CORNELL UNIVERSITY

Generation
Cultivating Plant Diversity for the Resource-Poor

Comparative Genomics Challenge Initiative: Improving Cereal Yields on Al Toxic and P Deficient Acid Soils

Leon Kochian
Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University

ars Agricultural Research Service

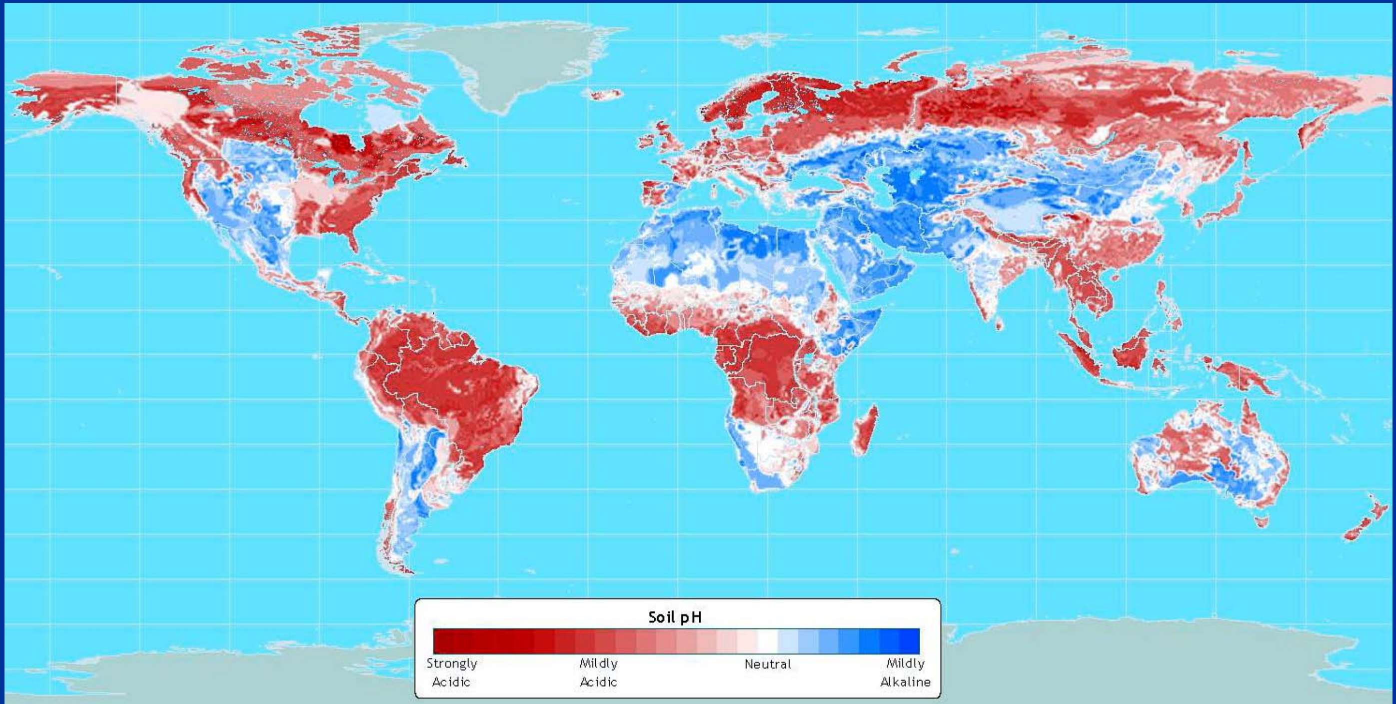
USDA
United States Department of Agriculture

