

Embrapa

Milho e Sorgo

# The effect of the $Alt_{SB}$ gene on root growth in nutrient solution and grain production of isogenic sorghum hybrids

## XVI International Plant Nutrition Colloquium: Plant Nutrition for Sustainable Development and Global Health



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# The effect of the *Alt<sub>SB</sub>* gene on root growth in nutrient solution and grain yield of isogenic sorghum hybrids

**A link between the molecular and field levels**

## Research Team

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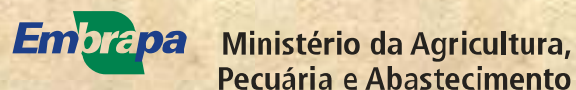
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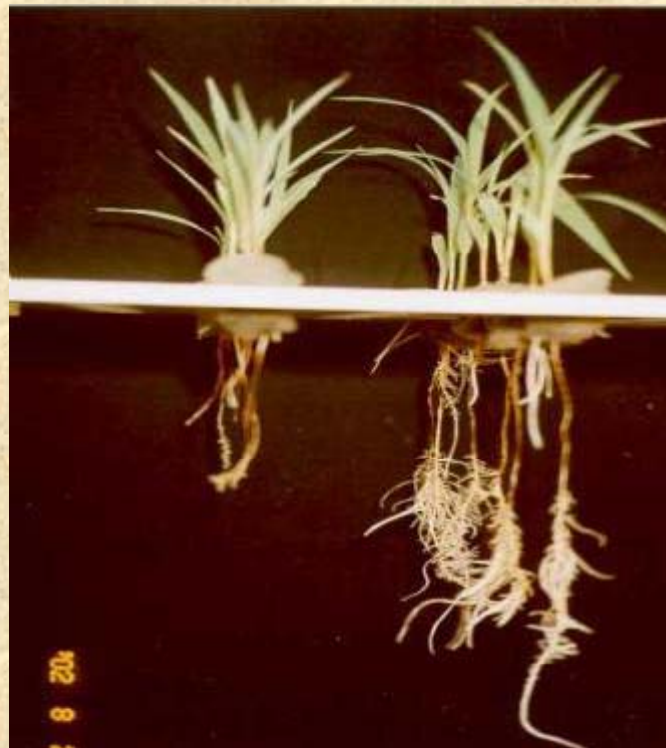
**1975 – Identification of Genetic Variation for Tolerance to Al Toxicity in Sorghum in Brazil**

**1976 – Identification of Sorghum Lines Contrasting for Tolerance to Al Toxicity in Sorghum in Brazil**

**BR007B**

**SC283**

**BR007B – Aluminum Susceptible Line**



**SC283 – Aluminum Tolerant Line from Sorghum Conversion Program**

**Sorghum seedlings seven days in nutrient solution with 27  $\mu$ M Al**

**1992 – Development of Two Pairs of Contrasting Isogenic Cytoplasmic Male Sterile Lines for Tolerance to AI toxicity from a Cross Between BR007B and SC283**



**ATF8B - AI Susceptible**

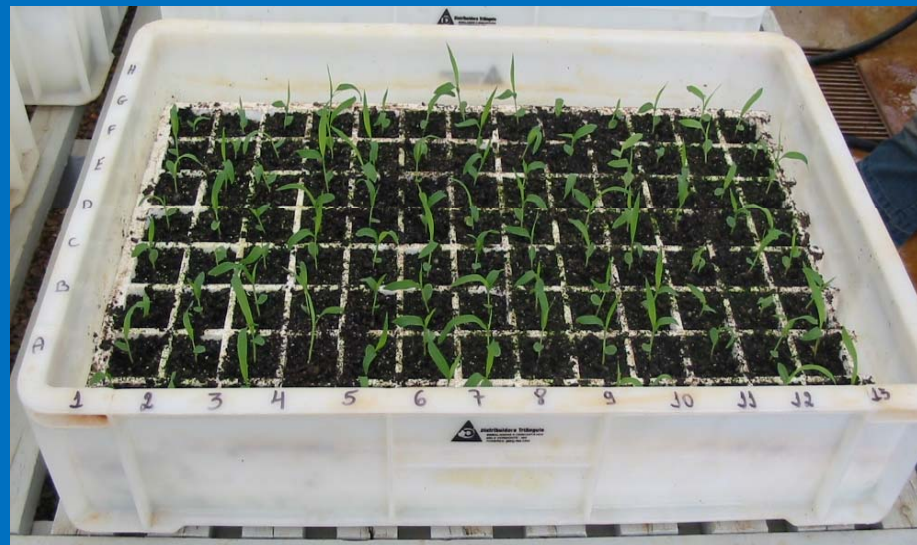
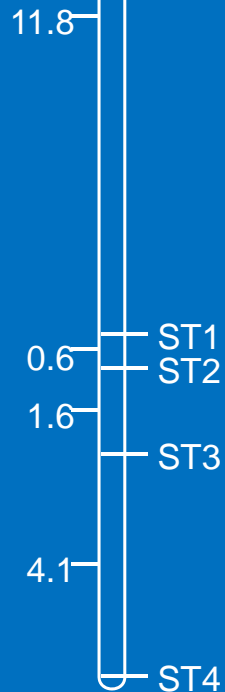
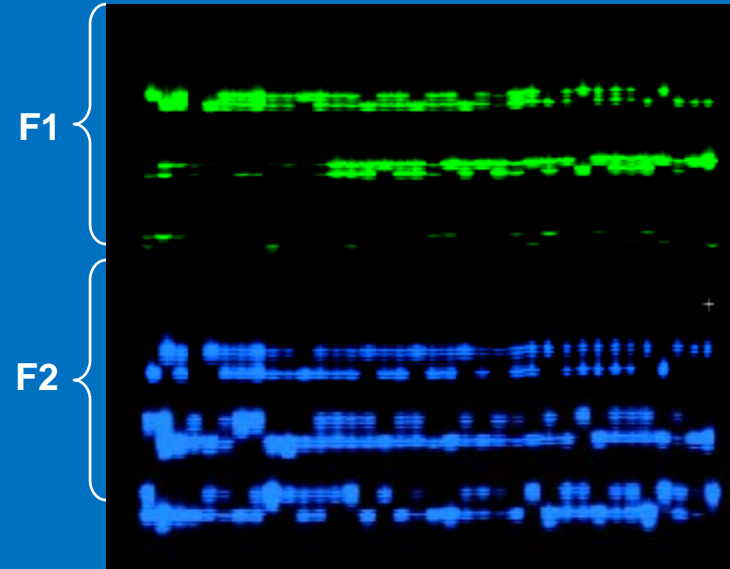
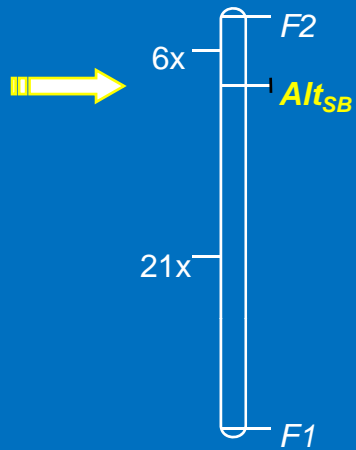
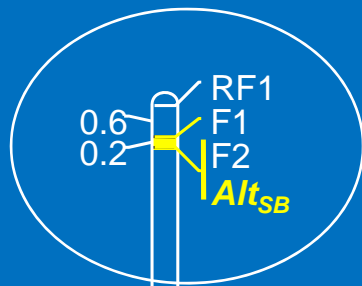
**ATF10B - AI Tolerant**



