New genomic regions associated with aluminum tolerance in a Kenyan corn population

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INTRODUCTION

Corn (*Zea mays* L.) is the most produced cereal in the world and of great economic and social importance, being used for food, animal and as a source of biofuel (Awika et al., 2011). In Kenya, the agriculture sector accounts for 1/3 of gross domestic product (Oluoch-Kosura, 1999). According to the Network of Hunger Warning Systems (2014), with the exception of few areas, the whole country suffers a situation of food insecurity, because in the last corn harvest, the basic food in Kenya was not enough to feed the entire population.

Of Kenya's total area, 16% is classified as high to medium potential for agricultural production, the other 84% being arid and semi-arid land. Acid soils are common in tropical regions, where grain production is low compared to the potential yield of crops (Oluoch-Kosura, 1999). In these soils, the presence of aluminum (Al) inhibits root growth, preventing the plant from obtaining water and nutrients necessary for its optimal development. This is because Al is solubilized in soil solution in the form of trivalent cation (Al3+), and is highly toxic to roots (Kochian, 1995).

Although there are agronomic strategies capable of increasing the pH of the soil, they are not effective in the subsurface layers and increase the cost of production. Thus, an economically viable way for agricultural development in acidic soils consists in the identification of genomic regions associated with Al tolerance, seeking to understand the molecular mechanisms that confer this characteristic (Foy, 1984).

Several Al tolerance genes have already been identified and characterized in plants, including organic acid transporters, ABC transporters, Al3+ transporters, and transcription factors. Members of the MATE family have been shown to mediate the exudation of Al-activated citrate in some plant species, including sorghum (Magalhães et al., 2007). A functional counterpart to the sorghum gene, Sbmate, was characterized in corn, *Zmmate1*, placing with a higher effect QTL for Al tolerance on chromosome 6 (Maron et al., 2010; 2013).

The population used in the present study was derived from lines of the corn improvement program in Kenya, representing a germplasm previously unexplored from the molecular-genetic point of view as a source of tolerance to Al. Thus, the objective of the present study was to identify genomic regions associated with Al tolerance in a segregating population of corn originating in Kenya.

MATERIAL AND METHODS

Genetic material- We used 180 F2:3 families derived from a cross between two contrasting corn strains for Al tolerance in Kenya, 203B-14 and SCH3. Lineage 203B-14 has a high tolerance to Al, equivalent to the tolerance of Cateto Al237, a lineage considered as a tolerance standard in Brazil. SCH3, on the other hand, is a lineage with agronomic attributes for Kenya's breeding program and has sensitivity to Al similar to L53, used as a sensitive control in Brazil.

Evaluation of aluminum tolerance in nutrient solution- The experiment was conducted in a growth chamber with controlled temperature 25/27 °C day/night and relative humidity of 75%. The seeds were disinfected with 0.5% sodium hypochlorite for 5 minutes, rinsed with deionized water and germinated for three days in rolls of moistened germination paper. The seedlings were transferred to polyethylene cups and maintained in nutritional solution described by Magnavaca et al. (1987) with pH 4.0 under continuous aeration. After acclimatization in complete nutritional solution without Al for 24 h, the initial length of the seminal root (CIRS) was measured on all seedlings, which were transferred to trays containing nourishing solution with activity {39} µM Al3+ or without Al, pH 4.0. After growing for five days, the final length of the seminal root (CFRS) was measured. Tolerance was evaluated as the seminal root relative growth (RNRG) measured as (CFRS - CIRS) with Al divided by (CFRS - CIRS) without Al.

The progeny were evaluated in six experiments in randomized blocks with three repetitions, and two lineages, L53 and Cateto Al237, were used as common witnesses in each experiment. The analysis of variance of the phenotypic data was performed using Proc GLM (SAS Institute, Inc., 1999). Heritability was calculated on the basis of genotype averages.

Molecular markers- DNA was isolated from young leaves using the CTAB method (Saghai-Maroof et al., 1984). PCR reactions to non-fluorescent microsatellite markers were performed according to Ninamango-Cárdenas et al. (2003), and the amplified fragments were separated by electrophoresis in gels of 10% polyacrylamide and stained with silver. Images were taken under white light in a Kodak imaging system. Initiator sequences and genomic localization of Ssrs were obtained in Maize Genetics and Genomic Database (http://www.maizegdb.org/). Snps were mapped into the population using Kompetitive Alelle-Specific PCR, called the KASP assay through the LGC Genomics company (www.lgcgenomics.com). The sequence information and physical position of Snps are available in Panzea (http://www.panzea.org/db/searches/webform/marker_search).