REDUCED CROP YIELDS ON ACID SOILS

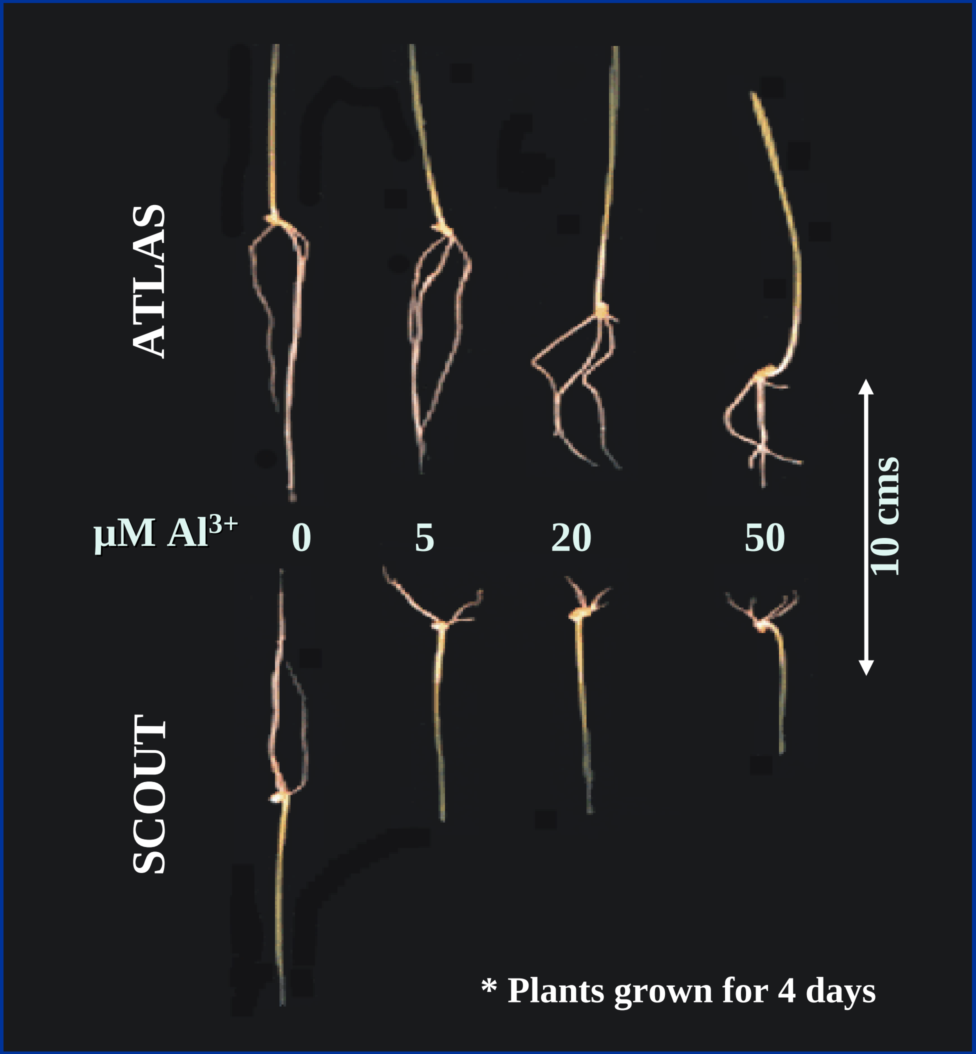
- Over 50% of world’s potentially arable soils are acidic
- At soil pH values below 5, Al^{3+} solubilized into the soil solution
- Al $^{3+}$ inhibits root growth
- Acid soils P deficient due to P fixation on Al and Fe hydroxides

- Crop yields on acid soils reduced due to Al toxicity and P deficiency

Need to identify genes underlying Al tolerance & P efficiency (tolerance to P deficiency)



ALUMINUM TOXICITY IN HIGHER PLANTS



* Plants grown for 4 days

Al^{3+} is the major toxic species

Dramatic inhibition of root growth, occurring within minutes

Root apex must be exposed to Al to 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

On acid soils, because Al toxicity results in a damaged stunted root system, drought stress is a major factor in reduced yields.

Rice P Deficiency

P deficiency occurs on acid, alkaline and neutral pH soils. In this example, mild drought co-occurred - combined stress is quite severe

Al Toxicity Reduces Crop Yields

Tolerant and sensitive RILs grown on highly acidic oxisol soil in Brazil – courtesy of Dr. Vera Alves, Embrapa Maize and Sorghum

Thus, there is considerable interest in identifying Al tolerance genes and associated physiological mechanisms