**ATF13B - AI Susceptible**

**ATF14B – AI Tolerant**

# 2006 - Positional Mapping of Sorghum AI Tolerant Gene ( $Alt_{SB}$ ) from SC283

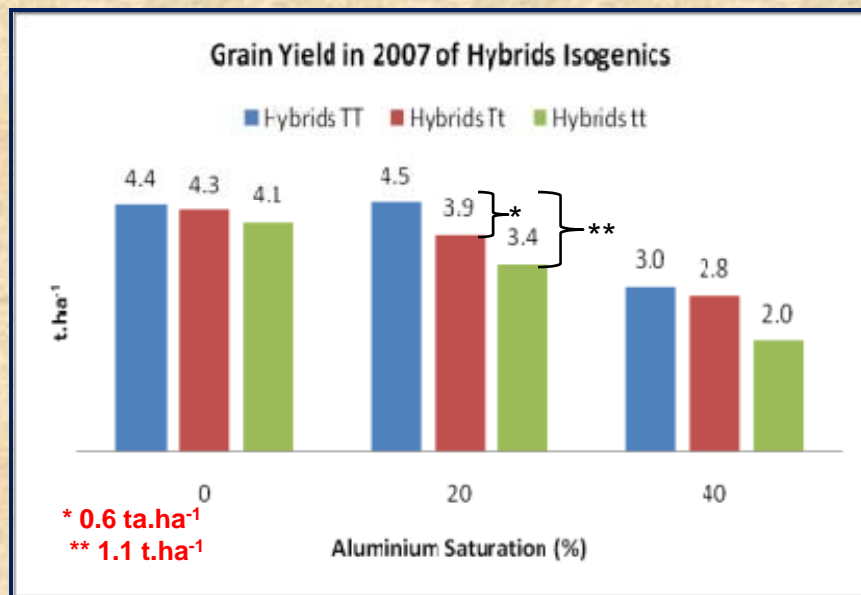
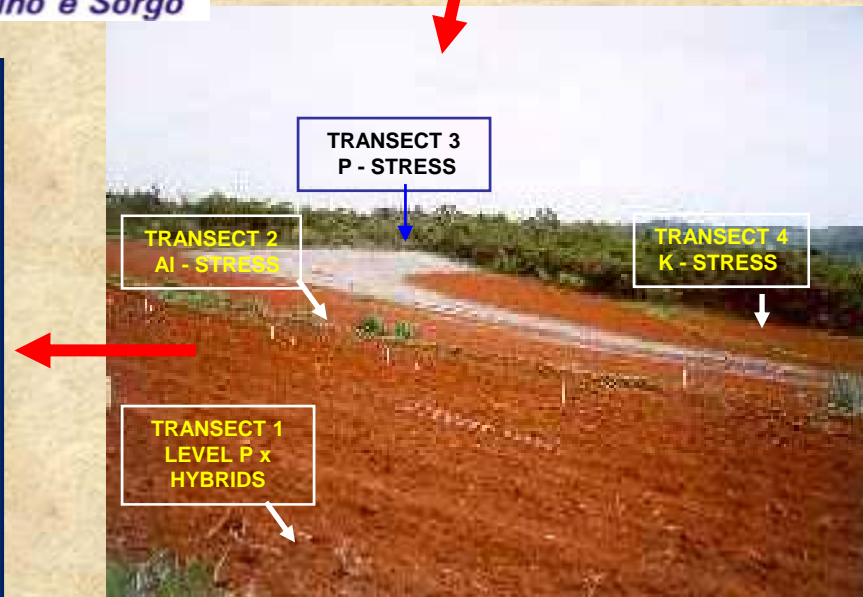
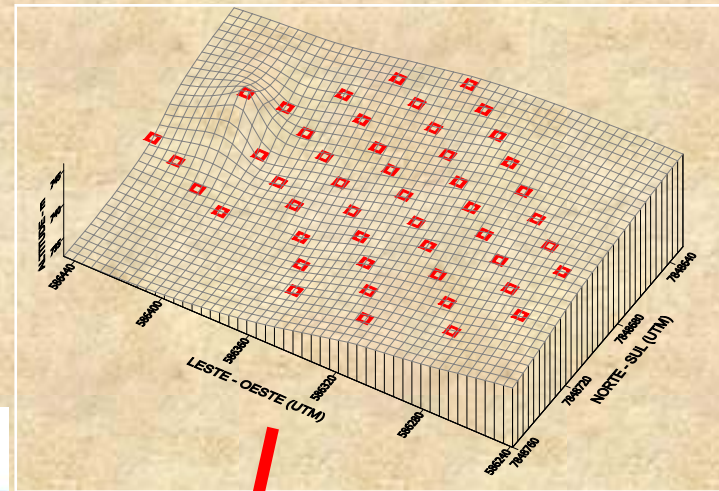


# 2006 – Development of Aluminum Toxicity Phenotyping Site

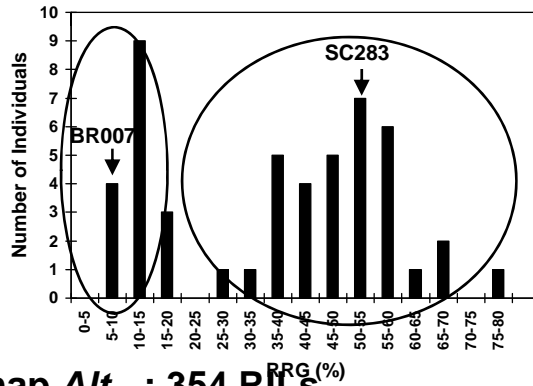
*Planning of Test Areas Using Precision Agriculture Technology to Reduce Soil Spatial Variability for Evaluation of Abiotic Stresses in Crops*



Three Levels of Al Saturation



# 2007 - Positional Mapping and Cloning of *Alt<sub>SB</sub>*

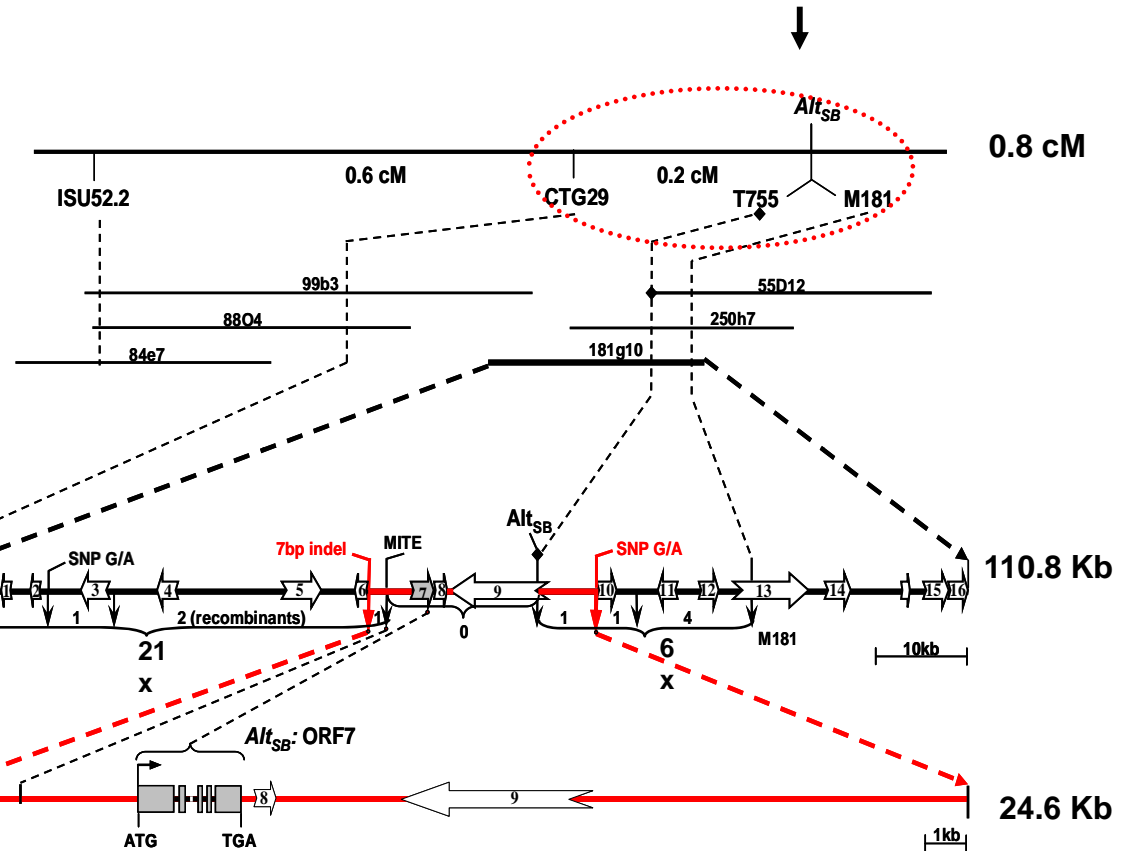


Genetic map *Alt<sub>SB</sub>*: 354 RILs

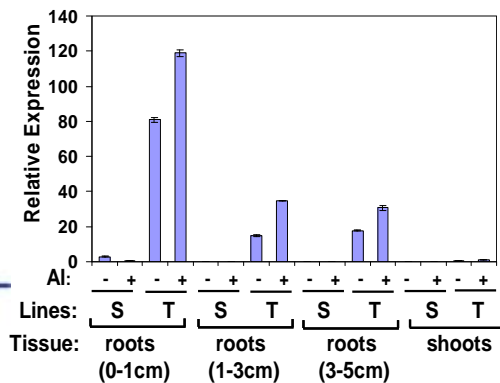
Physical map (Texas A&M)

