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Chromosome-anchored QTL conferring aluminum tolerance in hexaploid oat

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Abstract Aluminum (Al) toxicity is a major constraint on crop production in acid soils around the world. Hexaploid oat (*Avena sativa* L.) possesses significant Al tolerance making it a good candidate for production in these environments. Genetic improvement for Al tolerance in oat has traditionally been achieved through conventional plant breeding and could be enhanced by marker-assisted selection. The objectives of this study were to develop a chromosome-anchored genetic map for an oat recombinant inbred population and to identify SNP markers linked to quantitative trait loci (QTL) affecting root growth response to Al. Three QTL on chromosomes 7C-17A, 13A, and 19A conferring Al tolerance were identified using primary root regrowth of recombinant inbred lines derived from the cross between UFRGS 17 (Al tolerant) and UFRGS 930598-6 (Al sensitive). Localization of each QTL onto the sequenced rice genome revealed the genetic region on chromosome 13A might be associated with a putative malate transporter locus (LOC_Os06g15779). Studies of root apex tissue indicated that exudation of malate was increased in the Al-tolerant parent UFRGS17 and not in the sensitive parent. Based on these data, the malate transporter might be a candidate gene responsible for one of the Al tolerance QTL identified in this study.

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Introduction

Aluminum (Al) is the third most prevalent metal in the earth's crust and is predominately insoluble and non-toxic to plants at a soil pH above 5.5. At a pH below 5.5, Al becomes soluble, especially as the Al^{3+} cation, and becomes toxic to plants (Kobayashi et al. 2013; Merino-Gergichevic et al. 2010) by reducing cell wall, plasma membrane, and cytoskeleton stability and inhibiting cell division and elongation in root (Ryan et al. 2011; Sivaguru et al. 2000). Stunted roots result in reduced nutrient and water uptake, thereby restricting plant growth (Foy et al. 1978). Overall, Al toxicity in low pH soils has a major impact on crop production.

Surface liming is a common practice for ameliorating topsoil acidity in Al-laden soils. This approach is limited under no-tillage agriculture where lime is applied only to the soil surface. In this situation, acidic conditions will occur in the subsoil layers (Rossiello and Netto 2006) resulting in Al toxicity. Development of more tolerant crops to Al could provide a more comprehensive and economical alternative. In several crop species, genetic variability for Al tolerance has been exploited to develop Al-tolerant varieties and to study the function of the genes involved in the tolerance (Collins et al. 2008).

Two mechanisms have been reported for Al tolerance in plants including exclusion from the root apex by secretion of organic acids and cellular tolerance (Kochian et al. 2005). In wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor* L. Moench), Al tolerance has been attributed to the action of a single dominant gene, coding proteins that participate in the transport of organic acid (Delhaize et al. 2007; Magalhães et al. 2007; Minella and Sorrells 1997). However, in the wheat cv. Chinese Spring, three quantitative trait loci (QTL) that enhanced root growth under Al stress were identified, suggesting that inheritance of Al tolerance is a more complex (Ma et al. 2006). Similar to *Arabidopsis thaliana* and maize (*Zea mays* L.), in rice (*Oryza sativa* L.) and soybean (*Glycine max* L.), a polygenic inheritance is suggested by the identification of nine and five Al tolerance QTL, respectively (Bianchi-Hall et al. 2000; Maron et al. 2010; Nguyen et al. 2001). Genetic studies suggest the involvement of one or two genes in response to Al tolerance in oat (*Avena sativa* L.) (Castilhos et al. 2011; Nava et al. 2006; Sanchez-Chacon et al. 2000; Wagner et al. 2001). Oat, an allohexaploid species, with $2n = 6x = 42$ chromosomes, originated from the aggregation of three ancestral diploid genomes AA, CC, and DD (Rines et al. 2006). Oat is an important cereal crop worldwide with a majority of the production focused in temperate climates of Europe and North America (Murphy and Hoffman 1992). In subtropical environments, oat plays an important role for grain production during the winter season in no-tillage rotation with soybean. It is considered an Al-tolerant species.

The identification of DNA markers tightly linked to Al tolerance genes or QTL in mapping populations provides efficient tools for the application of marker-assisted selection (MAS) in breeding programs and may help identify the mechanism associated with tolerance (Silva-Navas et al. 2012; Wang et al. 2007). The availability of genetic markers for oat is limited, because of the difficulty in developing linkage maps due to the large size and complexity of the genome (Arumuganathan and Earle 1991; Portyanko et al. 2001; Wight et al. 2003). In addition, numerous chromosome rearrangements disrupted the expected colinearity among oat sub-genomes (Jellen et al. 1994). Recently, substantial effort was directed to the generation of high-resolution maps for oat. A significant contribution in oat molecular biology was delivered in 2011 with the development of first oat single nucleotide polymorphism (SNP) markers. This achievement was followed by the development of the first Illumina GoldenGate SNP array in 2013, which facilitated the first physically anchored map for hexaploid oat representing all 21 chromosomes (Oliver et al. 2013). The GoldenGate SNP array was upgraded to the Illumina Infinium array targeting 4975 loci across the oat genome (Tinker et al. 2014). The objectives of this study were to develop a chromosome-anchored genetic map of a Brazilian oat population using SNP markers and to identify markers linked to QTL that affect the response to Al tolerance in oat.

