obtained as $[(crgr_{1d}) = flc - ilc]$. The root growth rate under Al exposure over the 5-day period ($Alrgr_{5d}$) was then calculated as $Alrgr_{5d} = flAl - flc$ and Al inhibition of root growth was calculated relative to the control root growth: RRG (% relative root growth) = $[Alrgr_{5d}/(crgr_{1d} \times 5)] \times 100$.

Seedlings of parents and F_2 progeny were also qualitatively scored for visual symptoms of root damage caused by Al and for root apical Al accumulation using hematoxylin staining (Polle *et al.* 1978) as described by Tang *et al.* (2000). The combination of differences in mean percentage relative root growth inhibition (RRG), visual root damage, and hematoxylin staining pattern between Al-tolerant and Al-sensitive parents was used to classify F_2 progeny as Al tolerant or sensitive.

Progeny testing of F_2:BR007 × SC283 was completed on $F_{2:3}$ families, using visual symptoms of root damage and the RRG family means. Twelve F_3 plants from each F_2 individual were used for progeny testing, which for a dominant single gene model assures a probability of >95% for correctly classifying a heterozygous parent. Additionally, when only one plant in a family exhibited sensitivity to Al, testing was repeated with 20 progeny to ensure proper genotypic classification.

DNA isolation and restriction fragment length polymorphism analysis: Genomic DNA was isolated from ∼4 g of leaf tissue using the protocol described by Riede and Anderson (1996). Parental survey membranes for DNA blot analysis were prepared according to Tang *et al.* (2000), but with restriction digestions consisting of 10 μg of DNA and 10 units of restriction enzyme (18 different restriction endonucleases in total). For restriction fragment length polymorphism (RFLP) analysis, cloned inserts were isolated by restriction digestion and labeled with [^{32}P]dCTP by the random hexamer method (Feinberg and Vogelstein 1984), denatured at 100° for 10 min, and hybridized to parental membranes at 65° overnight as described in Bernatzky and Tanksley (1986). Membranes were sequentially washed at 65° for 30 min with 2× SSC, 1× SSC, and 0.5× SSC or 30 min with 2× SSC and 20 min with 1× SSC [for hybridization with genomic clones or cloned amplified fragment length polymorphism (AFLP) fragments]. All wash solutions also contained 0.1% (w/v) SDS.

For comparative mapping of sorghum *vs.* Triticeae for Al tolerance genes, progeny membranes containing subsets of the F_2 progeny derived from BR007 × SC283 ($n = 23$) and of the F_2 progeny from BR007 × SC566 ($n = 25$) were hybridized with a set of genomic and cDNA clones located in the Triticeae group 4 chromosomes and linked to Alt_{BH} in wheat (Riede and Anderson 1996), *Alp* in barley (Tang *et al.* 2000), and *Alt3* in rye (Miftahudin *et al.* 2002), as well as clones located elsewhere on barley chromosome 4H (Langridge *et al.* 1995) and sorghum linkage group C (Boivin *et al.* 1999).

Bulked-segregant analysis with AFLP markers: For bulked-segregant analysis (Michelmore *et al.* 1991) in the F_2 generation of BR007 × SC283, equal amounts of DNA from 10 tolerant and 10 sensitive progeny were combined to produce a tolerant bulk (TB) and a sensitive bulk (SB). The bulks were then screened for polymorphisms by AFLP analysis (Vos *et al.* 1995) using the GIBCO BRL AFLP Analysis System I kit (Life Technologies, Gaithersburg, MD) according to the manufacturer's recommendations. A total of 128 pairwise combinations between 8 *Eco*RI primers and 16 *Mse*I primers (primers described in the GIBCO BRL protocol and M-CCA, M-CCT, M-CGA, M-CGT, M-CCC, M-CGC, M-CCG, and M-CGG) were assayed. After progeny testing for Al tolerance, another TB was assembled that eliminated heterozygous F_2 individuals,

