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Identification of molecular markers for aluminium tolerance in diploid oat through comparative mapping and QTL analysis

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Abstract The degree of aluminium tolerance varies widely across cereal species, with oats (*Avena* spp.) being among the most tolerant. The objective of this study was to identify molecular markers linked to aluminium tolerance in the diploid oat *A. strigosa*. Restriction fragment length polymorphism markers were tested in regions where comparative mapping indicated the potential for orthologous quantitative trait loci (QTL) for aluminium tolerance in other grass species. Amplified fragment length polymorphism (AFLP) and sequence-characterized amplified region (SCAR) markers were used to provide additional coverage of the genome. Four QTL were identified. The largest QTL explained 39% of the variation and is possibly orthologous to the major gene found in the Triticeae as well as *Alm1* in maize and a minor gene in rice. A second QTL may be orthologous to the *Alm2* gene in maize. Two other QTL were associated with anonymous markers. Together, these QTL accounted for 55% of the variation. A SCAR marker linked to the major QTL identified in this study could be used to introgress the aluminium tolerance trait from *A. strigosa* into cultivated oat germplasm.

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Introduction

Many abiotic factors affect the growth and yield of oat and other cereal crops worldwide. One of these is the level of free aluminium present in acid soils. Although soil composition plays a role, aluminium tends to dissociate from soil colloids and come into solution if the pH falls below 5.5. If toxic levels are reached, this aluminium restricts root growth, reducing a plant's ability to take up water and nutrients, and affecting yield and grain quality (Foy 1992). The Food and Agriculture Organization of the United Nations (FAO) lists aluminium toxicity as affecting 14% of all soils worldwide, with the level greater than 50% in many countries (http://www.fao.org/ag/agl/agll/terrastat/wsr.asp#terrastatdb).

Genetic variability for aluminium tolerance has been documented in a number of species, and so developing aluminium-tolerant cultivars may be an effective way of increasing the productivity of acid soils. However, different plant species react to acidic soil conditions in different ways. The primary mechanism of aluminium tolerance in many systems seems to be the exudation of organic acids from root tips into the soil, although other aluminium tolerance mechanisms, such as the control of Ca^{2+} homeostasis, may be involved (Kochian et al. 2004; Mossor-Pietraszewska 2001). The more tolerant plant species, such as rice, may use a combination of different mechanisms, and many different genes in different species do show altered expression under aluminium stress (e.g., summary of Rodriguez Milla et al. 2002); however, these changes may or may not represent the primary response of the plants to aluminium stress.

In wheat, one major gene (A*lt*1) has been shown to account for most of the differences in aluminium tolerance between cultivars (Delhaize et al. 1993a). It was proposed that the mechanism of action of *Alt*1 is the release of malic acid from roots (Delhaize et al. 1993b). More recent evidence suggests that this gene, may,

indeed, encode an aluminium-activated malate transporter (Sasaki et al. 2004).

*Alt*1 is most likely the same locus identified as *Alt*2 in a study by Luo and Dvorák (1996), in which physical mapping was used to assign the gene to chromosome 4D. Using molecular marker mapping, Riede and Anderson (1996) associated a gene, which they called Alt_{BH}, with RFLP (restriction fragment length polymorphism) markers on the same chromosome.

The same markers that were linked to the wheat Alt_{BH} gene have been linked to an aluminium tolerance gene designated *Alp* on chromosome 4H in barley (Raman et al. 2003; Tang et al. 2000), which co-segregates with a QTL for the secretion of citrate under aluminium stress (Ma et al. 2004). Different molecular markers were used to identify a single gene on sorghum chromosome 3 (Magalhaes et al. 2004). Using physical mapping, aluminium tolerance loci have been identified on triticale chromosome 3RS (Ma et al. 2000) and three rye chromosomes: 3R, 4R, and 6R (Aniol and Gustafson 1984). The genes on chromosomes 4R and 6R have also been mapped using molecular markers (Miftahudin et al. 2002, 2005; Gallego et al. 1998a, b).

Quantitative trait locus (QTL) studies have identified aluminium tolerance loci on all 12 rice chromosomes, although the number and locations vary depending on the cross or species used (Ma et al. 2002; Nguyen et al. 2001, 2002, 2003; Wu et al. 2000). Epistatic effects have also been observed (Wu et al. 2000). In maize, QTL have been found on chromosomes 2, 6, 8, and 10; again, the number and locations vary depending on the cross (Ninamango-Cárdenas et al. 2003; Sibov et al. 1999).

A survey of 3,500 oat accessions from the USDA World Oat Collection identified the *Avena strigosa* Schreb. line CIav 9011 as having the highest tolerance to aluminium (S. Kibite, unpublished). The genetics of this tolerance is unknown; however, one independent study of nine hexaploid oat crosses found that one dominant gene contributed to the aluminium tolerance phenotype (Sánchez-Chacón et al. 2000). Another study of three hexaploid oat crosses found that one or two genes contributed to the aluminium tolerance phenotype, and epistatic effects were also observed (Wagner et al. 2001).

