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CROP PRODUCTION

Evaluation of grain sorghum hybrids for aluminum tolerance in nutrient solution

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ABSTRACT. Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereal crops in the world. In Brazil, the acreage of grain sorghum during off-season is quite expansive. Most of this area is the Cerrado, a Brazilian biome that is similar to a Savannah and is characterized by high acidity and soluble aluminum at toxic levels for plants. The aluminum acts as a limiting factor in achieving high yields. The purpose of this work was to phenotype sorghum hybrids for aluminum tolerance. Eighteen hybrids were evaluated in a nutrient solution containing {0} or {27} μM Al^{3+}. The work was carried out in a growth chamber at the Embrapa Maize and Sorghum, from April 4 to May 30, 2014. The lines ATF 13B (susceptible) and ATF 14B (tolerant) were used as check cultivars. Based on the Net Root Growth after 120 hours (NRG_{120}), Net Root Growth (NRG_{168}) after 168 hours and Relative Net Root Growth after 168 hours ($RNRG_{168}$), it was possible to distinguish tolerant hybrids from susceptible ones. The high aluminum saturation reduced root growth by 70%. The hybrids BRS 310 and BRS 373 were tolerant to aluminum stress under nutrient solution. The hybrid BRS 330 was clustered in an intermediate group, with an approximately 50% root growth reduction. The other hybrids were susceptible with significant root reduction.

Keywords: *Sorghum bicolor*; abiotic stress; soil acidity; plant breeding.

Avaliação de híbridos de sorgo granífero para tolerância a alumínio em solução nutritiva

RESUMO. Os solos do cerrado são caracterizados por apresentarem elevada acidez e alumínio solúvel em níveis tóxicos para as plantas, o que assume um papel de fator limitante ao aumento da produtividade destas culturas. A toxidez causada pelo alumínio afeta o desenvolvimento das plantas e em particular, inibe o crescimento das raízes e a absorção de água e nutrientes pela planta. Com o presente trabalho objetivou-se fenotipar híbridos de sorgo granífero quanto à tolerância ou susceptibilidade ao alumínio tóxico. O estudo foi realizado em câmara de crescimento na Embrapa Milho e Sorgo, entre os dias 04/04 a 30/05/2014. Foi realizado um experimento em solução nutritiva testando-se dois níveis de Al (0 e 27 μM), em 18 híbridos de sorgo, utilizando delineamento de blocos ao acaso, com duas repetições. Como testemunhas foram utilizadas as linhagens ATF 13B (suscetível) e ATF 14B (Tolerante). Com base nos caracteres avaliados: crescimento líquido de raiz após 120 horas, crescimento líquido de raiz após 168 horas e o crescimento relativo de raiz seminal após 168 horas foi possível distinguir os híbridos tolerantes dos suscetíveis. Os híbridos BRS 310 e BRS 373 apresentaram tolerância significativa, com redução no crescimento abaixo de 30% quando submetidos ao estresse de alumínio. O híbrido BRS 330 foi classificado em grupo intermediário com redução de tamanho de raiz em torno de 50%. Todos os outros híbridos foram suscetíveis com reduções de crescimento de raiz muito afetado pelo alumínio.

Palavras-chave: *Sorghum bicolor*; estresse abiótico; acidez do solo; melhoramento vegetal.

Introduction

The Cerrado region of Brazil is one of the most important grain production areas in the world. This region is responsible for over 50% of the country's soybean, corn, sorghum, coffee and beef production. The soils of the Cerrado are characterized by high acidity, high aluminum saturation, low availability of nitrogen, phosphorus, potassium, calcium, magnesium, zinc, boron and copper (Silva & Malavolta, 2000; Yamada, 2005). Aluminum toxicity is the main limiting factor for crop production on these soils.

Aluminum reduces root growth in sensitive plants by affecting both root elongation and cell division. Under such stress, plants cannot uptake water and nutrients from the subsoil due to their surface rooting, which makes them less productive and more susceptible to drought (Miguel et al., 2010).

The limitation for crop production in acid soils could be overcome by either the addition of chemical amendments such as lime and fertilizers or the use of species or cultivars that can tolerate soil acidity constraints. The second approach has practical application in many parts of the world because chemical amendments are costly for many farmers. The identification of cultivars that can tolerate soil acidity limitations is needed. These superior acid-tolerant cultivars will assist in not only increasing the area under sorghum production but also enhancing the yield per unit area. Acid-tolerant genotypes will aid breeders in producing superior cultivars that not only can adapt to acidic soils but also have high-yield potentials. Field trials and nutrient solutions have clearly shown the existence of variability for Al stress in sorghum (Carvalho Jr et al., 2016; Menezes et al., 2014).