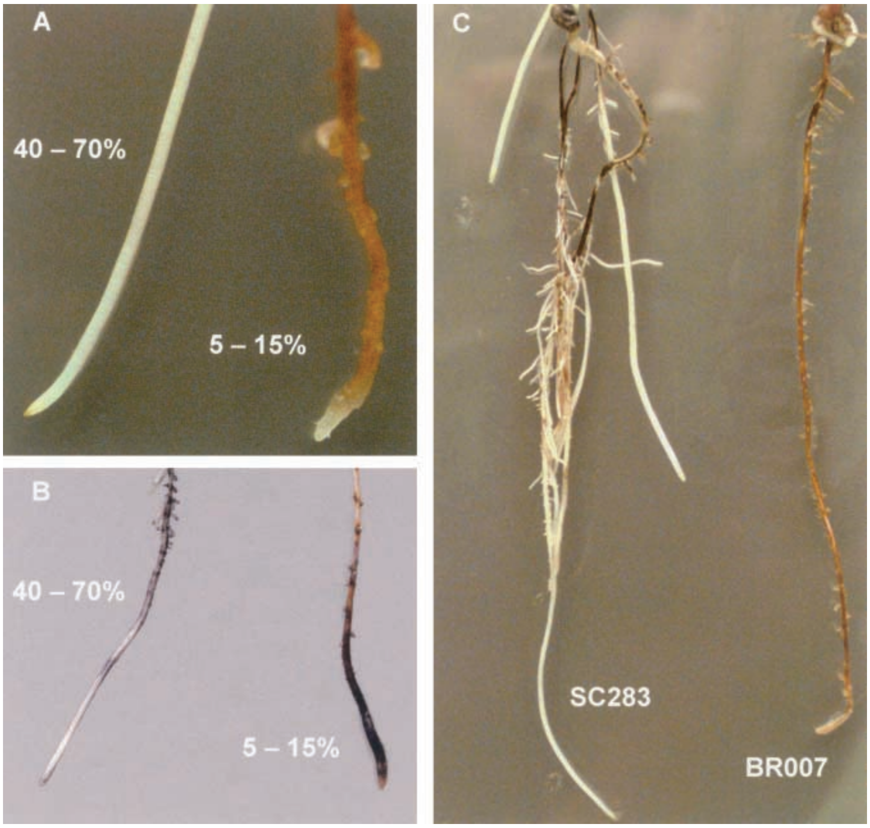

FIGURE 1.—Phenotypic analysis of Al tolerance. Comparisons of root damage and hematoxylin staining in Al-tolerant *vs.* Al-sensitive sorghum F_2 seedlings grouped in the 5–15% RRG *vs.* 40–70% RRG classes after growth in nutrient solution containing {27} μM Al^{+3} for 5 days. (A) Visual symptoms of Al toxicity *vs.* Al tolerance. (B) Hematoxylin staining patterns showing differential Al accumulation in roots. (C) Visual symptoms of root damage of the Al-tolerant (SC283) and Al-sensitive (BR007) parents subjected to the same Al activity and exposure period.

and the resulting homozygous TB and SB were screened with *Hin*dIII/*Mse*I primers using adapter and primer sequences described by KASUGA *et al.* (1997). A total of 256 pairwise combinations between *Hin*dIII (H-ACT, H-AGC, H-AAC, H-AAG, H-ACA, H-ACC, H-ACG, H-AGG, H-AAA, H-AAT, H-AGA, H-AGT, H-ATA, H-ATC, H-ATG, and H-ATT) and *Mse*I primers (same as those used for *Eco*RI/*Mse*I amplifications) were assayed. Primer combinations revealing polymorphisms were subsequently tested on individual F_2 progeny to test for linkage to Al tolerance genes.

Cloning and conversion of AFLP markers: AFLP fragments linked to Al tolerance genes were excised from gels, rehydrated in 100 μl of TE buffer overnight at 4°, reamplified, cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA), and sequenced. The identity of the cloned fragments was verified by electroblotting to Hybond N+ (Amersham, Arlington Heights, IL) membranes followed by hybridization as described by PIERRE *et al.* (2000). Sorghum parental survey membranes were hybridized with the cloned AFLP markers to identify RFLPs differentiating the parents, and complete genotypic classification for the AFLP markers was obtained with progeny membranes.

Linkage analysis: Genetic linkage maps were constructed using the Mapmaker Macintosh program V2.0 (LANDER *et al.* 1987), and genetic distances were estimated from recombination frequencies using the Kosambi function (KOSAMBI 1944). The two-point analysis with the "group" command (LOD = 3 and maximum recombination frequency, $\theta = 0.4$) was used to infer linkage groups. Three-point analysis was used to calculate the likelihoods of possible orders of each linked triplet, and multipoint analysis with "First Order" and "Compare" commands was used to verify the results of the three-point analysis. Finally, "Ripple" was used to confirm the correct order of all triplets in the context of the final order.

RESULTS

Inheritance of Al tolerance in sorghum—BR007 × SC283: *Analysis of F_2 progeny:* Individuals exhibiting between 5 and 15% RRG suffered severe root damage due to Al exposure and were heavily stained by hematoxylin (Figure 1, A and B), which was a phenotype similar to that displayed by the sensitive parent BR007 (Figure 1C). In contrast, the progeny with 40–70% RRG exhibited minimal hematoxylin staining and negligible visual symptoms of root damage (Figure 1, A and B) that were similar to the tolerant parent SC283 (Figure 1C). Thus, progeny in the RRG range between 5 and 15% were considered Al sensitive, while those in the 40–70% RRG range were classified as Al tolerant.

Analysis of $F_{2:3}$ families: The frequency distribution of mean RRG values in $F_{2:3}$ families (Figure 2) was bimodal, with a discontinuity present at the 20–25% RRG class. The $F_{2:3}$ families derived from Al-sensitive F_2 individuals uniformly exhibited strong visual symptoms of root damage after treatment with Al and low RRG means with a small variance, indicating that the F_2 parents were true breeding for sensitivity. Individuals within $F_{2:3}$ families derived from Al-tolerant F_2 plants were either