This Challenge Initiative Builds Upon Progress from Previous GCP Projects

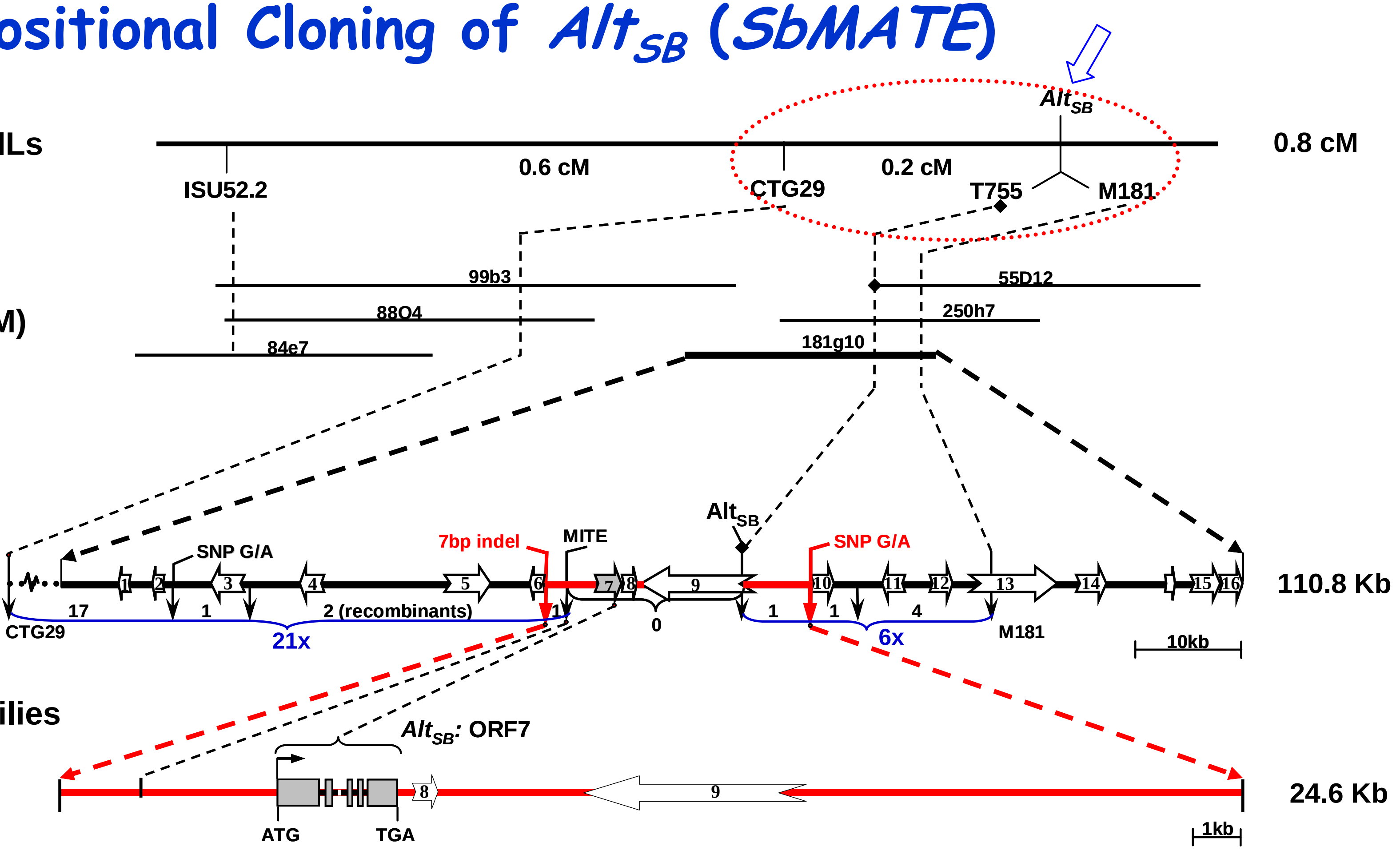
- Cloning of major sorghum Al tolerance gene
  - Isolation and Characterization of Aluminum Tolerance Genes in the Cereals: An Integrated Functional Genomic, Molecular Genetic and Physiological Analysis
  - Tailoring Superior Alleles for Abiotic Stress Genes for Deployment into Breeding Programs: A Case Study Based on Association Analysis of Alt_{SB}, a Major Aluminum Tolerance Gene in Sorghum
  - Improving grain yield on acid soils by the identification of genetic factors underlying drought and aluminum tolerance in maize and sorghum
- Identification and high resolution mapping of a major rice P efficiency QTL
  - Revitalizing Marginal Lands: Discovery of Genes for Tolerance of Saline and Phosphorus□Deficient Soils to Enhance and Sustain Productivity
  - Drought from a Different Perspective: Improved Tolerance in Rice through Phosphorus Acquisition
  - Application and Validation of the Major QTL Phosphate Uptake 1 (Pup1)

Positional Cloning of *Alt$_{SB}$* (*SbMATE*)

Alt$_{SB}$ genetic map: 354 RILs

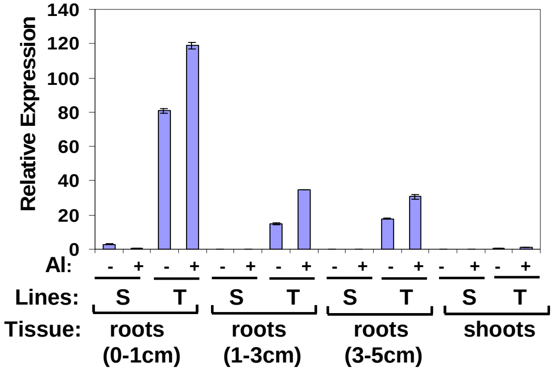
Physical Map (Texas A&M)

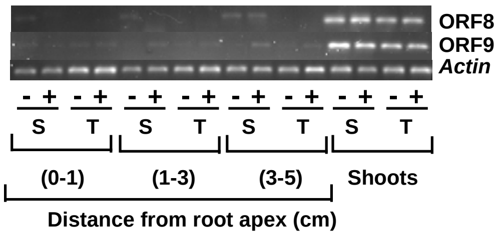
High resolution map:



- **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}**

RT-PCR analysis of ORF7:
<u>M</u>ultidrug and <u>T</u>oxic
<u>C</u>ompund <u>E</u>xtrusion
Family: MATE