The microsatellite amplification reaction with fluorescent initiators was performed with 50 ng of DNA, 1X buffer, Mgcl2 2.5 mM, 166 one of each dNTP, 0.2 mM of each initiator and 0.5 U of Taq DNA polymerase (Invitrogen) in a total volume of 15 µl. The amplification cycles consisted of an initial denaturation at 95 °C for 2 minutes, followed by eight cycles at 94 °C for 20 seconds, 60 °C (-1 °C/cycle) for 1 minute and 72 °C for 1 minute, followed by 35 amplification cycles at 94 °C for 20 seconds, 53 °C for 1 minute and 72 °C for 1 minute, with a final extension step at 72 °C for 5 minutes. After PCR, the reactions were diluted in water (1:100), being mixed 2 µl of each of the samples together with 0.3 µl of Genetab500 (Geneid) and 9.7 µl of Tween 20 0.1%. The mixture was denatured at 94 °C for 5 minutes and transferred to an ice bath before application. Capillary electrophoresis was performed in Megabace 1000 (Amersham Biosciences), using injection with 3kV for 45 seconds and running at 10kV for 75 minutes. The results were evaluated in the software Fragment Profile 1.2 (Amersham Biosciences).

Genetic Map- The markers were tested for the expected segregation of 1:2:1 using chi-square statistics ($p < 0.05$). The genetic map was constructed using the software MAPMAKER / EXP 3.0b (Lander et al., 1987) with minimum LOD of 3 and maximum recombination fraction of 0.40. The recombination fraction was converted into genetic distance by means of the Kosambi function (Kosambi, 1944).

Qtls-mapping Was performed using multiple interval mapping

(MIM) proposed by Kao et al. (1999) and implemented by QTL Cartographer 2.5 for Windows (Wang et al., 2012). The final MIM model was selected after several rounds of adjustments of the main effects and interactions between Qtls based on the Bayesian Information Content *(BIC)* criterion. The position, effects and proportion of genetic variance explained by the individual Qtls, as well as the total R2 of the model, were obtained by MIM. Confidence intervals were established as a LOD support interval (Lander & Botstein, 1989).

RESULTS AND DISCUSSION

Aluminum tolerance (Al) based on seminal root relative growth (RNRG) presented a wide genetic variability, with low coefficient of variation (8.82%) and high heritability (97.07%). These results indicate that the target population is suitable for QTL analysis and that phenotypic data have high reliability (Table 1).

Table 1. Analysis of variance grouped with common witnesses for Al tolerance in the mapping population.

Source of Variation	DF	Squared Medium
Rep/Experiment	12	26,86
Experiment(E)	5	1650,29**
Control X E	5	103,66**
Adjusted genotypes	183	715,48**
Controls	1	50912,12**
Genotypes/E	176	357,79**
(Control x Genotype) /E	6	2841,73**
Residue	376	10,49
Média Geral		36.72
Average of the Genotypes		35,34
Mean of the Controls		57,61
CV(%)		8.82
Heritability (%)		97.07

** significant at 1% by F test

The 132 polymorphic markers were ordered over the 10 binding groups and covered 1164.7 centiMorgans (cm) of the corn genome, with an average of one marker every 9 cm. Of these markers, 117 were Snps and 15 Ssrs (Figure 1).

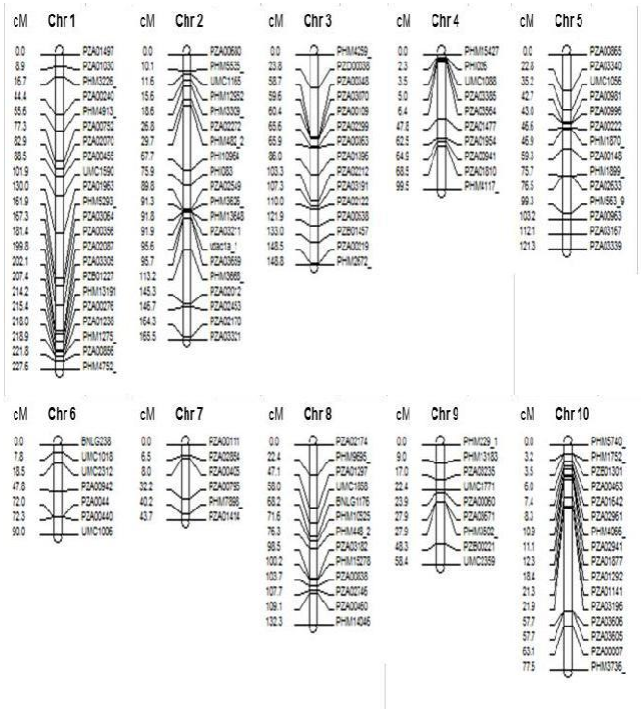

Figure 1. Genetic map constructed with 132 molecular markers.

