Title
The effect of the AltSB gene on root growth in nutrient solution of isogenic sorghum hybrids

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INTRODUCTION

Even though sorghum is the fifth most important crop in the world after maize, wheat, rice and barley, it is the most important source of food for millions of inhabitants in Sub-Sahara Africa. Crop production in many eco-systems of the tropics, including Sub-Sahara Africa, is constrained by acid soils and toxic levels of exchangeable aluminum. The toxic levels of aluminum inhibit cell division and elongation, resulting in a shallow and reduced root system limiting both water and nutrient uptake. Tolerance to aluminum toxicity in sorghum was first reported by Schaffert et al. (1975) in field trials in the acid savanna or *cerrado* of Brazil. The principal mechanism of tolerance to aluminum toxicity in sorghum is the exudation of citric acid in the root apex that complexes with the exchangeable aluminum (Magalhães et al. 2007) forming a nontoxic complex. These authors identified and cloned the gene Alt_{SB} in sorghum that underlies the physiological mechanisms of Al tolerance based on Al-induced citrate release. The gene, a member of the *Multidrug and Toxic Compound Extrusion* (Mate) family is expressed mainly in the root apex of sorghum and functions as a citric acid transporter in the plasma membrane.

The objective of this study was to investigate the allele dosage effect of Alt_{SB} in isogenic hybrids of sorghum. The results of this study will orient the sorghum breeder and sorghum seed industry to the necessity and desirability of including the gene for aluminum tolerance in either one parent or both parents of sorghum hybrids.

MATERIAL E METHODS

Development of Genetic Materials

Isogenic female sorghum lines were developed by crossing the aluminum tolerant non-fertility restoring B-line (SC283) to the commercial non-fertility restoring B-line (BR007B). Selection was performed to identify agronomically desirable aluminum tolerant and susceptible plants that were segregating in F5 families derived from single $F_{2:4}$ plants. These segregates were self pollinated to generate near-isogenic lines contrasting and homozygous for the Alt_{SB} allele. Two contrasting isogenic pairs, ATF8B/ATF10B and ATF13B/ATF14B, susceptible and tolerant to Al respectively, were identified and used to make contrasting male sterile female lines by six cycles of backcrossing to the male sterile A-line, BR007A. Isogenic male sterile, restoring lines (R-lines) were developed by marker assisted backcrossing (MABC) using the aluminum susceptible elite line BR012R as the recurrent parent with two sources of aluminum tolerant R-lines, SC549 and CMSXS225. Three tolerant isogenic R-lines were developed after six MABC cycles and selection for homozygous tolerant plants. These four isogenic R-lines, one susceptible (BR012R), and three tolerant (BR012R(SC549), BR012R(CMSXS225)1 and BR(012R(CMSXS225)2 were used to pollinate the four isogenic female lines to develop sixteen isogenic hybrids with zero (tt), one (Tt) and two (TT) alleles for tolerance alleles.

Evaluation of Seedling Root Growth in Nutrient Solution

A trial was conducted in controlled conditions at Embrapa Maize and Sorghum to evaluate root growth in nutrient solution with five aluminum levels ({0}, {11}, {20}, {27} and {39}µM, where brackets indicate free Al^{3+} activity). The aluminum activity of {27}µM is normally used to detect differences between sorghum tolerant and susceptible genotypes. Highly sensitive sorghum genotypes normally show a strong negative effect on seedling root growth at an aluminum concentration of {11}µM. The seeds were scarified in sterile sand to break seed dormancy and sterilized with sodium hypochlorite (0.525%) for 10 minutes with agitation and rinsed eight times with sterile water. The seeds of the 16 isogenic hybrids were germinated in rolled paper towels for four days in a growth chamber with a 12 hour day period at an average temperature of 27±3°C and a 12 hour night period at an average temperature of 20±3°C. Seedlings with uniform root length were transferred to small plastic recipients with a small whole for the seminal root. The small recipients were placed in a support containing 49 recipients and placed in square tubs with 8.5 l nutrient solution Magnavaca et al. (1987) for 24 hours. The five aluminum treatments were obtained by adding $AlK(SO_4)_2.12H_2O$ to reach the desired aluminum concentration of at pH of 4.0. Two replications and seven plants per replication were adopted. The initial seedling root length (IRL) was recorded after a 24 hour adaptation period in nutrient solution and the nutrient solution was replaced with a solution with the appropriate aluminum activity. Root length of each seedling was measured and recorded after zero, 72, 120 and 168 hours (0, 3, 5 and 7 days) of Al exposure. Relative seminal root growth (RSRG) was calculated by dividing the root growth in seven days by the initial root length and multiplying by 100. The RSRG relative to zero aluminum was calculated by dividing the RSRG in each aluminum treatment by the RSRG with no aluminum and multiplying by 100.