A number of molecular marker maps exist to facilitate genetic analysis in oat, including one for the diploid oat cross *A. atlantica* Baum and Fedak × *A. hirtula* Lag. (AH) (O'Donoughue et al. 1992; Van Deynze et al. 1995a) and one for the hexaploid oat cross *A. byzantina* C. Koch 'Kanota' × *A. sativa* L. 'Ogle' (KO) (Wight et al. 2003). Both of these maps are integrated with the international grass databases Gramene (http://www.gramene.org; Ware et al. 2002) and Graingenes (http://wheat.pw.usda.gov/GG2/index.shtml), where queries and tools are available to facilitate comparative mapping among related species and genera. The objectives of this study were to identify genomic regions in diploid oat suspected to contain genes and QTL that are orthologous to those affecting aluminium tolerance in other grass species, and to use this information to identify genetic markers linked to QTL affecting aluminium tolerance in oat.

Materials and methods

Population development

A cross was made between the *A. strigosa* Schreb. lines CIav 2921 (Al sensitive) and CIav 9011 (Al tolerant), obtained from the USDA World Oat Collection. While most accessions in this collection were sensitive to aluminium and had an aluminium tolerance index (ATI) of 30–40, CIav9011 had an ATI of 86.9 (S. Kibite, unpublished). Recombinant inbred lines (RILs) from this cross, designated LAG-211, were established by advancing a random population of 88 F_2 lines to the F_6 generation by single seed descent.

Aluminium tolerance screening protocol

Thirty-two seedlings from each RI line were screened for aluminium tolerance as follows. Sixteen seedlings of each line were grown in each of two 2644 $in.^2$ flow-trays: one was designated for aluminium treatment (ca. 300 μM Al) and the other was for the aluminium-free control. Each flow-tray could accommodate 30 root trainer trays, and each root trainer could accommodate seedlings from four RI lines. The root trainers were filled with perlite and the seeds planted directly into this medium. The flow-trays were flooded with the appropriate nutrient culture solution for one hour out of every three. The basic nutrient solution contained 1 mM Ca, 300 μM Mg, 2.9 mM NO_3, 300 μM NH_4, 100 μM SO_4, 34 μM Cl, 20 μM Na, 10 μM Fe, 6 μM B, 2 μM Mn, 0.5 μM Zn, 0.15 μM Cu, and 0.1 μM Mo. Because aluminium precipitates in the presence of phosphate and/or high pH, the aluminium-toxic nutrient culture solution contained 300 μM of Al, no phosphorous and had a pH of 4.5; the aluminium-free solution contained 0 μM of Al, 100 μM of PO_4 and had a pH of 6.5.

After 14 days of growth, the root length of each seedling was measured. For each line and each treatment, the root lengths of the ten seedlings with the longest roots were averaged. The relative root lengths (RRLs; calculated as 100% × mean root length in Al solution/mean root length in Al free solution) were used as an index of aluminium tolerance. Lines were included in up to three tests, with two replicates per test, and the RRLs for each line averaged for QTL analysis.

Comparative mapping

An extensive literature review and comparative mapping study was conducted to identify orthologous regions

conferring aluminium tolerance across grass species. Sources of comparative mapping data are presented in supplementary Table S1. Each cereal chromosome region identified in the literature as containing an aluminium tolerance QTL or gene was assigned to one of the seven diploid oat linkage groups using the AH diploid oat map as a reference. The most plausible assignments of the different chromosome regions to the AH linkage groups were made after first consulting the work of Moore (1995), Moore et al. (1995), Van Deynze et al. (1995a, b), and Sorrells et al. (2003) (see also http://wheat.pw.usda.gov/pubs/2003/Sorrells/). The comparative mapping feature of the Gramene database (CMap; http://www.gramene.org/cmap/) was also used extensively.

Only one of the maps previously used for mapping aluminium tolerance, the rice map of Ma et al. (2002), was available in the Gramene database. Because of that, and because of a lack of common markers between maps, the aluminium tolerance loci were first localized on "bridging" maps (Table S1) before being assigned to their most likely locations on the AH linkage groups. This was facilitated by transferring map information to a locally available comparative mapping program, C2maps (available as an upgrade of the M5 mapping program (Tinker 1999)).

DNA purification, restriction digestion, Southern blotting, restriction fragment length polymorphism (RFLP) analysis, and amplified fragment length polymorphism (AFLP) analysis

Large-scale DNA purification, restriction digestion, Southern blotting, and RFLP analysis were performed as described in Wight et al. (2003). AFLP analysis was performed as described in De Koeyer et al. (2004). Seventy RFLP clones were screened for polymorphism, and ten pairs of primers were used to generate AFLP data.

Sequence-characterized amplified region (SCAR) marker use

The SCAR primers developed by Gallego et al. (1998a) to mark aluminium tolerance in rye were synthesized by Sigma Genosys (The Woodlands, TX, USA). Gradient PCR performed in an Eppendorf Mastercycler Gradient machine was used to determine the optimal annealing temperature for these primers in oat under the following PCR conditions: 3 min at 94°C; 35 cycles of (1 min at 94°C, 1 min at 50–55°C, 2 min at 72°C), 10 min at 72°C, hold at 4°C. PCR reactions were conducted in a volume of 25 µl containing 1–2 mM genomic DNA, 200 nM of each primer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 U of *Taq* DNA polymerase (Invitrogen or MBI Fermentas), and 1X PCR buffer, as supplied by the manufacturer. Amplified fragments were separated in 1.8% agarose gels and visualized by ethidium bromide staining.