Materials and methods

Plant material

A genetic population of recombinant inbred lines (RILs) derived from the cross between UFRGS 17

(COR[2]/CTZ[3]/PENDEK/ME1563/76-29-76-23/75-28/
CI833) and UFRGS 930598-6 (UFRGS15/
UFRGS881920) was analyzed in this study. The
population consisted of 162 F_5-derived RILs devel-
oped by single-seed descent. This population was
selected based on the differential response to Al
toxicity of the parental lines (Nava et al. 2006;
Oliveira et al. 2005). Parental lines UFRGS 17 (Al
tolerant) and UFRGS 930598-6 (Al sensitive) were
developed by the oat breeding program at the Federal
University of Rio Grande do Sul (UFRGS), Brazil.

Phenotypic screening for Al tolerance

Parental lines and RILs from the cross UFRGS
17 × UFRGS 930598-6 were evaluated in hydro-
ponic solutions under controlled conditions as
described in previous study (Nava et al. 2006). In
brief, the root regrowth after Al exposure of 159
RILs was evaluated in a complete randomized
design. Each replicate consisted of a sample of
about 10 seedlings per RIL. The hydroponic solu-
tions consisted of an Al treatment and Al-free
control. The Al-free solution contained 4 mM
$Ca(NO_3)_2 \cdot 4H_2O$, 2 mM $MgSO_4 \cdot 7H_2O$, 4 mM
KNO_3, 0.435 mM $(NH_4)_2SO_4$, 0.5 mM KH_2PO_4,
2 µM $MnSO_4 \cdot 1H_2O$, 0.3 µM $CuSO_4 \cdot 5H_2O$, 0.8 µM
$ZnSO_4 \cdot 7H_2O$, 30 µM NaCl, 0.1 µM $Na_2MoO_4 \cdot 2H_2$-
O, and 10 µM H_3BO_3. The iron source was a high-
performance iron chelate, comprised of 6 % of
chelate iron and 92 % of an orthoisomer, with
0.9 µM of iron in the solution. The Al solution was
identical to the Al-free solution with the exceptions
of KCl in place of KH_2PO_4 to prevent Al precipita-
tion and a tenfold reduction in the overall concen-
tration. The Al source was aluminum sulfate
$(Al_2(SO_4)_3 \cdot 18H_2O)$ at the final concentration of
740 µM.

Oat seedlings were first grown for 48 h in the
hydroponic Al-free solution and then transferred to the
aluminum solution for 48 h. After Al treatment, the
seedlings were transferred back to the Al-free solution
for an additional 72 h. Root growth reinitiated after
removal from Al solution, and root regrowth of the
primary root from each seedling was measured
starting at the point of root thickening. The availability
and activity of Al^{3+} in the solution was calculated
using the software Visual MINTEQ 3.0 (Gustafsson
2010).

Aluminum localization in root apex across sections

Seeds from both parental lines with similar size were
selected for Al localization studies. Lemma and palea
tissues were manually removed, and the seeds were
soaked for 1 min in a solution containing 0.75 %
sodium hypochlorite and 0.05 % Tween 20. Seeds
were washed five times with ddH_2O, laid on germi-
nation paper, and incubated at 21 °C in the dark. After
germination, seedlings with similar root size (2 mm)
were transferred to a hydroponic solution containing
either 0 or 500 µM Al. After 14 days, the roots were
washed three times with ddH_2O for 20 min and stained
with hematoxylin 2 g L^{-1} and KIO_3 0.2 g L^{-1} for
30 min. The excess of dye was removed by successive
washes with ddH_2O, and the roots were fixed in
ethanol and stored in 50 % glycerol. Root tips were
sectioned and analyzed using an optical microscope
model Olympus BX41. Three independent biological
replicates were used, each one containing 10 plants.

Aluminum and malate quantification

Seedlings from each parental line were transferred to
small pots containing 40 mL of Al-free hydroponic
solution and incubated for 5 days to allow root
development. Seedlings were then transferred to either
a new Al-free solution or a solution containing
500 µM Al. After 24 h, the hydroponic solutions were
collected, frozen, lyophilized (L101, Liotop) to con-
centrate the solutes, and used to measure malate
secreted by the plants with the L-malic acid enzymatic
assay kit (Megazyme, Wicklow, Ireland). The roots
and shoots from each plant were washed with ddH_2O,
separated, and macerated, and the amount of Al was
measured by inductively coupled plasma mass spec-
trometry (ICP-MS). Three independent biological
replicates were used, each one containing 20 plants.

Statistical analysis

The data compiled were submitted to analysis of
variance (ANOVA), and the differences between the
means were compared by t test at $P \leq 0.05$.