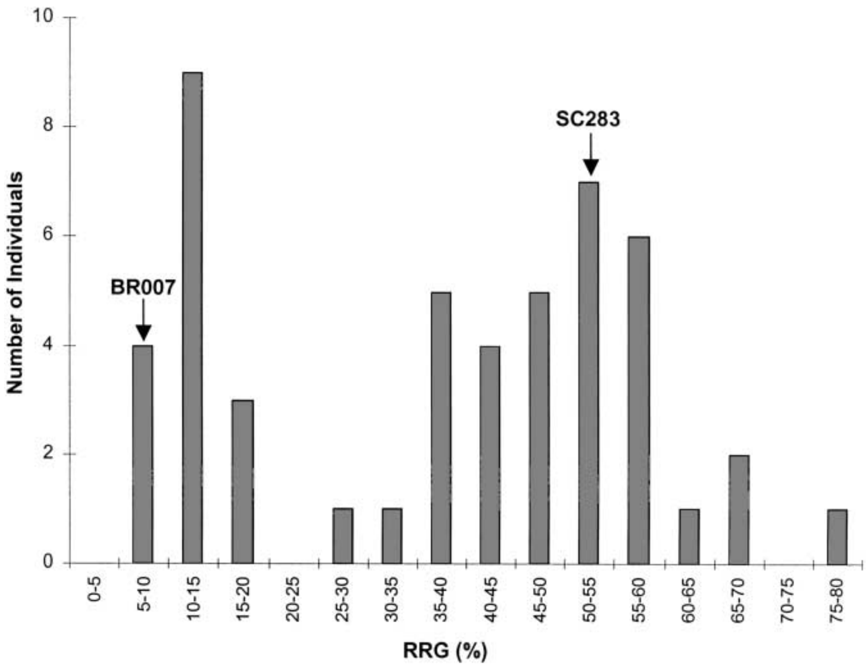

Figure 2.— Segregation for Al tolerance in BR007 × SC283. Mean percentage RRG frequency distribution for 49 $F_{2:3}$ families (12 individuals per family) grown in nutrient solution containing {27} μM Al^{+3} for 5 days. RRG mean values and standard deviations were 8.7 ± 0.96 for BR007 (n = 12) and 52.6 ± 19.71 for SC283 (n = 19).

uniformly undamaged by Al treatment or segregated for root damage symptoms. The data obtained from the $F_{2:3}$ families conformed to a 1:2:1 monogenic segregation model ($0.10 < P < 0.25$), thus indicating that SC283 harbors a single major Al tolerance gene henceforth designated Alt_{SB}.

Gene action of Alt_{SB}: Table 1 shows that in both the F_2 and the F_3 generations, the RRG mean of the heterozygous (*Tt*) class fell between the homozygote midclass mean [(*TT* + *tt*)/2] and the mean of the homozygous tolerant (*TT*) class. A similar observation was made from comparisons of the mean of the *Tt* class to the midpar-

TABLE 1

Estimates of gene action for Alt_{SB}

Genotypic class	Mean RRG (%)	SD	SEM	a[a]	d[b]	d/a[c]
SC283[d]	52.6	19.7	4.40			
BR007[d]	8.7	0.96	0.27			
(SC283 + BR007)/2	30.6	—	—			
$F_2 - TT$[e]	50.6	14.48	3.74			
$F_2 - Tt$	42.4	16.27	3.83			
$F_2 - tt$	13.9	5.75	1.48			
$F_2 - (TT + tt)/2$	32.2	—	—			
F_2				18.3	10.2	0.55
$F_{2:3} - TT$[f]	55.0	9.23	2.38			
$F_{2:3} - Tt$	41.9	9.66	2.28			
$F_{2:3} - tt$	12.0	2.92	0.75			
$F_{2:3} - (TT + tt)/2$	33.5	—				
F_3				21.5	8.4	0.39

[a] a denotes additive effects [$a = (TT - tt)/2$].

[b] d denotes dominance effects {$d = Tt - [(TT + Tt)/2]$}.

[c] Degree of dominance.

[d] Mean RRG for the parents SC283 (n = 19) and BR007 (n = 12).

[e] For the F_2 generation, RRG values for all individuals in a given genotypic class were used to calculate RRG means for the three genotypic classes: homozygous tolerant (*TT*, n = 15), homozygous sensitive (*tt*, n = 15), and heterozygous (*Tt*, n = 18).

[f] For the F_3 generation, RRG values for 12 individuals within an $F_{2:3}$ family were averaged to obtain family means. $F_{2:3}$ RRG means were then averaged within each genotypic class (n = 15 for the *tt* and *TT* classes; n = 18 for the *Tt* class).