RESULTS

Figure 1 demonstrates the clear distinction of seedling root development for the tolerant (TT) and susceptible (tt) isogenic female lines. Likewise in Figure 2 a clear distinction in seedling root development is also demonstrated between the tolerant (TT) and susceptible (tt) isogenic male lines. Figure 2C suggests that there is a discrete difference between the three isogenic aluminum tolerant male lines with BR012R(CMSXS225)1 exhibiting some degree of inferiority in seedling root development. A summary of the average daily root growth and RSRG relative to zero aluminum for the isogenic hybrids contrasting for zero (tt) and one (Tt) tolerance allele is presented in Figure 3. Figure 4 shows the average daily root growth and RSRG relative to zero aluminum for the isogenic hybrids contrasting for one (Tt) and two (TT) tolerance alleles. The results are summarized for the periods of 0-3, 3-5, and 5-7 days. Typically the termination of root growth in any one period is associated with severe root damage and eventual root death. A summary of the average daily root growth between the 5^{th} and 7^{th} day of the 16 isogenic hybrids is presented in Table 1.

The root growth of the susceptible isogenic or traditional hybrids (tt) in Figure 3 is significantly reduced after the period of 0-3 days for the 20, 27 and 39µM aluminum treatments where as the heterozygous hybrids with one tolerance allele has normal root development at 20µM aluminum and only a slight reduction at 27 µM aluminum. This clearly demonstrates the positive effect of the Alt_{SB} gene on root development in nutrient solution with toxic aluminum. A significant difference of root growth between the isogenic hybrids with one and two alleles (Figure 4 and Table 1) was observed in some cases but not all cases, indicating only partial dominance of the Alt_{SB} .