Further work was conducted using the primers $OPA08_{415}$-F (5′-GTGACGTAGGGTGCGTATGCA-3′) and $OPA08_{415}$-R (5′-GTGACGTAGGCAGGCTG-TAAG-3′), which generate the marker designated SCA08 by Gallego et al. (1998a). PCR was performed as described above, using an annealing temperature of 50°C.

Genes identified as candidates for conferring aluminium tolerance in plants were identified in the literature. Primers for these genes were developed in multiple steps. First, the GenBank (http://www.ncbi.nlm.nih.gov/) and TIGR (http://www.tigr.org/) sequence databases were searched for cDNA and genomic sequence data for each gene. When possible, only the information from cereal genes was used. Then, the program "MegAlign" (DNASTAR, Inc.) was used to compare the cDNA sequences from different species. If genomic DNA information was available, the program "EST2genome" from the European Molecular Biology Open Software Suite (EMBOSS (Rice et al. 2000); http://ngfnblast.gbf.de/cgi-bin/emboss.pl?_action=input&_app=est2genome) was used to detect the intron sequences in each gene. Finally, the program "Primer3" (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used to generate a number of primers for each gene. These were designed to flank the different intron sequences as well as target sequences that were conserved amongst the different cereal species. Gradient PCR was performed as described above.

Molecular marker and QTL mapping

Molecular mapping was performed using the programs "GMendel" (Holloway and Knapp 1993) and "Mapmaker v. 3.0" (Lander et al. 1987) as described in Wight et al. (2003). QTL were detected by simple interval mapping using the program "MQTL" (Tinker and Mather 1995). The experiment-wide false-positive rate for QTL main effects was estimated based on 10,000 random permutations, and QTL effects were estimated based on partial regression coefficients in a multi-locus linear model as described by Tinker et al. (1996). The proportions of phenotypic variance explained by each QTL and by the final multi-locus model were estimated using the R^2 statistics in the individual and multi-locus linear regressions.

Results

Phenotyping the LAG-211 population

Phenotypic data for the LAG-211 population are presented in Fig. 1. The histogram describes an approximately normal curve, suggesting that the aluminium tolerance trait in the LAG-211 diploid oat population is

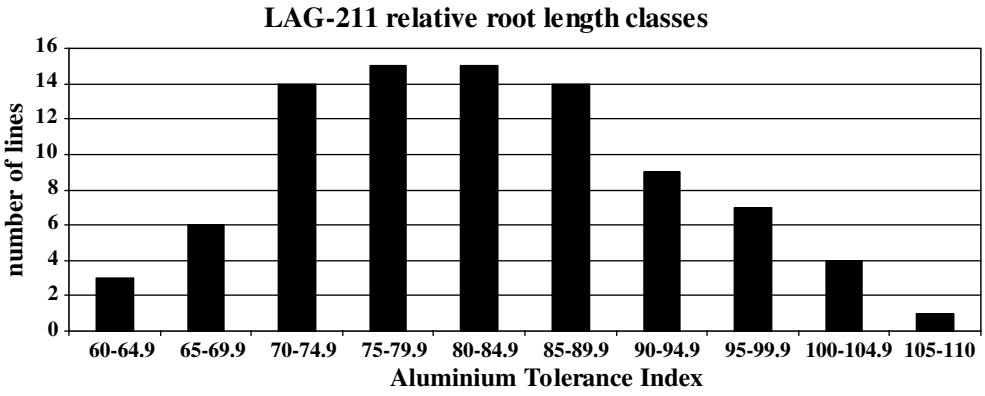

Fig. 1 Relative root length classes determined for the LAG-211 *A. strigosa* population. Aluminium tolerance index is defined as (mean root length in Al solution/mean root length in Al free solution)×100

controlled by more than one gene and/or is strongly affected by environment.

Comparative mapping of aluminium tolerance loci

Table 1 summarizes the regions of the AH genome from which RFLP markers were selected to test for linkage to the aluminium tolerance trait in the LAG-211 population. The comparative cereal QTL regions represented by them are also listed. Supplementary Fig. S1 illustrates details of the comparative mapping process and describes the results.

Markers for candidate genes

Although 74 primer pairs for ten candidate genes were tested, the only primers that generated polymorphic products in the LAG-211 population were those designed based on alignments for DNA sequences related to one form of calreticulin. Primers derived from a consensus calreticulin gene sequence were named calretB1L (5′-GAAAAGGAGCGAAGGGAAAG-3′) and calretB3R (5′-CAATCCATGGGATCTTCCAT-3′). Amplifications of genomic DNA using these primers were performed using an annealing temperature of 52°C. Although the calreticulin primers produced many bands