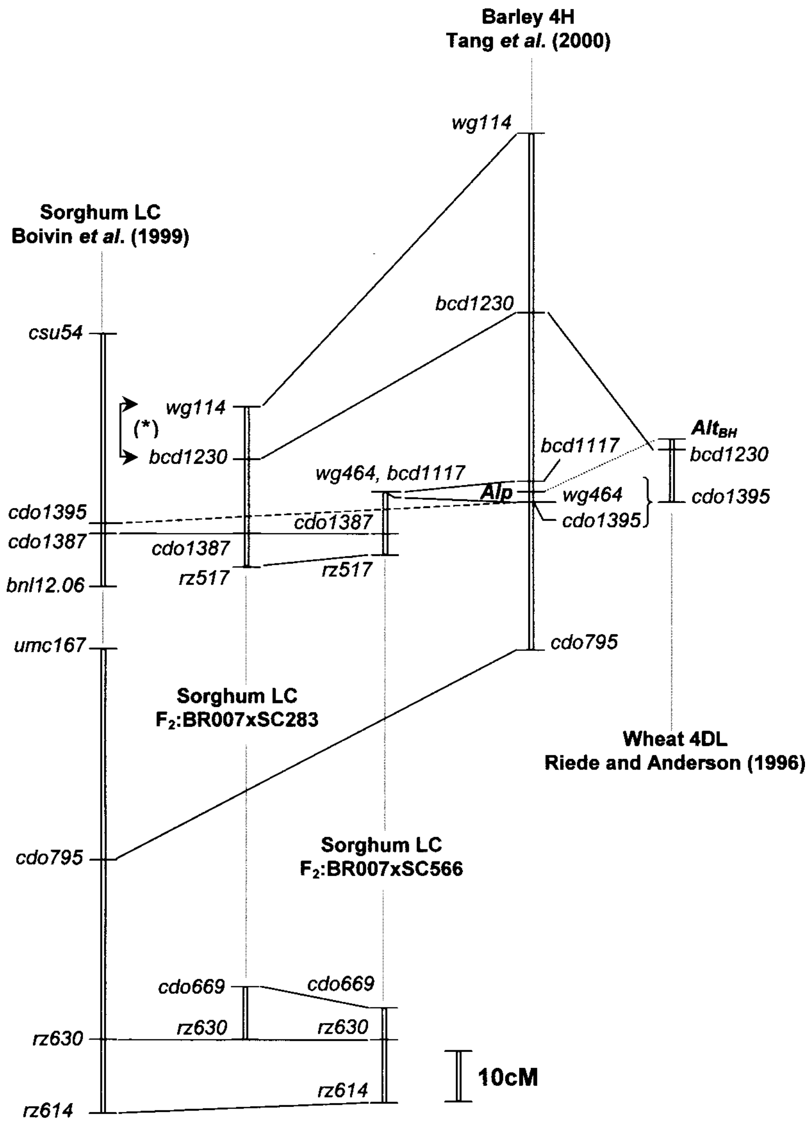

FIGURE 3.—Syntenic relationships for sorghum LC (BOIVIN *et al.* 1999 and BR007 crosses), barley 4H (TANG *et al.* 2000), and wheat 4DL (RIEDE and ANDERSON 1996). Sizes of the two selected progeny subsets were 23 for F$_2$:BR007 × SC283 and 25 for F$_2$:BR007 × SC566; markers were ordered with LOD > 3. The double arrow denoting *Xbcd1230* and *Xwg114* (*) in F$_2$:BR007 × SC283 indicates that the relative order of these two markers could not be determined.

ent [(SC283 + BR007/2)] RRG mean. These results suggest that Alt_{SB} is partially dominant under the experimental conditions for which the phenotype was assessed here. The addition of a tolerance allele from SC283 [additive effect (a)] increased RRG by ~20%, and the degree of dominance (d/a, where d is the dominance effect) was estimated as ~0.5.

Molecular mapping of Alt_{SB}: *Comparative mapping of Alt_{SB} vs. major Al tolerance genes in the Triticeae:* To determine if Alt_{SB} is orthologous to the major Al tolerance locus located on group 4 chromosomes in members of the Triticeae, we searched for evidence that molecular markers previously found to be tightly linked to the Triticeae Al tolerance genes Alt_{BH} and *Alp* were also linked to Alt_{SB} (Figure 3). This analysis also included the rye Al tolerance gene *Alt3* on chromosome 4R (MIFTAHUDIN *et al.* 2002), which is not depicted in Figure 3, but is most likely orthologous to Alt_{BH} in wheat 4DL and to *Alp* in barley 4H as all three Al tolerance loci are linked to the marker *Xbcd1230*. The marker loci *Xbcd1230*, *Xbcd1117*, and *Xwg464*, which are closely linked to the putative Triticeae orthologs Alt_{BH}, *Alp*, and *Alt3*, mapped to a single conserved region on sorghum LC (see F$_2$:BR007 × SC283 and F$_2$:BR007 × SC566 in Figure 3) that also contains *Xcdo1395* (BOIVIN *et al.* 1999). This finding indicated that sorghum LC is the counterpart to the Triticeae group 4 chromosomes as suggested by GALE and DEVOS (1998). However, a χ^2 analysis of goodness-of-fit to an independent segregation model did not support linkage to Alt_{SB} for any of these markers, including *Xbcd1230* in the F$_2$:BR007 × SC283 map (Figure 3). Additionally, the positions of *Xwg464*, *Xbcd1117*, and *Xcdo1395* in the conserved region of LC (Figure 3) indicate that those markers are also not linked to Alt_{SB} in F$_2$:BR007 × SC283 (data not shown). This finding suggests not only that Alt_{SB} resides elsewhere in the sorghum genome, but also that it may not be orthologous to the Triticeae Al tolerance genes.

AFLP markers for Alt_{SB} and anchoring on the sorghum map: Amplification of bulked DNA pools with E-ACG/M-CTA

