



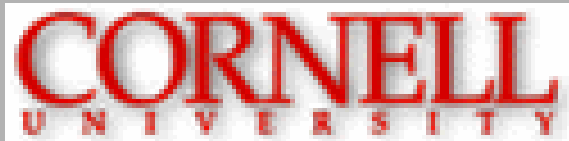
United States Department of Agriculture



# Aluminum Tolerance in Sorghum: Molecular, Genetic and Physiological Basis

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# Isolation and Characterization of Aluminum Tolerance Genes in the Cereals: An Integrated Functional Genomic, Molecular Genetic and Physiological Analysis (#16)

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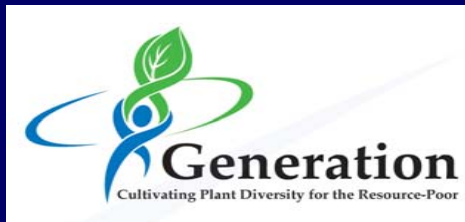
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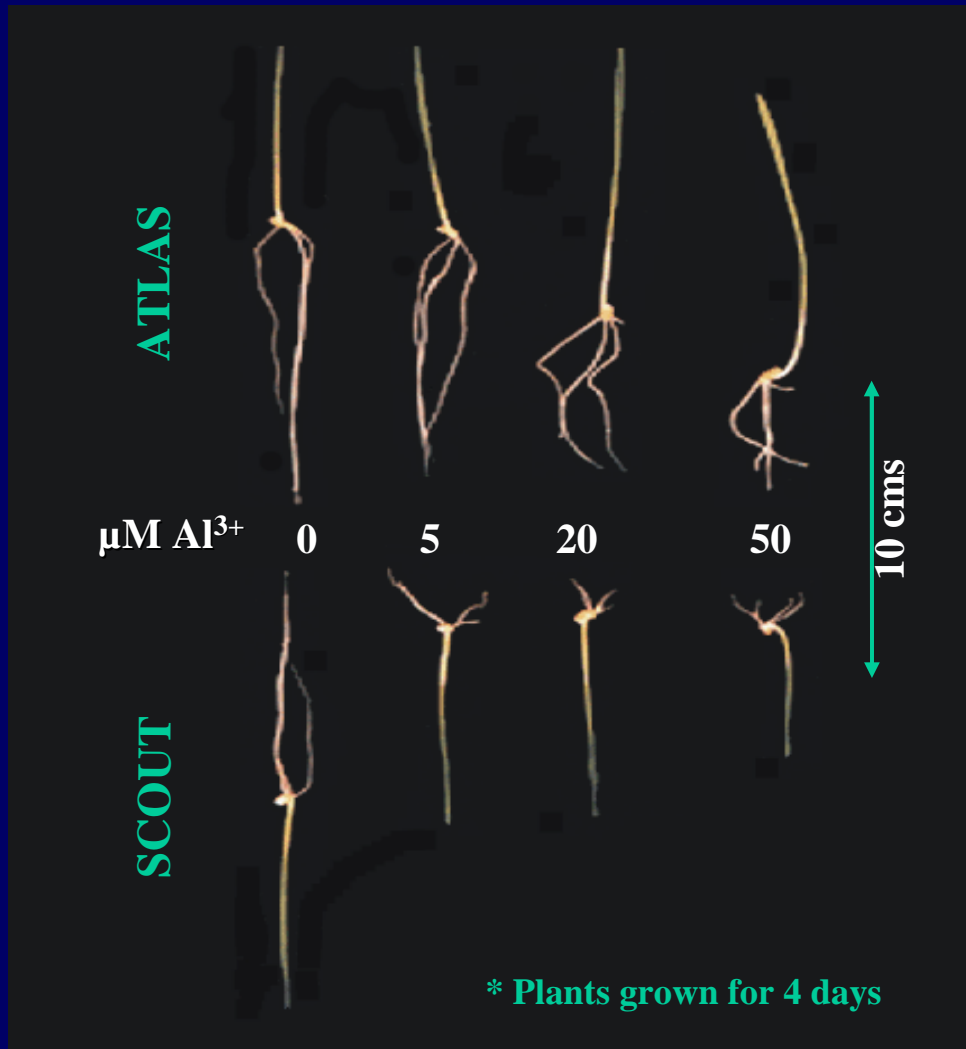
Annual GCP Meeting: Benoni, South Africa 9/12/07 - 9/16/07

# ALUMINUM TOXICITY AND ACID SOILS

- At soil pH values below 5,  $\text{Al}^{3+}$  solubilized into the soil solution
- $\text{Al}^{3+}$  inhibits root growth and function
- Over 50% of world's potentially arable soils are acidic
- Al toxicity is an important limit to agricultural productivity worldwide
- Thus, there is considerable interest in isolating Al tolerance genes and identifying associated physiological mechanisms