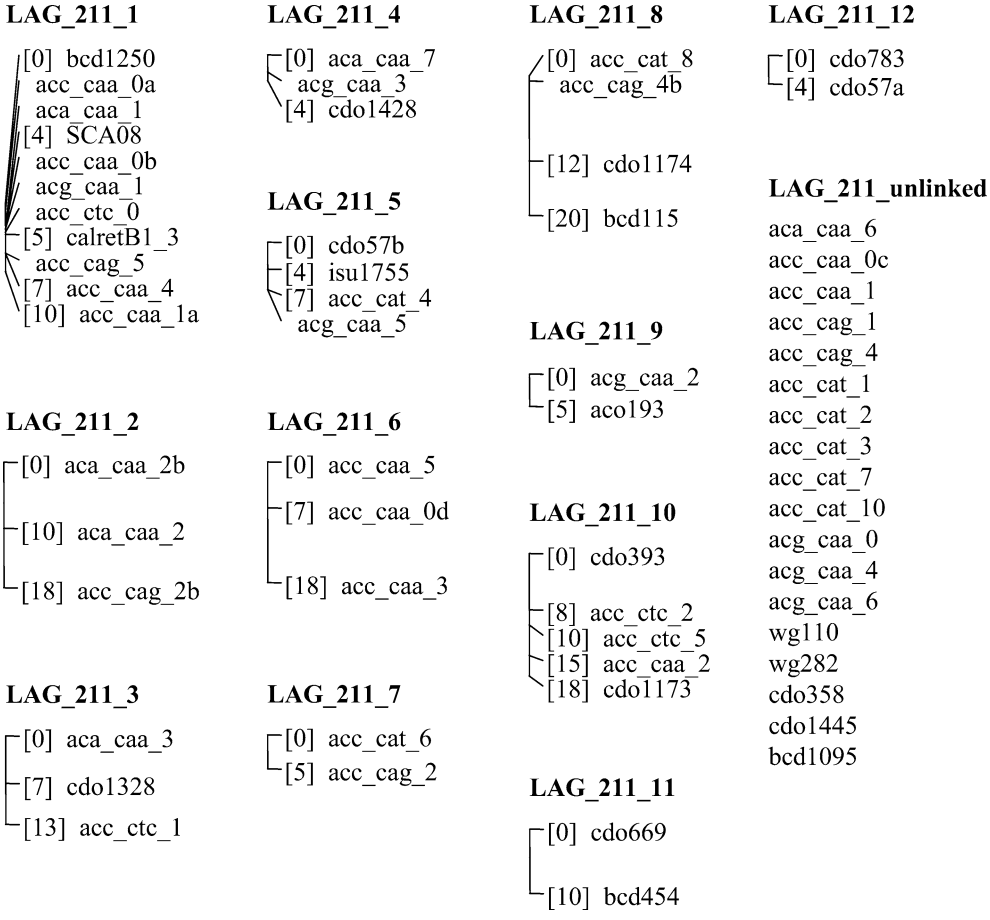

Fig. 2 Molecular marker map of the LAG-211 *A. strigosa* population. Framework linkage groups were drawn in the manner of Wight et al. (2003). All placed markers are named

Table 1 Regions of the *Avena atlantica* Baum and Fedak × *A. hirtula* Lag. (AH; O'Donoughue et al. 1992) diploid oat reference map chosen to represent aluminium tolerance genes and quantitative trait loci (QTL) from other species

AH regions chosen			QTL regions represented			
AH linkage group	Region chosen	Length (cM)	Species	Chromosome	Gene or QTL region	Reference
A	cdo580-cdo312 (including cdo1173.1)	8	Rice	5	bcd454-rg470	Nguyen et al. (2001)
			Rice	10	em16_9-g333	Nguyen et al. (2002)
			Maize	6	p_bnlg238	Ninamango-Cárdenas et al. (2003)
				6	mmc0241-nc013	Ninamango-Cárdenas et al. (2003)
				6	(*Alm*2)-csu70-umc59	Sibov et al. (1999)
				8[a]	csu155-p_bnlg162	Ninamango-Cárdenas et al. (2003)
A	cdo1173.2 (duplicated locus)	0	(As above)	(As above)	(As above)	(As above)
B	bcd1829a-bcd1882	30	Rice	2	cdo395-cdo1417	Nguyen et al. (2001)
			Rice	4	rg449	Nguyen et al. (2002)
			Rice	7	rg650-rz626	Nguyen et al. (2003)
			Rice	7	me4_3-em15_11	Nguyen et al. (2002)
			Maize	2	umc139-p_mag1a01	Ninamango-Cárdenas et al. (2003)
			Maize	10	(*Alm*1)-umc130-npi232	Sibov et al. (1999)
C	cdo460	0	Rice	1	me7_4	Nguyen et al. (2002)
C	cdo1174-rz69	10	Rice	1	c86	Ma et al. (2002)
			Rice	1	rz252	Nguyen et al. (2003)
			Rice	1	me10_14-cdo345	Nguyen et al. (2002)
			Rice	1	rg780-wg110	Nguyen et al. (2001)
			Rice	1	rg381-rg323	Wu et al. (2000)
			Rice	4	rg449	Nguyen et al. (2002)
			Maize	8	csu155-p_bnlg162	Ninamango-Cárdenas et al. (2003)
			Maize	8	p_bnlg1031-mace01c01	Ninamango-Cárdenas et al. (2003)
			Sorghum	3	*Altsb*	Magalhaes et al. (2004)
D	bcd1872-rz242	27	Rice	6	s1520-g200	Ma et al. (2002)
			Rice	8	rg28-rm223	Nguyen et al. (2003)
			Rice	8	c1121-me5_3	Nguyen et al. (2002)
E	cdo795-bcd1643	9	Rice	3	me8_2-cdo122	Nguyen et al. (2002)
			Rice	11	rz53-rg1094	Nguyen et al. (2001)
			Maize	2	umc139-p_mag1a01	Ninamango-Cárdenas et al. (2003)
E	cdo457-rz614	30	Rice	3	rz142-rg996	Nguyen et al. (2001)
E	cdo412-cdo57	6	Rice	9	rg667-rm215	Nguyen et al. (2002)
			Rice	9	rm201-wali7	Nguyen et al. (2003)
			Rice	9	rg667-agg_cag11	Wu et al. (2000)
F	bcd1230-cdo127	4	Rice	3	cdo1395-rg391	Nguyen et al. (2003)
			Rice	3	acc_ctg2-agc_cag4	Wu et al. (2000)
			Rice	12	rg98-rg341	Nguyen et al. (2001)
			Rice	12	aac_ctc5-agg_ctg1	Wu et al. (2000)
			Rice	12	me2_9	Nguyen et al. (2002)
			Maize	10	csu359(*Alp*)	MaizeGDB
			Maize	10	(*Alm*1)-umc130-npi232	Sibov et al. (1999)
			Wheat	4DL	*Alt_bh*	Riede and Anderson (1996)
			Barley	4H	*Alp*	Tang et al. (2000)
			Barley	4H	*Alp*	Raman et al. (2003)
			Barley	4H	Alt gene	Raman et al. (2002)
			Rye	4R	*Alt*3	Miftahudin et al. (2002)
G	wg110-cdo1495	4	Rice	1	c86	Ma et al. (2002)
			Rice	1	rz252	Nguyen et al. (2003)
			Rice	1	me10_14-cdo345	Nguyen et al. (2002)
			Rice	1	rg780-wg110	Nguyen et al. (2001)
			Rice	1	rg381-rg323	Wu et al. (2000)
			Rice	2	r2510-r2460	Ma et al. (2002)
G	cdo1428b-cdo59	11	Rice	2	c1408-c1419	Nguyen et al. (2002)
			Rice	2	cdo395-rz273	Nguyen et al. (2001)
			Rice	7	rg650-rz626	Nguyen et al. (2003)
G	Uncertain	NA	Rye *via* Wheat[b]	6R *via* 6A[b]	*Alt*1	Gallego et al. (1998b)