DNA isolation and purification

Etiolated shoots of each parental line and RIL were
harvested 7 days after planting, frozen in liquid

nitrogen, and ground. DNA extraction was performed using a cetyl trimethyl ammonium bromide (CTAB)-based protocol as follows: Ground tissue was homogenized with 0.5 mL of extraction buffer containing 0.15 M sorbitol, 0.22 M Tris–HCl pH 8.0, 23 mM EDTA pH 8.0, 0.8 M NaCl, 0.8 % (w/v) CTAB, and 1 % (w/v) sarcosine, at 65 °C for 25 min. DNA was extracted with 0.3 mL of chloroform/isoamyl alcohol solution (24:1), precipitated with chilled 70 % isopropanol, washed with ethanol 70 % twice, dried overnight, and resuspended in TE pH 8.0. DNA was quantified using NanoDrop (ND 1000 Spectrophotometer) and diluted to 150 ng L^{-1}.

KASPar-based SNP analysis

Single nucleotide polymorphism markers (SNPs) from the Illumina GoldenGate Oligonucleotide Assay (Oliver et al. 2013) were used for genotyping the UFRGS 17/UFRGS 930598-6 mapping population based on KASPar technology (LGC, UK). A total of 960 contigs containing SNP loci were selected based on the polymorphism across the original oat diversity panel. KASPar SNP assays were then designed using the primer designer software PrimerPicker (KBioscience Ltd., Hoddesdon, UK) with default parameters. SNP genotyping was performed on the Fluidigm (Fluidigm Corp., South San Francisco, CA) nanofluidic 96.96 dynamic array (Wang et al. 2009) using competitive allele-specific PCR specified by the KASPar genotyping chemistry (KBioscience Ltd., Hoddesdon, UK). For genotyping on the 96.96 dynamic array chip, a 5-µL sample mix, consisting of 2.25 µL genomic DNA (20 ng/µL), 2.5 µL of 2× KASP reagent mix (KBioscience Ltd.), and 0.25 µL of 20× GT sample loading reagent, was prepared for each DNA sample. Similarly, a 4-µL 10× KASP assay, containing 0.56 µL of the KASP assay primer mix (allele-specific primers 12 µM, common reverse primer 30 µM), 2 µL of 2× assay loading reagent, and 1.44 µL DNase-free water, was prepared for each SNP assay. The assay mix and sample mix were then loaded onto a 96.96 dynamic array chip, mixed, and subjected to polymerase chain reaction (PCR) using an IFC Controller HX and FC1 thermal cycler (Fluidigm Corp., South San Francisco, CA) according to the manufacture's protocols. PCR consisted of an initial denaturing step (70 °C—30 min; 25 °C—10 min), a hot-start Taq polymerase activation step (94 °C—15 min),

followed by a touchdown amplification protocol as follows: 10 cycles of 94 °C for 20 s, 65 °C for 1 min (decreasing 0.8 °C per cycle), 26 cycles of 94 °C for 20 s, 57 °C for 1 min, hold at 20 °C for 30 s. Endpoint fluorescent images of the chip were acquired on an EP-1 imager (Fluidigm Corp., South San Francisco, CA) and the data analyzed with Fluidigm SNP Genotyping Analysis Software.

Polymorphisms were scored across 153 of the 162 RILs. Chi-square tests were performed on each locus for goodness of fit to the expected 1:1 segregation ratio. Markers that deviated significantly ($P < 0.05$) from the expected segregation ratio were excluded for linkage analysis and map construction.

Linkage mapping

A linkage map was developed for the mapping population using MapMaker/EXP version 3.0 software (Lander et al. 1987). Mapping information was available for 115 polymorphic markers that were known to belong to 15 of the 21 chromosomes of the oat genome, from the oat consensus map (Oliver et al. 2013). Markers were anchored to their corresponding chromosome, according to the reference map, and the software command compare was used to calculate all possible marker order within each group and the maximum likelihood of the map. After the calculation of optimized distances between markers, 11 of those 115 markers were removed because they were causing distortion. Linkage groups were recalculated with 30 unanchored markers using LOD 3, and the Kosambi's mapping function was used to convert the recombination frequency into centimorgans (cM), and twelve of those were integrated into the map, totalizing 116 integrated markers in the map. Chromosomes 11A, 14D, 17A-7C, and 19A contained gaps and were broken into two linkage groups each to prevent distortion in the QTL analysis due to the large interval between markers. The final map was visualized using Mapchart 2.2 (Voorrips 2002).

QTL analysis

Quantitative trait loci (QTL) associated with primary root regrowth were detected using the single marker analysis and the simplified composite interval mapping procedure (sCIM) in MQTL (Tinker and Mather 1995). Stepwise regression with a threshold value of

$P = 0.05$ was used to select cofactors for sCIM. Parameters for analysis were an interval of 0.5 cM and an experiment-wise Type 1 error rate of 5 %. In order to identify significant QTL, an experiment-wide false-positive rate was established by running 10,000 random permutations, for details see Churchill and Doerge (1994). The test statistic was then converted to a LOD score by multiplying with 0.22 (Tinker and Mather 1995). QTL intervals were assigned to the area within one LOD score from the QTL peak.