# ALUMINUM TOXICITY IN HIGHER PLANTS



$\text{Al}^{3+}$  is the major toxic species

Dramatic inhibition of root growth, occurring within minutes

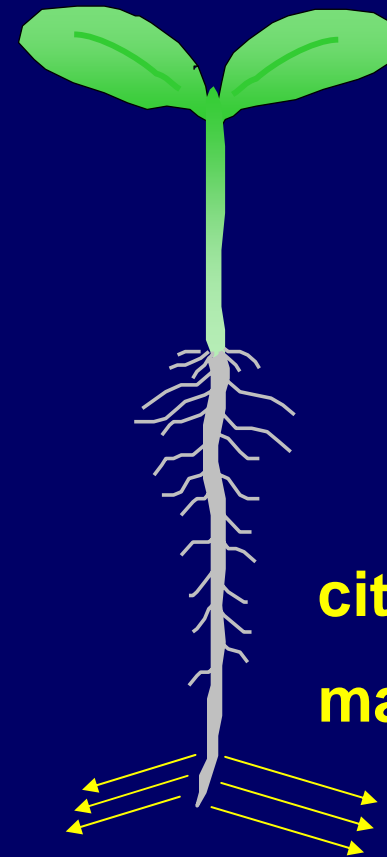
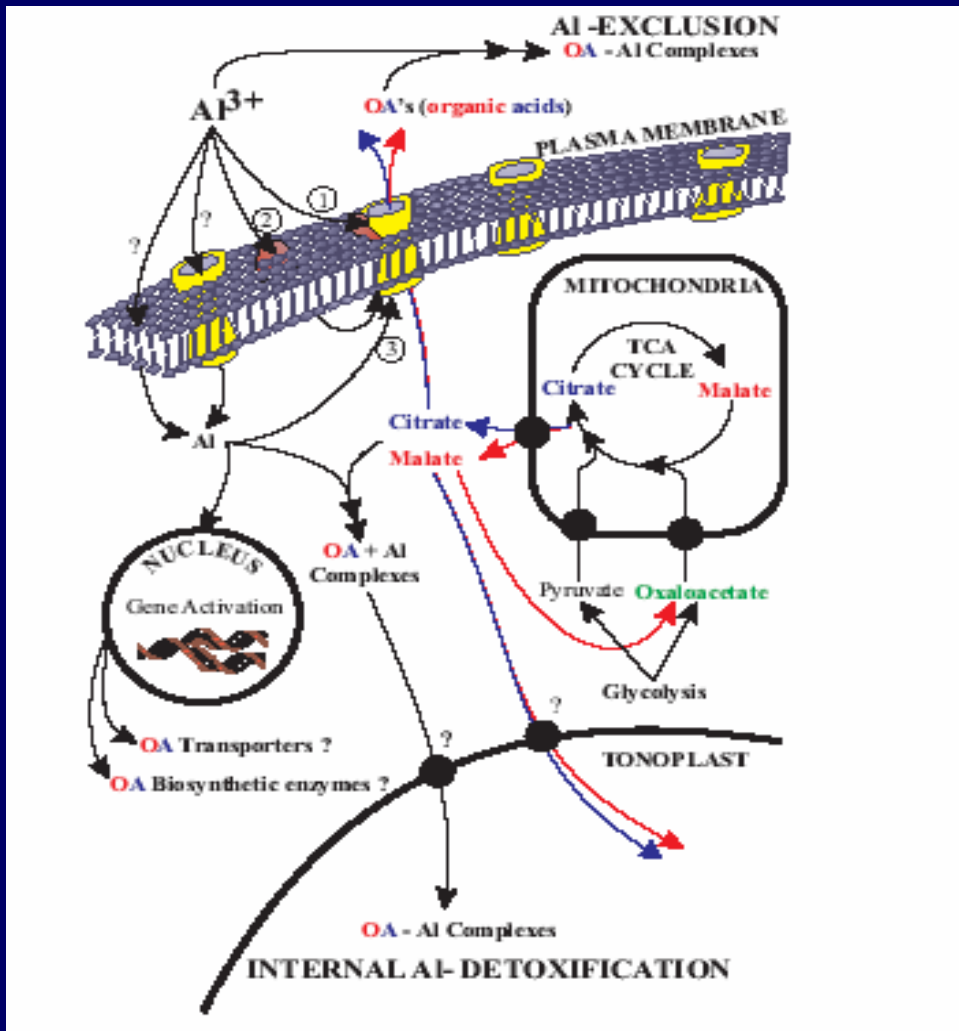
Root apex must be exposed to Al to inhibit root growth

Root apex is the site of toxicity

Genetic variation in Al tolerance has led to identification of adaptive mechanisms for plant growth on acidic, Al-toxic soils

Because Al toxicity results in greatly reduced water acquisition, it must be an important aspect of the drought syndrome on acid soils.

# Cellular Basis of Aluminum Tolerance



(*Alt<sub>SB</sub>*, Andropogoneae)

**Al-citrate**

**citrate + Al<sup>3+</sup>**

**malate + Al<sup>3+</sup>**

**Al-malate**

(*ALMT1*, Triticeae & Arabidopsis)

# ALMT1: First Al Tolerance Gene

*The Plant Journal* (2004) **37**, 645–653

doi: 10.1111/j.1365-313X.2003.01991.x

## A wheat gene encoding an aluminum-activated malate transporter

Takayuki Sasaki<sup>1</sup>, Yoko Yamamoto<sup>1</sup>, Bunichi Ezaki<sup>1</sup>, Maki Katsuhara<sup>1</sup>, Sung Ju Ahn<sup>1,†</sup>, Peter R. Ryan<sup>2</sup>, Emmanuel Delhaize<sup>2</sup> and Hideaki Matsumoto<sup>1,\*</sup>

<sup>1</sup>Research Institute for Bioresources, Okayama University, Chuo 2-20-1, Kurashiki, Okayama 710-0046, Japan, and

<sup>2</sup>CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Received 8 September 2003; revised 27 October 2003; accepted 19 November 2003.

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†Present address: Donald Danforth Plant Science Center, 975 North Warson Road, St Louis, MO 63132, USA.

- ALMT1 describes a new family of membrane proteins
- An Al-activated malate efflux transporter
- ALMT1 homologs responsible for Al tolerance in *Arabidopsis* (Hoekenga et al. 2006. PNAS) and *Brassica napus* (Ligaba et al. 2007. Plant Physiology).

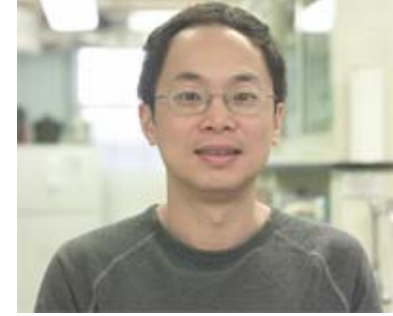
# Identification and Characterization of *Alt<sub>SB</sub>*: A Novel Sorghum Aluminum Tolerance Gene

Magalhaes et al. 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nature Genetics* 39:1156