[a]Underlined chromosome numbers identify QTL regions that could be matched to more than one AH region
[b]The wheat chromosome 6A map was used to establish the connection between the rye chromosome 6R and AH group G maps

Table 2 Summary of QTL affecting aluminium tolerance in the *Avena strigosa* population LAG-211

Linkage group	Peak marker	*P*(Type-1)[a]	Additive effect[b]
1	SCA08	< 0.0001	−11.37
10	acc_ctc_2	< 0.005	−4.45
Unlinked	acc_cat_2	0.01	−4.38
Unlinked	acg_caa_0	< 0.0005	−3.82

[a]Experiment-wide type-I error rate for simple interval mapping based on 10,000 random permutations of the full experiment
[b]Estimated using partial regression coefficients based on a multi-locus linear model containing four QTLs

under the PCR conditions used, the polymorphic band was larger than predicted based on DNA sequences.

Mapping in the LAG-211 cross

The molecular marker map generated for the LAG-211 population is presented in Fig. 2. Of the 70 RFLP clones tested, 18 (identified with asterisks in Fig. S1) identified polymorphisms in this cross, representing 9 of the 11 potential QTL regions. Sixty-one markers were mapped: 19 RFLP markers, 41 AFLP markers, and 2 SCAR markers (SCA08 and calretB1_3). Twelve linkage groups were formed and 18 markers remained unlinked.

The AFLP markers were used to provide additional coverage of the genome. Seven LAG-211 linkage groups contained AFLPs in addition to other types of markers, 3 groups contained only AFLP markers, and 13 AFLP markers remained unlinked. Both of the SCAR markers, SCA08 and calretB1_3, mapped to LAG-211 group 1.

QTL associations

Table 2 presents the QTL association results from the LAG-211 population. Four QTL were found using simple interval mapping (SIM). The QTL with the largest effect was associated with LAG-211 group 1, containing the AH group F-related marker bcd1250 as well as both SCAR markers. A second QTL was associated with LAG-211 group 10, which contains the AH group A markers cdo393 and cdo1173. The two remaining QTL were associated with unlinked AFLP markers. No QTL were found to be associated with any of the RFLP markers representing other AH regions.

When tested individually, the QTL associated with LAG-211 group 1 accounted for 39% of the phenotypic variation, the LAG-211 group 10 QTL accounted for 16%, the ACC_CAT_2 QTL accounted for 14%, and the ACG_CAA_0 QTL accounted for 20%. Together, the four QTL accounted for 55% of the total phenotypic variation in aluminium tolerance. The tolerant parent, CIav9011, contributed the positive allele in each case. No significant epistasis was detected between these QTL, or between these QTL and other genomic regions.

Utility of SCAR markers SCA08 and calretB1_3 in hexaploid oat germplasm

Figure 3 presents the results obtained when the SCAR markers SCA08 and calretB1_3 were tested across hexaploid oat germplasm. The calretB1_3 band associated with aluminium tolerance was present in all twelve hexaploid oat lines tested (Fig. 3a, ten lines not shown), while the SCA08 band was not present in any of the 12 hexaploid oat lines tested (Fig. 3b).

