

## ARTICLE

# Combining ability of grain sorghum lines selected for aluminum tolerance

Cicero Beserra Menezes<sup>1\*</sup>, Geraldo Afonso de Carvalho Júnior<sup>2</sup>, Lidianne Assis Silva<sup>3</sup>, Karine Costa Bernardino<sup>2</sup>, Vander Fillipe Souza<sup>4</sup>, Flávio Dessaune Tardin<sup>1</sup> and Robert Eugene Schaffert<sup>1</sup>

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**Abstract** – The purpose of this study was to estimate combining ability of 58 sorghum lines previously selected for Aluminum (Al) tolerance. One hundred sixty-five hybrids were evaluated at three levels of Al saturation (0%, 20% and 50%) at the same season. General Combining Ability (GCA) effects were significant for female lines for all three traits. GCA effects for male lines were significant only for plant height. Specific Combining Ability (SCA) effects were significant only for flowering time. The ratio GCA to SCA was greater than the unity, indicating the prevalence of additive effects for the control of Al tolerance. F7, F14, F17, F20, F21, F24, F29, F31, F41, F42, F48, F51, F54 and F55 lines contributed to increase yield, while F29, F48 and F51 also contributed to reduce flowering time. M2 was the best male line since it contributed to increase yield and plant height, and to reduce flowering time.

**Key words:** Sorghum bicolor, soil acidity, Aluminum stress, line selection.

## INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench.] is widely grown throughout the world (Asia, Africa, Europe, North, Central and South America) for food, feed and fodder. In South America, grain sorghum is grown particularly in Argentina and Brazil, and it is used primarily for livestock feed. In Brazil, sorghum is grown in different agro-ecological zones, but predominantly in the central and southeastern plains, which cover 86% of the total area of the country, mainly as succession crop after soybean (IBGE 2012). In the last ten years, Brazilian harvested area has doubled; and despite this expressive expansion, crop productivity is still low when compared either with main worldwide producers, or experimental trials.

Grain sorghum yield is limited by chemical constraints, such as high levels of phytotoxic minerals in the soil. The most of the soil in this area is acidic in nature, and aluminum (Al) has been recognized as the major problem in such situation (Ryan et al. 2011). Aluminum causes a rapid inhibition of root growth that leads to a reduced and stunted root system; thus, it directly affects the ability of a plant to get both water and nutrients (Cruz et al. 2011).

There are wide genetic variations among sorghum germplasm for tolerance to aluminum, indicating the potential for developing new sorghum cultivars that may be better adapted to acid conditions (Caniato et al. 2007). Selection of the appropriate parents to be used in artificial crosses is one of the main decisions faced by plant breeders, since it facilitates the exploitation of maximum genetic variability and production of superior recombinant genotypes. Breeding hybrid parents are essentially a balance of selection for performance *per se* and for combining ability. In a plant breeding program, the knowledge about the combining ability of the lines is essential to understand the inheritance of the characters (Bertan et al. 2007), and it facilitates the selection of those better lines to continue the program. Information of combining ability of sorghum germplasm is limited and incomplete; much less information is available on combining ability of tropical germplasm. Considering sorghum lines which are well-adapted under Brazilian conditions, information on combining ability is also very scarce.

The objective of this work was to estimate combining ability of tropical sorghum lines, previously selected for aluminum tolerance, aiming at parental selection and

<sup>1</sup> Embrapa Milho e Sorgo, Rod. MG 424, km 45, 35.702-098, Sete Lagoas, MG, Brazil. \*E-mail: cicero.menezes@embrapa.br

<sup>2</sup> Universidade Federal de Viçosa, Departamento de Genética, 36.570-900, Viçosa, MG, Brazil

<sup>3</sup> Universidade Federal do Acre, BR 364, km 04, 69.920-900, Rio Branco, AC, Brazil

<sup>4</sup> Universidade Federal de São João del-Rei, Rod. MG 424, km 47, 35.701-970, Sete Lagoas, MG, Brasil )

germplasm improvement for breeding programs to increase grain yield.