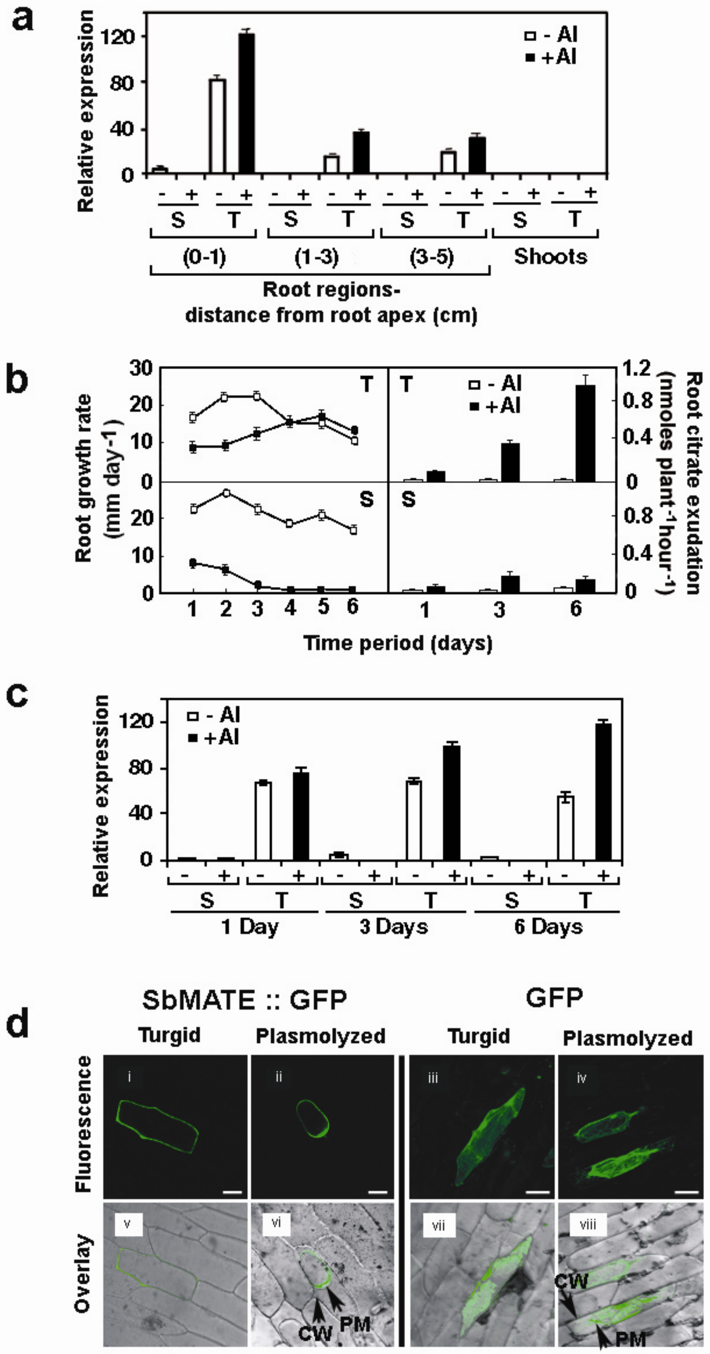


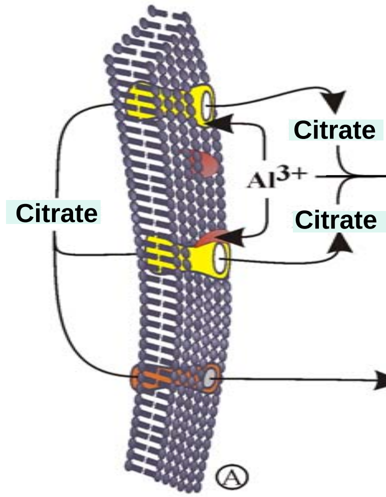


RT-PCR analysis of ORFs 8 and 9

Physiological Mechanism Encoded by *SbMATE*

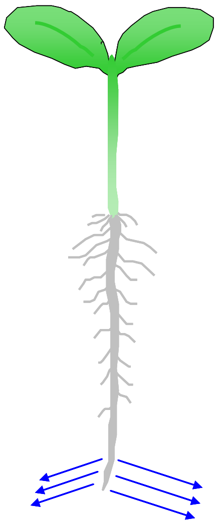
- ➢ Member of the Multidrug and Toxin Efflux (MATE) family
- ➢Al-induced root citrate exudation





Al-citrate

Nature Genetics
39:1156-1161 (2007)