High resolution map

- 2085 F<sub>2</sub>:BR007xSC283
- 27 recombinants
- Region of 24.6 Kb
- 513 Kb cM<sup>-1</sup>



RT-PCR  
*Alt<sub>SB</sub>*  
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Transgenic Arabidopsis  
expressing *Alt<sub>SB</sub>*

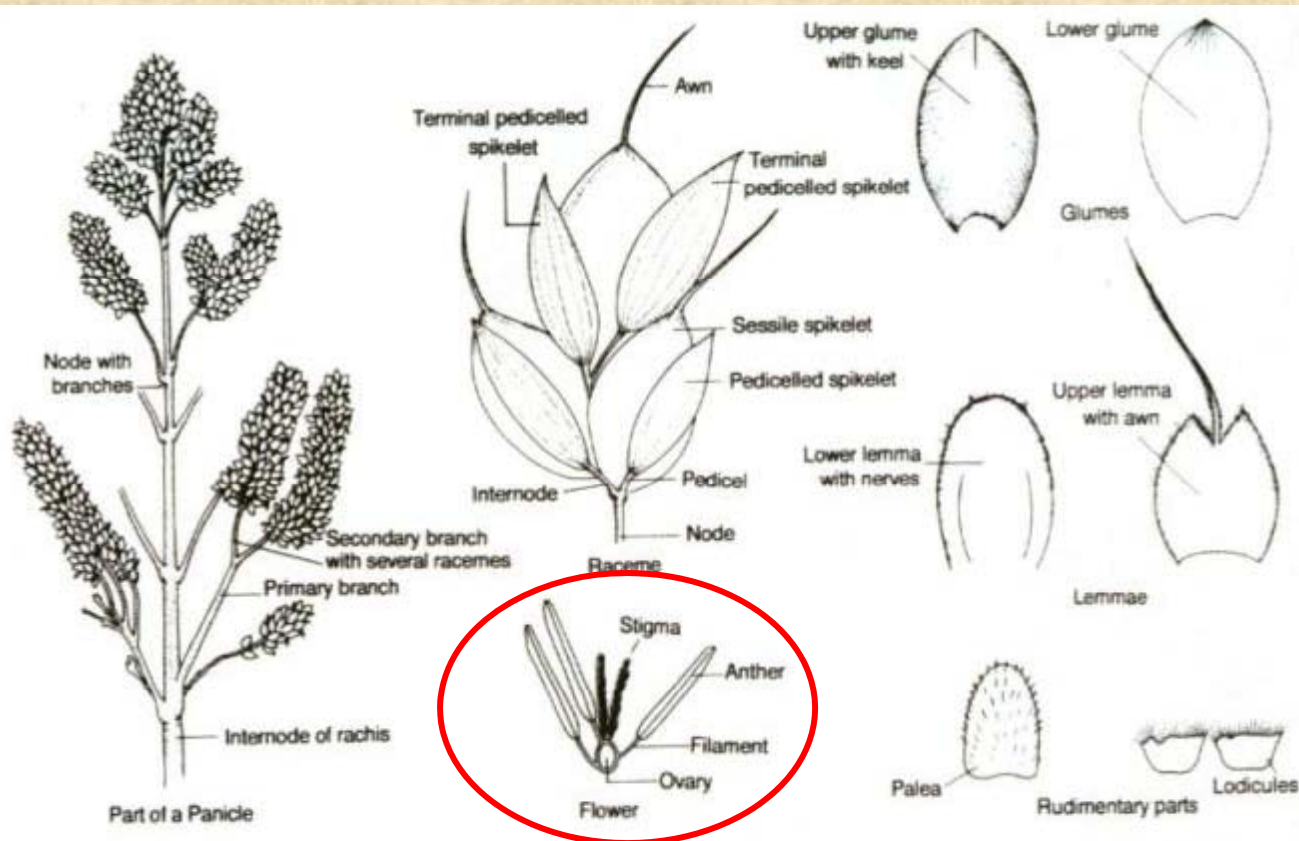
Magalhaes et al. Genetics, 2004

Magalhaes et al. Nature Genetics, 2007

# Summary of $Alt_{SB}$ from SC 283

- Aluminum Tolerance in Sorghum is Basically Monogenic;
- $Alt_{SB}$  Is Partially Dominant;
- Al  $Alt_{SB}$  Regulates Citrate Transport;\
- Tolerance in Sorghum is the Result of Citrate Exudation in the Root Tip and;
- Citrate Complexes with Al in the Soil Rhizosphere Forming a Nontoxic Compound

# Diagram of the sorghum flower



As the sorghum flower is perfect, that is, containing both male (anther) and female (ovary) organs within the glumes, hybridization is accomplished by using hand emasculation or cytoplasmic male sterility

Raceme with Spikelets

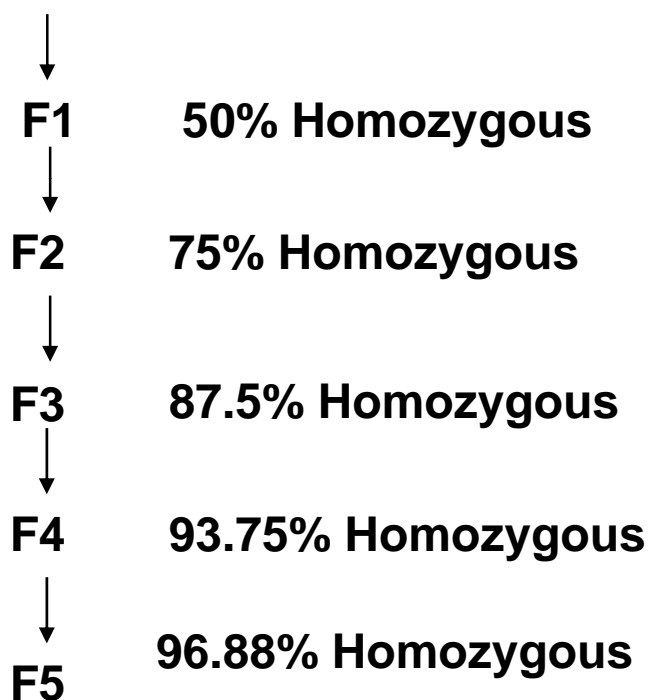
# Hand emasculating and pollination to produce hybrid seed – limited quantity



# Each of the Two Pairs of Contrasting Isogenic Cytoplasmic Male Sterile Lines Were Derived from Segregating F5 Families from the Cross Between BR007B and SC283