Jurandir Magalhaes

# Positional Cloning of $Alt_{SB}$



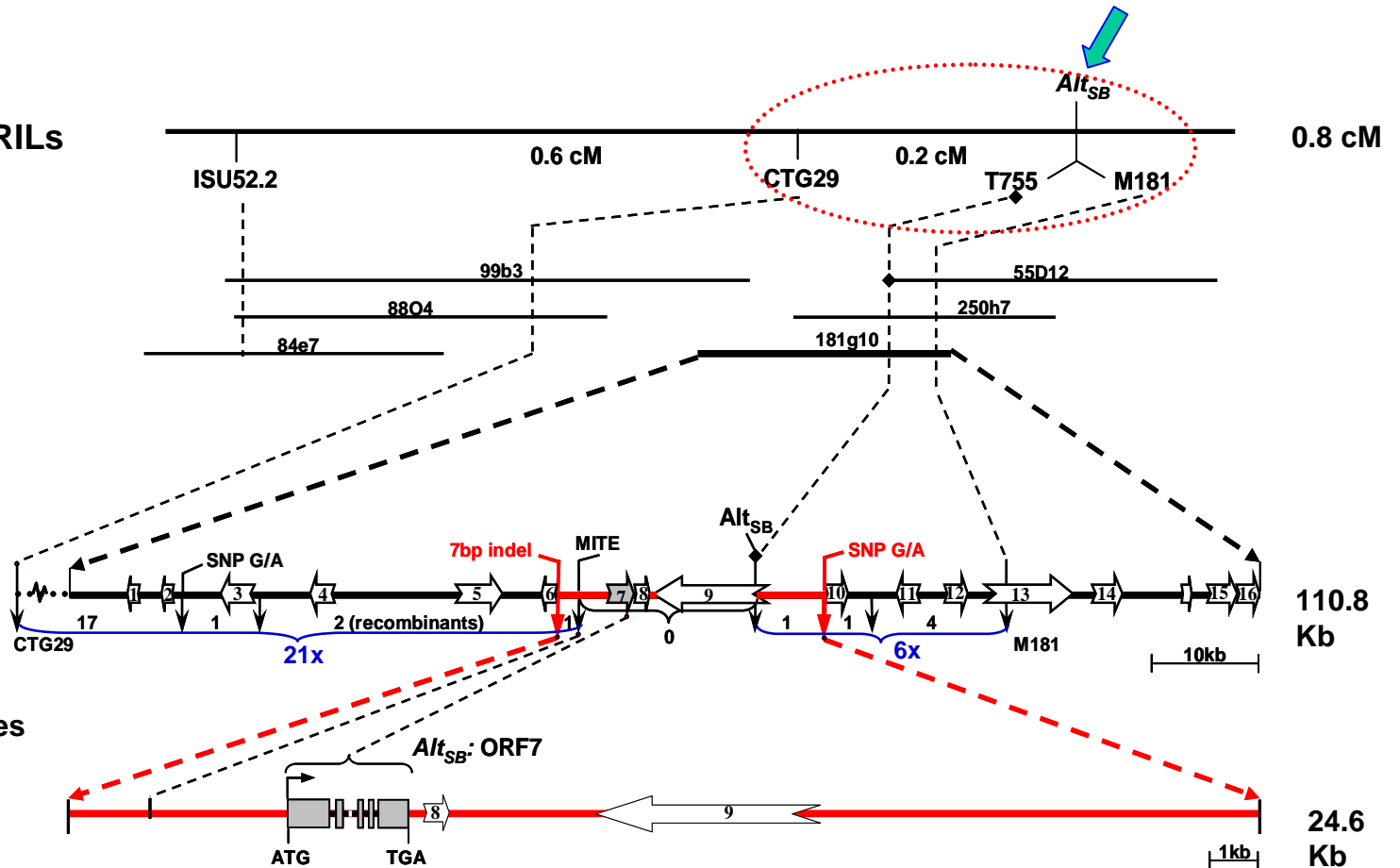
Jiping Liu

$Alt_{SB}$  genetic map: 354 RILs

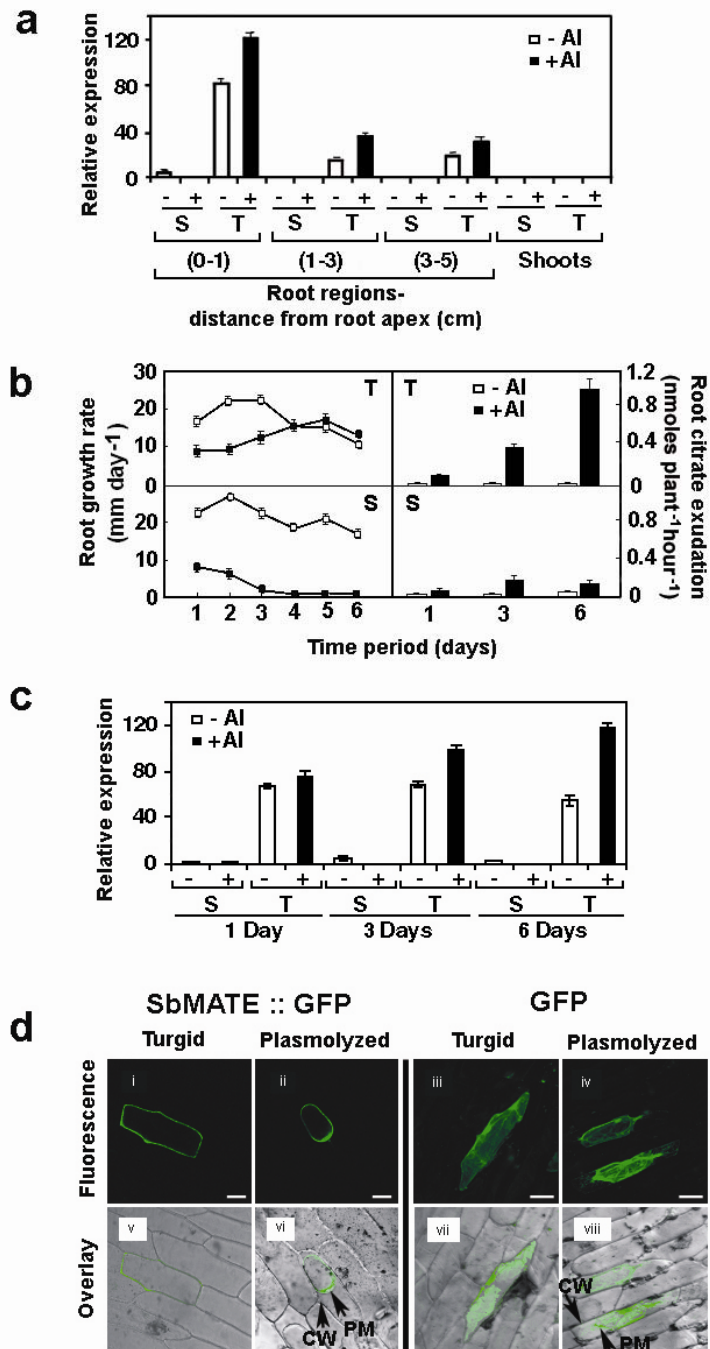
Physical Map (Texas A&M)