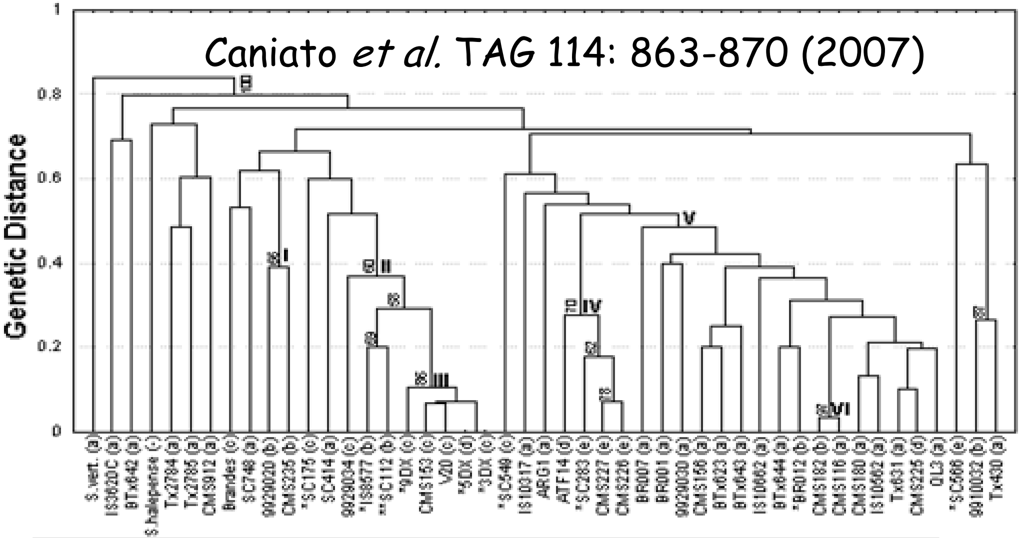
(Non-toxic)
Citrate : Al^{3+}

↑

Citrate + Al^{3+}
(phytotoxic)

Genetic Diversity for Al Tolerance in Sorghum

- ➢ 210 sorghum accessions from CIRAD diversity panel – Generation Challenge Programme: geographical and racial origins, photoperiod sensitivity, production systems
- ➢ 46 sorghum lines from the Embrapa breeding program: enriched for Al tolerance



Caniato *et al.* TAG 114: 863-870 (2007)

See talk by Jurandir Magalhaes on sorghum Al tolerance on Monday at 11:45 AM in "Cereals Research-Diversity" parallel session

Deu *et al.* Genome 49:168-180 (2006)

Involvement of *SbMATE* Homologs in Maize Aluminum Resistance

Maize Al Tolerance

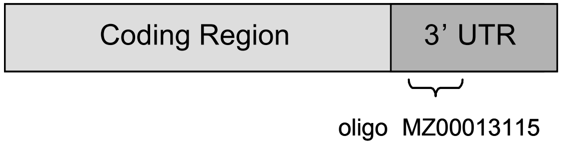
- Maize Al tolerance is a complex genetic trait
- We have found that Al-activated root citrate exudation is a key mechanism of maize Al resistance
  - Pineros et al. 2002. The physiology and biophysics of an aluminum tolerance mechanism based on root citrate exudation in maize. Plant Physiol 129: 1194.
- However, we subsequently found that Al-activated root citrate exudation does not explain all of the variation in maize Al tolerance. Thus other, non-organic acid efflux mechanisms must be functioning in maize.
  - Pineros, et al. 2006. Aluminum tolerance in maize cannot be solely explained by root organic acid exudation. A comparative physiological study. Plant Physiol 137: 231.

Joint Linkage-Expression Analysis of Maize MATE Genes

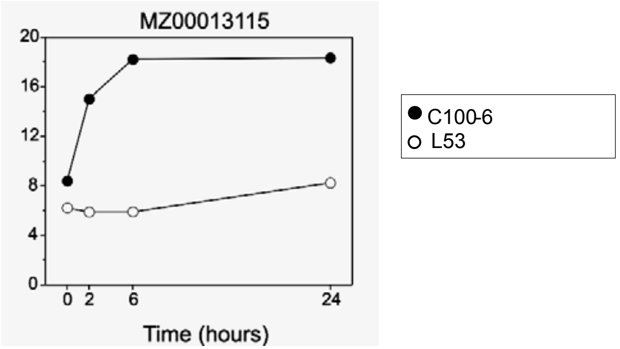
Lyza Maron
Chunzao Mao

Maron et al. 2007. New Phytologist 179: 116

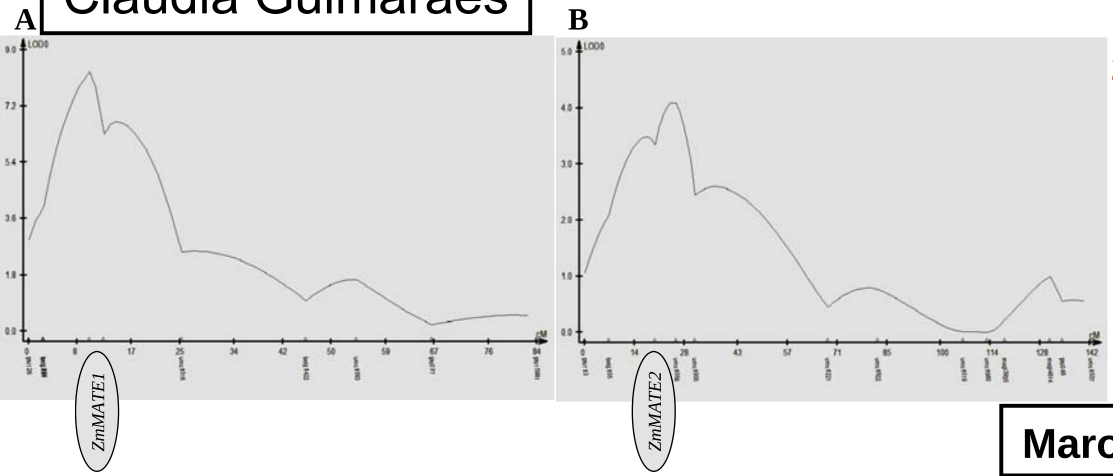
- **Maize EST NP667103 :**



1. **Two MATE oligos were found to be much more highly expressed in root tips of Al tolerant maize lines versus Al sensitive lines using maize long-oligo microarrays**



