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Integrating genomic, molecular genetic and physiological approaches to identify plant aluminum tolerance genes and their associated physiological mechanisms

Leon Kochian[1)], Owen Hoekenga[1)], Jurandir Magalhaes[2)], Miguel Pineros[1)], Vera Alves[2)], Lyza Maron[1)], Paul Mason[1)], Claudia Guimares[2)], and Robert Schaffert[2)]

[1)] *US Plant, Soil and Nutrition Laboratory, USDA-ARS, Cornell University, Ithaca, NY 14853 (lvk1@cornell.edu)*

[2)] *Embrapa Maize and Sorghum, Sete Lagoas – Minas Gerais, Brazil*

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Abstract

Aluminum (Al) toxicity is one of the most significant limitations to crop production worldwide, reducing yields on the acidic soils that comprise up to 50% of potentially arable lands. Breeding for Al tolerance and agronomic practices aimed at ameliorating soil acidity have been productive avenues for improved crop production on acid soils. However, it is widely recognized that biotechnology is a very important avenue for further future improvements in Al tolerance, especially for farmers without the economic resources to take advantage of improved agronomic practices. Hence a number of the laboratories around the world are working to identify and characterize Al tolerance geness in plants. The Kochian lab has been taking an interdisciplinary approach integrating approaches from genetics, genomics and molecular biology, and physiology to identify Al tolerance genes and the associated mechanisms in 3 model plant systems: sorghum, maize and *Arabidopsis thaliana*.

Introduction

It is well known that aluminum (Al) toxicity is an important agronomic trait, limiting crop production on acid soils that comprise up to 50% of the world's potentially arable lands. It has also been widely documented that significant genetic variation in Al tolerance exists in both crop plants and Arabidopsis. The exploitation of this genetic variation to breed crops with increased Al tolerance has been a productive and active area of research; however the underlying molecular, genetic and physiological bases are still not well understood. Because of the agronomic importance of this problem, this is an area that has attarcted significant interest from a number of molecular biology and physiology laboratories around the world. Only very recently was the first Al tolerance gene, *ALMT1*, isolated in wheat and shown to be a novel Al-activated malate transporter (Sasaki *et al.* 2004). Work in the Kochian lab laboratory has focused on using integrated genomic (gene and protein expression profiling), molecular genetic and physiological approaches to identify novel Al tolerance genes and the physiological mechanisms they control in the cereal crops maize and sorghum, and also in Arabidopsis. This talk will describe the recent progress made towards identifying Al tolerance genes in these 3 model plant systems (two of which that are also important food crops).

Results

As shown in Figure 1, in sorghum we had previously shown that Al tolerance is the result of a single locus, Alt_{SB}, which maps to the top of sorghum chromosome 3 in a region totally distinct from where the major Al tolerance gene maps in wheat and other related members of the Triticeae (Magalhaes *et al.* 2003).

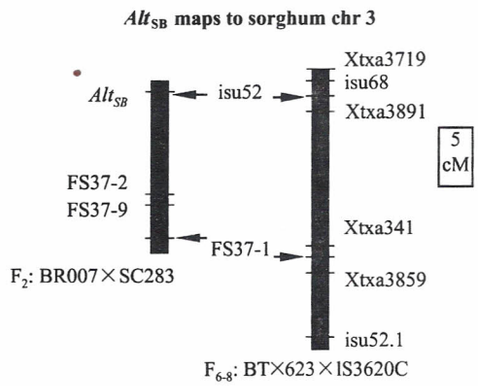

Figure 1. Chromosomal location of the sorghum Al tolerance gene, Alt_{SB}, on chromosome 3 showing markers linked to the tolerance locus (*isu52, FS37-1, 2, and 3*) and markers delineating this region of the chromosome.

Very recently, we have used map-based cloning techniques in sorghum to clone Alt_{SB} and have found it is a novel Al tolerance gene. In our presentation we will present a molecular characterization of the Alt_{SB} gene and also the physiological mechanism of sorghum Al tolerance it controls.