High resolution map:

- 2085  $F_2$ :BR007xSC283
- 27 single recombinant  $F_2$ s
- Progeny testing of  $F_{2:3}$  families
- Target 24.6 Kb region
- 513 Kb  $cM^{-1}$

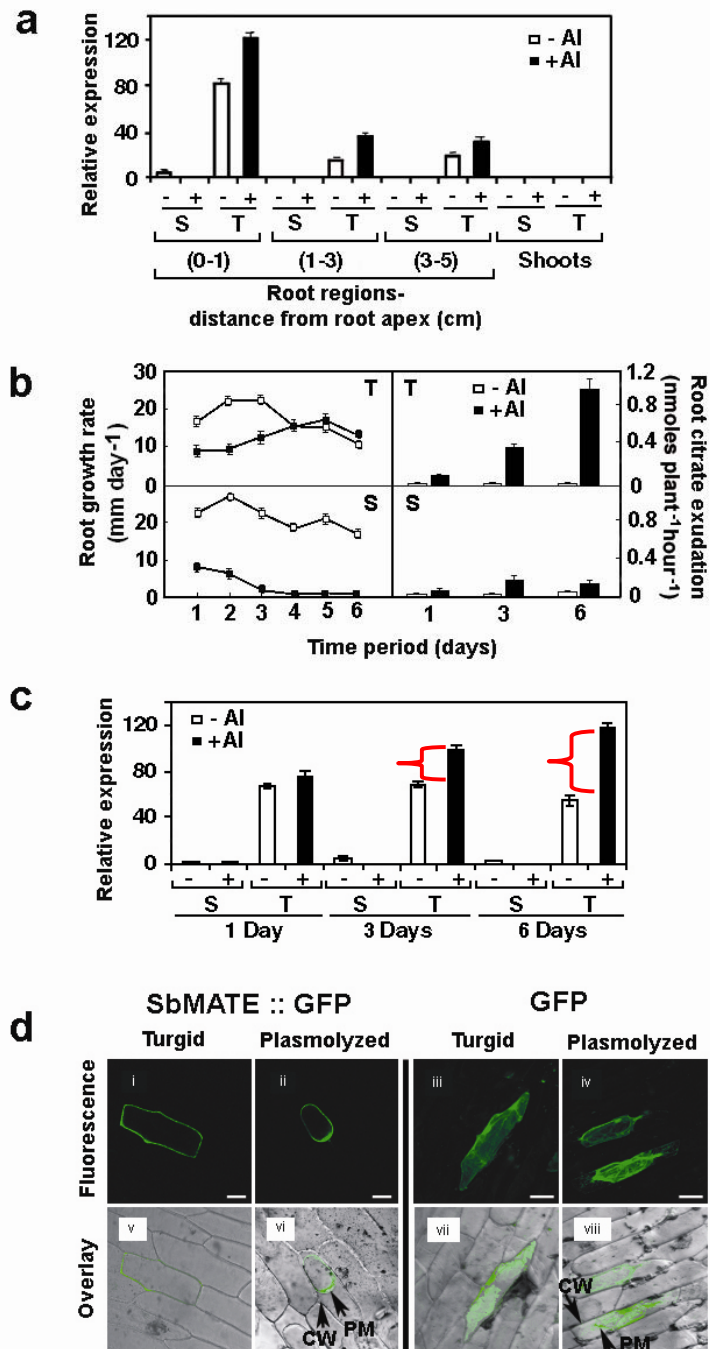


## Does *MATE* Underlie *Alt*<sub>SB</sub> Locus?



- *MATE* expressed only in Al tolerant NIL
- *MATE* expression localized to root tip (site of Al toxicity)
- Al tolerance and Al-activated root citrate exudation inducible – both increase substantially after 3-6 days of Al exposure
- *MATE* expression is also Al-inducible over same time period (but what about constitutive *MATE* expression?)
- *MATE* protein is localized to plant cell plasma membrane
- What about *MATE* expression in transgenic plants? (Sorghum is difficult to stably transform)

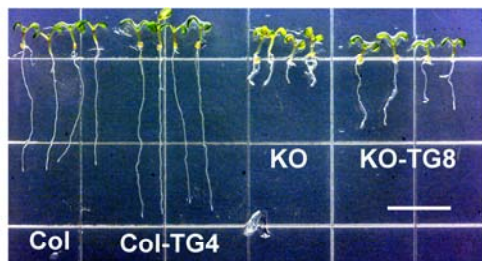
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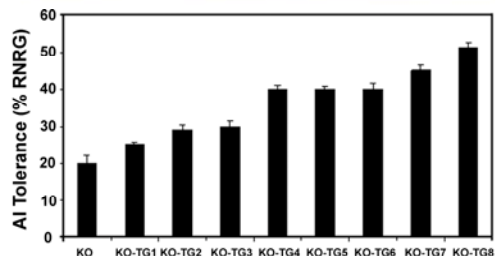
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T<sub>3</sub> lines