In addition, the soil improving capacity with chemical amendments does not go beyond the surface layers of the soil, which stops the normal growth of the root system of plants. Plants need a greater volume of soil to explore, mainly in depth, to be able to absorb the necessary nutrients and water (Malavolta, Vitti, & Oliveira, 1997; Cançado et al., 2001).

Sorghum breeding programs have developed aluminum-tolerant cultivars that can be used to explore soils with subsurface acidity and high levels of aluminum, which may substitute or complement the use of limestone and increase the grain yield in acid soils (Magalhaes et al., 2007; Carvalho Jr et al., 2016). These strategies are extremely important from an environmental standpoint because they enable a reduction in the use of inputs, such as fertilizers and water (Borém & Miranda, 2009).

The genetic control of aluminum toxicity tolerance has warranted special attention in the scientific community. According to Hartwing et al. (2007), Al tolerances are divided into two main classes: the exclusion mechanisms that act after absorption or blocking its entry into the root system and those that are involved in detoxification and complex the Al in specific organelles, mainly in the vacuoles. In many species, physiological mechanisms have been reported to be responsible for the activation of organic acids (mainly citrate and malate) that act as Al chelating agents, but many of these processes are not yet understood. In sorghum, a gene that accounts for 80% of the phenotypic variation of aluminum tolerance was mapped by Magalhães et al. (2004) and called Alt_{SB}. This gene encodes a citrate carrier that is localized in the plasmatic membrane of the cells located in the root tips, which confer tolerance via a physiological mechanism that is based on the citrate exudation of aluminum in the rhizosphere (Magalhaes et al., 2007; Caniato et al., 2007).

Considering the rapid expansion of agricultural frontiers into the Cerrado biome, the selection of genotypes of sorghum with aluminum tolerance provides an alternative for the better exploitation of these areas. Grain sorghum is an excellent option for succession cultivation after summer soybean. Among the most planted cereals, sorghum stands out for its drought tolerance and lower production cost (Menezes, Silva, & Tardin, 2015).

The present work aimed to phenotype grain sorghum hybrids for their tolerance to aluminum saturation in nutrient solution and to select those hybrids that exhibit better root growth under stress, which may support the expansion of the crop into acid soils.

Material and methods

Eighteen sorghum hybrids from different companies were used for this study. These hybrids are among the highest cultivated sorghum in the Biome Cerrado in Brazil and serve as succession crops after soybean.

The trials were carried out in a growth chamber at the Embrapa Maize and Sorghum. The inhibition of seminal root growth elicited by Al in nutrient solution was used to quantify Al tolerance using the basal nutrient solution described by Magnavaca, Gardner, and Clark (1987) at pH 4.0.

Seeds were gently sand-scarified inside cloth bags, surface sterilized with 0.525% NaOCl for 10 min. under continued stirring and finally rinsed eight times with 18 mΩ H_2O. Seeds were then allowed to germinate for 4 days on moistened germination paper rolls in a growth chamber with 27°C day and 20°C night temperatures, a light intensity of 330 μmol photons m^{-2} s^{-1} and a 12-h photoperiod.

The seminal roots from the seedlings were inserted through the mesh bottoms of polyethylene cups placed into polyethylene containers filled with 8.5 L of nutrient solution under continuous aeration (49 seedlings $container^{-1}$). Seedlings were given a 24-h acclimation period in a nutrient solution without Al ({0} μM Al^{3+}), after which the initial length of each seedling's root growing in the control solution was measured. The solution was then replaced with a nutrient solution that had an identical composition but contained either no Al or Al supplied as $AlK(SO_4)_2.12H_2O$ at the desired final Al activities ({0 or 27} μM Al^{3+}); the pH was adjusted to 4.0. The activity of 27 μM Al^{3+} has been shown to adequately differentiate sorghum genotypes for Al tolerance (Magalhaes et al., 2004; 2007; Caniato et al., 2007; 2011).