Results

Phenotypic screening for Al tolerance

Twenty-eight percent of the Al in our experiments was in the Al^{3+} form (52.8 μM). The parental lines displayed very diverse response to Al. Roots of UFRGS 17 (tolerant) were significantly longer (*t* test, $P \leq 0.05$) than those of UFRGS 930598-6 (sensitive). The mean root regrowth of UFRGS 17 was 22.9 mm, the minimum regrowth observed was 16 mm, and the maximum regrowth observed was 26 mm, whereas UFRGS 930598-6 displayed mean root regrowth of 2.6 mm, the minimum regrowth

observed was 1.2 mm, and the maximum regrowth observed was 4.0 mm. The primary root regrowth distribution from the UFRGS 17/UFRGS 930598-6 mapping population varied from 1.2 to 48 mm (Fig. 1) with a mean and standard deviation of 10.6 and 9.5 mm, respectively. The heritability of primary root regrowth after Al exposure for this inbred population was 0.90.

UFRGS 930598-6 accumulates more aluminum in root apex than UFRGS 17

The concentration of Al was higher (*t* test, $P \leq 0.05$) in the roots of UFRGS 930598-6 and UFRGS 17 (1001.63 and 616.25 μg g^{-1}, respectively) than in the shoots (209.07 and 113.00 μg g^{-1}, respectively), after 24 h of Al exposure. Less Al was observed by hematoxylin stain in the UFRGS 17 root apex than in the UFRGS 930598-6 apex after 14 days of exposure to 500 μM Al. External portions of roots were colored by oxidation of the hematoxylin–Al^{3+} complexes except the meristem region of UFRGS 17 roots (Supplementary Figure S1a). The analysis of sequential cross sections (1 mm from the root tip) showed difference in the Al localization between the parental lines. In UFRGS 17, the Al was restricted to
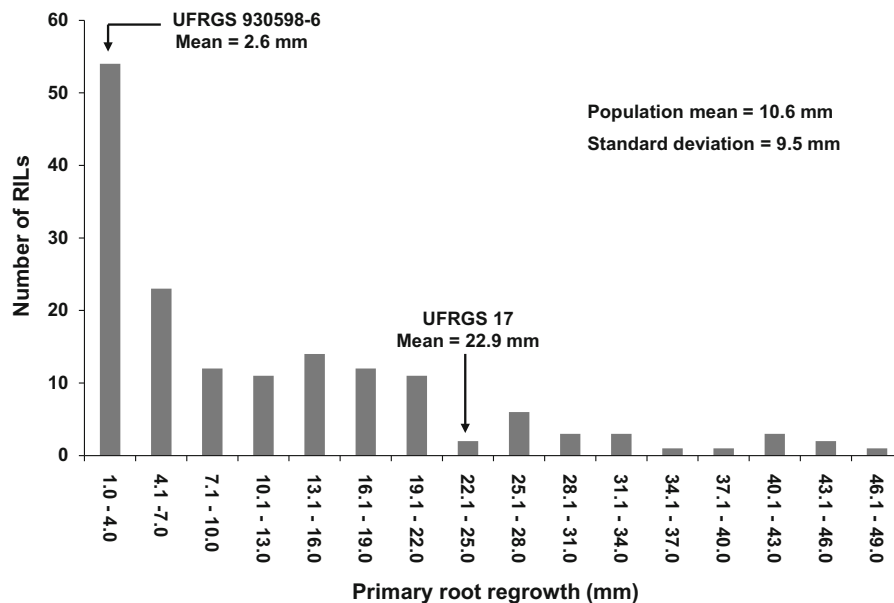
Fig. 1 Distribution of mean primary root regrowth from the oat mapping population UFRGS 17 × UFRGS 930598-6 evaluated in a hydroponic solution under controlled conditions. Means of parental lines tested adjacent to RILs are indicated with *arrows*

outer cells, while it was observed throughout the cortex tissue in UFRGS 930598-6 (Supplementary Figure S1b).

UFRGS 17 exudes malate in response to aluminum

UFRGS 17 roots exuded significantly higher (*t* test, $P < 0.001$) amounts of malate after 24 h of exposure to Al than UFRGS 930598-6. UFRGS 17 exuded 49.33 ± 9.45 nmol/plant of malate in response to Al, whereas no measurable malate amounts were exuded by UFRGS 930598-6. Measurable malate exudation was not detected in the Al-free control for either parental line even in the absence of phosphorus.