**Susceptible** x **Tolerant**

**BR007B** x **SC283**

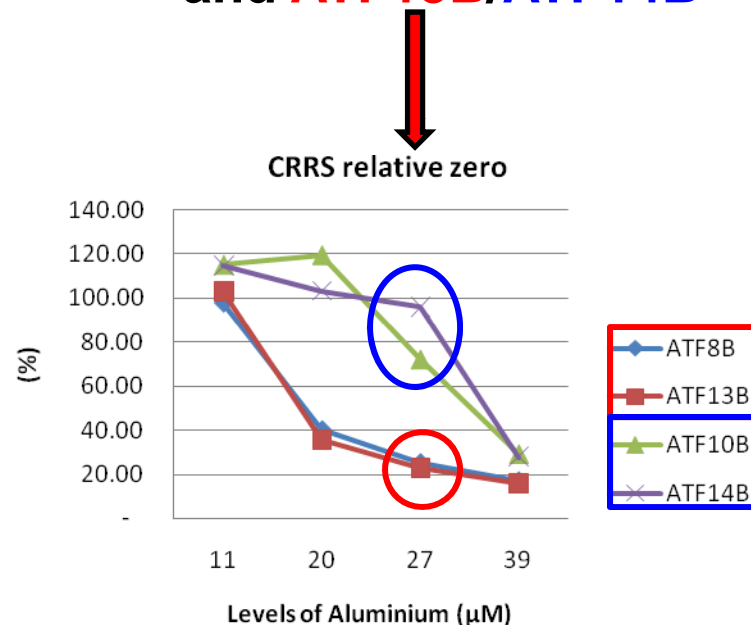


**ATF 8B**  
**ATF 13B**

**ATF 10B**  
**ATF 14B**

} **Two isogenic pairs**

**ATF8B/ATF10B**  
and **ATF13B/ATF14B**



**Observation:** Both Isogenic pairs were derived from the same F3 plant, ATF14B is slightly superior to ATF10B

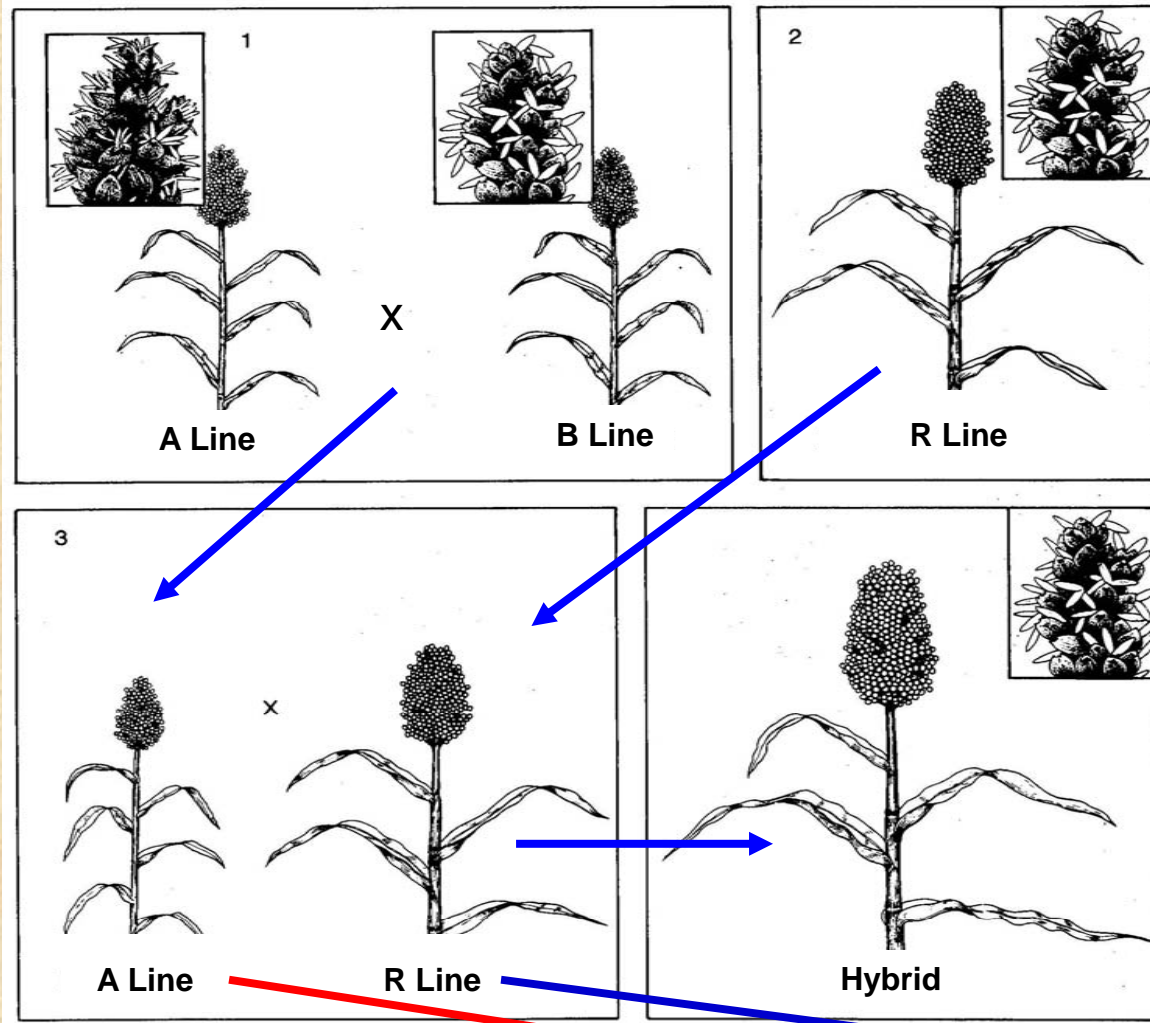
# 2007 - Marker Assisted Backcrossing to Develop Isogenic Breeding Lines Tolerant to Al Toxicity in Sorghum. (Phenotypic Marker – Seedling Root Growth in Nutrient Solution with Al)

	Recurrent Al Susceptible Line	x	Tolerant Source
Cross	tt	x	TT
F1		x	Tt
BC1 (Recurrent Parent) x F1	(tt	x	Tt)
BC1F1	$\frac{1}{2}$ Tt		( $\frac{1}{2}$ tt Eliminated in Nutrient Solution Test)
BC2 (Recurrent Parent) x BC1F1	(tt	x	Tt)
BC2F1	$\frac{1}{2}$ Tt		( $\frac{1}{2}$ tt Eliminated in Nutrient Solution Test)
.			
.			
.			
BC4F1	$\frac{1}{2}$ Tt		( $\frac{1}{2}$ tt Eliminated in Nutrient Solution Test)
BC4F2	$\frac{1}{4}$ TT		( $\frac{1}{2}$ Tt and $\frac{1}{4}$ tt Eliminated in Nutrient Solution Test)
BC4F3	100% TT		and 97% Equal to Recurrent Parent

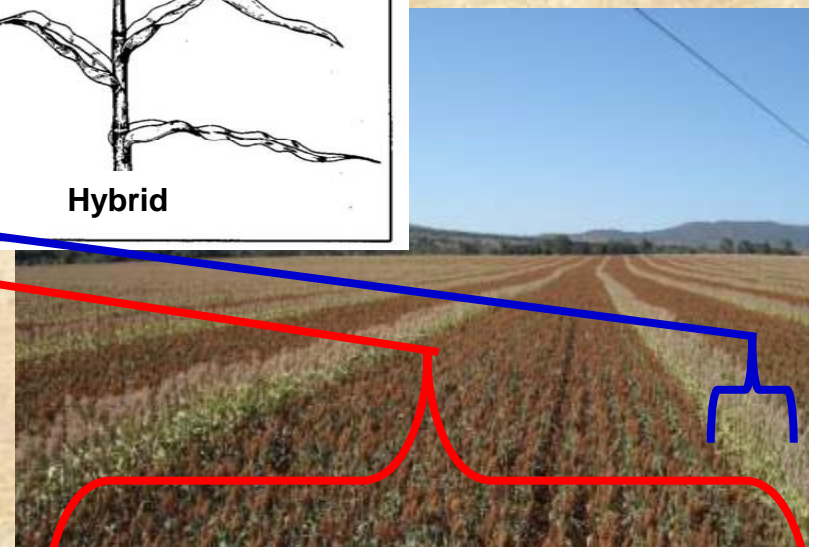


# Commercial Sorghum Hybrid Seed Production

Isogenic A & B lines have been developed by selecting contrasting plants in advanced generations of segregating families from single seed descent

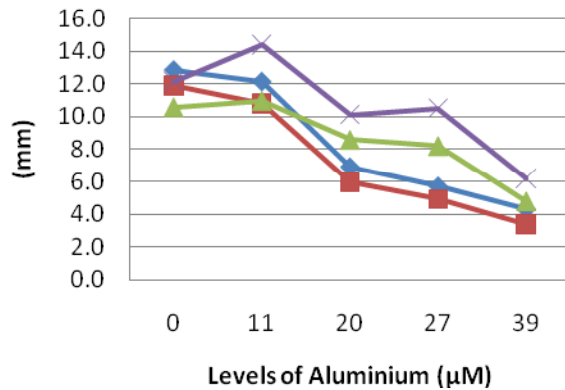


Isogenic R Lines for AI tolerance have been developed by Marker Assisted Backcrossing