Claudia Guimaraes

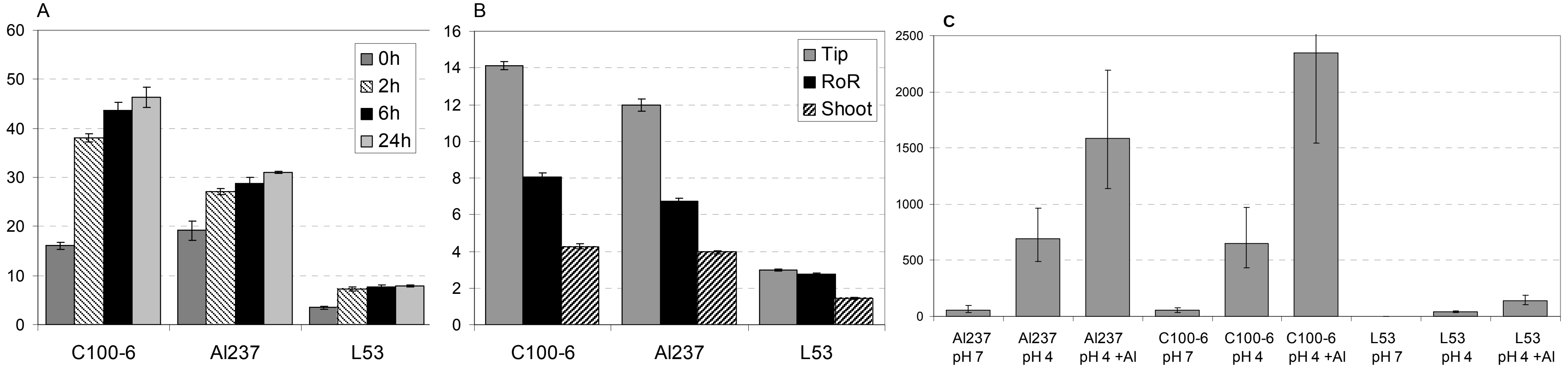


2. ***ZmMATE1* maps to the most significant Al resistance QTL on maize chromosome 6 (A), while *ZmMATE2* maps to the second most significant Al resistance QTL on maize chromosome 5 (B).**

Maron et al. 2009. Plant J (submitted)

ZmMATE1 Is the SbMATE Homolog and Root Citrate Transporter in Maize

1) *ZmMATE1* expressed preferentially in root tip of Al resistant maize and strongly upregulated by Al stress.



Gene expression analysis by quantitative real-time PCR in Al tolerant (C100-6 and Al237) and Al sensitive (L53) maize. **(A)** time course of *ZmMATE1* expression in root tips exposed to 39μM Al^{3+}. **(B)** *ZmMATE1* expression in different parts of the plant: root tip, rest of root (RoR) and shoot. **(C)** Shift from neutral to low pH also induces *ZmMATE1* expression in tolerant lines, and this expression is increased further when Al is added to low pH media

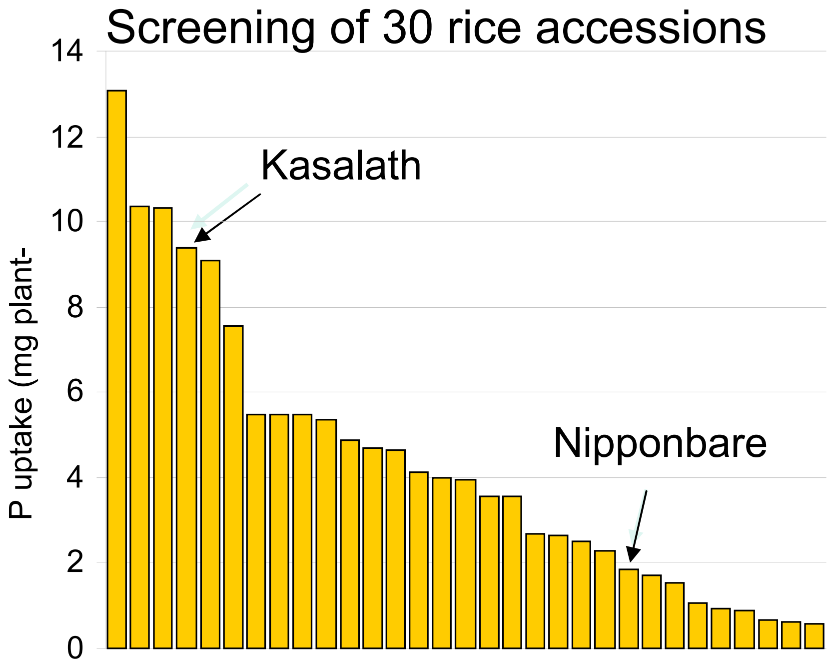
2) Electrophysiological analysis of ZmMATE1 expressed in *Xenopus* oocytes showed it is a citrate transporter and has identical transport properties to SbMATE.