## MATERIAL AND METHODS

Fifty-five male sterile and three restorer lines (Table 1), previously selected for aluminum tolerance, were crossed in a diallel system through controlled hand pollination. The resulting hybrids (165) were grown in field with three levels of Al saturation (0%, 20% and 50%). Hybrids were sowed on January 28<sup>th</sup>, 2008, comprising the main growing season for sorghum in Brazil.

The area was divided in three homogeneous areas (see Figures 1 and 2) with 0%, 20% and 50% of Al saturation. Dolomitic limestone with 33% CaO and 10% MgO was broadcasted over the entire area and disked to 20 cm depth to achieve exchangeable Al saturation of 0% and 20%. No limestone was needed in the area with 50% of Al saturation. Soil samples from 0-20cm and 20-40cm of depth were

collected two months after liming, for assessment of Al saturation (Table 2 and Figures 1 and 2). Fifty one, 54 and 169 points from areas of 0%, 20% and 50% of Al saturation, respectively, were sampled. At each point, soil was collected in triplicate samples and mixed to compose a final sample per point. The assessment of Al saturation was performed using 1M KCl method (EMBRAPA 1997), which displaces the aluminum from the cation exchange sites with potassium, providing a measure of exchangeable Al. Aluminum saturation was calculated as the ratio of exchangeable Al divided by the sum of basic cations plus Al, expressed in  $\text{Cmol kg}^{-1}$  (Fox 1979). Figures 1 and 2 represent the Al Tolerance Phenotyping Site and the distribution (0-20 and 20-40cm) of Al saturation.

Starter fertilizers were applied according to soil analysis, using  $350 \text{ kg ha}^{-1}$  of 08-28-16+Zn. At sixth-leaf stage,  $250 \text{ kg ha}^{-1}$  of urea was side dressed in the area with 0% and 20% of Al saturation, and  $400 \text{ kg ha}^{-1}$  of Ammonium Sulfate in the area with 50% of Al saturation.

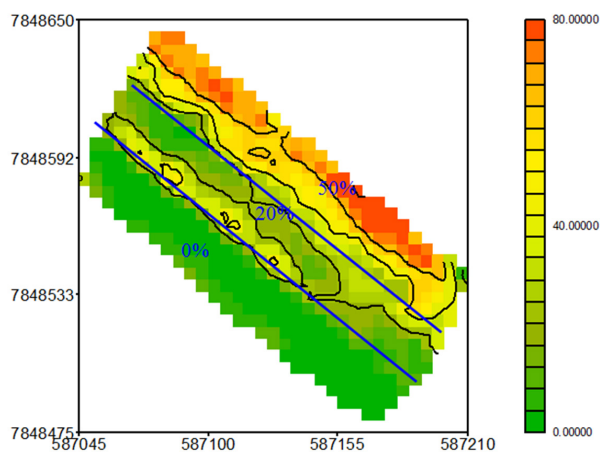
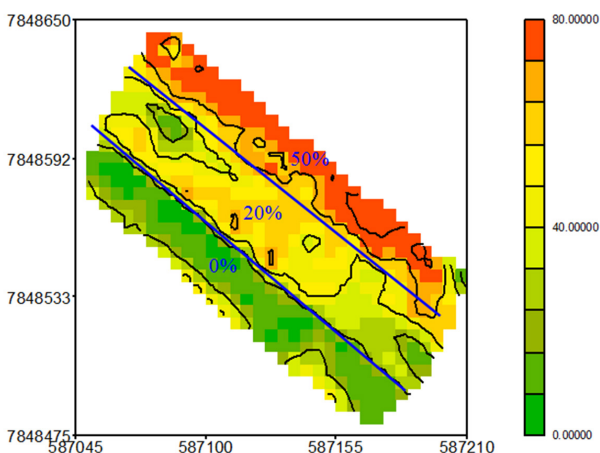
**Table 1.** Pedigree of the fifty-eight parental lines used in diallel crossings