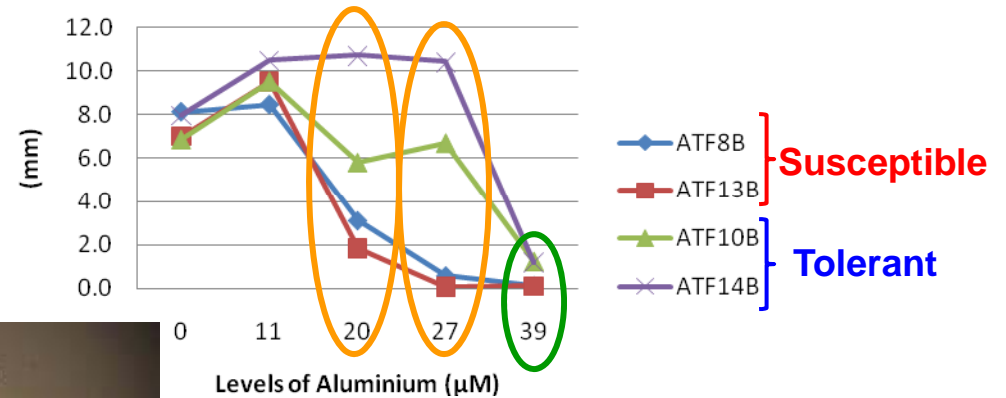


# Root Growth Response of Isogenic Female Sorghum Lines to Increasing Levels of Aluminum in Nutrient Solution

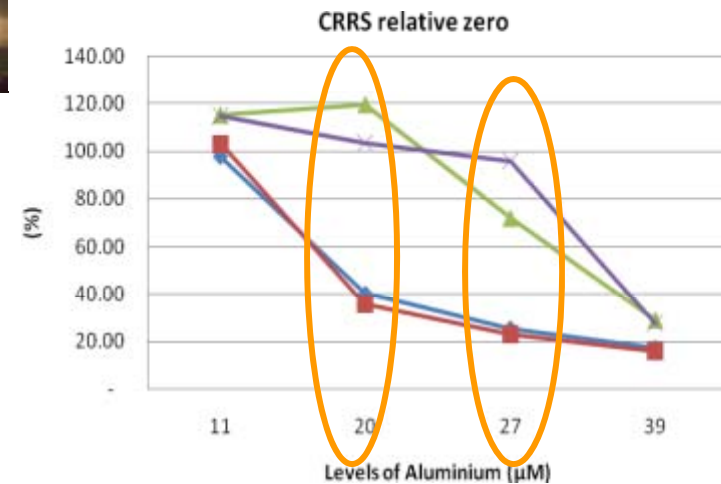
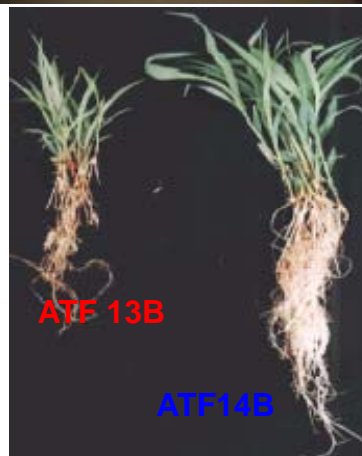
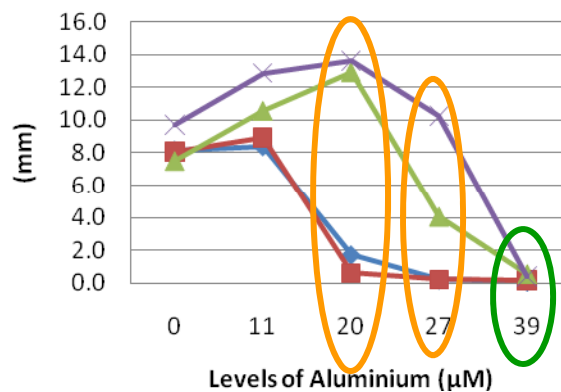
Daily Root Growth 0-3 Days of inbred lines



Daily Root Growth 3-5 Days of inbred lines



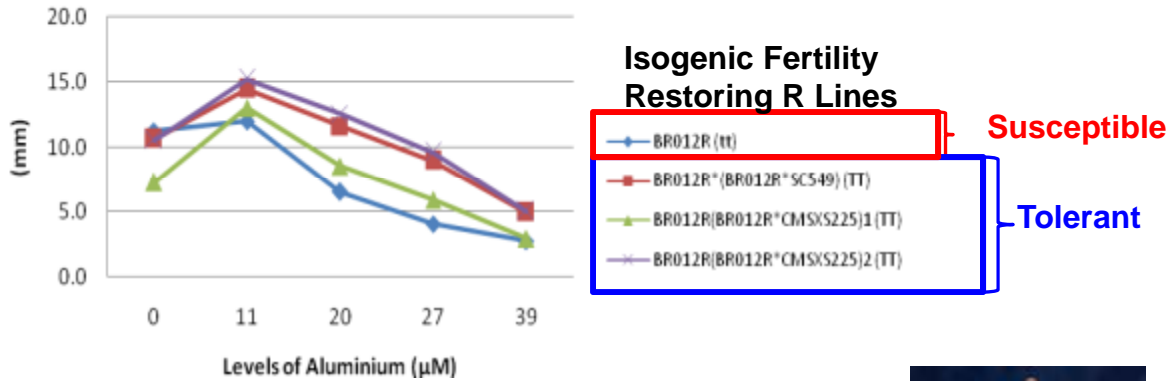
Daily Root Growth 5-7 Days of inbred lines



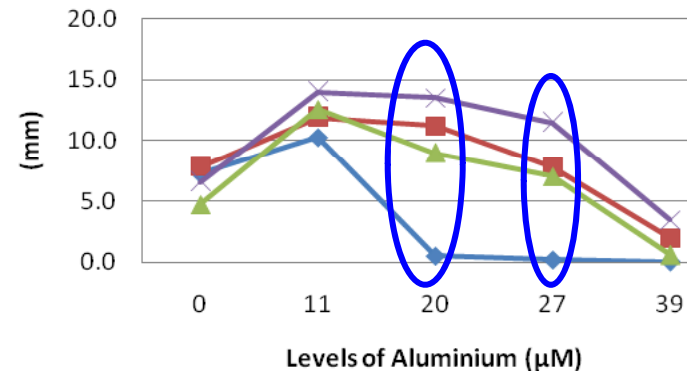
- Daily root growth of zero indicates that the root has died
- ATF14B is slightly superior to ATF10B

# Root Growth Response of Isogenic Male Sorghum Lines to Increasing Levels of Aluminum in Nutrient Solution

Daily Root Growth 0-3 Dias Isogenic Lines

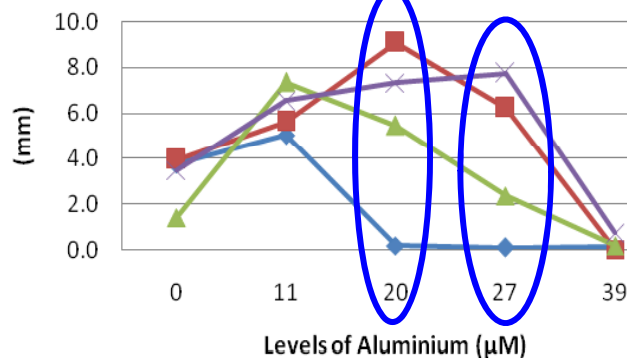


Daily Root Growth 3-5 Days Isogenic Lines



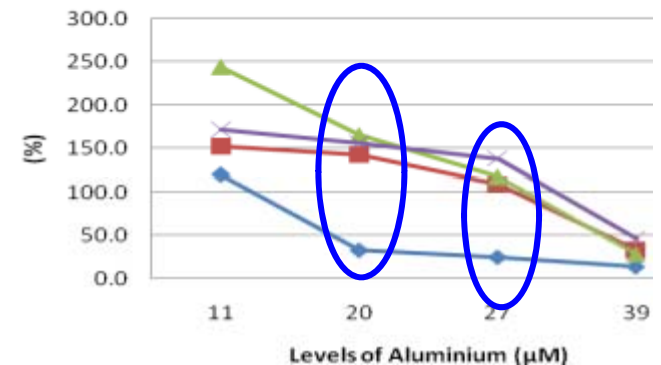
BR012R RC3F5 (SC549)

Daily Root Growth 5-7 Days Isogenic Lines



BR012R RC3F5 (CMSXS 225)

Relative Seminal Root Growth to Zero Al<sup>3+</sup>



Obs. selection 2 of CMSXS225 is slightly superior to selection 1

# 2007 - GENERATION OF ISOGENIC HYBRIDS

Large quantities of seed of sixteen isogenic hybrids were generated by crossing each of the four male sterile A lines with four isogenic fertility restoring male line

## Male Sterile Female A-Lines

ATF8A – Susceptible  
 ATF10A – Tolerant  
 ATF13A – Susceptible  
 ATF14A – Tolerant