KASPar SNP analysis and linkage map

A total of 960 SNP markers were evaluated in this study. From these, 145 were polymorphic among UFRGS 17/UFRGS 930598-6 RILs. Due to distortion in the Mendelian segregation analysis, 29 of the 145

polymorphic markers were removed. The 116 remaining SNP loci (80 %) were assembled into 19 linkage groups, which corresponded to 15 chromosomes on the physically anchored oat map (Table 1). Of the 15 corresponding groups, 1C, 3C, 4C, 5C, 6C, 7C-17A, 8A, 9D, 11A, 12D, 13A, 14D, 16A, 19A, and 20D were represented. Some linkage groups were very small, like in the linkage group 7C-17A, where the markers found GMI_ES15_444 and ES17_c18155_185 cosegregated with BA_grs_14655_201. The largest linkage group was chromosome 5C, which covered 180 cM and contained 22 markers (Fig. 2). Based on the marker location in the oat consensus map and to prevent distortion in the QTL analysis due to large interval between markers, two independent linkage groups were associated with each one of the chromosomes 7C-17A, 11A, 14D, and 19A. Overall, the linkage map assembled in this study covered 32, 62, and 28 % of the genomes A, C, and D, respectively, when compared to the oat consensus map (Table 1).

Table 1 Genetic map statistics of linkage group length, number of markers per linkage group, chromosome, and genome

Linkage Group	Size (cM)	Markers	Chromosome[a]	Genome
1	17	7	8A	A
2	45	5	11A	A
3	2	2	11A (2)	A
4	61	7	13A	A
5	10	7	16A	A
6	7	2	19A	A
7	53	3	19A (2)	A
Total	195	33		
8	66	9	1C	C
9	50	8	3C	C
10	27	8	4C	C
11	180	22	5C	C
12	14	4	6C	C
13	0	3	7C-17A	C
14	66	6	7C-17A (2)	C
Total	403	60		
15	25	4	9D	D
16	57	5	12D	D
17	35	9	14D	D
18	1	2	14D (2)	D
19	20	3	20D	D
Total	167	23		
Total	765	116		

[a] Chromosome anchoring based on the oat consensus map (Oliver et al. 2013)

Fig. 2 Molecular map for the oat RIL population UFRGS 17 × UFRGS 930598-6 and QTL controlling aluminum tolerance. *Thick bar* represents QTL region and dot QTL peak
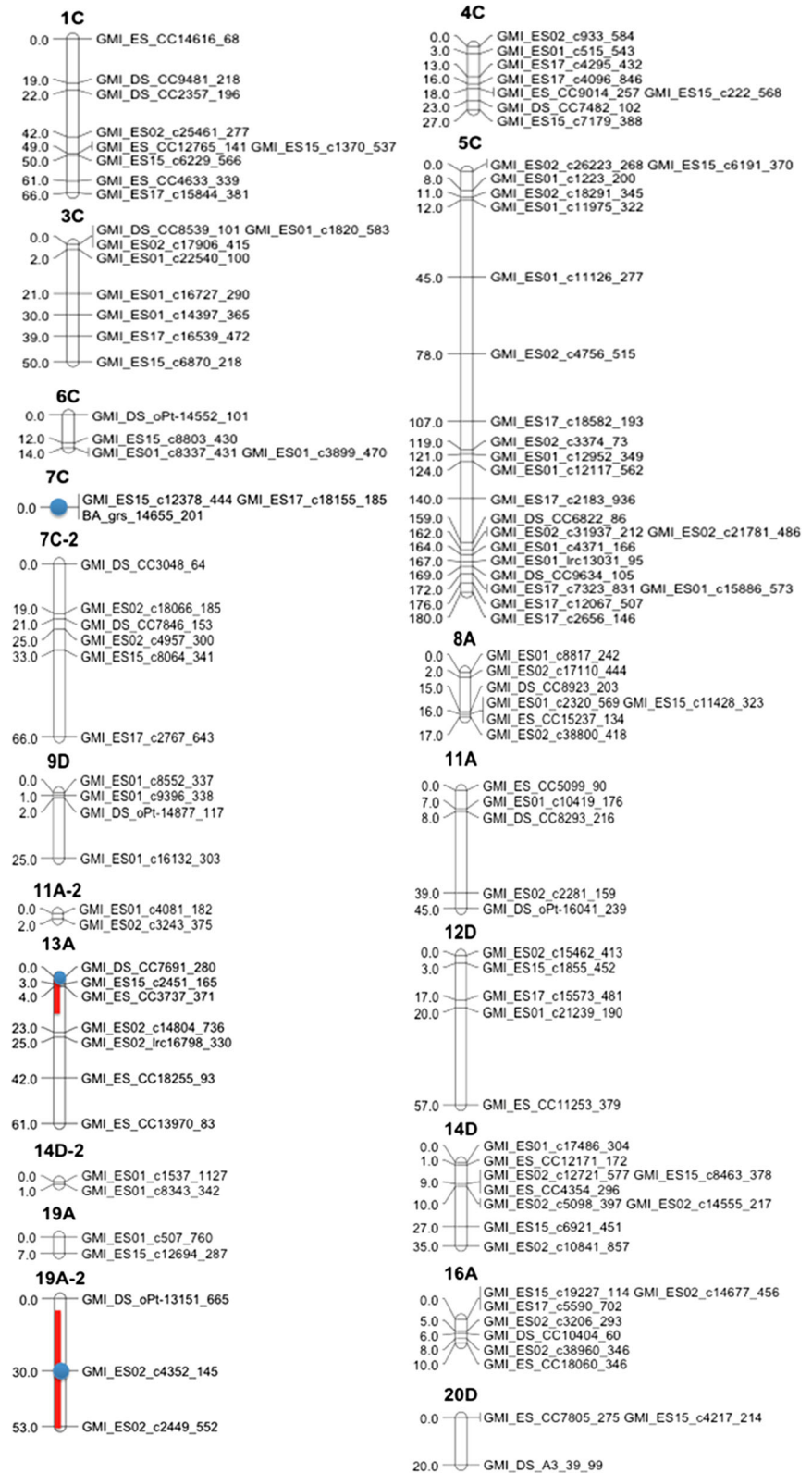
Locations and estimations of QTL