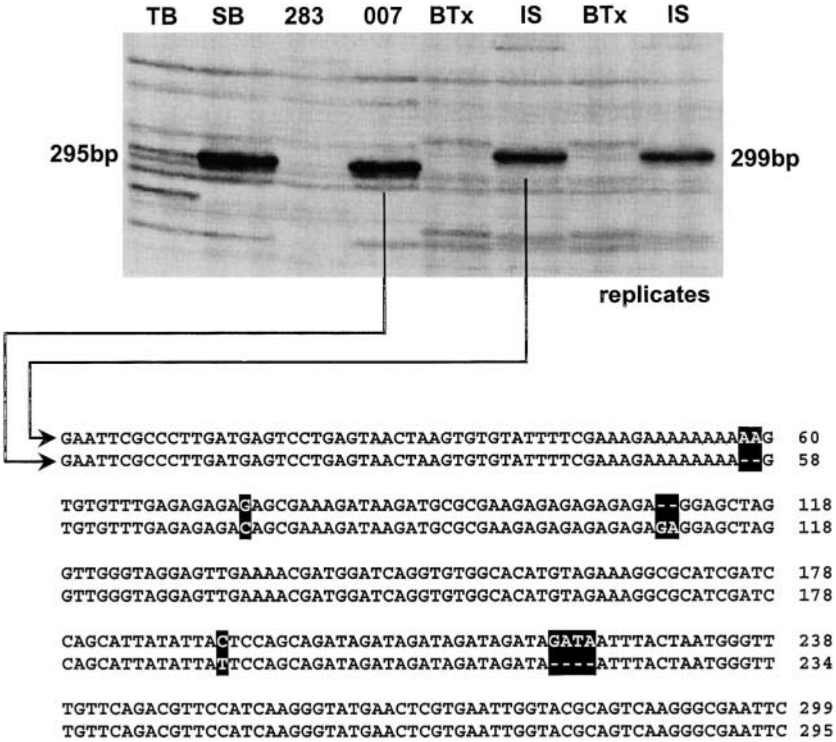

Figure 4.—DNA sequence comparison of *AFS37-1* and its allele in IS3620C. The AFLP patterns obtained from the amplification of the tolerant bulk (TB), sensitive bulk (SB), the tolerant parent SC283 (283), the sensitive parent BR007 (007), BTx623 (BTx), and IS3620C (IS) are shown on top. Sequence alignment between the 295-bp fragment amplified in the sensitive bulk and BR007 (=*AFS37-1*) and the 299-bp fragment amplified in IS3620C is shown below, with polymorphic repeats and nucleotide substitutions differentiating the alleles highlighted.

generated a 295-base-pair (bp) fragment (Figure 4) differentiating the tolerant and sensitive bulks and linked in repulsion to Alt_{SB}. This marker, designated *AFS37-1*, was found to be a single-copy sequence when the cloned fragment was used as a probe in Southern analysis (data not shown).

When DNA template from parents of a reference sorghum mapping population, BTx623 and IS3620C (Peng *et al.* 1999; Menz *et al.* 2002), was amplified with E-ACG/M-CTA, a putative allele of *AFS37-1* was identified in IS3620C but not in BTx623, and this was verified by sequence alignment to *AFS37-1* from BR007 (Figure 4). We then utilized the BTx623 × IS3620C recombinant inbred line (RIL) population and the corresponding mapping data set of Menz *et al.* (2002) to map *AFS37-1* by AFLP analysis to the terminal region of sorghum linkage group C (LG-C; Menz *et al.* 2002) depicted in Figure 5A. LG-C in the Menz *et al.* (2002) map corresponds to linkage group G (LG) in Boivin *et al.* (1999) rather than to LC. This confirmed that Alt_{SB} is located on a sorghum chromosome that is not homeologous to that harboring the major Al tolerance locus in sev-

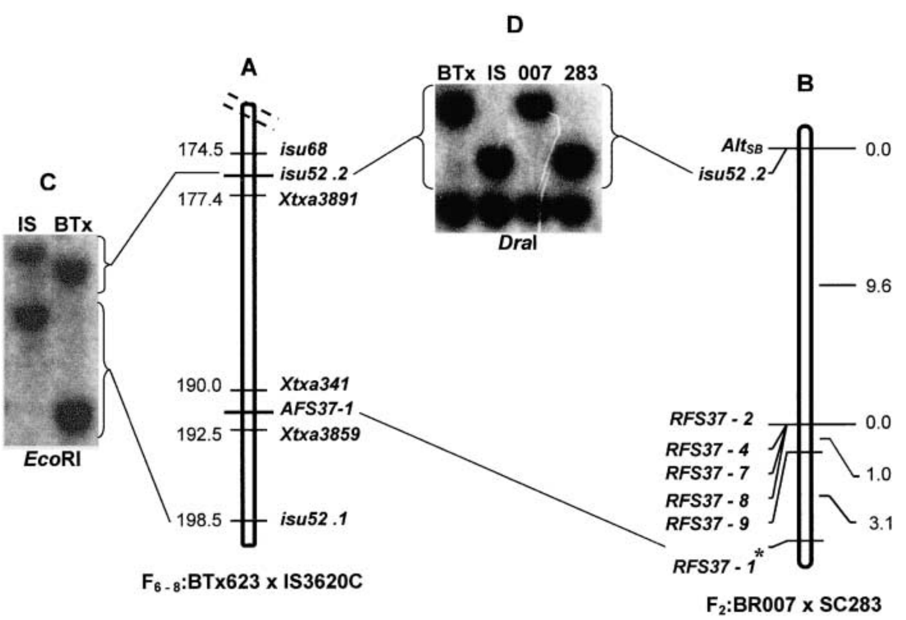

Figure 5.—Chromosomal location of Alt_{SB}. (A) Position of marker *AFS37-1* on sorghum chromosome 3 (LG-C in Menz *et al.* 2002). (B) Alt_{SB} linkage map in F_2: BR007 × SC283 (*RFS37-1–9* are RFLP markers originated from the conversion of AFLP markers). Asterisk denotes that *RFS37-1* refers to the cloned AFLP marker *AFS37-1* that was anchored onto sorghum chromosome 3 (Menz *et al.* 2002). (C) RFLP profile of IS3620C (IS) and BTx623 (BTx) DNA restricted with *Eco*RI and hybridized with *isu52.* (D) RFLP profile of BTx623 (BTx), IS3620C (IS), BR007 (007), and SC283 (283) DNA restricted with *Dra*I and hybridized with *isu52.* Revised positions of the markers shown in A in the context of the complete sorghum chromosome 3 data set are found by selecting linkage group C at http://sorghum genome.tamu.edu. Genetic distances are shown in centimorgans.