In Arabidopsis, we have previously shown that Al tolerance is a quantitative trait and have identified two major Al tolerance QTL on chromosomes 1 and 5; we showed these genes both function to confer tolerance via Al activated root malate release (Hoekenga *et al.* 2003). We found that a member of the Arabidopsis gene family that is a close homolog to wheat *ALMT1* maps near the largest tolerance QTL on chromosome 1 and have also

found this gene encodes the Al-activated malate transporter involved in Arabidopsis Al tolerance. However, we have clear molecular genetic and functional evidence that this gene is not responsible for the nearby QTL and can not explain the variation in Al tolerance in our Col x Ler RIL mapping population. Fine scale mapping of this QTL indicates it actually could harbor two different Al tolerance genes, which we are in the process of identifying. We are speculating that one or both of the proteins encoded by these genes interacts with the Al-activated malate transporter encoded by the nearby *ALMT1* homolog.

In maize, where Al tolerance is a complex trait, we have identified 5 QTL that explain 60% of the variation in Al tolerance in the IBM population of maize RIL. It is generally accepted that maize Al tolerance is conferred by Al activated citrate exudation (Pellet *et al.* 1995; Jorge and Arruda, 1997). In both of these studies, this assertion is based on the correlation of rates of Al activated citrate exudation with differences in Al tolerance between a single Al tolerant genotype and one or two Al sensitive lines. More recently, we conducted a similar analysis with a broader panel of Al tolerant and sensitive maize lines from Brazil and North America (Pineros *et al.* 2004). These genotypes ranged from the extremely Al tolerant Brazilian hybrid, Cateto, to very Al sensitive N. American lines Mo17 and B73, and S. American maize lines L53 and 11 x 723. The differences in Al tolerance appear to be due to Al exclusion, as the ability to exclude Al from the maize root tip correlated well with the differential Al tolerance. Subsequently, rates of Al-activated root citrate exudation were quantified in the six maize genotypes at a range of Al activities. As seen in Figure 2, the very Al tolerant Brazilian line, Cateto, exhibited a high rate of Al-activated citrate release, as would be expected. However, several of the Al sensitive lines from Brazil and

A

Al3+ (µM)	Cateto	Pioneer	Mo17	B73	L53	11x726
0	0 ± 0	18 ± 10	91 ± 22	0 ± 0	0 ± 0	11 ± 11
5	33 ± 4	67 ± 40	173 ± 116	5 ± 5	0 ± 0	48 ± 26
10	41 ± 1	105 ± 23	675 ± 183	42 ± 12	28 ± 19	53 ± 15
20	203 ± 57	137 ± 44	511 ± 225	61 ± 40	301 ± 41	139 ± 9
40	368 ± 95	221 ± 82	829 ± 332	17 ± 10	309 ± 15	297 ± 99
80	**324** ± 70	769 ± 168	**911** ± 172	**44** ±15	249 ± 059	**284** ± 67

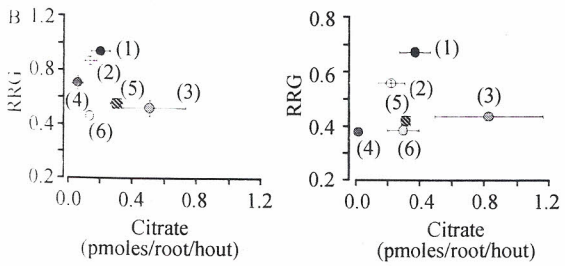

Figure 2. A: Table of rates of root citrate exudation in the 6 maize genotypes in response to Al activities ranging from 0 to 80 µM Al^{3+}. The circled citrate exudation rates indicate that although Cateto maintains a significant rate of Al-activated citrate release, the Al sensitive maize inbred, Mo17, exhibits a much higher citrate exudation and citrate exudation in Al sensitive 11x726 is close to that of Cateto. B: Relationship of Al tolerance measured as Relative Root Growth (RRG) and root citrate exudation, showing the lack of correlation between the two.

North America also exhibited high rates of Al-activated citrate release. In fact, the North American maize inbred, Mo17, exhibited the highest rate of citrate exudation even though it is relatively Al sensitive.

These findings, along with the quantitative genetic nature of maize Al tolerance, suggest multiple mechanisms and genes are involved in tolerance in this species. To help clarify this, we are in the process of constructing near isogenic lines for each of the 5 QTL and these will be an excellent resource for identifying the additional maize tolerance mechanisms as well as isolating the tolerance genes responsible for these mechanisms via a combined genomic and molecular gene-tic approach. Furthermore, we are in the process of determining if any of the maize Al tolerance QTL are encoded by homologs of wheat *ALMT1*, sorghum Alt_{SB}, or the Arabidopsis Al tolerance genes we are close to identifying.

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