## Male Fertility Restoring R-Lines

BR012R – Susceptible\*  
 (BR012R(SC549) – Tolerant  
 (BR012R(CMSXS225)1 – Tolerant  
 (BR012R(CMSXS225)2 – Tolerant

X

Susceptible (tt)	X	Susceptible (tt)	→	Susceptible Hybrid (tt)
Susceptible (tt)	X	Tolerant (TT)	→	“Tolerant” Hybrid (Tt)
Tolerant (TT)	X	Susceptible (tt)	→	“Tolerant” Hybrid (Tt)
Tolerant (TT)	X	Tolerant (TT)	→	Tolerant Hybrid (TT)

The hybrid ATF8A x BR012R is isogenic to the hybrid ATF10A x BR012R

- ATF13A x BR012R has no tolerant alleles (tt)
- ATF14A x BR012R has one tolerant allele (Tt)
- ATF8A x BR012R has no tolerant alleles (tt)
- ATF10A x BR012R has one tolerant allele (Tt)

The hybrid ATF8Ax(BR012RSC549) is isogenic to the hybrid ATF10Ax(BR012RSC549)

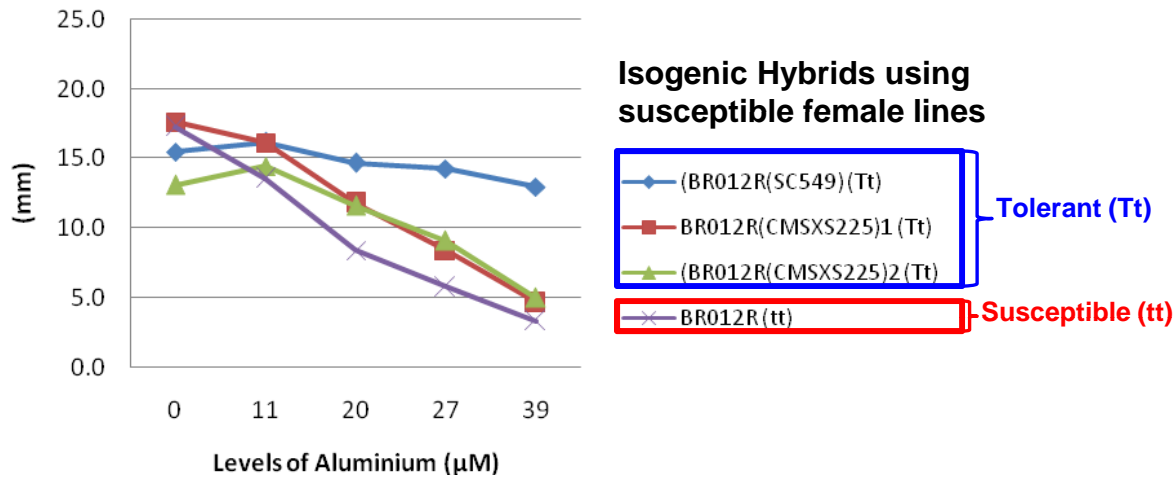
- ATF8A x (BR012RSC549) has one tolerant alleles (Tt)
- ATF10A x (BR012RSC549) has two tolerant allele (TT)
- ATF13A x (BR012RSC549) has one tolerant alleles (Tt)
- ATF14A x (BR012RSC549) has two tolerant allele (TT)

Likewise for isogenic R-lines BR012R(CMSXS225)1 and BR012R(CMSXS225)2

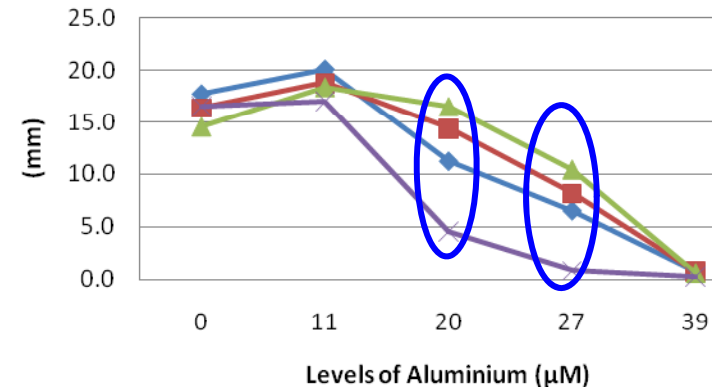
Observation: As ATF8B and ATF13B are susceptible and related the results have been pooled in the field experiments, likewise as ATF10B and ATF14B are tolerant and related the results have been pooled in the field experiments.

# Comparison of Isogenic Hybrids Using Susceptible Female Lines (Zero vs. One Allele)

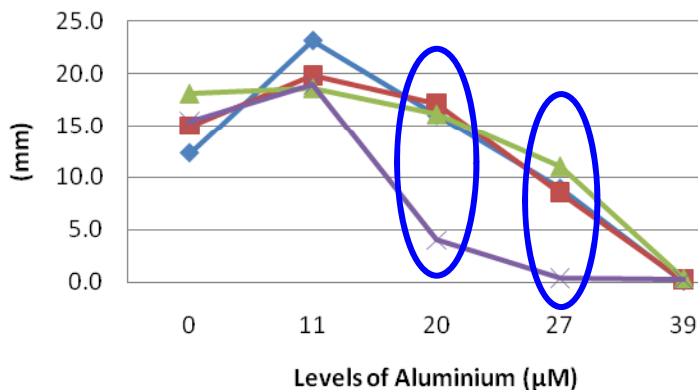
Daily Root Growth 0-3 Days Isogenic Hybrids



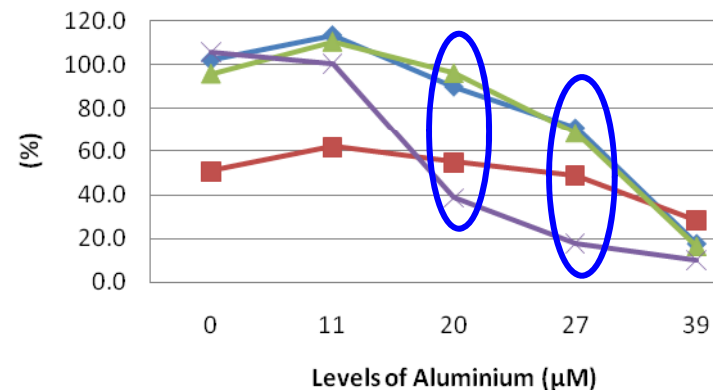
Daily Root Growth 3-5 Days Isogenic Hybrids