The statistical test established the threshold for QTL significance (LR) as 11.5 (LOD = 2.5). Three QTL were associated with Al tolerance based on the phenotypic variation in the root regrowth. The QTL were located on chromosomes 7C-17A, 13A, and 19A(2) (Fig. 2). Chromosome 7C-17A contained the QTL peak at the marker BA_grs_14655_201 with a test statistic (LR) of 16.9 (LOD = 3.72) and explaining 20.2 % of the phenotypic variation for root regrowth among assessed lines. The linkage group only consisted of three markers that were tightly linked ($\leq$1.5 cM). A second QTL (LR of 15.7 or LOD = 3.45) explaining 17 % of variation was located between 0 and 4.1 cM on chromosome 13A with the nearest marker GMI_ES15_c2451_165. The third QTL was located between 12 and 53 cM on chromosome 19A (LR of 21.4 or LOD = 4.71) and explained 33.8 % of the phenotypic variation (Table 2). Since this linkage group had only three markers with a large distance from each other, the position of this QTL is less precise, and its peak was at the marker GMI_ES02_c435_145.

Discussion

In order to develop Al-tolerant oat varieties to cope with the negative effects caused by soil acidity, it is important to understand the genetic and molecular factors involved. Currently, the mechanism of Al tolerance in higher plants is only partially understood. In wheat, the tolerance is based on the expression of a malate transporter gene, ALMT1 (Sasaki et al. 2006). In barley, a citrate transporter gene HvAACT1 is required for tolerance (Zhou et al. 2013). A 1-kb DNA insertion upstream of the HvAACT1 gene in some Al-tolerant genotypes (Fujii et al. 2012) and a 21-bp indel have been suggested as causes of the difference between tolerant and sensitive barley genotypes (Bian et al. 2013). In oats, however, the genes related to Al tolerance have not yet been identified.

Al^{3+} activity in a hydroponic solution has efficiently discriminated tolerance in the parental lines UFRGS 17 and UFRGS 930598-6 as measured by root regrowth in this study. Root regrowth demonstrates the resistance of the root apical meristem to the toxic effects of Al. In fact, this trait is well correlated with plant Al tolerance in field; therefore, it represents an efficient and fast screening methodology (Nava et al. 2015). UFRGS 17 had significantly higher root regrowth than UFRGS 930598-6. This result was expected since Al tolerance in UFRGS 17 has been reported previously (Castilhos et al. 2011; Hervé et al. 2013; Nava et al. 2006). Earlier studies suggested that Al tolerance in UFRGS 17 may involve an external mechanism reducing the uptake of Al by the root apex as well as the induction of an antioxidant mechanism to cope with the oxidative stress caused by Al (Castilhos et al. 2011; Pereira et al. 2013).

Our results showed higher Al concentration in the roots than in the shoots for both parental lines. The root meristem region of UFRGS 17, however, seemed to be more protected from Al entrance. This was suggested by the reduced amount of Al in internal cells over UFRGS 930598-6 based on color formation from the hematein/Al complex. Possible Al detoxification mechanisms may include: (1) selective permeability of the plasmatic membrane to reduce Al uptake (Archambault et al. 1997), (2) proteins that are connected to Al secretion (Basu et al. 1999), (3)

Table 2 Quantitative trait loci for primary root regrowth after aluminum exposure in UFRGS 17 × UFRGS 930598-6 RIL population and graphic representation

QTL	Flanking Markers	LR	Additive effect	Interval cM	QTL peak cM	Max. LOD	QTL effect %	Chromosome
1	GMI_DS_CC7691_280 GMI_ES15_c2451_165	15.7	−9.5	0–4.1	2	3.45	17	13A
2	GMI_ES15_c12378_444 BA_grs_14655_201	16.9	−8.6	0–1.5	1.5	3.72	20.2	7C-17A
3	GMI_DS_oPT-13151_665 GMI_ES02_c2449_552	21.4	−15.9	12–53	26–27	4.71	33.8	19A-2

phosphate efflux (Huan-Xin et al. 2009; Pellet et al. 1996; Zheng et al. 2005), (4) secretion of phenolic compounds (Ofei-manu et al. 2001), and (5) organic acids (Liu et al. 2009), which chelate Al ions. Initially, citrate was considered the main organic acid exudate for Al tolerance (Zheng et al. 1998) by *A. sativa* roots; however, recent studies have shown only malate to increase its secretion in response to Al (Radmer et al. 2012). Our data showing malate exudation by Al in roots of UFRGS 17 and not UFRGS 930598-6 roots support this recent work.

