Mapping Aluminum Tolerance QTLs in Maize

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INTRODUCTION

The identification, development and use of maize and sorghum genetic resources tolerant to Al toxicity and more efficient in P acquisition and utilization will increase production sustainability across tropical areas where Al toxicity and low P availability are principal constraints. The development of more refined genetic standards to study these constraints, as recombinant inbred lines (RILs) are essential tools to generate superior genotypes for cultivation in acid soil regions. These resources allow the elucidation of the physiological mechanisms and inheritance patterns of the underlying genes controlling these traits. This research also helps developing molecular markers for marker-assisted selection in plant improvement.

The objective of this study was to identify molecular markers associated to Al tolerance in a maize RIL population. Once identified, these regions can be used to assist breeding programs to accelerate the efficiency of cultivar improvement. Additionally, this result will be very useful for physiological studies and to identify candidate genes.

MATERIAL AND METHODS

The genetic materials were 130 (S8) RILs derived from a cross between maize inbred lines L53 (Al susceptible) and L1327 (Al tolerant - Cateto Al 237/67).

RILs were evaluated for Al tolerance in nutrient solution using a randomized complete design with 2 replicates and 7 plants per replicate. Seeds of each family were germinated for five days in paper towel rolls and the most uniform seedlings, without visible seminal root damages were selected. The initial seminal root length (ISRL) was measured, and the seedlings were transferred to plastic containers with aerated nutrient solution. Each family was evaluated after growth for seven days in a nutrient solution with and without 222 µM Al^{+3} supplied as $KAl(SO_4)_2$ according to Magnavaca et al. (1987). The final seminal root length (FSRL) was measured for each plant and the parental lines, which were used as controls.

To evaluate aluminum tolerance in nutrient solution, the Relative Seminal Root Length (RSRL) = (FSRL – ISRL with Al)/(FSRL – ISRL without Al) was estimated, using the seminal root growth without Al as an internal control. The Net Seminal Root Length (NSRL) = (FSRL – ISRL) under Al treatment was also used as a second phenotypic measure of Al tolerance. The Seminal Root Growth without Al was also measured (SRG) = (FSRL – ISRL) in order to eliminate interferences related to intrinsic differences in rates of root growth or seed vigor. The analysis of variance, heritability based on family means (h^2_m) and coefficients of genetic and experimental variation were estimated using Genes software (Cruz, 1997).

Genomic DNA of each inbred line was extracted from the leaf tissue according to Saghai-Maroof et al. (1984), quantified on agarose gels and stored at -20ºC. Microsatellite reactions were performed according to Ninamango-Cárdenas et al. (2003). Association analyses among molecular markers and the phenotypic indices were performed using single and multiple regression models by the Jump software (SAS Institute, Inc.).

RESULTS AND DISCUSSION

In the current work we will use two phenotypic indices in the QTL mapping analysis, RSRL (obtained with a control without Al) and NSRL (only in the presence of Al). The issue of which is the "best" phenotypic measurement for maize Al tolerance in hydroponic conditions is a matter of substantial controversy.. Ninamango-Cárdenas et al. (2003) used the Net Seminal Root Length for QTL mapping, but the effect of intrinsic differences in root growth rates among the lines might have confounded the final tolerance scores. In order to overcome this potential interference, the use of root growth without aluminum as a control (Relative Seminal Root Length) has been indicated to evaluate Al tolerance in maize and rice (Ma et al. 2002).

According to the analysis of variance for RSRL and NSRL and the respective frequency distribution in the progeny, both phenotypic indices and also that for seminal root growth means without Al showed significant genetic variability (Table 1) and goodness-of-fit to a normal distribution by Lilliefors test (Figures 1). These results support our QTL results for maize Al tolerance that will be shown next.

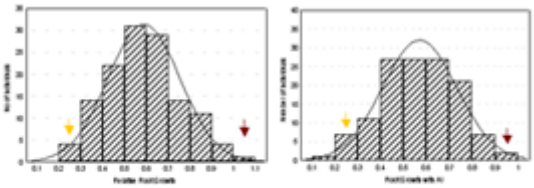

FIGURE 1. Distribution of the phenotypic indices Relative and Net Seminal Root Length used to evaluate aluminum tolerance under nutrient solution in the maize RIL population. The yellow arrow indicates the location of the susceptible parental line L53 and the brown arrow indicates the location of the Al tolerant line L1327.