Discussion

Development of comparative mapping strategy

Given the accumulated evidence of intergeneric conservation of aluminium tolerance loci within the grasses, a comparative mapping strategy was developed to identify candidate genomic regions for the multiple aluminium tolerance loci expected in the LAG-211 population. No prior mapping information was available for this population, and it was reasoned that this strategy could identify informative loci more quickly than a strategy based on anonymous markers. More meaningful intergeneric comparisons could also be made through the use of RFLP-based markers.

Although a substantial body of information was available in the literature for comparative mapping (Table S1), a number of complicating factors needed to be considered when interpreting these data. Firstly, it is unlikely that any single experiment will detect all (and only) the relevant genes that are segregating in a population. Secondly, although aluminium tolerance is generally measured as the degree of damage to the root system, different methods are used to inflict and assess this damage. Thirdly, different studies applied different statistical methods and error tolerances in their QTL analyses. Lastly, comparative mapping is complicated by evolutionary history as it pertains to changes in ploidy level, gene loss, and gene duplication. Barley, rye, and diploid oat contain seven chromosomes, diploid sorghum contains ten, and rice contains 12, while maize, an ancient tetraploid, contains 10, and hexaploid wheat and oat contain 21. A theoretical "ancestral" genome map of cereals has been assembled (Moore et al. 1995), and the accumulated evidence for genomic conservation is substantial. However, any region of apparent homology could be interrupted by major or minor regions of undetected differences, as was found to be the case by Miftahudin et al. (2005) in their attempt to clone the *Alt3* gene in rye using information from rice sequences.

Because of these factors, the comparative mapping results presented in the supplementary material (Fig. S1) need to be interpreted carefully. Nevertheless, we submit that these results provide a useful summary and a detailed guide for those interested in expanding these studies in any grass species.

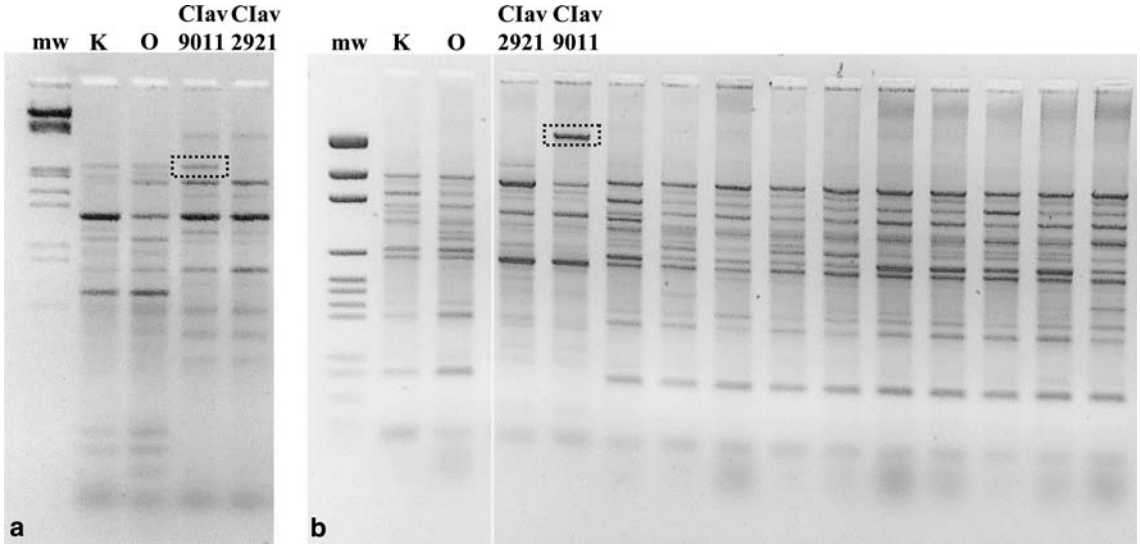

Fig. 3 Presence of SCAR marker bands in hexaploid oats. **a** The PCR results obtained using the calretB1_3 SCAR; **b** the results obtained using the SCA08 SCAR. The bands linked to aluminium tolerance in the LAG-211 population are highlighted. K and O contain DNA from the hexaploid oat varieties Kanota and Ogle. CIav2921 is the aluminium-sensitive *A. strigosa* parent, and CIav9011 is the tolerant parent. Unnamed lanes contain DNA from ten other hexaploid oat varieties or breeding lines

Selection of target regions for RFLP mapping

The AH oat map was used for the selection of regions to screen for aluminium tolerance QTL in diploid oat. A second diploid oat map, for the cross *A. strigosa* Schreb. × *A. wiestii* Steud. (Portyanko et al. 2001), was available for comparison; however, it contains more than seven linkage groups, seems to be missing a region expected to be homologous with AH group C, and was not available as part of the Gramene database.

As was expected, some of the relationships between the rice, maize, and diploid oat chromosomes were fairly easy to discern, while other relationships proved to be more complex. Six QTL regions, three from rice (on chromosomes 1, 4, and 7) and three from maize (on chromosomes 2, 8, and 10) showed homology with two different AH linkage groups (Table 1; Fig. S1, panels i, ii, iii, v, vii, and viii).