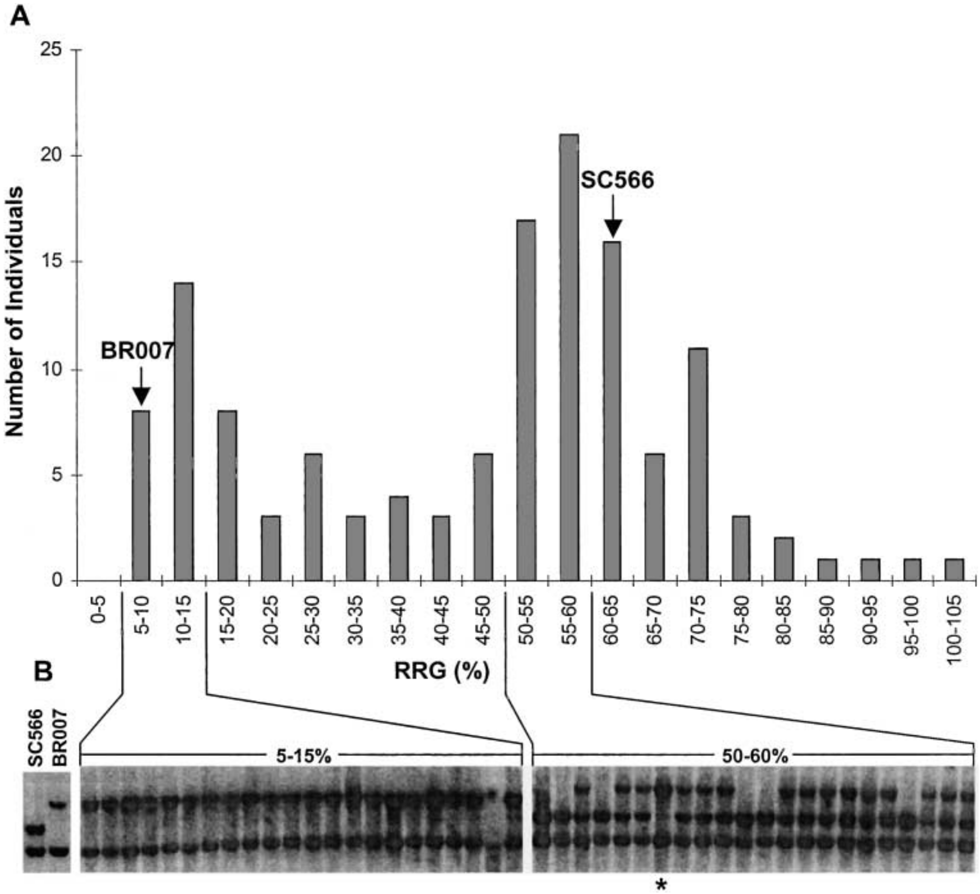

FIGURE 6.—Molecular genetics of Al tolerance in SC566. (A) Frequency distribution for percentage RRG of 135 F_2:BR007 × SC566 individuals exposed to a nutrient solution containing {27} μM Al^{+3} for 5 days. The RRG means for BR007 (5–10% RRG class) and SC566 (60–65% class) represent the mean of 12 and 38 individuals per parent, respectively. (B) Association between the SC566 Al tolerance gene and *isu52.2*. Shown are RFLP profiles of Al-tolerant and -sensitive parents SC566 and BR007, Al-sensitive F_2 progeny (5–15% RRG), and Al-tolerant progeny in the 50–60% RRG range digested with *Dra*I and hybridized to *isu52*. The RFLP profiles for the Al-tolerant progeny shown are representative of all Al-tolerant F_2 progeny (35–105% RRG), with only three recombinants detected (asterisk denotes recombinant individual).

eral Triticeae species. Sorghum LG-C (MENZ *et al.* 2002) is now being referred to as sorghum chromosome 3 (KLEIN *et al.* 2003), a designation that will be adopted here. The final Alt_{SB} linkage map was constructed in the BR007 × SC283 population solely with codominant segregation data (Figure 5B). To do that, *AFS37-1* and additional linked AFLPs were cloned and used as probes for RFLP analysis (as RFLPs, loci designated as *RFS37-x*). These markers spanned a genetic distance of 13.7 cM in the vicinity of Alt_{SB}.

Validation of the chromosomal location for Alt_{SB}*:* The segregation of *isu52*, a marker located at position 198.5 cM on sorghum chromosome 3 (MENZ *et al.* 2002; Figure 5A), was scored in F_2:BR007 × SC283 using the restriction enzyme *Dra*I. No recombinants were detected between *isu52* and Alt_{SB} (Figure 5B), and for this reason minimum linkage distances were estimated as 3 cM (p-0.05) and 4.6 cM (p-0.01) by the method of HANSON (1959).