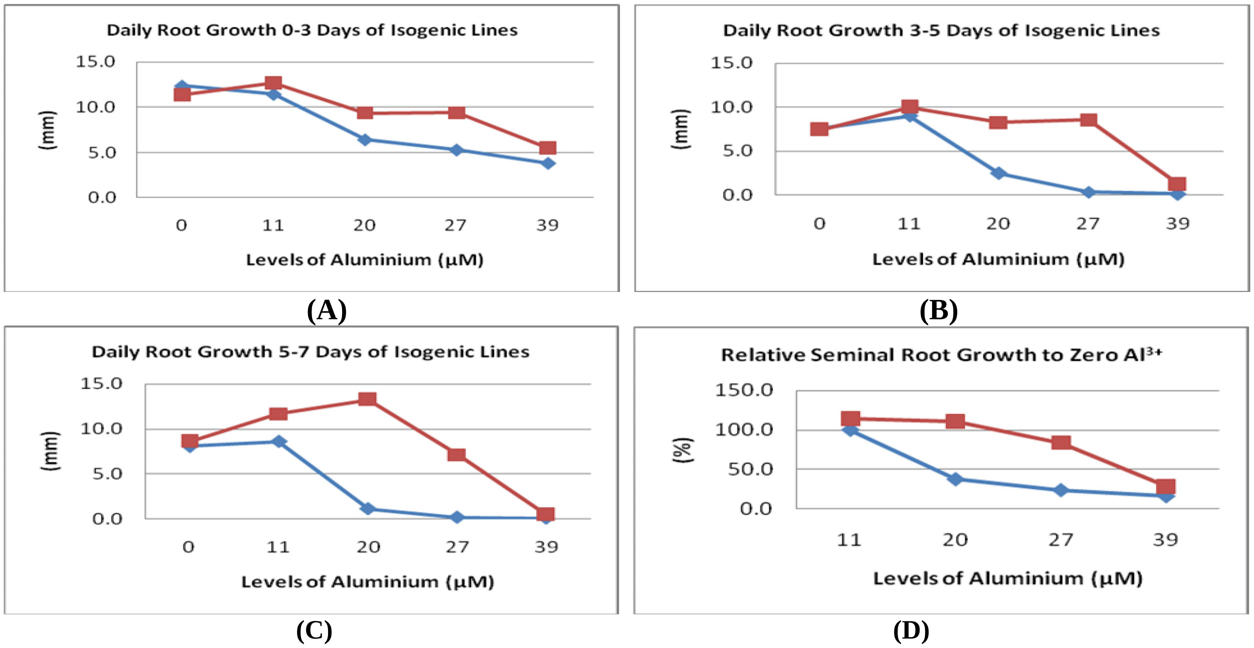

Figure 1: Comparison of average daily root growth, between aluminium tolerant (TT) and susceptible (tt) isogenic sorghum female lines in nutrient solution with five levels of aluminium; daily root growth between 0-3 days(A), 3-5days(B), 5-7 days (C) and RSRG relative to zero aluminium (D). (ATF8B e ATF13B (tt) ATF10B e ATF14B (TT))

