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

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A link between the molecular and field levels

Research Team

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Embrapa  McKnight Foundation  Generation Challenge Program
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 – Aluminum Susceptible Line

SC283 – Aluminum Tolerant Line from Sorghum Conversion Program

Sorghum seedlings seven days in nutrient solution with 27 μM Al
1992 – Development of Two Pairs of Contrasting Isogenic Cytoplasmic Male Sterile Lines for Tolerance to Al toxicity from a Cross Between BR007B and SC283
2006 - Positional Mapping of Sorghum Al 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

Grain Yield in 2007 of Hybrids Isogenics

- Hybrids TT
- Hybrids Tt
- Hybrids tt

0 20 40
Aluminium Saturation (%)

1.1 t.ha⁻¹
0.6 t.ha⁻¹

* 0.6 t.ha⁻¹
** 1.1 t.ha⁻¹
2007 - Positional Mapping and Cloning of $Alt_{SB}$

Genetic map $Alt_{SB}$: 354 RILs

Physical map (Texas A&M)

High resolution map

- 2085 F$_2$:BR007xSC283
- 27 recombinants

- Region of 24.6 Kb
- 513 Kb cM$^{-1}$

Transgenic Arabidopsis expressing $Alt_{SB}$

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
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.
Hand emasculation 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

<table>
<thead>
<tr>
<th>Generation</th>
<th>Homozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>50%</td>
</tr>
<tr>
<td>F2</td>
<td>75%</td>
</tr>
<tr>
<td>F3</td>
<td>87.5%</td>
</tr>
<tr>
<td>F4</td>
<td>93.75%</td>
</tr>
<tr>
<td>F5</td>
<td>96.88%</td>
</tr>
</tbody>
</table>

Observation: Both Isogenic pairs were derived from the same F3 plant, ATF14B is slightly superior to ATF10B
Marker Assisted Backcrossing to Develop Isogenic Breeding Lines Tolerant to Al Toxicity in Sorghum. (Phenotypic Marker – Seedling Root Growth in Nutrient Solution with Al)

<table>
<thead>
<tr>
<th>Cross</th>
<th>tt x TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Tt</td>
</tr>
<tr>
<td>BC1 (Recurrent Parent)</td>
<td>½ Tt (½ tt Eliminated in Nutrient Solution Test)</td>
</tr>
<tr>
<td>BC1F1</td>
<td>½ Tt (½ tt Eliminated in Nutrient Solution Test)</td>
</tr>
<tr>
<td>BC2 (Recurrent Parent)</td>
<td>½ Tt (½ tt Eliminated in Nutrient Solution Test)</td>
</tr>
<tr>
<td>BC2F1</td>
<td>½ Tt (½ tt Eliminated in Nutrient Solution Test)</td>
</tr>
<tr>
<td>BC4F1</td>
<td>½ Tt (½ tt Eliminated in Nutrient Solution Test)</td>
</tr>
<tr>
<td>BC4F2</td>
<td>¼ TT (½ Tt and ¼ tt Eliminated in Nutrient Solution Test)</td>
</tr>
<tr>
<td>BC4F3</td>
<td>100% TT and 97% Equal to Recurrent Parent</td>
</tr>
</tbody>
</table>
Isogenic A & B lines have been developed by selecting contrasting plants in advanced generations of segregating families from single seed descent.
Root Growth Response of Isogenic Female Sorghum Lines to Increasing Levels of Aluminum in Nutrient Solution

- 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

Isogenic Fertility Restoring R Lines

- BR012R (tt)
- BR011R:BR012R:SC549 (SC549)
- BR011R:BR012R:CMSXS225 [3 (TT)]
- BR011R:BR012R:CMSXS225 [2 (TT)]

Daily Root Growth 3-5 Days Isogenic Lines

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

Daily Root Growth 5-7 Days Isogenic Lines

Relative Seminal Root Growth to Zero Al^3+

Levels of Aluminium (µM)

Levels of Aluminium (µM)

BR012R RC3F5 (SC549)

BR012R RC3F5 (CMSXS 225)
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

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

[Graph showing root growth over 0-3 days with isogenic hybrids using susceptible female lines]

Daily Root Growth 3-5 Days Isogenic Hybrids

[Graph showing root growth over 3-5 days with isogenic hybrids using susceptible female lines]

Daily Root Growth 5-7 Days Isogenic Hybrids

[Graph showing root growth over 5-7 days with isogenic hybrids using susceptible female lines]

CRRS

[Graph showing CRRS over levels of aluminium (µM)]
2009 - Comparison of Isogenic Hybrids Using Tolerant Female Lines (One vs. Two Alleles)

Observation: Small difference between TT and Tt hybrids because of partial dominance
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$^{-1}$) to hybrids with just one allele.
**Observation:**

- The presence of a single AltSB allele improved grain yield productivity at both 20 and 40% Al Saturation by nearly 1.5 t.ha⁻¹
- The presence of a second AltSB allele contributed an additional 200 to 400 kg.ha⁻¹
- 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 with 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