3) Expression of *ZmMATE1* in transgenic Arabidopsis greatly increased Al resistance.

The locus Pup1 (P uptake 1) confers tolerance to P deficiency

Experimental conditions at Tsukuba: Upland = rainfed field, but optional irrigation, particularly during seedling stage.
Soil: volcanic ash, highly P fixing, Bray-II P <10ppm (Al-P, Fe-P, org-P)

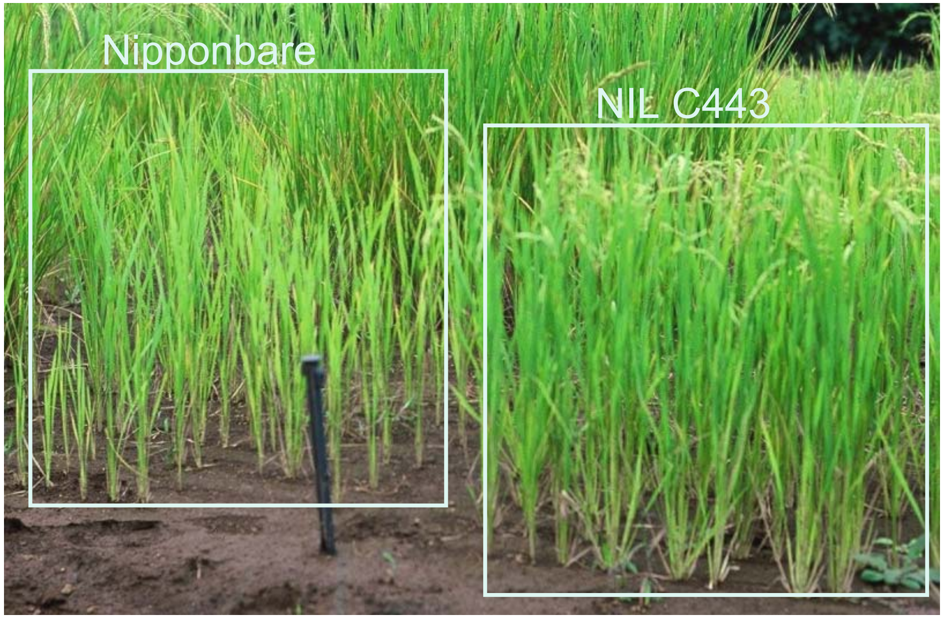
A Major P Uptake Efficiency QTL, *Pup1*, Identified – Mapped Here in a P-Fixed Field Site in Japan Under Rainfed Conditions (Matthias Wissuwa, JIRCAS)


Screening of 30 rice accessions

P-uptake:	LOD 10.7 (28%)
dry weight:	LOD 10.5 (27%)
tiller number:	LOD 7.9 (21%)

(P-use efficiency: LOD 6.6 (19%) NB allele)

