QTL and Selection Mapping for Al Tolerance in Tropical Maize

Claudia T Guimarães[1]*, Jurandir V Magalhães[1], Silvia N Jardim[1], Ramon V Almeida[1], Lyza Maron[2], Sidney N Parentoni[1], Vera MC Alves[1], Ubiraci GP Lana[1], Elto EG Gama[1], Owen Hoekenga[2], Edilson Paiva[1] and Leon V Kochian[2]

1. Embrapa Maize and Sorghum, Sete Lagoas, Brazil, 35701-970.
2. USDA-ARS, Cornell University, Ithaca, NY 14853, USA.
* Corresponding author: claudia@ cnpms. embrapa. br

ABSTRACT

This work aimed at validating the chromosomal location of aluminum tolerance QTL mapped in a maize RIL population using allelic frequency shifts from a drift model, across three cycles of recurrent selection for this trait. A genetic linkage map composed by 162 markers was used to detect six QTLs explaining around 50% of the aluminum tolerance. Two major QTLs were located at chromosomes 6 and 5, explaining 25% and 12% of the phenotypic variation. Five SSR markers distributed along chromosome 5 were evaluated in three selection cycles under aluminum stress. Among those, only the closest marker to the aluminum tolerance QTL located on chromosome 5. 02 showed significant shifts in allelic frequencies. QTL mapping together with the results of selection mapping support the presence of genetic factors controlling aluminum tolerance on maize chromosome 5. Additionally, co-localization with sequences homologous to Alt_{SB}, which is a major aluminum tolerance gene in sorghum, suggests that maize and sorghum share a common Al tolerance mechanism.

INTRODUCTION

Aluminum toxicity is one of the major constraints for agriculture on acid soils, which occupy large regions of the world's agricultural area. Cultivars genetically adapted to acid soils may offer an environmental compatible solution, conducive for a sustainable agriculture system. Root exudation of organic acids such as malate, citrate, and oxalate has been considered the most important aluminum tolerance mechanism (Kochian et al., 2004). The organic acids are believed to chelate ionic forms of Al near the root apex, which is the most sensitive region for Al stress (Ryan et al., 1993), thus providing Al tolerance. However, Piñeros et al. (2005) reported that citrate efflux could not completely explain the difference in Al tolerance in some maize cultivars, suggesting that additional mechanisms may exist. Recently, the *ALMT1* (*Aluminum-activated Malate Transporter*) gene that encodes a malate transporter activated by aluminum was cloned and found to provide wheat Al tolerance (Sasaki et al., 2004). Magalhães et al. (2007) have also cloned an aluminum-activated citrate transporter that underlies the major sorghum aluminum tolerance locus, Alt_{SB}, confirming the role of organic acid exudation conferring Al tolerance in grasses.

However, tolerance to Al in maize seems to display a quantitative inheritance, controlled by a small number of genomic regions. Sibov et al. (1999) identified two QTLs associated with this trait on maize chromosomes 6 and 10, while Ninamango-Cárdenas et al. (2003) mapped five QTLs located on chromosomes 2, 6, and 8. The aim of this work was to identify genomic regions associated with Al tolerance in maize, using QTL and selection mapping strategies. In addition, comparative genomics was applied using the Al tolerance genes and QTL already identified in sorghum, wheat and rice, in order to support the location of maize genomic regions associated with Al tolerance.

MATERIALS AND METHODS

A population of 118 RILs derived from a cross between two contrasting maize inbred lines for Al tolerance L53 (Al susceptible) and Cateto237/67 (Al tolerant) was evaluated using the net seminal root length (NSRL) in nutrient solution according to Magnavaca et al. (1987). The phenotypic index NSRL was measured as total root length under 222 $\mu mol \cdot L^{-1}$ of Al (concentration) divided by root length of plants grown without aluminum according to (Ninamango-Cárdenas et al. 2003).

DNA isolation and molecular marker analyses were performed according to Ninamango-Cárdenas et al. (2003). The linkage map was constructed using 162 markers including three RFLP, 11 STS and 148 SSR with a minimum LOD of 2. 0 and maximum recombination fraction of 0. 3. QTL mapping was performed using multiple interval mapping using the software QTL Cartographer 2. 5 for Windows.

Three selection cycles of the Synthetic-Aluminum, derived by intercrossing Cateto237/67, SLP181/71, L1154 and L3, were used to evaluate shifts in allelic frequency. Forty two individuals of each cycle (C0, C1 and C2) were

sampled and the selection was performed under 222 μmol · L^{-1} followed by 398 μmol · L^{-1} for the first and second cycle, respectively. Allelic frequency shifts were evaluated using the critical value of the chi-square test according to Waples (1989).

RESULTS AND DISCUSSION

A total of 162 markers were mapped along 1657 cM using the maize RILs population. The population showed significant genetic variability and six QTL were mapped on chromosomes 3, 5, 6 and 8, which explained around 53% of the phenotypic variation. Two genomic regions could be considered as harboring major QTL in this population, as they explained from 25% to 12% of the aluminum tolerance phenotype, while others explained around 5%. Three of these QTLs co-localized with homologs of the sorghum Al tolerance gene, Alt_{SB}, suggesting that maize and sorghum share a common Al tolerance mechanism based on citrate release. Additionally, while Alt_{SB} is a single copy gene in sorghum, its homologues in maize have evolved as a multigene family, with several functional members. The QTL mapped on chromosomes 6 and 8 were coincident with the ones detected by Ninamango-Cárdenas et al. (2003), using the $F_{3:4}$ families from the same population. In addition, one of the two QTLs located on chromosome 6 was coincident with the *Alm2* QTL mapped by Sibov et al. (1999) in other tropical maize population.

In order to test the allelic frequency shifts, five SSR markers distributed along the maize chromosome 5 were evaluated in three cycles of the Synthetic-Aluminum selected under aluminum stress. Only one SSR closest the QTL located at bin 5.02 showed significant shifts in allelic frequency using the neutral test proposed by Waples (1989). As the Waples test considers in the model that only stochastic processes such as sampling error or genetic drift affect the population effective number, significant frequency shifts suggest that other factors as selection may have taken place. The allele derived from Cate to 237/67, the most tolerant line, increased its frequency from 6.4% to 33% between the cycles C0 and C1, where the highest genetic gain for the aluminum tolerance (45%) was achieved. This result associated with the presence of an aluminum tolerance QTL strongly suggests the presence of genetic factors controlling aluminum tolerance in this genomic region.

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