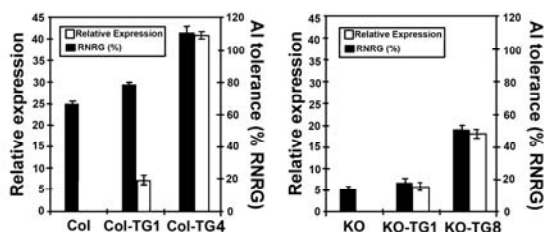
a



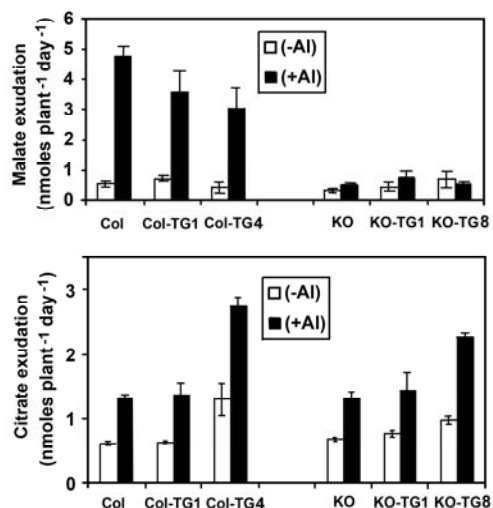
b



c



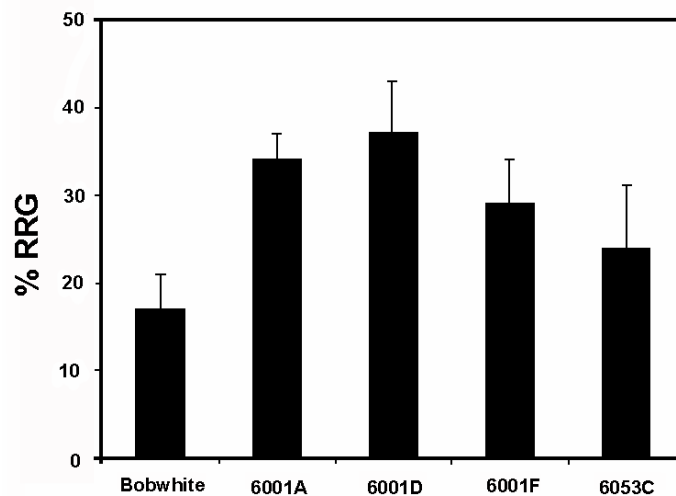
d



# Alt<sub>SB</sub> Expression Increases Al Tolerance in Transgenic Arabidopsis

- See significant increases in tolerance when expressed both in WT (Col), and Col where major Al tolerance gene, *AtALMT1*, is knocked out (ALMT1 is Al-activated malate transporter)
- Level of transgene expression correlates with level of increased tolerance.
- *Alt<sub>SB</sub>* expression confers root citrate release.

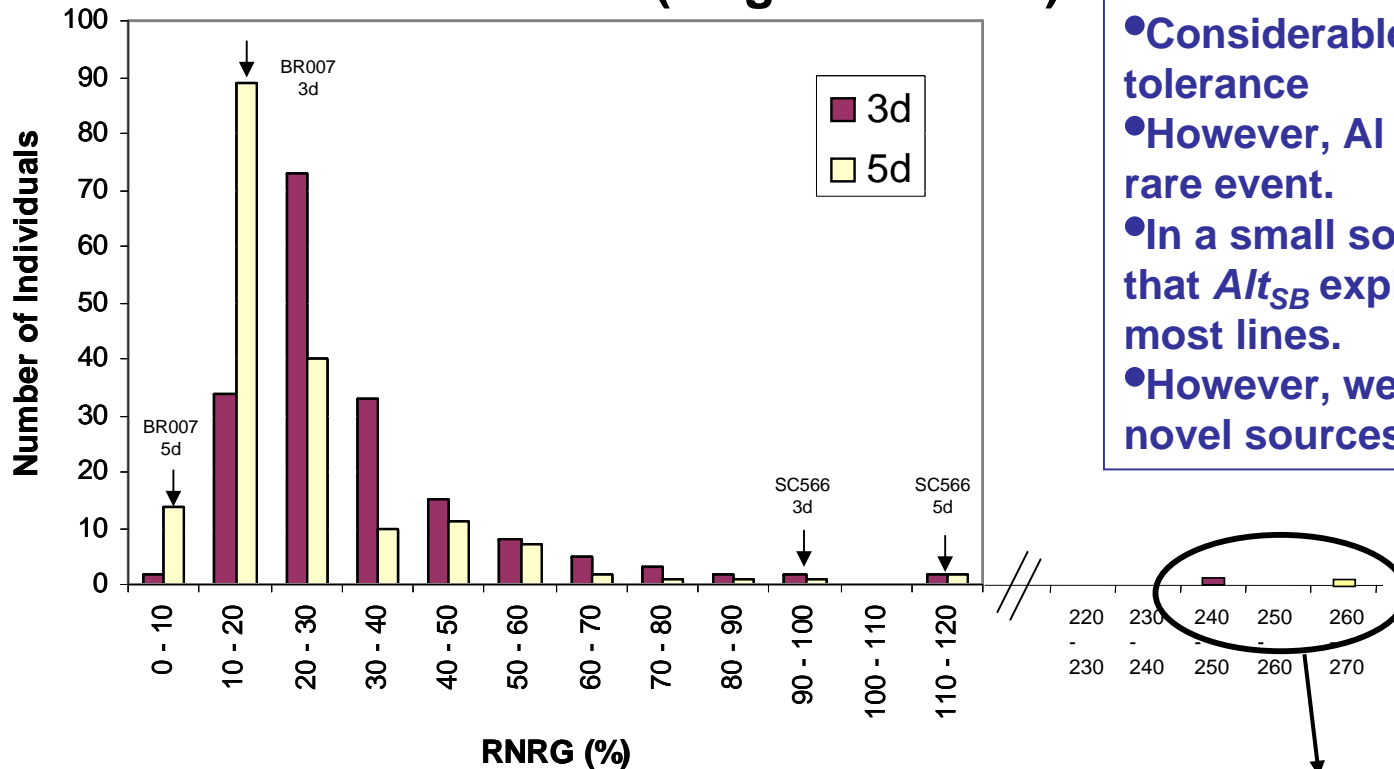
## Alt<sub>SB</sub> Expression Increases Al Tolerance in Transgenic Wheat