Daily Root Growth 5-7 Days Isogenic Hybrids

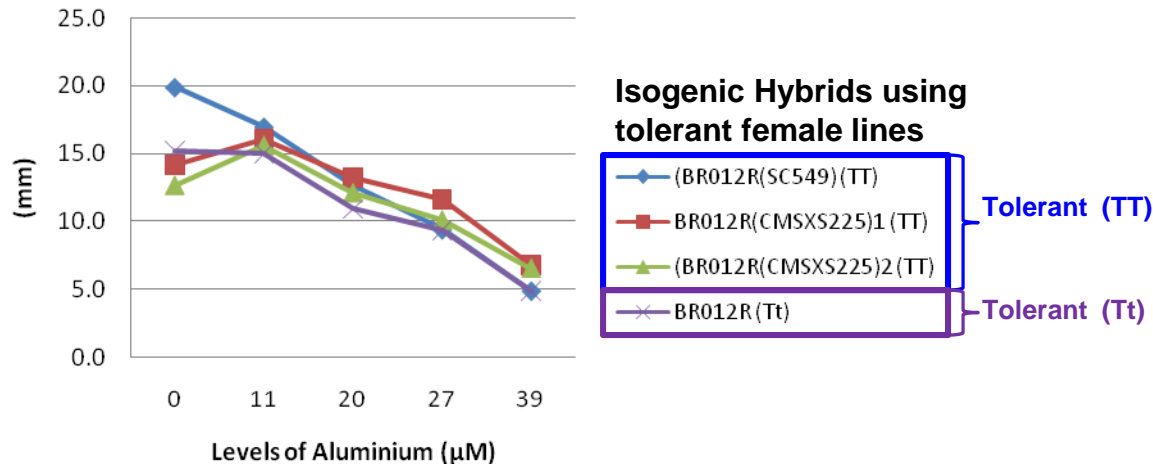


CRRS

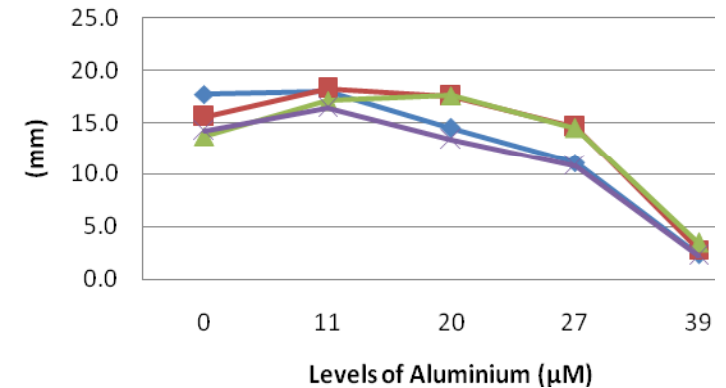


# 2009 - Comparison of Isogenic Hybrids Using Tolerant Female Lines (One vs. Two Alleles)

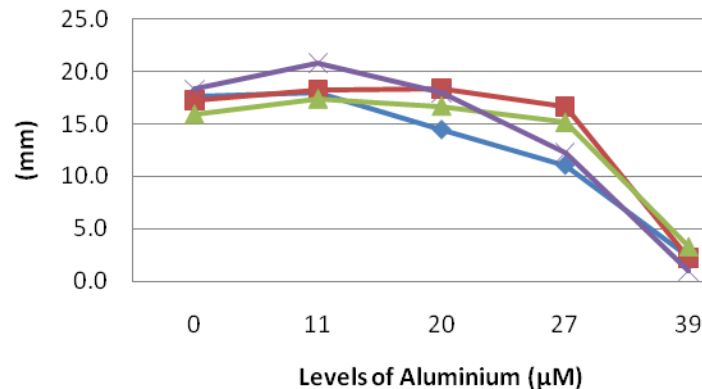
Daily Root Growth 0-3 Days Isogenic Hybrids



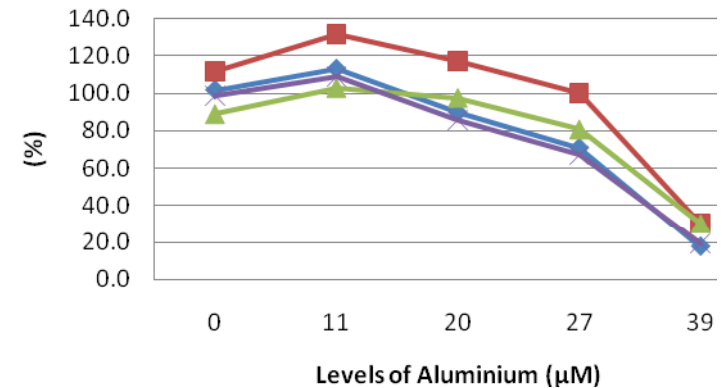
Daily Root Growth 3-5 Days Isogenic Hybrids



Daily Root Growth 5-7 Days Isogenic Hybrids

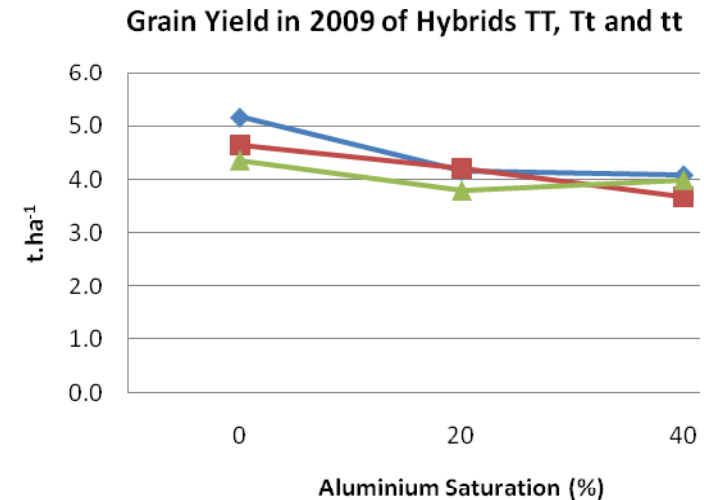
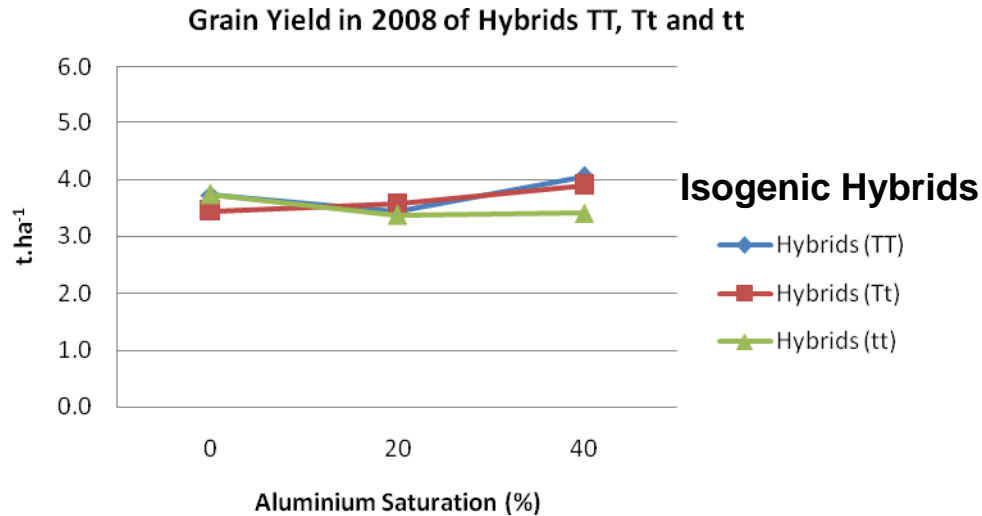


CRRS



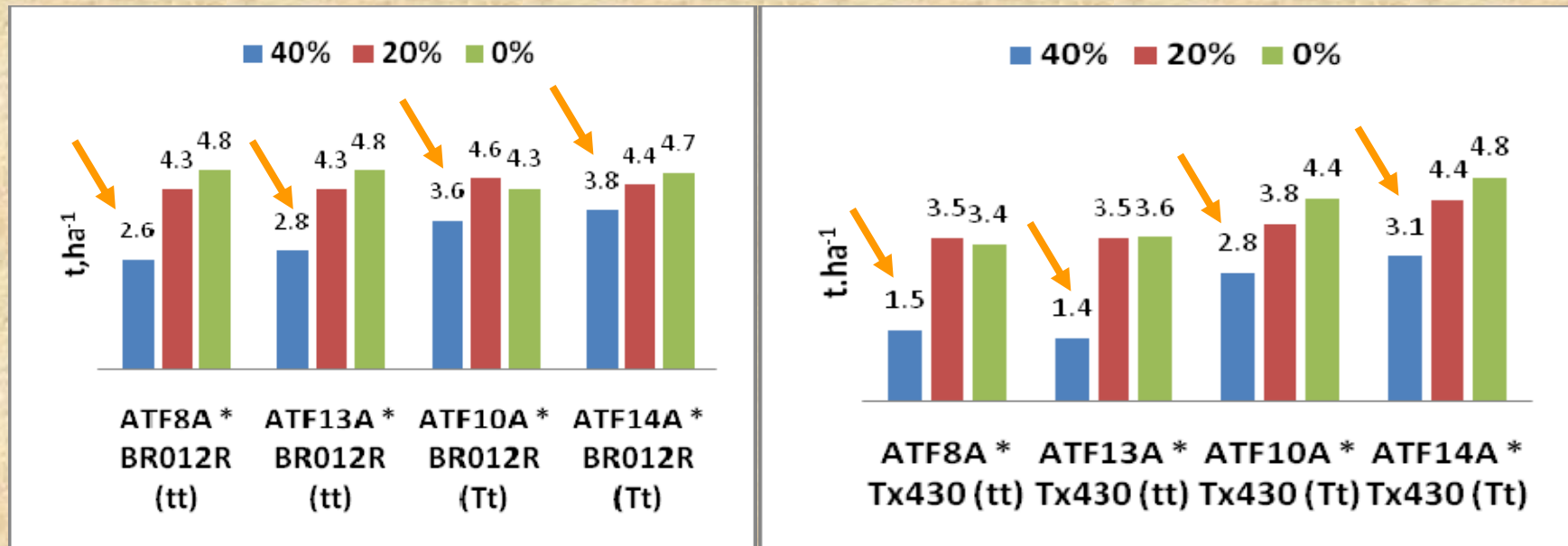
**Observation: Small difference between TT and Tt hybrids because of partial dominance**