Lines*	Pedigree	Lines*	Pedigree
F1	[(Tx623B*ATF54B)6-1]-54-C-1	F30	[(Tx623B*ATF54B)6-1]-561-C-1
F2	[(Tx623B*ATF54B)6-1]-54-C-2	F31	[(Tx623B*ATF54B)6-1]-561-C-2
F3	[(Tx623B*ATF54B)6-1]-54-C-3	F32	[(Tx623B*ATF54B)6-1]-567-C-1
F4	[(Tx623B*ATF54B)6-1]-54-C-4	F33	[(Tx623B*ATF54B)6-1]-567-C-2
F5	[(Tx623B*ATF54B)6-1]-54-C-5	F34	[(Tx623B*ATF54B)6-1]-596-C-1
F6	[(Tx623B*ATF54B)6-1]-61-C-1	F35	[(Tx623B*ATF54B)6-1]-596-C-2
F7	[(Tx623B*ATF54B)6-1]-61-C-2	F36	[(Tx623B*ATF54B)6-1]-45-C-1
F8	[(Tx623B*ATF54B)6-1]-64-C-1	F37	[(Tx623B*ATF54B)6-1]-48-C-1
F9	[(Tx623B*ATF54B)6-1]-64-C-2	F38	[(Tx623B*ATF54B)6-1]-49-C-1
F10	[(Tx623B*ATF54B)6-1]-64-C-3	F39	[(Tx623B*ATF54B)6-1]-09-C-1
F11	[(Tx623B*ATF54B)6-1]-64-C-4	F40	[(Tx623B*ATF54B)6-1]-09-C-2
F12	[(Tx623B*ATF54B)6-1]-144-C-1	F41	[(Tx623B*ATF54B)6-1]-10-C-1
F13	[(Tx623B*ATF54B)6-1]-206-C-1	F42	[(Tx623B*ATF54B)6-1]-10-C-2
F14	[(Tx623B*ATF54B)6-1]-206-C-2	F43	[(Tx623B*ATF54B)6-1]-10-C-3
F15	[(Tx623B*ATF54B)6-1]-207-C-1	F44	[(Tx623B*ATF54B)6-1]-93-C-1
F16	[(Tx623B*ATF54B)6-1]-236-C-1	F45	[(Tx623B*ATF54B)6-1]-122-C-1
F17	[(Tx623B*ATF54B)6-1]-240-C-1	F46	[(Tx623B*ATF54B)6-1]-122-C-2
F18	[(Tx623B*ATF54B)6-1]-240-C-2	F47	[(Tx623B*ATF54B)6-1]-124-C-1
F19	[(Tx623B*ATF54B)6-1]-263-C-1	F48	[(Tx623B*ATF54B)6-1]-136-C-1
F20	[(Tx623B*ATF54B)6-1]-263-C-2	F49	[(Tx623B*ATF54B)6-1]-140-C-1
F21	[(Tx623B*ATF54B)6-1]-263-C-3	F50	[(Tx623B*ATF54B)6-1]-186-C-1
F22	[(Tx623B*ATF54B)6-1]-268-C-1	F51	[(Tx623B*ATF54B)6-1]-197-C-1
F23	[(Tx623B*ATF54B)6-1]-295-C-1	F52	[(Tx623B*ATF54B)6-1]-197-C-2
F24	[(Tx623B*ATF54B)6-1]-295-C-2	F53	[(Tx623B*ATF54B)6-1]-584-C-1
F25	[(Tx623B*ATF54B)6-1]-298-C-1	F54	[(Tx623B*ATF54B)6-1]-589-C-1
F26	[(Tx623B*ATF54B)6-1]-497-C-1	F55	[(Tx623B*ATF54B)6-1]-589-C-2
F27	[(Tx623B*ATF54B)6-1]-497-C-2	M1	(BR012R*CMSXS225)-1
F28	[(Tx623B*ATF54B)6-1]-508-C-1	M2	(BR012R*CMSXS225)-2
F29	[(Tx623B*ATF54B)6-1]-555-C-1	M3	BR012R*SC549

\* F: female lines; M: male lines.