T<sub>1</sub> lines segregating for transgene

# How Does $Alt_{SB}$ Confer Differential AI Tolerance in Sorghum?

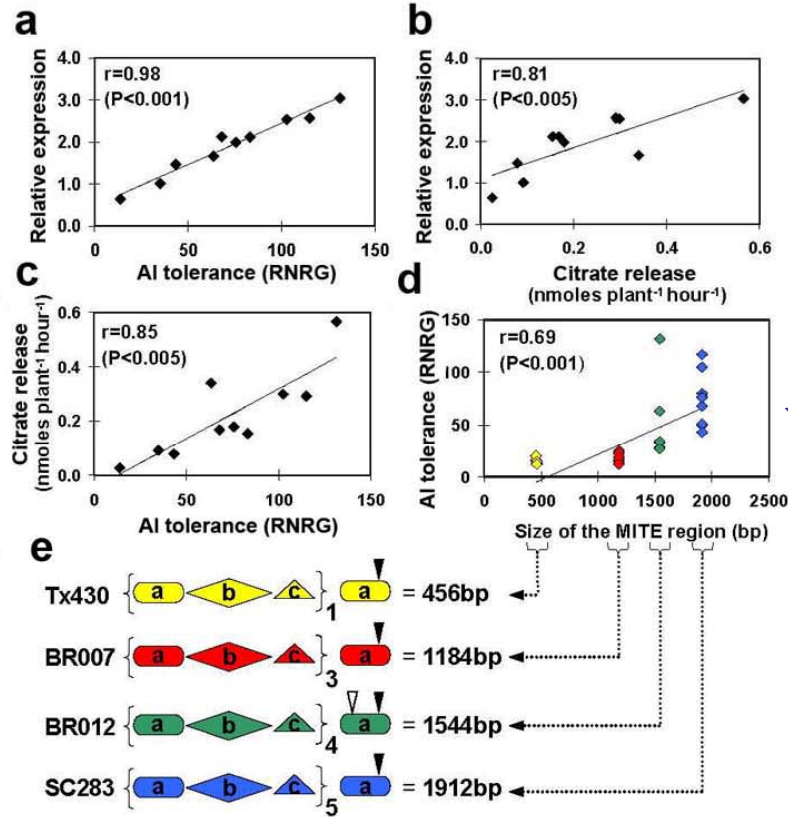
## Phenotyping the CIRAD Association Panel for AI Tolerance (Magalhaes Lab)



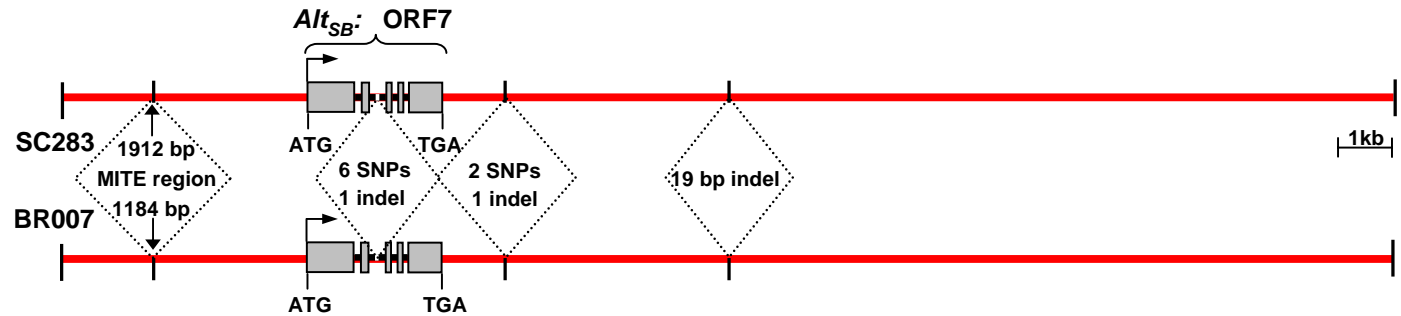
- Considerable diversity for sorghum AI tolerance
- However, AI tolerance is a relatively rare event.
- In a small sorghum diversity panel, find that  $Alt_{SB}$  explains tolerance variation in most lines.
- However, we have identified several novel sources of AI tolerance.

New sources of allelic diversity for  $Alt_{SB}$  or novel sources of tolerance?

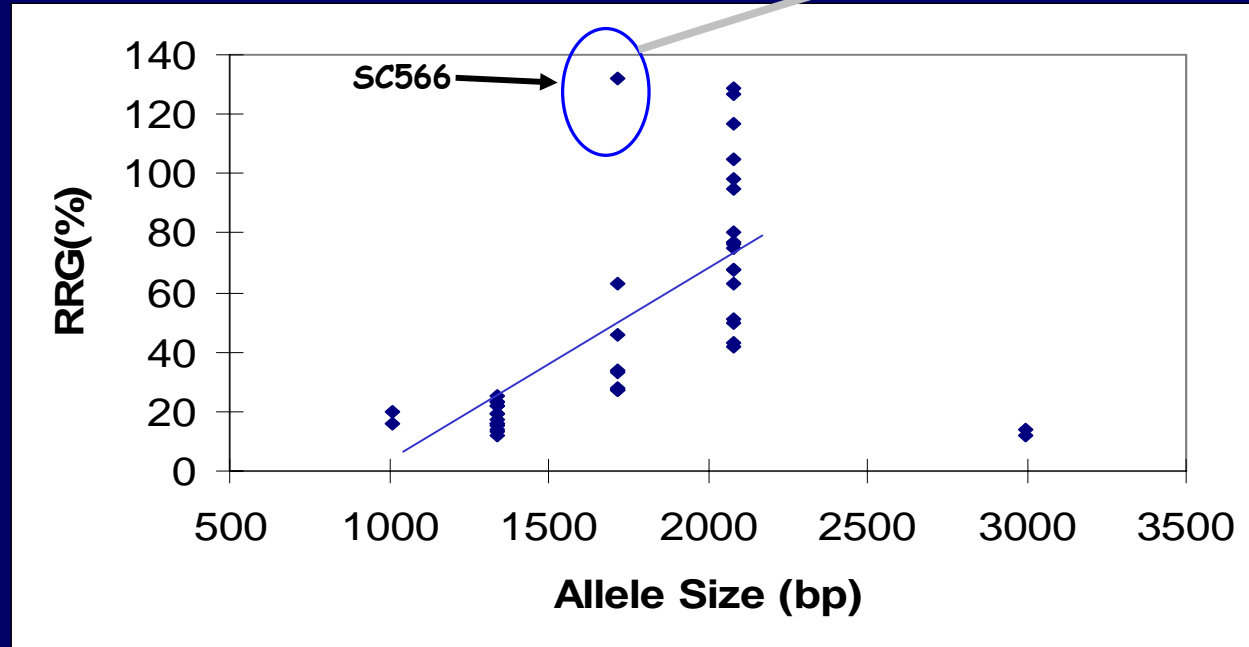
# Al Tolerance is Correlated with $Alt_{SB}$ Expression and Root Citrate Exudation