The amount of malate secreted by UFRGS 17 roots was similar to those detected from other tolerant oat genotypes in response to Al (Radmer et al. 2012). Therefore, UFRGS 930598-6 may have a defective malate transporter mechanism or a disruption in the Al-response pathway. The presence of other concomitant mechanisms cannot be discarded. Sensitive oat genotypes sharing the same hydroponic solution with tolerant ones do not differ from those grown in vessels containing only sensitive plants. It suggests that the organic acid secreted in the solution by the tolerant genotype is not enough to reduce the amount of toxic Al ions in the whole solution. Therefore, either there is a mechanism other than organic acid secretion involved in the tolerance or a significant amount of organic acid is retained in the apoplast and/or in the mucilage of tolerant genotypes.

Despite the phenotypic diversity observed between the parental lines as well as among the RILs, only a small number of polymorphic markers were found (15 %). This number is consistent with results from Oliver et al. (2013) where 16 % of the SNP loci interrogated by the Illumina GoldenGate assay were polymorphic across six different mapping populations on average. Low polymorphism is a common characteristic to species with large genomes (Kulcheski et al. 2010; Li et al. 2000; Yu and Herrmann 2006). It has been suggested that the low genetic polymorphism found in crops such as oat and wheat is due to the evolution of the species (Bryan et al. 1999). The polyploidy nature of the oat and wheat genomes could also be a major factor based on SNP assays binding to more than one genome. Illumina's platform was developed based on *Avena* spp., and choosing genotypes with significant genetic distance increases the usefulness of the platform. However, it is expected to have lower efficiency for comparison between genotypes from the same species and developed using the same germplasm.

About 20 % of the polymorphic SNP markers exhibited distorted segregation ratio within the genetic population. Distortion may occur from several reasons, such as preferential fertilization, DNA breakage during extraction, amplification of other genome regions or analysis error, and occasionally, it is a true biological phenomenon (Tan et al. 2001). Biological reasons for distortion include physical nearness between markers and genes involved in either gamete formation or genotype viability after fertilization (Vogl and Xu 2000). Because distorted ratios impact the association between markers and the segregation data for the phenotypic characteristic, causing false positives, it is recommended their exclusion from the data before the linkage map development. Our results indicate that the use of KASPar assays in place of the Illumina GoldenGate for scoring oat SNP markers was feasible and efficient. KASPar assays have the advantage of being cheaper and therefore speed up the gain of information about different populations.

The genetic map covered 736 cM (one marker every 6.3 cM, on average). This map coverage represents about 37 % of the total *Avena sativa* genome, based on the 2000 cM estimated by the consensus map (Oliver et al. 2013) and 25 % considering previous estimated sizes (2932 cM) (O'Donoughue et al. 1995). Despite the 19 linkage groups formed, 15 chromosomes were represented in our genetic map, when comparing the markers/chromosome assignments in the oat consensus map. The physically anchored consensus map was generated using monosomic lines to define the marker location in the hexaploid oat chromosomes (Oliver et al. 2013). In the same study, Oliver et al. (2013) found unexpected smaller numbers of markers located on chromosomes 10D, 17A-7C, and 21D, which were not represented in our genetic map. Both studies, Oliver's and ours, were based on markers derived from the same diversity panel. The lack of markers on 17A-7C may result from reciprocal translocation (Jellen and Beard 2000). Clearly, genome C was the best represented in our map. It covered 62 % of the genome C length regarding the oat consensus map size. The best coverage of genome C could be explained on the belief that the genomes A and D are very similar to each other. The new chip recently developed by Illumina containing 6000 SNP markers will be useful for increasing the map coverage (Tinker et al. 2014). It is foreseen that genotypes developed in a same

location would carry similar genetic adaptation blocks, not only as a result of similar background but also because these blocks are essential for adaptation to the local environmental conditions, reducing polymorphism in those regions. A more drastic example is the oat genome D, which seems to have higher genetic conservation than the other two, indicated by a lower level of polymorphism and the prevalence of major oat disease resistance genes (Oliver et al. 2013). The "genome asymmetry" suggests that one genome may have played a more important role than the others during evolution and domestication (Peng et al. 2003).

Three QTL for Al tolerance were identified on three different chromosomes in the UFRGS 17/UFRGS 930598-6 mapping population explaining 71 % of the phenotypic variation. Previous studies support our results by indicating Al tolerance is conferred by one or two dominant genes in *A. sativa* (Castilhos et al. 2011; Nava et al. 2006; Oliveira et al. 2005; Wagner et al. 2001). It is important to point out that root regrowth is not a discrete trait and a large variation has been observed among genotypes and individuals from the same genotype (Hervé et al. 2013). Root regrowth may be influenced by genes related to root growth rate and their interaction with the environment, including the plant response to phosphate limitation. Aluminum interacts with phosphate forming a precipitate interfering with Al availability; therefore, to analyze Al tolerance in hydroponic solution, phosphate must be omitted. As a result, phosphate starvation responses may have been induced as well. A phosphate starvation response can include reduction in root cell division and elongation. In the diploid species *A. strigosa*, four QTL were found affecting Al tolerance (Wight et al. 2003). Moreover, the Al tolerance in wheat was also considered a monogenic trait, and more than one QTL has been found suggesting a more complex inheritance, due to one or two major loci and several minor interacting loci (Navakode et al. 2009).