Perhaps the most important of these is the QTL region on rice chromosome 1 identified in five different rice populations (Ma et al. 2002; Nguyen et al. 2001, 2002, 2003; Wu et al. 2000). This region represents one of the major QTL for aluminium tolerance in rice. Because rice chromosome 1 has extensive homology to chromosome 3 of the Triticeae (Van Deynze et al. 1995b; Sorrells et al. 2003), this region may be orthologous to aluminium tolerance genes residing on sorghum chromosome 3, rye chromosome 3R, and chromosome 3RS in triticale. The importance of this region is further emphasized by the recent *in silico* mapping to this same QTL interval of two genes whose expression in rice is upregulated by exposure to aluminium (Mao et al. 2004). However, while this chromosome seems to be homologous with AH group C (Table 1, Fig. S1iii), the marker wg110, which is associated with this QTL in two of the rice populations and is present on chromosome 3 in both wheat and rye, does not reside on AH group C, which otherwise has extensive homology with chromosome 3 of the Triticeae. Rather, it is located on AH group G.

The relative locations of the QTL on maize chromosome 10 are also of some importance. One half of one maize chromosome 10 region containing an aluminium tolerance QTL shows homology with rice chromosome 4 and AH group B, while the other half shows homology with rice chromosome 12 and AH group F (Table 1; Fig. S1i and vii).

It is also uncertain whether there are one or two QTL on maize chromosome 10. Sibov et al. (1999) provided evidence of an aluminium tolerance QTL associated with the marker umc130, yet mapped *Alm*1 as a single gene 20 cM away, in the direction of the marker csu359. This csu359 marker has sequence homology to the *wali*7 aluminium-induced gene found in wheat (Richards et al. 1994) and has been designated *Alp* in the MaizeGDB database (http://www.maizegdb.org/; Lawrence et al. 2004). It is clearly in the region homologous to AH group F. Interestingly enough, a *wali*7 probe also highlights a QTL on rice chromosome 9 (Nguyen et al. 2003), which shows homology with AH group E (Fig. S1vi).

In addition to the situation with maize chromosome 10, there were three cases where it was difficult to decide whether one or more QTL were present on the chromosome (rice chromosomes 7, 8, and 12). While these were generally minor QTL, the case of rice chromosome 12 is noteworthy (Table 1, Fig. S1vii). One QTL was found on this chromosome in each of three studies, and the marker rg9 was mapped in all three populations. On the map of Wu et al. (2000), this marker defines the centre of the region containing the QTL. It is fairly close to, but outside of, a QTL region of greater length in the

population used by Nguyen et al. (2001), and it is also on the map of Nguyen et al. (2002), but at a distance of 85 cM from the QTL. Because different populations were used to generate these results, the question is: do these QTL actually represent different genes, or do chromosomal rearrangements resulting in changes in marker order mask the fact that the gene is the same?

Both rice chromosome 12 and the one end of maize chromosome 10 show homology with AH group F, and rice chromosome 3 represents portions of both AH groups E and F (Fig. S1v). AH group F contains the markers bcd1230 and cdo1395, one or both of which were found to link to the Alt_{BH} gene on wheat chromosome 4DL (Riede and Anderson 1996), the *Alp* gene on barley chromosome 4H (Raman et al. 2003; Tang et al. 2000), and the *Alt*3 gene on rye chromosome 4R (Miftahudin et al. 2002). The marker cdo1395 is also located on maize chromosome 10 in the region of the umc130 QTL, indicating that the *Alm*1/*Alp* gene(s) may be orthologous to those found in the Triticeae.

Rice chromosome 3 has extensive homology to chromosome 4 of the Triticeae (Van Deynze et al. 1995b; Sorrells et al. 2003), and one QTL found in two different populations is also linked to the cdo1395 marker (Fig. S1v); however, its effect on aluminium tolerance in rice is minor, in contrast to the importance of the gene in the Triticeae and maize (Nguyen et al. 2003; Wu et al. 2000). Nevertheless, this QTL may still represent an orthologous gene. As the QTL on rice chromosome 12 and the one from chromosome 4 (*via* maize chromosome 10) also show homology to this region, they may represent paralogous loci.

Because of the ambiguity concerning the placement of the QTL on rice chromosome 12, three regions corresponding to the three potentially different QTL on rice chromosome 12 were selected on the rice bridging map. One of these was in a region of chromosome 12 sharing no common markers with AH group F, and the other two identified one short region on AH group F. As the markers associated with the major aluminium tolerance gene in the Triticeae also mapped to this region, we were reassured that searching this small region was a good decision. However, this situation, as well as that concerning maize chromosome 10, serves to reinforce the idea that comparative mapping is not as straight forward a process as it may first appear.

LAG-211 map construction

The RFLP linkages in three LAG-211 groups (5, 11, and 12) highlight the difficulties of comparative mapping in oat; namely, the existence of duplicated regions and those that have been rearranged. LAG-211 group 11 contains markers expected to identify regions homologous to AH groups E (cdo669) and A (bcd454). LAG-211 groups 5 and 12 also contain markers from different AH groups; however, each of these groups contains a locus highlighted by the clone cdo57, which marks group E in AH, but links to markers for groups D (isu1755) and C (cdo783) in the LAG-211 population.