## 2009 - Comparison of Grain Yield of Isogenic Hybrids with **Zero (tt)**, **One (Tt)** and **Two (TT)** $Al/t_{SB}$ Alleles at Three Levels of Al Saturation in 2008 and 2009 at Embrapa Phenotyping Site



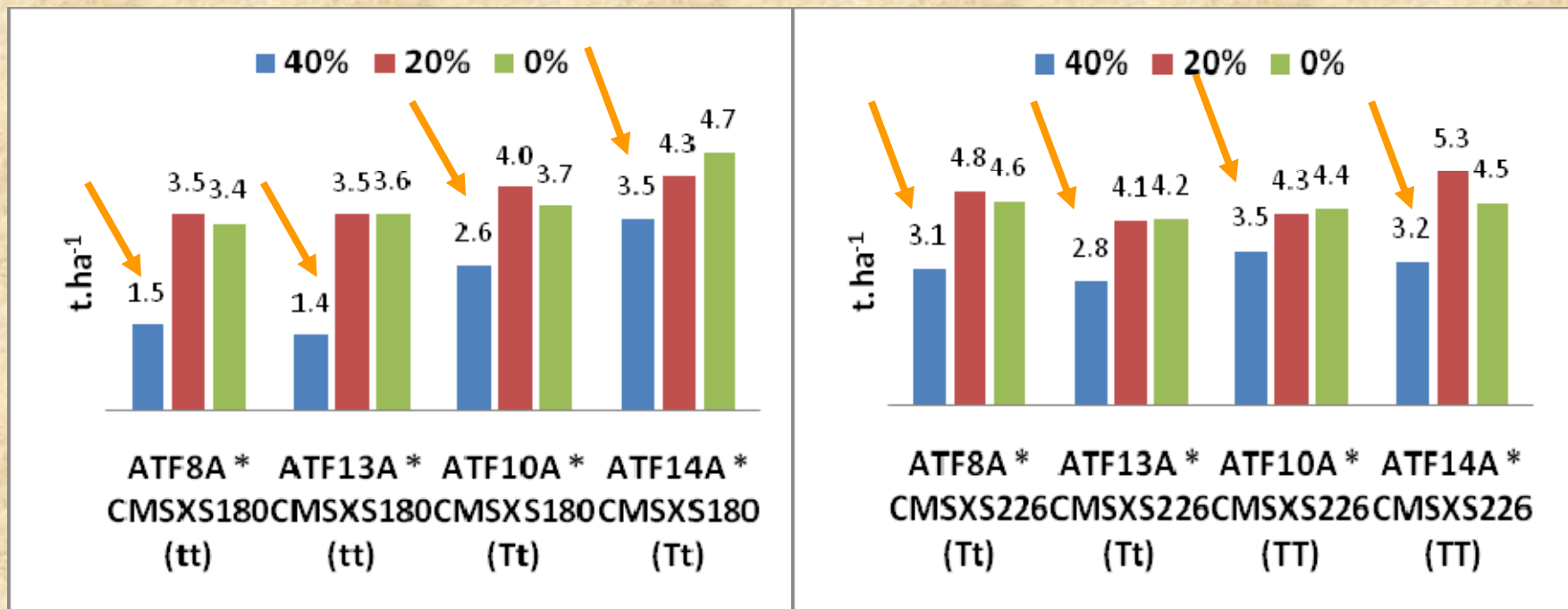
**Observation:** The constant level of productivity in 2008 and 2009 may be due to the partial tolerance of the recurrent parent (BR012R) and the lack of moisture stress in these trials

# Field Evaluation of Eight Pairs of Isogenic Hybrids for $Alt_{SB}$ at Three Levels of $Al^{+3}$ Saturation



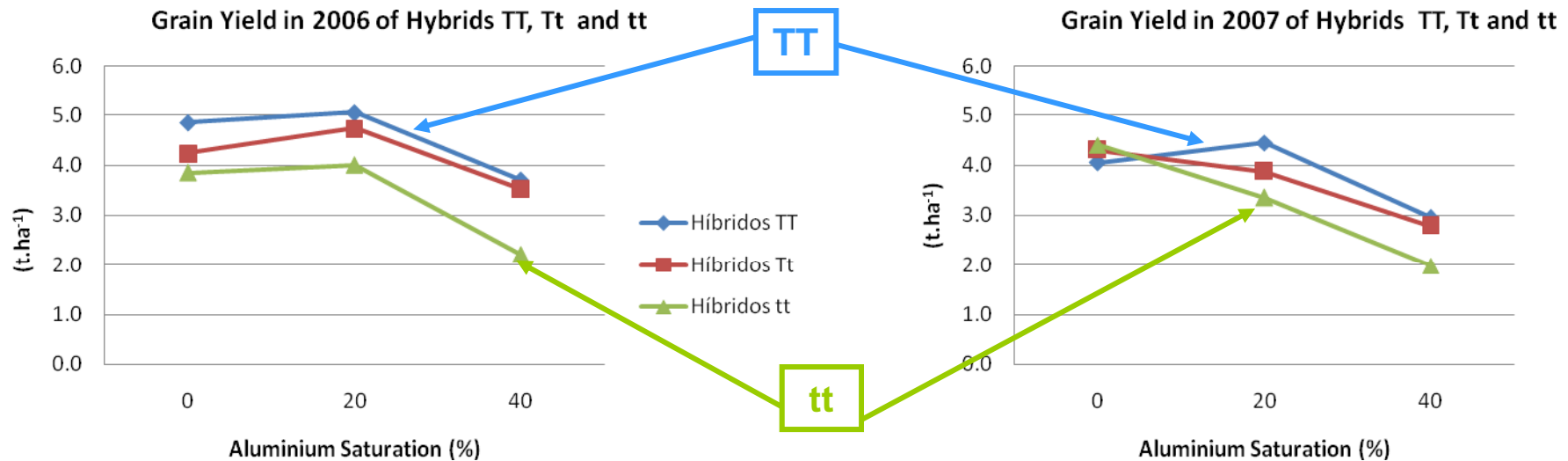
The advantage of the  $Alt_{SB}$  allele is highly significant in field conditions with Aluminum saturation above 20%. Hybrids with one allele are superior to hybrids with no allele.

# Field Evaluation of Eight Pairs of Isogenic Hybrids for $Alt_{SB}$ at Three Levels of $Al^{+3}$ Saturation



The advantage of the  $Alt_{SB}$  allele is highly significant in field conditions with Aluminum saturation above 20%. Hybrids with two alleles are slightly superior (200 – 400 kg.ha<sup>-1</sup>) to hybrids with just one allele.

# Comparison of Grain Yield of Eight Pairs of Isogenic Hybrids with Zero (**tt**), One (**Tt**) and Two (**TT**) $Alt_{SB}$ Alleles at Three Levels of Al Saturation in 2006 and 2007 at Embrapa Phenotyping Site



## Observation:

- The presence of a single  $Alt_{SB}$  allele improved grain yield productivity at both 20 and 40 % Al Saturation by nearly 1.5 t.ha<sup>-1</sup>
- The presence of a second  $Alt_{SB}$  allele contributed an additional 200 to 400 kg.ha<sup>-1</sup>
- Post flowering moisture stress occurred in 2006

# Conclusions

- The  $Alt_{SB}$  allele has a significant effect on root growth in nutrient solution.
- The  $Alt_{SB}$  allele has a significant effect on yield with acid soils and subsoils with toxic Al.
- The increase in yield associated the  $Alt_{SB}$  allele is quite probably a result of a deeper root system providing the plant with more efficient water and nutrient acquisition.
- Development of sorghum hybrids with the presence of the  $Alt_{SB}$  allele in both parents is recommended for regions of the world with acid soils or acid subsoils.
- AS citrate may also increase P solubilization, we are initiating research to study the effect of  $Alt_{SB}$  on P acquisition efficiency

**BRS 310 – An Aluminum Toxicity Tolerant Sorghum Hybrid in the Cerrado (acid savanna) of the Central-West of Brazil, planted as a second crop following soybeans**



**Thank You!**



# Sorghum Hybrid Production Field

**White Seeded  
Male Parent**

**White Seeded  
Male Parent**

**Red Seeded  
Female Parent**