A total of five Qtls was associated with Al tolerance on chromosomes 1, 5, 8, 9, and 10, explaining approximately 47% of the phenotypic variation for RNRG, including epistatic effects (Table 2).

Table 2. Qtls associated with Al tolerance using multiple interval mapping.

QTL	Cro	Marker	Position (cm)	Type	LOD	Effect	R^2 (%)
1	1	PZA00356_8	184.4	To	4.5	4.060	6.3
				D	1.9	-3.859	3.4
2	5	PZA00222_7	46.6	To	2.9	3.211	3.9
				D	1.3	2.878	1.5
3	8	PHM10525_11	71.6	To	0.1	-0.568	0.4
				D	2.0	-3.580	3.6
4	9	PHM229_15	2	To	2.3	-3.095	4.1
				D	1.1	-2.882	1.0
5	10	PHM1752_36	3.2	To	3.8	3,406	5.8
				D	0.2	1,130	0.3
Interactions							
1x3				DD	2.6	9.197	5.6
1x4				OF	2.2	6.549	4.8
1x5				OF	2.3	-6.155	4.2
1x5				AA	1.0	3.179	1.8
Total R2 (%)							**46.74**

Qtls with additive effects and due to dominance were identified, explaining

relatively small proportion of the genotypic variance of Al tolerance. Epistatic effects were significant and of high importance, being comparable to the main effects. The Qtls identified on chromosomes 5, 8 and 10 coincide with regions previously associated with Al tolerance in other studies (Sibov et al., 1999; Ninamango et al., 2003; Conceição et al., 2009). Among them, we can highlight the QTL mapped on chromosome 5, whose genomic region co-locates with the Zmnrat1 gene, previously *associated with* Al tolerance in corn by Guimarães et al. (2014) (Figure 2). The Zmnrat1 gene **is** homologous to Osnrat1, *which encodes* an Al3+ carrier associated with *Al* tolerance in rice. Interestingly, no Al tolerance QTL was detected on chromosome 6, where a larger effect QTL was mapped in a population of Rils derived from the Brazilian lineage Cateto Al237 (Guimaraes et al., 2014). However, new genomic regions were identified on chromosomes 1 and 9.

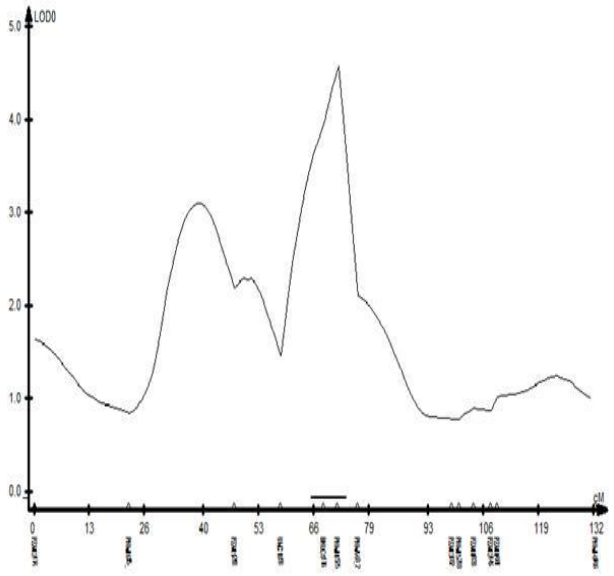

Figure 2. QTL of Al tolerance mapped on chromosome 5, co-located with the candidate gene Zmnrat1.

CONCLUSION

It was possible to identify new genomic regions associated with Al tolerance, and other Qtls, including co-location with the Zmnrat1 gene, will be investigated in *later studies*.

INTELLECTUAL PRODUCTION AND PARTICIPATION IN EVENTS/COURSES

Abstract presented at the 59th Brazilian Congress of Genetics in 2013: Genomic Regions Associated with Aluminum tolerance in a Kenyan maize line.

Abstract and seminar presented at the V PIBIC and BIC JUNIOR Scientific Initiation Seminar at Embrapa Corn and Sorghum in 2013: Mapping of Associated Qtls with Aluminum Tolerance in Corn,

Participation of the XXXIV Brazilian Congress of Soil Science in 2013.

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