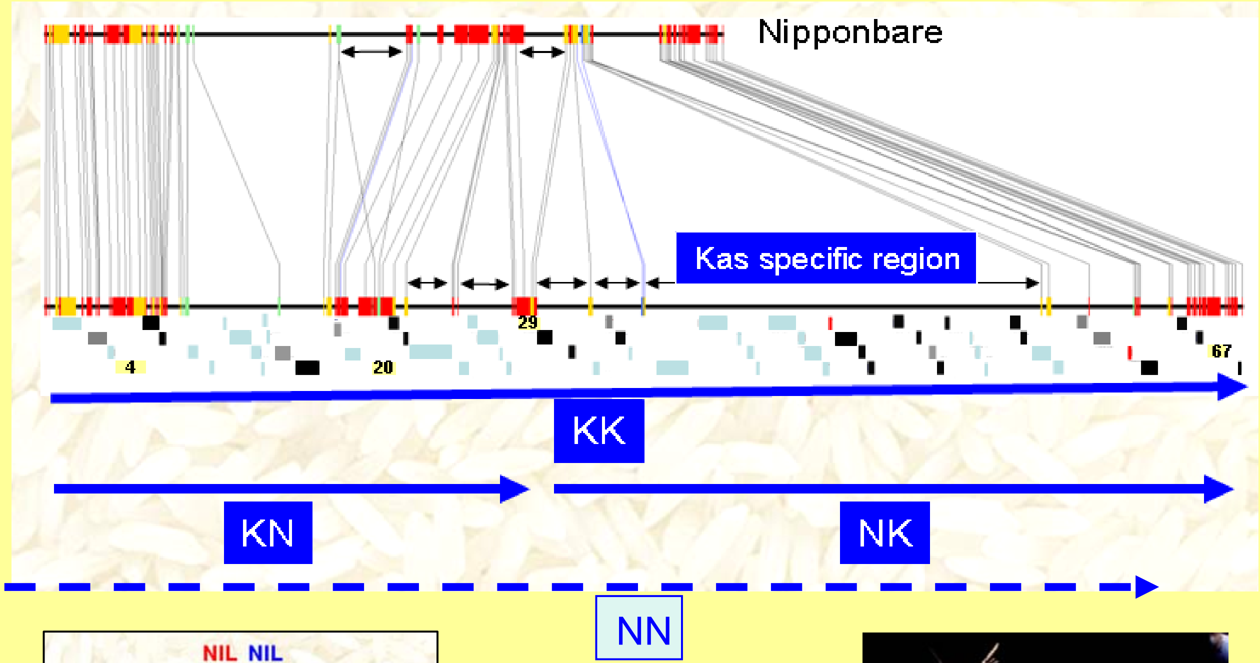
Pup1 near isogenic lines

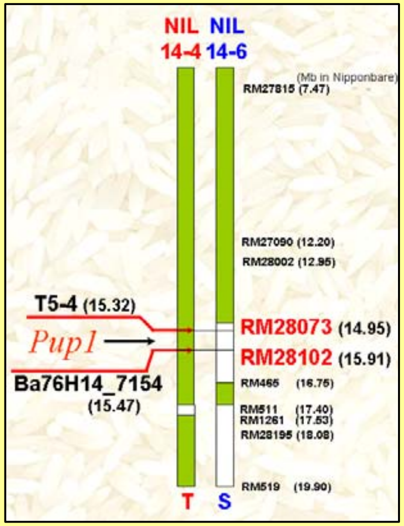


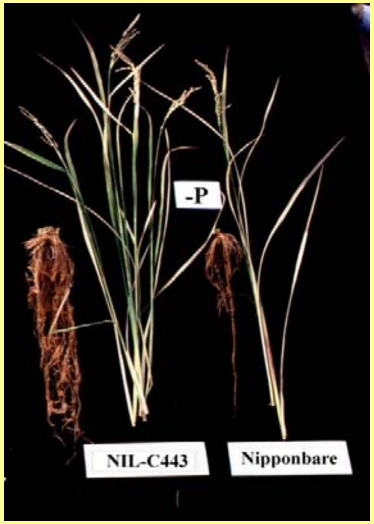
P uptake (mg root weight^{-1})

	+P	-P
Nipponbare	13.7	1.8
NIL-C443	13.9	3.2
Kasalath	10.9	3.2

Fine-Scale Mapping of *Pup1* QTL: Gene-Based *Pup1* Markers & *Pup1* NILs







IRRI

Current Short List Pup1 Candidate Genes

Gene name	Annotation	Gene in Nipponbare	model validated?	Gene expressed?
OsPup1K01-1	DOMON (DUF568)	no gene model in Nipponbare	+	no
OsPup1K04-1	fatty acid alpha DOX	highly conserved	+	yes
OsPup1K05-1	CutC or part of DOX?	no gene model in Nipponbare	still unclear	yes
OsPup1K20-1	dirigent	conserved	+	yes
OsPup1K29-1	hypothetical protein	corresponds to two genes in Nipponbare and 93-11	+	yes
OsPupK46-1	protein kinase	absent	+	yes
OsPup1K49-1	Zn-knuckle	absent	+	yes
OsPup1K67-1	aspartic proteinase	conserved	+	no
OsPup1K53-1	Zn-finger protein?	absent	+	no

See talk by Sigrid Heuer on *Pup1* on Wednesday at 11 AM in the Cereal Research – Breeding parallel session

Transgenic plants:

(i) **RNAi:**

Genes #5, #20, #29, #46

(ii) **Overexpression:**

Genes #20, #46, #5, #30,

Current CI: Improving Cereal Yields on Al Toxic and P Deficient Acid Soils via Comparative Genomics

- Using comparative genomics to identify *SbMATE* homologs in rice and maize in order to isolate rice/maize Al tolerance genes
  - Good progress already on *ZmMATE1* as a *bona fide* maize Al tolerance gene (the citrate efflux transporter)
  - If rice *SbMATE* homologs not functional, map-based cloning of novel and major Al tolerance QTL well under way.
- Using comparative genomics to identify *Pup1* homologs in maize and sorghum
  - If maize or sorghum *Pup1* homologs not functional, *Pup1* QTL that will be identified for verification of *Pup1* homologs will be introgressed into breeding lines for testing in Africa.
- These discoveries translated into improved rice, maize & sorghum acid soil tolerance via NARS partners in Kenya (maize), Mali & Niger (sorghum) and Indonesia (rice)

PARTNERS

- Sam Gudu, Moi University and KARI, Kenya
- Soumana Souley & Maman Nouri, INRAN, Niger
- Eva Weltzien, Bettina Haussman and Fred Rattunde, ICRISAT
- Masdiar Bustamam, ICABIOGRAD, Indonesia
- Sigrid Heuer and Abdel Ismail, IRRI, Philipines
- Matthias Wissuwa, JIRCAS, Japan
- Susan McCouch, Steve Kresovich, Theresa Fulton, Cornell University
- Leon Kochian, Jiping Liu, Adam Famoso, Miguel Pineros, Lyza Maron, USDA-ARS, Cornell University
- Jurandir Magalhaes, Bob Schaffert, Claudia Guimaraes, Sidney Parentoni, Joao Herbert M Viana, Alvaro Resende Vilela, Vera Alves, Embrapa Maize and Sorghum

IRRI