

Joint Linkage-Association Analysis of Aluminum Tolerance in Maize

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ABSTRACT

We undertook an experimental plan integrating genetic and genomic approaches, building upon our previous work on the physiological bases for Al tolerance in maize. We used comparative genomics to identify gene families related to known Al tolerance genes. Results were analyzed with respect to the physical and genetic maps of maize and in a comparative sense with the rice genome. Based on these data, we selected candidate Al tolerance genes to evaluate using association analysis. We identified several single nucleotide polymorphisms in multiple genes that are associated with Al tolerance. Results from association analyses were confirmed using linkage analysis.

INTRODUCTION

Aluminum (Al) toxicity is a profound limitation to crop production worldwide, reducing yields on up to 50% of potentially arable lands (von Uexküll and Mutert, 1995). Breeding for Al tolerance and agronomic practices aimed at ameliorating soil acidity have historically been productive avenues for improved crop production. However, it is widely recognized that additional improvements in crop Al tolerance will depend on biotechnology.

Traditional genetic analysis based on linkage is a powerful tool to identify genes that underlie agronomically important traits, such as Al tolerance. However, linkage analysis suffers from at least two limitations. First, only two alleles are tested, such that the phenotypic range for a trait of interest may not be fully represented. Second, it may take months or years of determined effort to identify the gene responsible for the trait using map-based cloning methods (Flint-Garcia et al., 2003). An alternative genetic strategy is called association analysis. This relies upon naturally existing diversity for the trait of interest, analyzing the correlations between particular molecular markers and the phenotype of interest, relative to an understanding of population structure (Flint-Garcia et al., 2003). Association analysis utilizes collections of diverse germplasm, which increase the number of alleles tested and thus may capture a broader phenotypic range than that observed in linkage analysis. As one is working at the molecular level, typically assessing polymorphisms observed in candidate genes, one could make rapid progress in determining whether a particular candidate gene is important for the trait of interest. However, association analysis can

suffer from low statistical power if the germplasm collection is not sufficiently large, if the phenotypic information is poor, or if the trait is poorly heritable. Importantly, linkage and association analyses are not mutually exclusive and thus can be combined to maximum benefit. We have employed a joint linkage-association to assess the importance of individual candidate maize Al tolerance genes that exist in multi-gene families, in the absence of insertion mutants or transgenic plants. The significance of these results and of this approach will be discussed. This work has been supported by NSF Plant Genome Award DBI #0419435, two grants from the Generation Challenge of the CGIAR, a McKnight Foundation Collaborative Crop Research Program and USDA-ARS base funds.

RESULTS AND DISCUSSION

Two Al activated organic acid transporters are known: ALMT, the Al activated malate transporter found in wheat and Arabidopsis (Hoekenga et al., 2006; Sasaki et al., 2004); *Alt_{SB}*, the citrate transporter found in sorghum and barley (Wang et al., 2007; Magalhães et al., 2007). We searched publicly available maize sequence databases and identified 14 *ZmALMT* (for *Zea mays* ALMT) and 8 *ZmASL* (for *ea mays Alt_{SB}-like*) genes. DNA sequencing was performed on each locus, evaluating polymorphisms observed within the maize association collection assembled by Prof. Buckler (Flint-Garcia et al., 2005); the importance of these polymorphisms was then assessed using a mixed-model ANOVA (Yu et al., 2006). For both gene families, a single member contained polymorphisms associated with Al tolerance. The detection of each gene was confirmed using linkage analysis in F₂ populations where the parents were

known to carry contrasting alleles (Figures 1, 2). In both cases, linkage analysis confirmed the association result. Thus, as in Arabidopsis, a single ALMT-related gene is important for Al tolerance in maize while other members of the transporter family apparently do not contribute to this trait (Hoekenga et al., 2006). This *ZmALMT* is not the gene identified as *ZmALMT1*, consistent with the report from Pineros et al. (2008). Both malate and citrate transporters are implicated as significant for Al tolerance in maize. Joint linkage-association analysis allowed us to make this determination without a comprehensive collection of insertion mutants and did not require plant transformation to confirm the association analysis result.

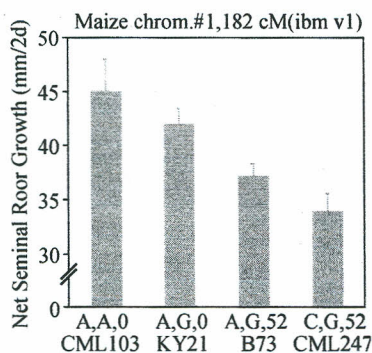


Figure 1 *ZmASLI* haplotypes

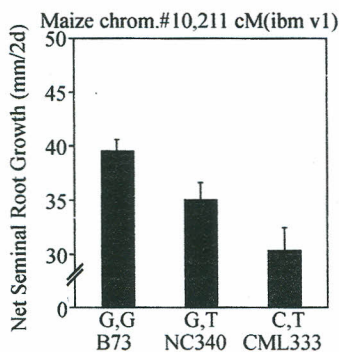


Figure 2 *ZmALMT2* haplotypes

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