Root lengths were measured at 0, 48, 120 and 168 hours after exposure to Al. A completely randomized design was used, with two replications and seven seedlings per replication. Each polyethylene container had six hybrids, and the central row of each container had four and three plants of the susceptible and tolerant check cultivars, respectively. The check cultivars were ATF 13B (Susceptible) and ATF 14B (Tolerant).

The characteristics evaluated were the initial root length at 0 (zero) hours (IRL_0), root growth after 48 hours (RG_{48}), root growth after 120 hours (RG_{120}) and root growth after 168 hours (RG_{168}). Using these data, it was possible to estimate the net root growth after 120 hours (NRG_{120}), calculated as $NRG_{120} = RG_{120}\text{-}IRL_0$; the Net Root Growth after 168 hours (NRG-168), calculated as $NRG_{168} = RG_{168} - IRL_0$; and the relative net root growth after 168 hours (RNRG), calculated as RNRG = $[(RNRG_{168} - IRL_0)/IRL_0]*100$.

The data were submitted to statistical analysis to compare the mean of the root length of the seven seedlings of each genotype. Statistical analyses were performed using the Genes software (Cruz, 2013), and the Scott & Knott test was used to group the hybrids and the levels of Al.

Results and discussion

The combined analysis of variance across the two levels of Al and the estimates of variance for the three traits (NRG_{120}, NRG_{168} and $RNRG_{168}$) are presented in Table 1. The effects of hybrids and interaction hybrids x levels of Al were significant for all traits evaluated (Table 1). Thus, there was variability among the hybrids, but the best hybrids at zero Al saturation were not the same as those observed at the high Al saturation. The Coefficients of Variation were approximately 13%, which is lower than the limit proposed for similar trials of sorghum.

Table 1. Analysis of variance for Net Root Growth after 120 hours (NRG_{120}), Net Root Growth after 168 hours (NRG_{168}) and Relative Net Root Growth after 168 hours ($RNRG_{168}$) at 0 and 27 μM of Al for 18 grain sorghum hybrids. Sete Lagoas, 2014.

FV	GL	NRG_{120}	NRG_{168}	$RNRG_{168}$
		Mean Square		
Rep (A)	2	6.02	9.17	2.03
Hybrids (H)	17	367.64**	800.04**	1255.92**
Al levels (A)	1	70919**	127142**	118836**
HxA	17	418.93**	1084.43**	951.27**
Error	34	63.37	103.48	96.07
Mean 0 Al		89.8	117.5	114.7
Mean 27 Al		27.1	33.5	33.5
Growth reduction (%)		69,6	71,5	70,7
CV%		13.62	13.47	13.23

**Significant at 1% of probability by the F test.

The IRG_0 at zero μM of Al varied from 75 to 129 mm, and at 27 μM of Al, it varied from 56 to 134 (data not shown). These results suggest that all analyses should be performed while using IRG_0 as the standard point.

The general means for the Initial Root Growth (IRG_0) of the hybrids at the two levels of Al (0 and 27) were similar (Figure 1D): the IRG_0 at zero and 27 μM of Al were 105 mm. The same was found for the check cultivars (ATF 13B and ATF 14B), which had similar root growth at zero Al saturation (Figure 1D).