- Coding sequence for  $Alt_{SB}$  alleles from parents of mapping population are identical.
- Sequence for entire 24.6 Kb region between flanking markers only contains 4 polymorphic regions – all in vicinity of  $Alt_{SB}$ .
- Most dramatic polymorphism is large indel in  $Alt_{SB}$  promoter (1.4 Kb from transcription initiation site).
- This polymorphism contains a Miniature Inverted Repeat Transposable Element or MITE.
- Size of this MITE insertion is positively correlated with the degree of Al tolerance in a panel of 60 diverse sorghum lines.
- Repeat elements in this MITE-containing region acting as enhancer of  $Alt_{SB}$  expression?



# Is $Alt_{SB}$ Allelic Variation Due to a Combination of Differences in Gene Expression and Protein Function?



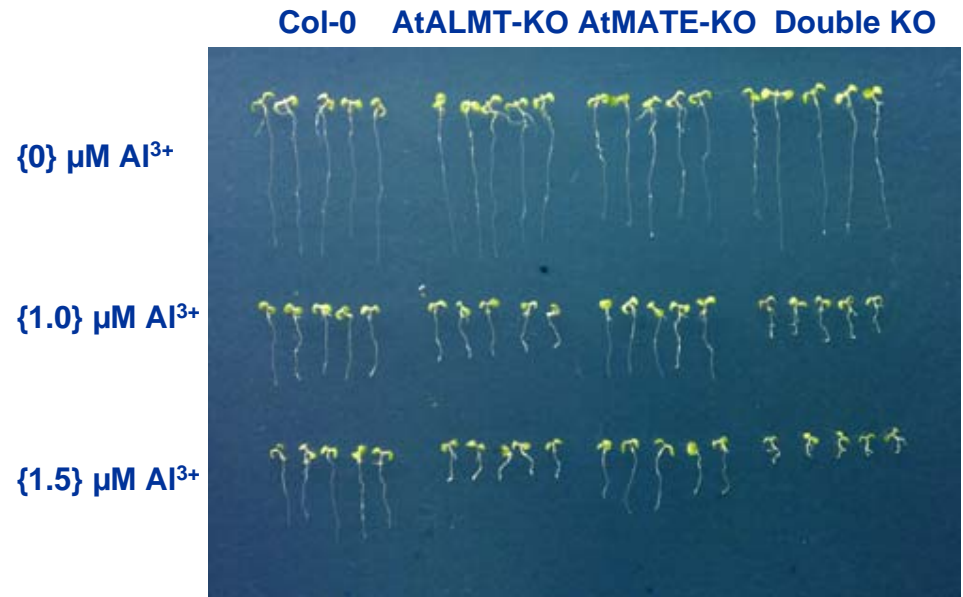
- SC566 is the most Al tolerant line in Embrapa's collection and exhibits the largest root citrate exudation
- Has a mutation in coding region that results in non-conservative amino acid substitution (histidine for leucine) in the first exon
- Thus extreme tolerance may be due to enhanced transport function and not high expression
- Allelic variation resulting from changes in protein function and expression?
- But, it also has very high expression even though the MITE region is smaller than in SC283.

# Do $Alt_{SB}$ Homologs Confer AI Tolerance in Other Plant Species?

- The MATE family is quite large and diverse (56 members in *Arabidopsis*), and a small subset of them are  $Alt_{SB}$ -like.
- In maize, we have identified an  $Alt_{SB}$  homolog that is a candidate AI tolerance gene.
- Via maize root microarray analysis, it was first identified via greater expression (+/-AI) in root tips of tolerant genotypes.
- Subsequently, it was mapped close to a major QTL on Chr 1 in Embrapa's maize RIL mapping population (AI237xL53).
- Quantitative real-time PCR analysis verified it is preferentially expressed in the root tip, and its AI-inducible expression is much larger in tolerant maize lines.
- In barley, a MATE gene appears to be the citrate transporter conferring barley AI tolerance.

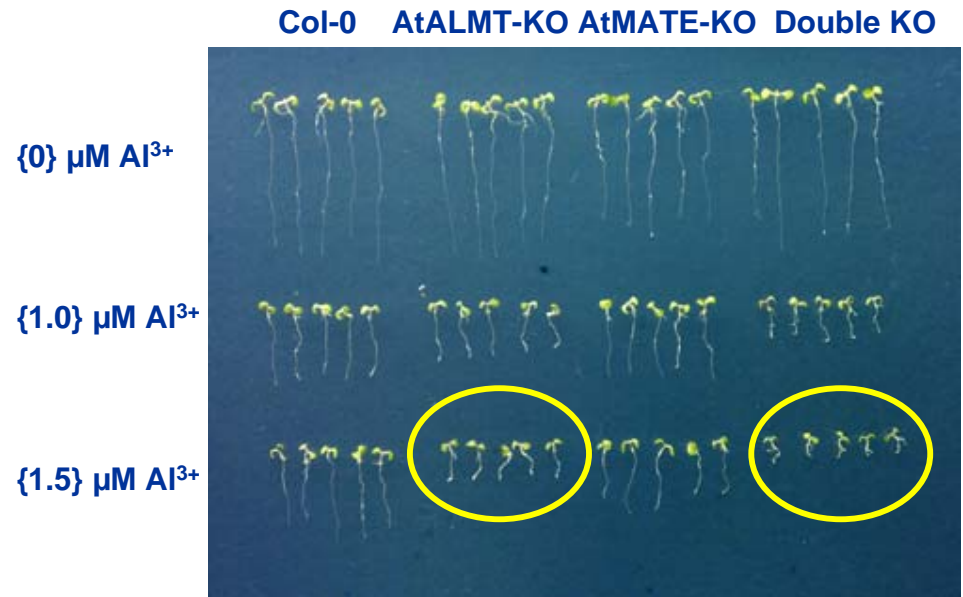
# An *Alt<sub>SB</sub>* Homolog is Involved in Arabidopsis Al Tolerance

- In Arabidopsis, we have shown that the major Al tolerance gene is an ALMT homolog that mediates root malate exudation (Hoekenga et al. 2006. PNAS).
- The large MATE family in Arabidopsis contains a clade of 5 *Alt<sub>SB</sub>*-like genes.
- When each of these were knocked out, only one mutant was more Al sensitive (seen most clearly in plants where *AtALMT* was already knocked out).
- This gene, we call *AtMATE*, is expressed preferentially in roots and its expression is Al-inducible.