Subsequently we sought to resolve an apparent discrepancy observed for the position of markers at the end of chromosome 3 where Alt_{SB} is located. The RFLP loci *isu52* and *isu68*, both originally scored as single copy markers, were found to be tightly linked by PEREIRA and LEE (1993), whereas these same markers were genetically unlinked according to PENG *et al.* (1999). To clarify this discrepancy, both markers were rescored in the BTx623 × IS3620C RILs, which is the same population used in the study by PENG *et al.* (1999) and MENZ *et al.* (2002). The RFLP profile obtained with *Eco*RI in this population revealed that *isu52* is duplicated on sorghum chromosome 3. One copy is located at position 198.5 cM (*isu52.1*; Figure 5A) and corresponds to the locus shown in Figure 5C that was also scored by Peng and co-workers (G. E. HART, personal communication). Segregation for the second copy, *isu52.2*, could be scored on the *Xba*I (not shown), *Eco*RI (Figure 5C), and *Dra*I membranes (Figure 5D) and mapped at ∼175 cM (Figure 5A). The marker *isu68* was found to be a single-copy marker and was tightly linked to *isu52.2* at position 174.5 cM (Figure 5A). Thus, *isu52.2* corresponds to *isu52* on the map of PEREIRA and LEE (1993). Because *Dra*I produced identical RFLP profiles with *isu52* in both the BTx623 × IS3620C and the BR007 × SC283 parents (Figure 5D), it is *isu52.2* (Figure 5A) that is tightly linked to Alt_{SB} (Figure 5B). A linear regression of $F_{2:3}$ RRG mean values as a function of the three genotypic classes for *isu52.2* showed that this marker alone explained 79% of the phenotypic variance for Al tolerance in the progeny ($r^2 = 0.79$, $P < 0.001$).

Molecular genetics of Al tolerance in SC566: The RRG frequency distribution of F_2 progeny derived from the cross of BR007 with a second Al tolerant parent, SC566, was clearly bimodal (Figure 6A). RRG values, symptoms of root damage caused by Al, and hematoxylin staining of F_2 progeny roots were compared to SC566 and BR007, and Al-sensitive individuals were identified in the 5–15% RRG classes while Al-tolerant progeny exhibited RRG values between 35 and 105%. Individuals exhibiting RRG values within intermediate classes (15–35% RRG) were also identified, but could not be unambiguously classified as tolerant or sensitive. To test whether Al tolerance in SC566 is due to the presence of Alt_{SB}, *isu52.2* segregation was scored in this population (Figure 6B). F_2 individuals that were sensitive to Al (5–15% RRG) were all homozygous for the BR007 allele of *isu52.2* (Figure 6B). In contrast, all but three of the Al-tolerant F_2 progeny (35–105% RRG) were either homozygous for the SC566 allele of *isu52.2* or heterozygous. If a major Al tolerance gene and a marker locus are unlinked, the expected frequency of double homozygous individuals is 0.0625. Thus, in theory just 8 such individuals should be present in the BR007 × SC566 population, rather than the 22 that were observed (Figure 6B). This strong linkage disequilibrium specifically with *isu52.2* indicates that SC566 harbors Alt_{SB} or an allele of this gene.

DISCUSSION

The grass family Poaceae is highly diverse and contains ~10,000 species (Kellogg 2001), many of which are our most important staple crops. The extremely broad adaptation of the grasses to diverse environments (Kellogg 1998), including adaptation to the widespread Al-toxic acid soils, raises the question whether adaptation to Al toxicity in different grass species is associated with mutations in a limited number of genes or whether a far more diverse range of genes contributes to Al tolerance in the grasses.

Our genetic analysis of Al tolerance in sorghum, a member of the tribe Andropogoneae, revealed that this trait was encoded by a single major locus, Alt_{SB}, which behaved in a semidominant fashion under our experimental conditions. Thus, to date, Alt_{SB} is the only major Al tolerance gene that has been mapped to the genome of a grass species not in the tribe Triticeae. In addition, because Alt_{SB} was identified in SC283, which is considered to be a standard for Al tolerance (Duncan *et al.* 1983; Furlani *et al.* 1987; Duncan 1988), it conditions perhaps the highest Al tolerance level within sorghum.

A molecular marker-based evaluation of intraspecific Al tolerance diversity in sorghum indicated that the single major Al tolerance loci in SC283 and SC566, another extremely tolerant sorghum cultivar, are the same. The fact that SC283 and SC566 exhibited very distinct morphological characteristics and that they were collected at different sites in Africa suggested that these cultivars may have different genetic origins. Indeed, the *caudatum* race to which SC566 belongs was proposed to have arisen from a domestication episode more recent than that from which the *guinea* race, which includes SC283, arose (Harlan 1975; Doggett 1988). Thus, the presence of a common Al tolerance locus in these two highly diverse sorghum cultivars indicates that the genetic basis for Al tolerance in sorghum may be quite narrow. This is similar to results of a comprehensive study of Al tolerance gene diversity in barley (Minella and Sorrells 1992), where different Al tolerance levels displayed by a large set of cultivars were found to be due to allelic variation at a common locus (*Alp*). These findings in both sorghum and barley suggest that in crop species that display single gene inheritance for Al tolerance, mutations in just one or a few genes may confer agriculturally significant levels of Al tolerance, although different alleles at a single locus may be present (Minella and Sorrells 1992). In such species, intraspecific gene pyramiding may not be a feasible strategy for enhancing Al tolerance. Alternatively, combining distinct Al tolerance genes from different species may hold greater potential for Al tolerance improvement, provided that such interspecific diversity exists, that the genes can be isolated, and that they function in other genetic backgrounds.