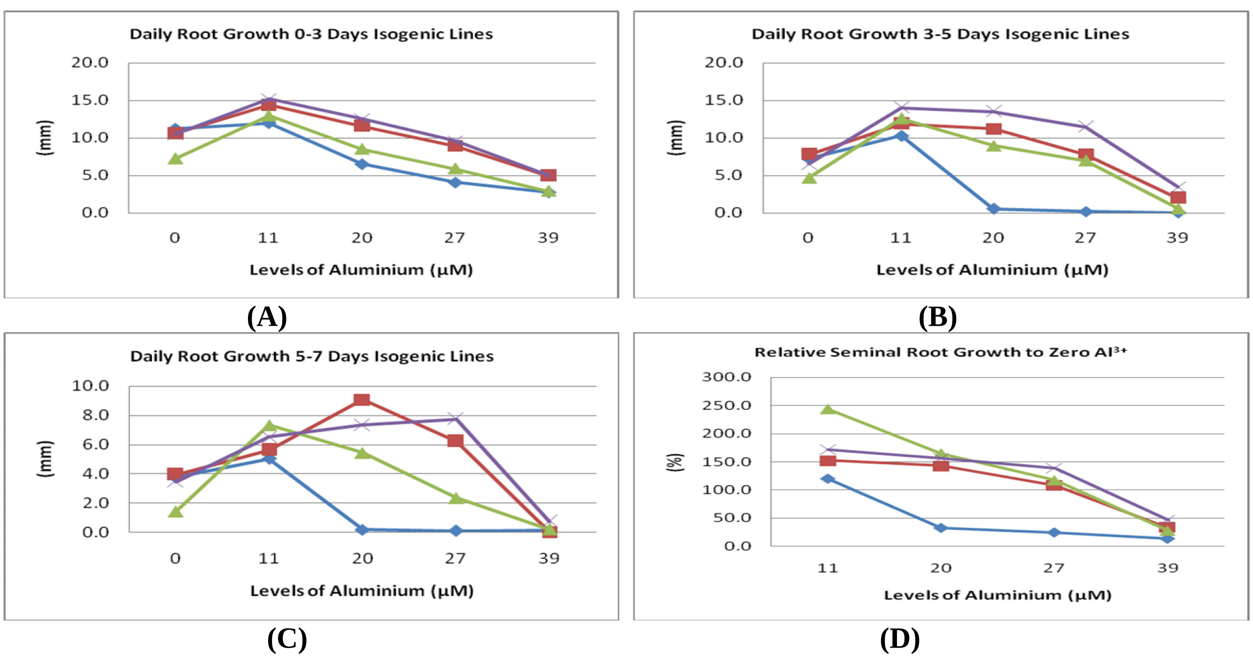

Figure 2: Comparison of average daily root growth, between aluminium tolerant (TT) and susceptible (tt) isogenic sorghum male lines in nutrient solution with five levels of aluminium; daily root growth between 0-3 days(A), 3-5days(B), 5-7 days (C) and RSRG relative to zero aluminium (D). (BR012R (tt) BR012R(SC549) (TT) BR012R(CMSXS225)1 (TT) BR012R(CMSXS225)2 (TT)).

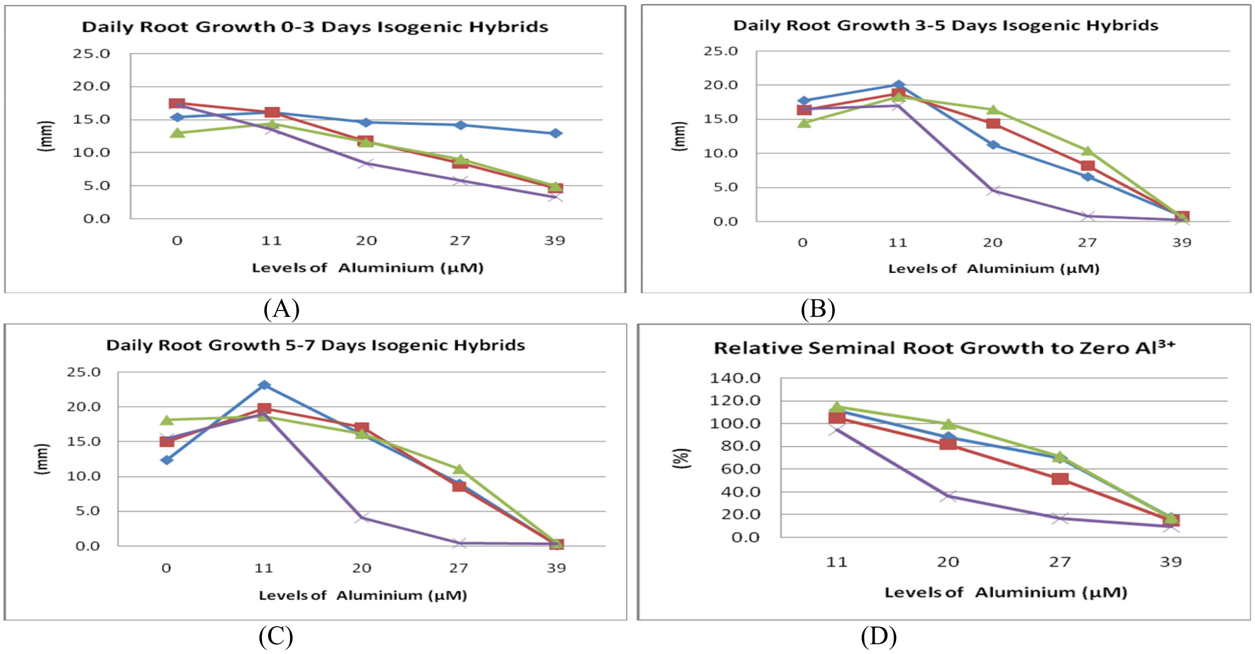

Figure 3: Comparison of root growth, between isogenic sorghum hybrids with zero (tt) and one (Tt) *Alt*$_{SB}$ allele, in nutrient solution with five levels of aluminium; daily root growth between 0-3 days, 3-5days (B), 5-7 days (C) and RSRG relative to zero aluminium (D). (Male parent of isogenic hybrids: ((BR012R(SC549) (Tt) BR012R(CMSXS225)1 (Tt) (BR012R(CMSXS225)2 (Tt) BR012R (tt))