BLAST was used to localize EST-based SNP loci from oat onto related species with sequenced genomes. SNP loci in two regions contained sequences similar to genes deposited in the NCBI, but none of them showed similarity to genes involved in Al tolerance in other species. GMI_ES15_c2451_165 and GMI_ES15_c12378_444 were similar to XM_003557226.1, an oxidase-like protein, and to XM_003568689.1, an ABA receptor PYL8-like from

Brachypodium distachyon, respectively. The only oat SNP marker orthologous to rice was GMI_ES15_c2451_165 on oat chromosome 13A. This locus shared 87 % homology to the orthologous sequence LOC_Os06g01490 on rice chromosome 6.

Currently, there is no expressive information about mechanisms, genes or even loci related to Al tolerance in oat. The only published work was on the diploid species *A. strigosa* and, as previously mentioned, identified four QTL associated with Al tolerance (Wight et al. 2003). Two of them mapped to homologous rice chromosomes 3, 5, 10, and 12 containing Al tolerance genes (Wight et al. 2003). Rice chromosome 3 shows microsynteny to Al tolerance important regions in wheat chromosome 4DL, where the gene *TaALMT1* is located, as well as in barley chromosome 4H, *HvMATE* location, and rye chromosome 7RS, where *ScALMT1* genes are situated (Collins et al. 2008; Raman et al. 2005; Wang et al. 2007). Comparative mapping allows the identification of orthologous regions affecting the trait across species and may help to identify the mechanism involved. Substantial macro-colinearity between oat and the model rice and *B. dystachion* genomes was identified recently (Oliver et al. 2013). The region of the QTL located at oat chromosome 7C-17A was collinear to the *B. dystachyon* chromosome 2, the region of the QTL at oat chromosome 13A was collinear to *B. dystachyon* chromosome 1 and rice chromosome 5, and finally, the one at oat chromosome 19A was collinear to *B. dystachyon* chromosome 2 and rice chromosome 1 (Oliver et al. 2013). At rice chromosome 1, a major QTL for Al tolerance exists and this region has extensive homology to chromosome 3 of Triticeae (Sorrells et al. 2003). Orthologous genes to the Al-related transcription factor, *STOP1*, were identified at *B. dystachyon* chromosome 2 and rice chromosome 1 (Genome sequencing and analysis of the model grass *Brachypodium distachyon* 2010; Project 2008; Consortium TRCS 2005). A MATE citrate transporter gene, *OsFRDL4*, conferring minor effects to Al tolerance was mapped to rice chromosome 1 (Yokosho et al. 2011). At rice chromosome 5, Nguyen et al. (2001) found a minor QTL for Al tolerance and the gene *STAR2/ALS3* (ABC transporter) was identified (Huang et al. 2009). An orthologous of *STAR2/ALS3* was found at *B. dystachyon* chromosome 2. Based on the parental lines differences in malate secretion in response to Al, we

expected that at least one of the QTL would locate in a region collinear to rice chromosome 3. *ALMT1* gene, the main malate transporter in the Triticeae, occurs in regions with high homology to rice chromosome 3 (Sorrells et al. 2003). Oat chromosome 19A also shows high homology to rice chromosome 3 (Oliver et al. 2013), however, not in the region of the identified QTL. Marker GMI_ES15_c2451_165 on oat chromosome 13A matched 87 % to orthologous sequence on the rice chromosome 6, where the gene LOC_Os06g01490 is located. This gene shows the same chromosome location as the gene LOC_Os06g15779, a putative malate transporter, and LOC_Os06g06440, an ABC transporter in rice genome. These gene families have been implicated in Al tolerance in other plant species; however, the distance between these genes in rice is large. LOC_Os06g01490 locates at chromosome 6 between 297 and 302 kbp, whereas LOC_Os06g06440 lies within 3001 and 3009 kbp and LOC_Os06g15779 within 8960 and 8964 kbp.

The identification of genetic markers near QTL for Al tolerance may facilitate the use of this tolerance in other crops. Introgression of Al resistance using marker-assisted backcrossing was obtained recently in barley (Soto-Cerda et al. 2013). There is a short distance between the markers associated with the QTL located at chromosomes 7C-17A and 13, which are candidates for further analysis. Furthermore, marker-assisted selection in oat has the potential to overcome the difficulties of conventional phenotypic selection, especially in environments that lack toxic levels of Al in the soil. The mapping may also help in the identification of the physiological mechanism involved in the Al tolerance, which is yet speculative in oats.

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