The correlation coefficient between Net and Relative Seminal Root Length means was significant (r=0.65**??) suggesting that at least some of the genomic regions identified using both phenotypic indices should be the same. However, the values for Relative Seminal Root Length were inversely correlated with Seminal Root Growth without aluminum (-0.32). According to the Figure 2, this negative correlation was mainly caused by the tolerant inbred line group , which showed a reduction in root growth rates without aluminum as the aluminum tolerance increased (r=-0.44**??). This result suggests that one possible mechanism of Al tolerance in maize may be related to the same events affecting the rate of root elongation.

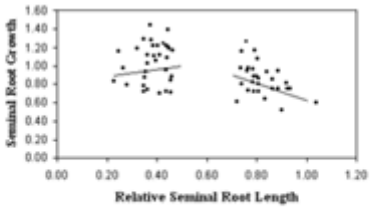

FIGURE 2. Correlation between Relative Seminal Root Length and Seminal Root Growth without aluminum in two groups composed of the 30 most Al sensitive lines and 30 most tolerant lines.

TABLE 1. Analysis of variance of the phenotypic indicesRelative and Net Seminal Root Length and Seminal Root Growth without Al (RSRL, NSRL and SRG, respectively).

Source of variation	D.F.	MS		
		RSRL	NSRL	SRG
Families	122	0.065 **	4.74 **	7.096 **
Error	123	0.005	0.18	0.262
Total	245			
Heritability (h^2_m)		0.92	0.96	0.96
Coef. of experimental variation (CV_e)		11.49	8.45	6.85
Coef. of genetic variation (CV_g)		26.71	30.24	23.38
CV_g / CV_e		2.33	3.58	3.41
Mean		0.64	4.99	6.85

** Significant at 1% of probability.

Analysis of variance using both phenotypic indices (NSRL and RSRL) and the seminal root growth without Al detected significant genetic variability in the population (Table 1). The high heritability values based on family means (h^2_m) and the low experimental coefficient of variation (CV_e) indicates that aluminum tolerance in maize is not a very complex trait, as initially proposed by Prioli (1987) and Torres et al. (1997). Additionally, the heritability value of the NSRL was very similar to the estimate obtained by Ninamango-Cárdenas et al. (2003) for the same phenotypic index using the $F_{3:4}$ population (0.97).

, Three mendelian markers were significantly ($P < 0.01$) associated with Relative Seminal Root Length under nutrient solution, explaining from 7.83 to 10.34% of the phenotypic variation (Table 2). However, the markers bnlg161 and bnlg238 showed an expressive increment in the regression coefficient using the Net Seminal Root Length under Al stress. As these two markers were located at chromosome 6 (bin 6.00), it was confirmed that this region is likely to harbor putative genes related to aluminum tolerance, as previously identified by Ninamango-Cárdenas et al. (2003) and the *Alm*-2 Al tolerance QTL detected by Sibov et al. (1999).

Only the marker mmc0241 was associated with the Seminal Root Growth without aluminum, which explained 5.81% of the phenotypic variation. As this marker is located on a region (bin 6.05) where no QTL for Al tolerance was detected, it would be possible to consider the Net Seminal Root Length as a reliable phenotypic index to evaluate the aluminum tolerance in the RIL population.

TABLE 2. Regression analysis showing associations between molecular markers and phenotypic indices used to evaluate the aluminum tolerance in the maize RIL population.

Phenotypic index	Molecular marker	Location (bin)	Single Regression	
			R^2	F
Relative Seminal Root Length	bnlg1179	1.01	7.83	10.43**
	bnlg238	6.00	8.92	11.96**
	bnlg161	6.00	10.34	14.60**
Net Seminal Root Length	bnlg1179	1.01	4.46	6.18*
	bnlg238	6.00	17.19	24.24**
	bnlg161	6.00	19.93	30.37**
Seminal Root Growth	mmc0241	6.05	9.81	13.19**

Based on this preliminary result, it may be suggested that the rates of root elongation with and without aluminum stress are controlled by different groups of genes that can be distinguished and mapped using molecular markers. However, other genomic regions still need to be investigated, because a significant proportion of the Al tolerance phenotypic variation still remains to be explained. Therefore, we will develop a better-saturated genetic map to validate the syntenic regions where there are strong indications for the presence of stable aluminum tolerance genes or QTLs in other crops..

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