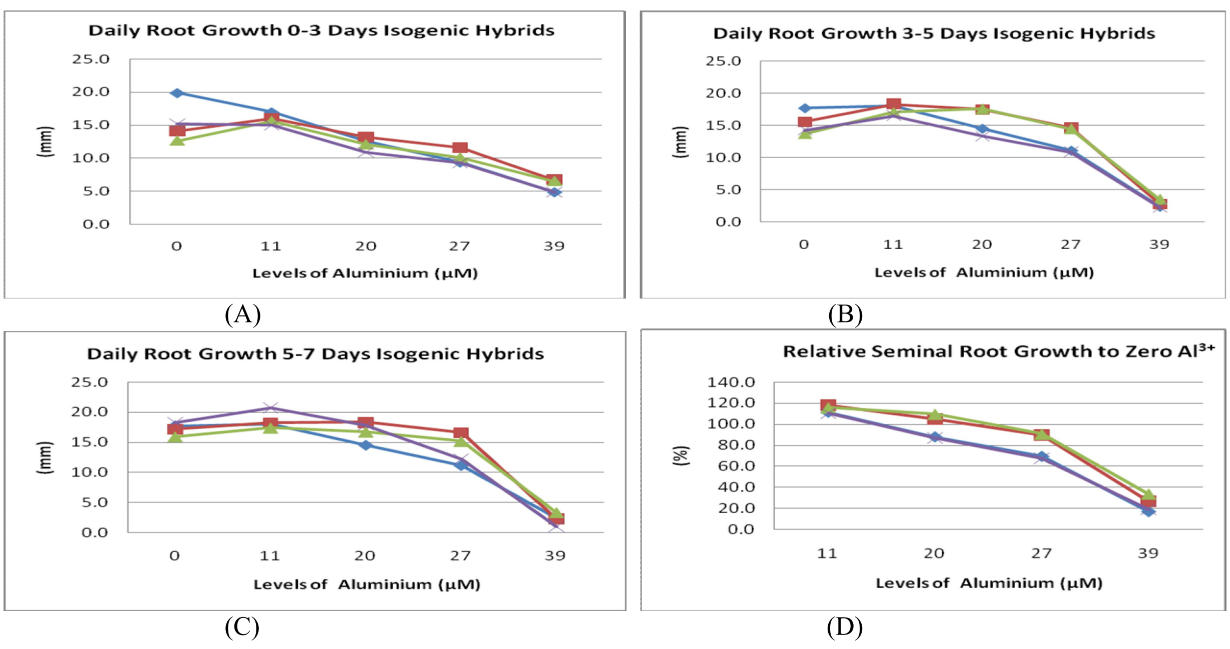

Figure 4: Comparison of root growth, between isogenic sorghum hybrids with one (Tt) and two (TT) *Alt*$_{SB}$ alleles, in nutrient solution with five levels of aluminium; daily root growth between 0-3 days, 3-5days(B), 5-7 days (C) and RSRG relative to zero aluminium (D) (Male parent of isogenic hybrids: ((BR012R(SC549) (TT) BR012R(CMSXS225)1 (TT) (BR012R(CMSXS225)2 (TT) BR012R (Tt))

Table 1: Average daily root growth (mm) of the interval between the 5th and 7th day of 16 isogenic hybrids at five aluminum concentrations, differences based on Tukey test.