Potential orthology between major Al tolerance genes in the Andropogonae and Triticeae was assessed by comparative mapping. Our results showed that while molecular markers linked to the Al tolerance loci on the Triticeae group 4 chromosomes mapped to the expected syntenic region in sorghum, Alt_{SB} mapped to sorghum chromosome 3, which is not homeologous to the Triticeae group 4 chromosomes. The absence of significant disruptions of macrocolinearity between the homeologous sorghum LC and Triticeae group 4 chromosomes in the region near the major Triticeae Al tolerance locus suggests that Alt_{SB} is a gene distinctly different from that identified in the Triticeae.

Interestingly, a wheat-rye chromosome 3R addition line showed a dramatic increase in tolerance (Aniol and Gustafson 1984). Considering that the Triticeae group 3 chromosomes are likely to be homeologous to sorghum chromosome 3 (Nelson *et al.* 1995; Gale and Devos 1998), it is possible that an Alt_{SB} ortholog is present and functioning in rye, but has not yet been mapped in this or other Triticeae species because of a lack of polymorphism among genotypes. Alternatively, because perturbations of gene colinearity caused by small-scale events such as gene duplications and deletions (Bennetzen and Ramakrishna 2002) occur in the grasses, and segmental translocations to nonhomeologous chromosomes have been found to disrupt colinearity between the sorghum genome and those of wheat and barley (Li and Gill 2002), we cannot rule out the possibility that Alt_{SB} is orthologous to the group 4 Triticeae Al tolerance genes and has been translocated to a nonhomeologous sorghum chromosome.

Alt_{SB} is located on sorghum chromosome 3, which is homeologous to rice chromosome 1 (Ventelon *et al.* 2001; Klein *et al.* 2003), and Al tolerance QTL have been repeatedly detected at the end of rice chromosome 1 (Wu *et al.* 2000; Nguyen *et al.* 2001, 2002, 2003). In particular, the major rice QTL detected by Nguyen *et al.* (2001) explained 25% of the phenotypic variance and was linked to *Xwg110*, which is located ~28 cM from *isu52* on rice chromosome 1 [see http://www.gramene.org: Rice-Cornell RFLP 2001-1 and Wilson *et al.* (1999) for marker positions in rice]. Interestingly, *isu68*, which we found to be tightly linked to *isu52.2* in the Alt_{SB} region of sorghum chromosome 3 (see Pereira and Lee 1993 and Figure 5A), is not tightly linked to *isu52* in rice and falls within the confidence interval for the rice *Xwg110* Al tolerance QTL (between *Xwg110* and *rg109* according to the Gramene database). A BLAST analysis (data not shown) indicated that *isu52* is present as a single-copy gene on rice chromosome 1, whereas in sorghum we found *isu52.2* tightly linked to Alt_{SB} and *isu52.1* ~24 cM from the sorghum Al tolerance gene. This implies that the rice *isu52* locus corresponds to *isu52.1* that is loosely linked to Alt_{SB} in sorghum and that the major rice Al tolerance QTL on chromosome 1 is likely to correspond to Alt_{SB} due to their common proximity to *isu68*.

Another major rice Al tolerance QTL has been identified on rice chromosome 3 (Wu *et al.* 2000; Nguyen *et al.* 2003), which is homeologous to the Triticeae group 4 chromosomes (Ahn *et al.* 1993; Van Deynze *et al.* 1995). Because this rice QTL and the Al tolerance genes on the Triticeae group 4 chromosomes are both linked to *Xcdo1395*, these loci may be orthologous (Nguyen *et al.* 2003). Thus, it appears that the more complex quantitatively inherited Al tolerance in rice (Oryzeae), one of the most Al-tolerant grasses (Ma *et al.* 2002; Nguyen *et al.* 2002), is in part due to the action of two major QTL, which in the Andropogoneae and the Triticeae act as two distinct major Al tolerance genes.

Our sorghum Al tolerance map data, when jointly analyzed with data obtained for both major Al tolerance genes or Al tolerance QTL in other domesticated grass crops from different tribes, suggest that the capacity to adapt to Al toxicity is associated with mutations in a small and common suite of genes, which is congruent with the results of Paterson *et al.* (1995) for traits involved in crop domestication. However, a likely pattern of gene conservation between distinct major Al tolerance genes in sorghum and the Triticeae and two major rice QTL was revealed only by a broad intertribe comparative analysis of Al tolerance genes.

The use of comparative mapping to integrate information from genomes of a range of plant species to a reference genome such as that of rice or Arabidopsis has become pivotal to modern plant genomics. However, as noted previously (Kilian *et al.* 1997), chromosomal rearrangements that disrupt colinearity (Tikhonov *et al.* 1999) may reduce the likelihood of finding an ortholog of a gene of interest in the expected syntenic position of a single given reference genome. Our results suggest that this issue may be mitigated through broader evolutionary comparisons among different members of a plant family. Because of its small genome size, relatively distant evolutionary relationship with rice, and growing genome resources, sorghum serves as a useful complement to the rice genome to foster comparative genomics in the grasses.

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