The SCAR marker SCA08 was identified in the literature as being loosely linked to an aluminium tolerance gene on rye chromosome 6R (Gallego et al. 1998b). However, it is linked in the LAG-211 population to the RFLP marker bcd1250, which is located on AH group F. Group F should be homologous to rye chromosome 4R. While SCA08 mapped to chromosome 6R in rye (Gallego et al. 1998a), the primers produced many bands under the conditions used here to amplify oat DNA, and the band mapped is larger than that found in rye (approximately 3 kb vs. 415 bp).

Calreticulin was chosen as a candidate gene as it may increase intracellular calcium stores during aluminium stress, triggering callose formation, the closure of plasmodesmata, and reducing symplastic intercellular transport (Sivaguru et al. 2000). Using comparative mapping and the "rice chromosome *vs.* cereal genes" feature of the Graingenes database (http://wheat.pw.usda.gov/cgi-bin/gbrowse/japonica), calreticulin genes were found to be associated with most of the QTL on rice chromosomes 1, 3, 4, 5, 7, and 8. Two forms of calreticulin were found in the NCBI database. One form of the gene is represented by the calretB1_3 primers. The other form is represented by the oat clone cdo678, which is located on AH group B, in the region where an aluminium tolerance QTL might be expected. Unfortunately, the restriction fragments revealed by the cdo678 clone were monomorphic in the LAG-211 population.

Identification of QTL for aluminium tolerance in oat

The major QTL found in the LAG-211 *A. strigosa* population was associated with markers in LAG-211 group 1, which includes the AH group F marker bcd1250. This suggests that the AH group F region of diploid oat surveyed contains a gene that is orthologous to the major aluminium tolerance genes found in wheat, rye, barley, and perhaps maize, as well as a minor QTL in rice. It is also intriguing that the calretB1_3 marker is so closely linked to the major QTL in diploid oat, although the current study provides no direct evidence for the involvement of calreticulin in conferring aluminium tolerance in this species.

One of the smaller QTL in LAG-211 was associated with group 10, which includes the AH group A markers cdo393 and cdo1173. This marker linkage suggests that the QTL is in the region of cdo1173.2, and not cdo1173.1, as might have been expected. While cdo1173 can be associated with the *Alm*2 gene found on maize chromosome 6, the peak marker for the QTL on LAG-211 group 10 was cdo393, and this marker is located at the opposite end of the chromosome from cdo1173 on both maize chromosome 6 and rice chromosome 5. The marker bcd454, associated with aluminium tolerance QTL on maize chromosome 6 and rice chromosome 5 but not in the LAG-211 population, is located in the

middle of the maize and rice chromosomes. Therefore, we can speculate with caution that the QTL associated with LAG-211 group 10 is orthologous to the *Alm2* gene. To highlight the complexities associated with molecular mapping in oat further, the AH group A clones cdo393 and cdo1173 and the AH group F clone bcd1230 define a region on KO group 5 that spans only 17 cM and contains a marker cluster defining the probable location of an AH group A /F translocation breakpoint (Wight et al. 2003).

No QTL were found to be associated with any of the RFLP markers chosen to represent regions of the other AH groups. This included wg110, as well as the other markers representing AH group C or G, indicating that the aluminium tolerance gene found on rice chromosome 1, chromosome 3R in rye and triticale, and sorghum chromosome 3 may not be important in diploid oat or may not be polymorphic in this germplasm.

There may be several reasons why the two QTL associated with anonymous AFLP markers did not link to RFLP markers from the targeted regions. Firstly, some regions defined by comparative mapping may have been incorrect, either because of differences in marker orders between maps (e.g. the locations of rg9 and wg110) or because of a low number of common markers between maps. Secondly, polymorphic RFLP markers were not identified for every region of the genome harbouring a potential QTL for aluminium tolerance. Thirdly, we may have identified aluminium tolerance QTL operating solely in oats, or not showing any contrast between the parents of the populations used in other studies.

Error control *versus* genome coverage

Genome mapping in this population was targeted towards regions where comparative mapping provided hypotheses for QTL presence. Although this provided practical advantages, it also provided increased statistical power for those regions that were tested. This advantage is a result of the reduced number of statistical tests that are applied through interval mapping, and the corresponding reduction in the significance threshold that must be met in order to control the experiment-wide error rate. The trade-off for increased statistical power is the possibility that QTL segregating in this population may not have been discovered because of incomplete genome coverage. However, given the amount of variance explained by the detected QTL, it is unlikely that additional QTL with major effects exist in this population.

Markers available for marker-assisted selection

While the diagnostic band produced by the calretB1_3 marker was present in all the hexaploid oat germplasm tested, it may yet prove useful in other oat germplasm or if the bands can be sequenced and new primers designed. The SCA08 marker, on the other hand, was not present in the hexaploid oat germplasm, and should prove useful for breeders wanting to introgress the major gene for aluminium tolerance from *A. strigosa* CIav9011 into elite, hexaploid oat germplasm. However, because the banding pattern is complex, future work will also include modifying the design of these primers.

This work has demonstrated the effectiveness of using different types of information from different species to identify the locations of genes for a complex trait in a diploid oat population previously uncharacterised by molecular mapping.

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