**Table 2.** Descriptive statistics for Al saturation (%) of soil data collected from 0%, 20% and 50% (0 – 20cm)

Statistics for Al saturation (%)	0%	20%	50%
Mean	2.1020	20.8328	55.9241
Median	0.4444	18.7513	60.3276
Standard deviation (SD)	5.0214	15.2209	18.3798
Minimum	0	0	1.9189
Maximum	29.4059	60.4155	88.6041
Mean – 1 SD	-2.9194	5.6120	37.5443
Mean + 1 SD	7.1235	36.0537	74.3039
Mean – 2 SD	-7.9408	-9.6089	19.1645
Mean + 2 SD	12.1449	51.2746	92.6837

**Figure 1.** Al saturation for 0, 20 and 50% (0 – 20 cm).**Figure 2.** Al saturation for 0, 20 and 50% (20 – 40 cm).

Weeds were controlled with atrazine (2-cloro-4-ethyl-amine-6-isopropylamine-s-triazine) at 3.0 kg ha<sup>-1</sup>. Hand hoeing was used when needed to control additional weeds during the study. All panicles from each harvested plot were bulked, air dried for minimum of seven days, threshed, and

grain yields determined. Grains were calculated at 13% seed moisture.

Aluminum tolerance from female lines was derived from SC283 accession, and the tolerance from male lines came from CMSXS225 and SC549. Populations derived from these three accessions indicated that *Alt<sub>SB</sub>* locus plays a role in controlling Al tolerance, although other minor genes in its background contribute to enhance tolerance (Magalhães et al. 2004, Caniato et al. 2007, Caniato et al. 2011). All female lines share some similarity, once all of them were twice backcrossed to ATF54B. CMSXS225 and SC549 were previously selected in nutrient solution by Caniato et al. 2007. These two lines were crossed and backcrossed to BR012R, which means they also share some similarity.

Sorghum hybrids were grown in a randomized complete block design, with two replications. Plots consisted of 1 row, 5 m long, with 45 cm between rows. Plots were thinned to 12 cm between plants (180,000 plants ha<sup>-1</sup>). Trials were carried out at the experimental area of Embrapa Maize and Sorghum, in Sete Lagoas, MG, Brazil.

Analyses of variance and genetic analysis followed the procedures described by Cruz and Regazzi (1997). General (GCA) and specific (SCA) combining ability effects were estimated according to Griffing's (1956) Method 4, adapted for partial diallel to Cruz and Regazzi (1997). Analyses were performed using Genes software (Cruz 2006).

## RESULTS AND DISCUSSION

Different screening methods have been used to evaluate Al tolerance in plants, such as cell and tissue culture, nutrient solution culture, soil bioassays and field evaluations. Laboratory and greenhouse based techniques for screening are widely used since they are quick, highly accurate, non-destructive, and can be applied at early developmental plant stages (Hede et al. 2001). Field based techniques are more laborious and time consuming, mainly when building the areas of Al saturation.

Figure 1 demonstrates the clear distinction of the three areas (0%, 20% and 50%) used to screen the hybrids. A total of 274 samples were collected to assess the Al saturation of the areas. Descriptive statistics for Al saturation are presented in Table 2. The mean Al saturation achieved for the three areas were 2.1%, 20.8% and 55.9% for 0%, 20% and 50%, respectively, being very close to the desired saturation.

The combined analysis of variance across the three environments and the estimates of variance for combining abilities are presented in Table 3. Significant differences among hybrids were detected for all three traits. Interaction hybrids x environments was significant only for plant height. Environment effects were significant for yield and

plant height. Thus, there is variability among the hybrids, but the best ones at low level of Al saturation were also the best on higher Al saturation, with some restriction for plant height.

Overall mean for yield, considering the three environments, was 5.01 ton ha<sup>-1</sup>, which is twice the Brazilian yield, that is 2.4 ton ha<sup>-1</sup> (Table 3). At 20% and 50% soil Al saturation, yield was reduced by 16% and 32%, respectively, compared to 0% Al-saturated soil. These data show that aluminum toxicity represents a limiting factor for yield in sorghum.

Flores et al. (1988) carried out an experiment to determine the variations in sorghum associated with soil Al saturation. They found no differences in yield for the acid tolerant genotypes grown at 40% or 60% Al saturation. However, the susceptible genotypes showed reduced yield in higher Al saturation compared to lower Al saturation. All sorghum genotypes grown at above 70% of Al saturation performed poorly. Baligar et al. (1989), while evaluating sorghum genotypes in field, showed that grain yields were reduced by an average of 24% at 41% Al saturation, as compared to 2% Al saturation; and at 64% Al saturation the reduction was 54%.