Genotype of Isogenic Hybrid	Level of Al (µM)				
	0	11	20	27	39
ATF8A*BR012R (tt)	18.2 abc	17.6 b	2.3 de	0.7 h	0.6 a
ATF13A*BR012R (tt)	12.7 bcd	20.4 ab	5.8 cde	0.2 h	0.0 a
ATF10A*BR012R (Tt)	19.1 a	21.8 ab	17.4 a	11.6 bcdef	1.0 a
ATF14A*BR012R (Tt)	17.4 abcd	19.7 ab	18.4 a	12.8 abcde	0.9 a
ATF8A* (BR012R(SC549) (Tt)	12.4 bcd	22.3 ab	16.4 a	8.7 def	0.1 a
ATF13A* (BR012R(SC549 (Tt)	12.3 bcd	24.0 a	15.7 a	9.2 cdef	0.1 a
ATF10A* (BR012R(SC549) (TT)	13.3 abcd	18.8 ab	19.3 a	15.9 ab	0.5 a
ATF14A* (BR012R(SC549) (TT)	11.8 d	21.0 ab	18.7 a	15.5 abc	0.6 a
ATF8A* (BR012R(CMSXS225)1 (Tt)	12.3 cd	22.1 ab	14.4 ab	6.3 fgh	0.2 a
ATF13A* (BR012R(CMSXS225)1 (Tt)	17.7 abcd	17.6 b	19.7 a	10.8 bcdef	0.3 a
ATF10A* (BR012R(CMSXS225)1 (TT)	16.7 abcd	17.4 b	17.1 a	18.0 a	2.3 a
ATF14A* (BR012R(CMSXS225)1 (TT)	17.8 abcd	19.1 ab	19.6 a	15.2 abc	2.2 a
ATF8A* (BR012R(CMSXS225)2 (Tt)	17.6 abcd	18.3 ab	17.3 a	12.2 abcdef	0.6 a
ATF13A* (BR012R(CMSXS225)2 (Tt)	18.6 ab	18.9 ab	14.9 ab	9.9 bcdef	0.3 a
ATF10A* (BR012R(CMSXS225)2 (TT)	16.1 abcd	18.2 ab	17.7 a	15.7 ab	2.9 a
ATF14A* (BR012R(CMSXS225)2 (TT)	15.8 abcd	16.7 b	15.8 a	14.7 abcd	3.7 a

Means followed by the same letter are not significantly different ($P<0.05$)

DISCUSSION

Previous results from our program (Caniato et al. 2007) indicate that one class of sorghum lines which includes the parental line (BR007B) of the isogenic female lines, has significant root growth inhibition at {11} µM aluminum in nutrient solution. The male fertility restorer line used in this experiment expresses normal root development at {11} µM aluminum indicating that it apparently has a small degree of aluminum tolerance other than that controlled by Alt_{SB}. Even with this slight degree of Al tolerance we were able to show the significant contribution of the Alt_{SB} allele to root development in the presence of toxic levels of aluminium. Our results have also shown (data not included) that when evaluating pairs of isogenic hybrids, derived with the female lines described in this study and the male restorer lines BR012R and Tx430R in the field with 40% aluminium saturation, the hybrids with the presence of one Alt_{SB} allele (from one parent only) out yielded the isogenic hybrid by 1.0 to 1.5 t/ha in grain production.

The results from Figure 2 indicate that even after six backcrosses where we expect more than 99% similarity in the isogenic lines, differences may still exist and the performance of isogenic lines developed by this procedure need to be verified before put into production. These results along with other results obtained at Embrapa Maize and Sorghum indicate that significant yield advantages can be obtained in using sorghum hybrids with only one parent tolerant to aluminium toxicity in areas with acid surface or subsoil acidity, especially in areas where moisture stress may occur and deep root systems are desirable. We also recommend using hybrids where both parents have tolerance to aluminium toxicity. Our plans are to field test these isogenic sorghum hybrids at several locations with subsoil acidity to validate the positive effect of the Alt_{SB} gene on grain production and yield stability in the field.

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