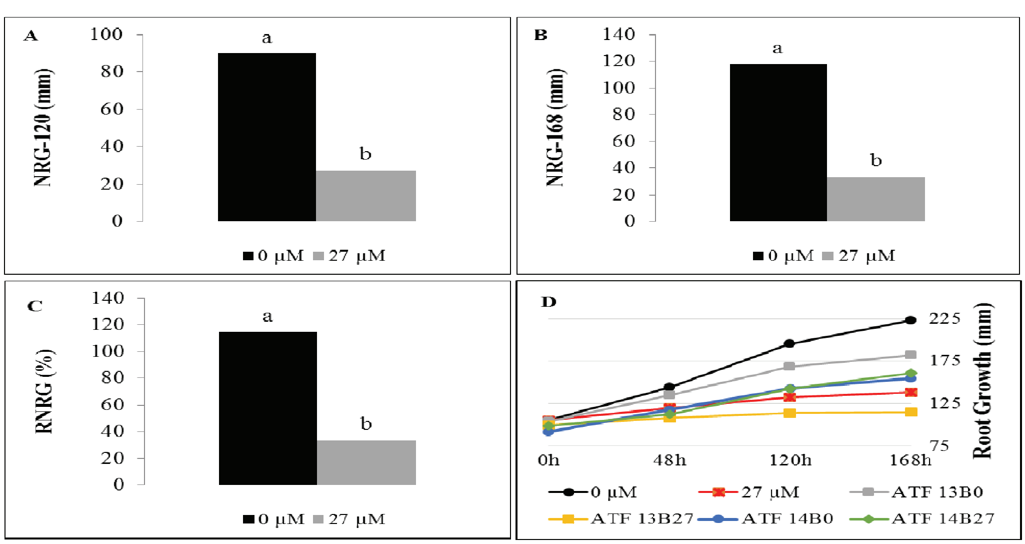

Figure 1. Evaluation of the Net Growth after 120 hours (A), Net Root Growth after 168 hours (B), Relative Net Root Growth after 168 hours (C) and Root Growth from zero to 168 hours (D) after stress, for eighteen hybrids of grain sorghum, grown under 0 and 27 μM of Al.

Toxic levels of aluminum inhibit cell division and elongation, resulting in a shallow and reduced root system that limits water and nutrient uptake. The effects of Al on plant metabolic processes can be observed within minutes after the onset of the stress syndrome and are followed by secondary effects that occur at later stages (Kochian, Hoekenga, & Pineros, 1995). In the present work, the damaging effect of Al in the root was very rapid: at 48 hours after changing the solution, it was already possible to detect root growth reduction (Figure 1D), and after 120 hours the difference between root growth at the two levels of Al increased.

The mean for the Net Root Growth after 120 hours (NRG_{120}) was 89.8 mm at zero μM of Al and 27.1 mm at 27 μM of Al, which indicates a growth reduction of 70%. The growth reductions of Net Root Growth after 168 hours (NRG_{168}) and Relative Net Root Growth after 168 hours ($RNRG_{168}$) were similar, at 71% reduction for both traits (Table 1; Figure 1). The RNRG varied from 13.2% to 105.4, which is much closer than that found by Caniato et al. (2007), who studied genetic diversity in sorghum.

Different levels of Al tolerance were observed among the hybrids that were subjected to increasing levels of Al toxicity in nutrient solution. Using an Al tolerance threshold of 70% RNRG, most of the hybrids were susceptible to Al stress. The best hybrids, considering all three traits, were BRS 310 and BRS 373, which had root reductions lower than 30% in both stress and non-stress solutions (Figure 2). The hybrid BRS 330 presented reductions of 54% in NRG_{120}, 42% in NRG_{168} and 50% in $RNRG_{168}$ when it was exposed to high Al saturation. The average grouping test clustered this hybrid into a second group, with a higher reduction than BRS 310 and BRS 373 but a lower reduction than the other hybrids, which had a reduction greater than 50% in all characters. The hybrids 50A70, Buster, 50A50 and 1G 282 presented a RNRG lower than 20% and showed a severe inhibition of root growth when they were exposed to Al stress (Table 2; Figure 2).

The female lines of the hybrids BRS 310, BRS 330 and BRS 373 are tolerant to Al stress. BRS 310 performed better than did the other two tolerant hybrids, which suggests a contribution from the other parent in the genetic control of the trait. Carvalho Jr. et al., (2016) developed isogenic sorghum hybrids with zero, one and two alleles for aluminum toxicity tolerance. One allele of the gene had a very significant effect on maintaining root growth up to a concentration of 27 μM of aluminum. A second allele of the gene continued to have a positive effect for some of the isogenic hybrids, thus confirming the effect of partial dominance for aluminum tolerance.

The means for ATF 14B at zero Al were lower than the means of the hybrids, which can be explained by the effects of heterosis in the hybrids. This finding is supported by the fact that ATF 14B is the parental line of the hybrids BRS 330 and BRS 373, both of which had root growth higher than that of the parental line. Hybrid vigor or heterosis may be related to the Al tolerance found in some hybrids. However, further studies are necessary to confirm if hybrid vigor is the factor responsible for the distinct response to Al saturation.

Table 2. Mean of Net Root Growth after 120 hours (NRG_{120}), Net Root Growth after 168 hours (NRG_{168}) and Relative Net Root Growth ($RNRG_{168}$) at 0 and 27 μM of Al of 18 grain sorghum hybrids grown in nutrient solution. Sete Lagoas, 2014.