# An $Alt_{SB}$ Homolog is Involved in Arabidopsis Al Tolerance

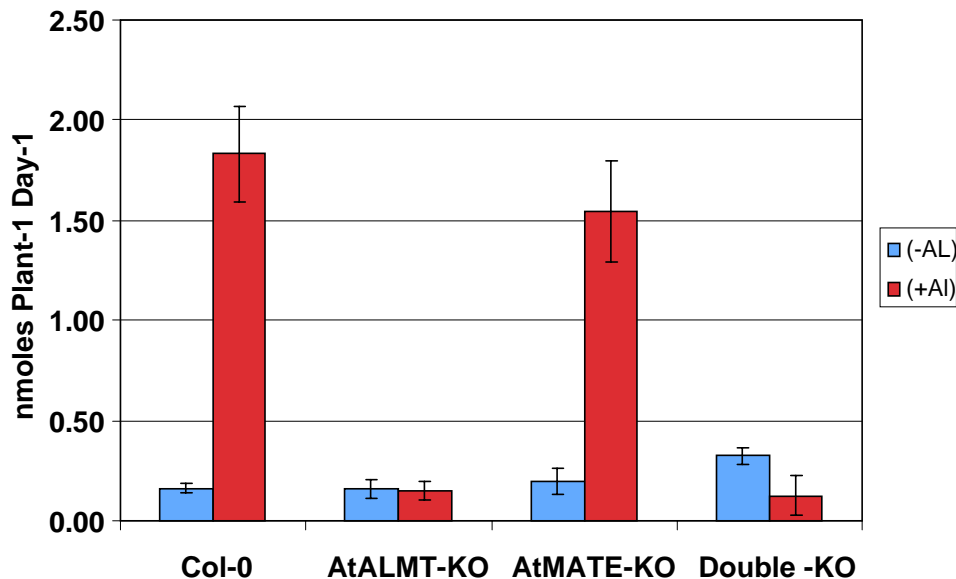
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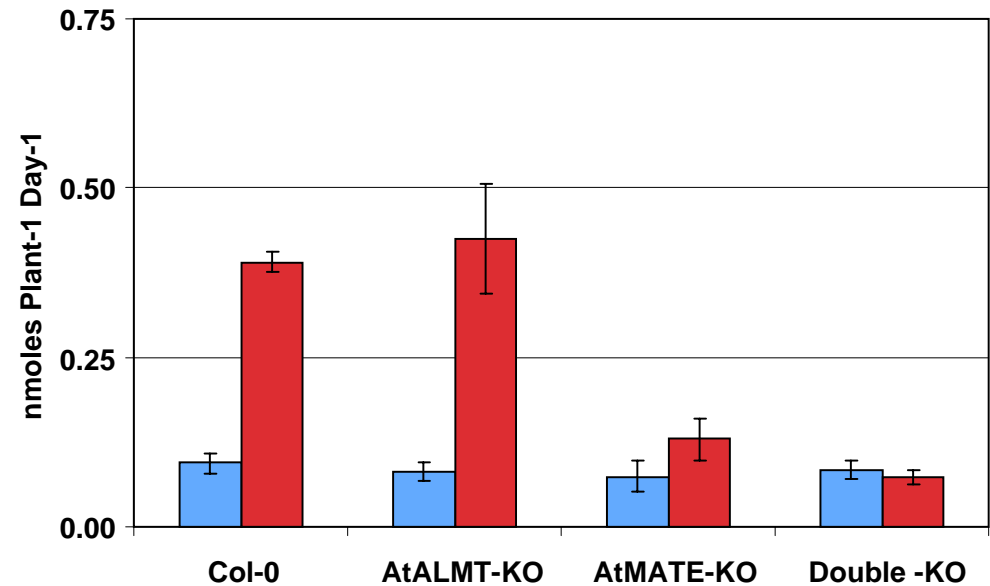
# Arabidopsis Pyramids $Alt_{SB}$ and $ALMT$ Genes to Confer AI Tolerance

- Arabidopsis AI tolerance is a quantitative trait, and appears to be due to the combined function of homologs of  $ALMT$  (major gene) and  $Alt_{SB}$  (minor gene).
- Physiological, this results in AI-activated root malate and citrate exudation, which appear to be controlled independently of each other.

Root Malate Exudation



Root Citrate Exudation



# Ongoing and Future Sorghum Research

- This information will now be used for improving sorghum Al tolerance for African agriculture via marker assisted breeding
  - New CGP project: “Tailoring superior alleles for abiotic stress genes for deployment into breeding programs” (PI: Jurandir Magalhaes). See plenary talk by Dr. Magalhaes tomorrow).
  - Also – Concept note submitted: “Assessment of the breeding value of superior haplotypes for  $Alt_{SB}$ , a major Al tolerance gene in sorghum: linking upstream genomics to acid soil breeding in Niger and Mali” (Co-PI’s: INRAN, ICRISAT, Embrapa, and Cornell)
- Use information to identify candidate tolerance genes in other species to more broadly facilitate increases in crop Al tolerance via molecular breeding. Homologs in maize, barley, rice and other grasses.
- Crop improvement via biotechnology - expression of tolerant sorghum  $Alt_{SB}$  allele in transgenic sensitive genotypes of sorghum, maize, barley and wheat.
- Analysis of  $Alt_{SB}$  Promoter
  - Identify cis elements involved in enhanced Al-inducible expression and localization of expression to root tip.
  - Search for associated trans-acting factors
- $Alt_{SB}$  candidate is a novel transporter – electrophysiological analysis following heterologous expression in oocytes, mammalian cells and transgenic plants to identify functional elements that enhance activity (Dr. Miguel Pineros)