Flowering time and plant height varied from 84 to 93 days and 118 to 167cm, respectively. Aluminum toxicity did not alter the flowering time. However, plants were shorter at higher Al saturation (132 cm) relative to lower Al saturation (143 cm). There is variability for both traits and hybrids with earlier maturity and plant height around 150cm should be selected.

Mean squares were significant for all three traits due to GCA I (female lines), and the interaction GCA I x E was

not significant. This indicated the importance of additive genetic variance for the expression of variability. Significant GCA II and SCA effects were observed only for plant height and days to flowering, respectively, indicating the importance of additive effects for the former trait, and dominant effects for the latter (Table 3). Ratio GCA/SCA was higher than the unity for all studied traits; this means that these traits are predominantly controlled by additive gene action. Results suggest that crossing two parents showing the highest general combining ability for yield may produce the best performing cross due to an increasing frequency of favorable genes. Flores et al. (1991) crossed acid-soil tolerant (AS-T) lines with acid soil-sensitive (AS-S) lines. They found that hybrids from AS-T x AS-S crosses were as tolerant as their AS-T parents, showing a dominant effect of the gene that controls Al tolerance. Both GCA and SCA effects were significant for acid-soil tolerance, but GCA effects were more important than SCA effects.

GCA II (male lines) mean square was significant only for plant height (Table 3). This can be explained by the similarity among male lines, once they have two backcrosses for the same parent (BR012R), and it is suppose that Al tolerance imparted by them are similar. Interactions GCA II x E were significant for all three traits (Table 3), indicating that there was difference among the performance of male lines in different environments.

Estimates of the GCA of a parent provide important indicators of its potential to generate superior lines. A low GCA estimate, whether positive or negative, indicates that the mean of a parent in crossing with the other does not differ greatly from the general mean of crosses. On the other hand, a high GCA estimate indicates that the parental mean is superior or inferior to the general mean. This gives

**Table 3.** Mean square from analysis of variances for combining ability for yield, flowering time and plant height, measured across three levels of aluminum saturation

Sources of Variation*	df	Mean Squares		
		Yield (t ha <sup>-1</sup> )	Flowering (days)	Plant Height (cm)
Hybrids (H)	164	3.5018**	12.3740**	621.6371**
GCA I	54	5.6151**	24.2756**	719.0563**
GCA II	2	50.695	33.5343	25673.7121*
SCA	108	1.5711	6.0313*	109.0002
Environments (E)	2	267.8396**	0.2101	12833.8636**
H x E	328	1.5959	5.6745	112.6238*
GCA I x E	108	1.5027	4.7564	97.7603
GCA II x E	4	23.9805**	94.8040**	1552.9167**
SCA x E	216	1.2280	4.4831	93.7603
Error	492	1.5360	5.6174	95.8863
Mean (ton ha <sup>-1</sup> )		5.01	86.32	139.09
CV (%)		24.97	2.75	7.04

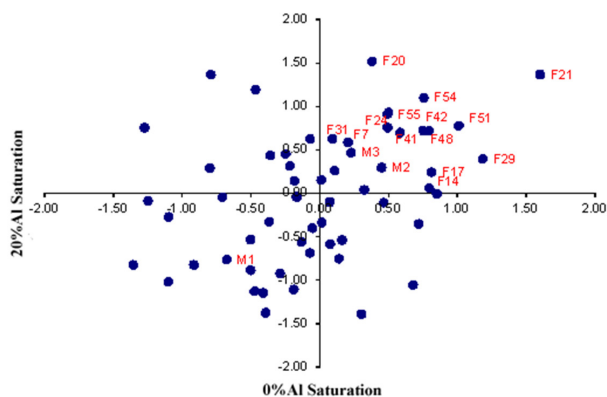
\* GCA I: female lines; GCA II: male lines.

information about the concentration of predominant genes with additive effects (Cruz and Regazzi 1997).