Hybrids	NRG_{120} (mm)		NRG_{168} (mm)		$RNRG_{168}$ (%)	
	0 μM	27 μM	0 μM	27 μM	0 μM	27 μM
1G 100	79.1 c A	27.7 c B	99.9 d A	31.4 c B	88.5 c A	27.0 c B
1G 282	80.6 c A	17.4 c B	110.6 c A	17.6 c B	84.2 c A	13.2 c B
50A50	98.1 b A	12.9 c B	130.9 b A	13.5 c B	124.2 b A	13.9 c B
50A70	77.1 c A	21.1 c B	98.9 d A	21.6 c B	77.4 c A	16.4 c B
80G80	90.1 c A	20.0 c B	116.6 c A	21.7 c B	133.8 a A	21.8 c B
A 9721R	59.1 c A	16.4 c B	76.6 d A	17.6 c B	93.6 c A	20.9 c B
AG 1040	110.4 a A	30.6 c B	146.1 a A	34.9 c B	116.4 b A	27.2 c B
AG 1060	115.3 a A	31.0 c B	158.5 a A	33.5 c B	138.6 a A	31.7 c B
BM 737	92.9 c A	24.9 c B	119.7 c A	29.5 c B	126.6 b A	31.2 c B
BRS 310	89.4 c A	63.0 a B	114.6 c A	97.6 a A	118.6 b A	105.4 a A
BRS 330	86.6 c A	40.0 b B	110.1 c A	64.1 b B	145.3 a A	73.2 b B
BRS 332	85.8 c A	18.9 c B	110.4 c A	19.1 c B	140.9 a A	26.2 c B
BRS 373	75.4 c A	54.6 a B	92.3 d A	75.0 b A	94.4 c A	78.2 b A
Buster	96.7 b A	16.1 c B	129.9 b A	16.5 c B	111.8 b A	14.6 c B
DKB 540	80.4 c A	27.1 c B	107.1 c A	30.0 c B	96.6 c A	26.2 c B
DKB 550	85.6 c A	28.0 c B	117.1 c A	37.4 c B	108.0 c A	34.6 c B
FOX	96.9 b A	17.8 c B	126.7 b A	18.6 c B	135.8 a A	20.5 c B
Jade	116.6 a A	20.1 c B	149.6 a A	23.2 c B	130.0 a A	20.1 c B
Mean	89.8 A	27.1 B	117.5 A	33.5 B	114.7 A	33.5 B
ATF 13B	73.0	14.1	84.6	15.3	98.0	17.2
ATF 14B	78.2	60.3	95.2	90.6	98.1	91.1

Means followed by the same lower case letters in the column and capital letters on the lines do not differ significantly by the Scott-Knott ($p < 0.05$)