Several female lines were identified with high GCA for yield, which should be useful in a practical breeding program. These included F20, F21, F48, F54 and F55 with GCA higher than 900 kg ha<sup>-1</sup> (Table 4, Figures 3 and 4). Sixty percent out of the hybrids with higher yield had one of these female lines as parental. Other lines that should be considered in further trial are F17, F24, F29, F41, F42 and F51. These lines contributed with more than 500 kg ha<sup>-1</sup> in the hybrids in which they took part. Superior combining ability of these lines could be exploited either involving them in hybridization programs or by recurrent selection.

Twenty seven female lines contributed to reduce days to flowering, but only three (F29, F48 and F51) contributed with those better for yield. This is explained because yield and hybrid maturity are positively correlated, once late maturity hybrids used to present superior yield. In Brazil,

sorghum is sowed as a rainfed crop, in late summer and early fall, being subjected to strong dry spells during its cycle. Therefore, early maturity hybrids are preferred. Sorghum growers are used to beginning sowing later hybrids and

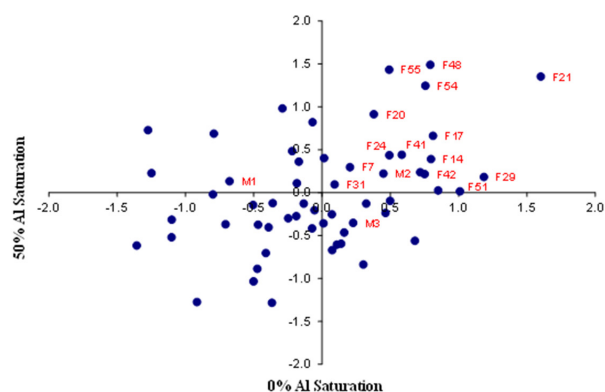


**Figure 3.** General Combining Ability for grain yield (ton ha<sup>-1</sup>) of sorghum lines evaluated at 0% and 20% AI saturation.

**Table 4.** Estimates of mean general combining ability effects for yield, flowering time and plant height of 55 female lines (GCA I), assessed in three AI saturation (0%, 20% and 50%)

Lines*	Yield (t ha <sup>-1</sup> )	Flowering (days)	Plant Height (cm)	Lines*	Yield (t ha <sup>-1</sup> )	Flowering (days)	Plant Height (cm)
F1	-0.562	-1.32	-12.98	F29	0.588	-0.987	4.798
F2	-0.807	-1.265	-10.202	F30	-0.177	-1.598	3.965
F3	-0.83	-1.154	-4.091	F31	0.271	-1.765	6.187
F4	0.049	-0.376	-9.646	F32	-0.031	-1.154	2.298
F5	0.027	0.846	-7.702	F33	-0.065	-1.154	1.187
F6	-0.523	1.458	-9.369	F34	-0.392	-0.542	-5.48
F7	0.363	0.402	-3.258	F35	-1.004	-0.82	-4.091
F8	-0.373	0.402	-7.146	F36	-0.643	-1.209	1.742
F9	-0.723	0.013	-3.813	F37	-0.079	-0.487	-2.146
F10	-0.394	0.735	-4.924	F38	-0.932	-1.098	-6.591
F11	-0.881	0.791	-4.091	F39	-0.371	0.124	-1.869
F12	0.022	-0.542	4.242	F40	-0.016	1.069	-7.98
F13	0.444	1.291	-3.813	F41	0.575	2.346	3.965
F14	0.415	-0.82	0.909	F42	0.563	0.069	3.131
F15	0.459	-1.32	5.631	F43	-0.22	-0.987	5.354
F16	0.117	-0.265	-1.035	F44	0.193	0.013	3.131
F17	0.573	0.291	-2.146	F45	-0.094	0.18	-2.98
F18	0.289	1.68	5.076	F46	-0.281	0.291	-4.924
F19	0.07	2.513	12.298	F47	-0.275	-0.598	4.798
F20	0.938	2.569	10.354	F48	0.999	-0.598	-4.091
F21	1.44	2.791	12.02	F49	-0.754	-0.265	-3.535
F22	0.2	-0.654	4.798	F50	0.078	-0.709	-7.424
F23	0.42	1.346	10.354	F51	0.601	-1.098	-1.035
F24	0.561	2.346	7.576	F52	-0.077	-1.098	-6.591
F25	-0.312	0.013	5.631	F53	0.04	0.624	-6.035
F26	-0.402	0.291	3.965	F54	1.033	0.235	4.798
F27	-0.659	0.124	7.298	F55	0.945	0.569	12.854
F28	-0.394	-1.542	0.631				

\* F: female line.