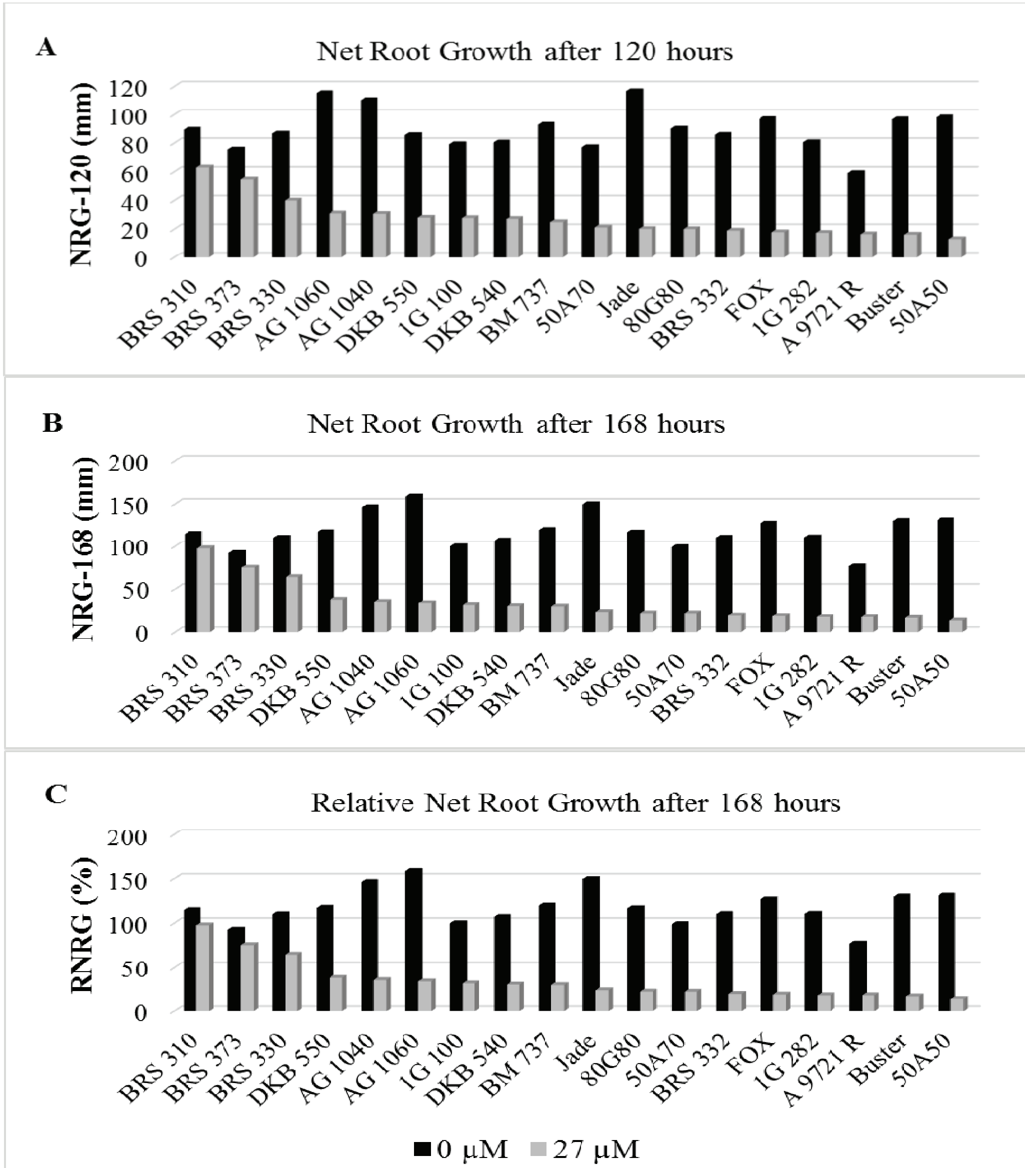

Figure 2. Mean of Net Root Growth in 120 hours (A), Net Root Growth in 168 hours (B) and Relative Net Root Growth (C), for eighteen hybrids of grain sorghum, grown at 0 and 27 μM of Al.

In fields with 40% Al saturation, the hybrid BRS 330 performed better than BRS 310 did, and both hybrids performed better than did the susceptible hybrids BRS 304 and 332 (Menezes et al., 2014), thus confirming the effect of tolerance in grain yield of sorghum.

The tolerance to Al present in the hybrids BRS 310, BRS 330, and BRS 373 is derived from the line SC283, which possesses the Al-tolerance gene Alt_{SB} (Magalhães et al., 2004). Alt_{SB} is a major gene of the Multidrug and Toxic Compound Extrusion (Mate) family that confers tolerance to aluminum in sorghum (Magalhães et al., 2007). This gene is a transporter gene that is responsible for the exudation of citric acid in the presence of toxic levels of aluminum in the soil. The citrate complexes with the toxic aluminum forming a nontoxic compound.

Studies have confirmed that the Alt_{SB} gene in sorghum cultivars used in regions with acid soils or subsoils contributes to the development of better and deeper root systems and promotes greater and more sustainable yields. Carvalho Jr. et al. (2016) evaluated isogenic hybrids and found a stronger effect of Alt_{SB}, with a yield advantage of 0.5-ton ha^{-1} in hybrids arising from one Al-tolerant Alt_{SB} allele. Evaluating sorghum in field conditions, Menezes et al. (2014) observed a reduction of 30% in grain yield at 40% soil Al saturation, showing that Al saturation is very important in soil and can reduce the crop yield significantly.

The number of hybrids evaluated in this study is limited to cover all cultivar variability in the national market, but this study suggests that breeding companies are not currently addressing aluminum tolerance, as only BRS hybrids are Al-tolerant. Further studies should be done with new cultivars to confirm that statement.

Conclusion

Aluminum stress has a significant effect on the sorghum cultivars evaluated and significantly reduces root growth in susceptible genotypes.

The hybrids with the best root growth under Al stress were BRS 310 and BRS 373, followed by BRS 330.

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