**Figure 4.** General Combining Ability for grain yield (ton ha<sup>-1</sup>) of sorghum lines evaluated at 0% and 50% Al saturation.

ending its season with earlier ones.

Plant height and yield are also significantly correlated. Most of the lines that presented positive GCA for yield showed the same for plant height.

When comparing GCA estimates at the environment with lower Al saturation and the environment with higher Al saturation, the best lines should be those with positive GCA in both environments. Figures 3 and 4 present a dispersion of the estimates of GCA for 0%, 20% and 50% of Al saturation. Twenty lines showed positive GCA in 0% and 20% of

Al saturation (Figure 3), while eighteen presented positive GCA in 0% and 50%. Fifteen of these lines are similar, i.e. presented positive GCA in all three environments (F7, F14, F17, F20, F21, F24, F29, F31, F41, F42, F48, F51, F54, F55 and M2), showing a good consistence of the data. These lines are classified as tolerant (high Al saturation) and responsive to soil improvement (low Al saturation). These lines will be selected and used as standard lines for Al tolerance in future crosses.

On the other hand, several female lines presented negative GCA in both low and high Al saturation environments (Table 4, Figures 3 and 4); moreover, eleven of them reduced yield around 500 kg ha<sup>-1</sup> in the crosses they took part (F1, F2, F3, F9, F11, F27, F35, F36, F38 and F49). These lines should be dropped from the breeding program for grain yield in acid soils.

Among male lines, M1 presented negative GCA for low Al saturation and positive GCA for high Al saturation (Table 5, Figures 3 and 4). On the other hand, M3 presented positive GCA for low Al saturation and negative GCA for high Al saturation (Table 5, Figures 3 and 4). Their performance explained the interaction of GCA x environments. M2 presented positive GCA for yield and plant height; and negative value for days to flowering. Thus, M2 can be considered the best male line due to its contribution to increase yield and plant height, and to reduce days to flowering.

**Table 5.** Estimates of general combining ability effects for yield, flowering time and plant height, of three male lines (GCA II), assessed in three Al saturation (0%, 20% and 50%)

Lines*	Al saturation	Yield (t ha <sup>-1</sup> )	Flowering (days)	Plant Height (cm)
M1	0%	-0.677	-0.800	-5.500
	20%	-0.764	0.361	-2.636
	40%	0.132	-0.158	-10.000
M2	0%	0.450	-0.727	10.773
	20%	0.296	0.215	9.727
	40%	0.221	0.006	9.864
M3	0%	0.228	1.527	-5.273
	20%	0.468	-0.576	-7.091
	40%	-0.353	0.152	0.136

\* M: male line.

## Capacidade combinatória de linhagens de sorgo tolerantes ao Alumínio

**Resumo** – O objetivo deste trabalho foi estimar a capacidade combinatória de 58 linhagens parentais de sorgo previamente selecionadas para tolerância ao Alumínio (Al). Cento e sessenta e cinco híbridos obtidos a partir do cruzamento destas linhagens foram avaliados em três níveis de saturação de Al. A saturação de Al reduziu a altura das plantas de sorgo, mas não alterou a data de florescimento dos híbridos. A Capacidade Geral de Combinação (CGC) das linhagens fêmeas foi significativa para todas as características. A CGC dos machos foi significativa somente para altura de plantas. A Capacidade Específica de Combinação (CEC) foi significativa somente para dias para florescimento. As linhagens com maior CGC para produção de grãos foram F7, F14, F17, F20, F21, F24, F29, F31, F41, F42, F48, F51, F54, e F55, sendo que F29, F48 e F51 também contribuíram para reduzir a data de florescimento dos híbridos. M2 foi a melhor linhagem-macho.

**Palavras-chave:** Sorghum bicolor, sorgo granífero, solos ácidos, tolerância ao Alumínio.

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