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TOLERANCE TO ALUMINIUM TOXICITY IN TANZANIAN SORGHUM GENOTYPES

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ABSTRACT

Aluminium (Al) toxicity is a major abiotic constraint on grain sorghum (*Sorghum bicolor* L. Moench) production on acid soils in East Africa. Aluminium in acidic soil inhibits water and mineral uptake from and consequently, reduces plant vigour and yield. A study was done to determine genetic diversity of Tanzania's sorghum for response to Al toxicity. Five day old seedlings of 98 sorghum genotypes were subjected to 0, 148 or 222.25 moles of Al^{3+} supplied as Al2 (SO4)3.16H2O in Hoagland's nutrient solution. Seedlings were raised in a growth chamber for five days, after which root lengths were recorded. Net root growth was used to discriminate the germplasm into phenotypic groups. The genotype MCSR T33 exhibited highest net root length and was classified as tolerant. *Wahi*, MCSR T69 and MCSR T11 were moderately tolerant, while the rest were susceptible.

Key Words: Genetic diversity, root length, *Sorghum bicolor*

RÉSUMÉ

La toxicité aluminique est une contrainte majeur à la production du sorhgo (*Sorghum bicolor* L. Moench) sur les sols acides en Afrique de l'est. L'aluminium (Al) des sols acides inhibe l'assimilation d'eau et de minéraux du sol, et réduit par conséquent la vigueur des plantes et le rendement.Une étude était faite pour déterminer la diversité génétique du sorgho de la Tanzanie en réponse à la toxicité aluminique. Les plants agés de 5 jours issus de 98 génotypes de sorgho étaient soumis à 0, 148 ou 222.25 moles de Al^{3+} fournis sous forme de Al2 (SO4)3.16H2O dans une solution de nutriment de Hoagland. Les plantules étaient plantées dans la chambre de croissance pendant 5 jours après lesquels la longueur des racines était mesurée. La croissance nette des racines était utilisée pour séparer les racines en groupes phénotypiques. Le génotype MCSR T33 avait exhibé une longueur nette plus élevée des racines et était classifié comme tolérant. *Wahi*, MCSR T69 et MCSR T11 étaient modérément tolérant, alors que les restes étaient susceptibles.

Mots Cles: Diversité génétique, longueur des raciness, *Sorghum bicolor*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the important staple cereals in the semi-arid regions of the world (Rohrbach *et al*., 2002). It is an important food and feed crop, and is becoming an industrial crop used in biofuels and brewing. It is an appropriate crop for cultivation in semi-arid lands of Eastern Africa because of its relative tolerance to drought. Moreover, it performs better under low soil fertility than the other locally grown cereals. It has been identified as a crop that can improve livelihoods of the vulnerable communities (World Bank, 2005) living in the arid environments. However, lack of superior cultivars arising from limited research on sorghum improvement in the region, coupled with drought and acidity often result in low yields (800 kg^{-1} compared to 2-3.0 t ha^{-1} world average) (INTSORMIL/ USAID, 2006).

Aluminium toxicity often occurs in acidic soils and is one of major abiotic stresses that limit sorghum productivity worldwide (Magalhaes *et al.*, 2004). Moreover, over 40% of the arable lands are acidic (Von Uexkull and Mutert, 1995). Most sorghum production in East Africa occurs on soils with pH<5.5. In fact, in some parts, Al saturation is high (4-55%) and dramatically affects the availability of phosphorus (Kanyanjua *et al*., 2002). Acid soils cover more than 15% of the agricultural land in Tanzania (MARI, 2006), and over 7.5% in Kenya of arable land (Kanyanjua *et al*., 2002).

Previous studies indicate that Al tolerance in plants is largely influenced by a putatively orthologous series of at least two major loci that are inherited as major Al tolerance genes in sorghum and wheat (Magalhaes *et al*., 2007). Tolerance to Al in sorghum is controlled by a major gene Alt_{SB}, located on chromosome 3 (Magalhães *et al*., 2004). The quantitative trait locus (QTL) located on chromosome 1 of rice is orthologous to the Alt_{SB} sorghum gene, while the QTL found on chromosome 3 of rice is orthologous to the Alt_{BH} wheat genes (chromosome 4DL) and to barley *Alp* on chromosome 4H (Magalhães *et al*., 2004). The *ALMT1* gene, which encodes a malate transporter activated by Al, was cloned by Sasaki *et al.* (2004); and was found to be related to Al tolerance in wheat. In rice (*Oryza sativa*), Al tolerance is a quantitative trait and QTL studies identified Al tolerance loci in all the 12 rice chromosomes (Nguyen *et al.*, 2003).

Several techniques have been developed for more rapid evaluation of tolerance to soil acidity. Among those is bioassay that includes nutrient solutions (Duncan *et al*., 1983; Magnavaca *et al.*, 1987). Screening sorghum genotypes for tolerance to Al toxicity has been done through Al-induced root growth inhibition (Magalhaes *et al.*, 2004), callose production and Al-content in root tips in nutrient solution (Baligar *et al*., 1989). Solution culture is cheap, fast and the most commonly used method in Al toxicity screening experiments. It provides easy access to root systems, tight control over nutrient availability and pH, and non-destructive measurement of tolerance (Carver and Ownby, 1995). It has been applied for Al tolerance analysis in alfalfa (Baligar *et al.*, 1989), cowpea (Paliwal *et al.*, 1994); barley (Ma *et al*., 1997); maize (Conaado *et al.*, 1999); tomato and rape (Luo *et al.*, 1999); Soybean (Villarcia *et al.*, 2001) and in sorghum (Magalhaes *et al.*, 2004). The inhibition of seminal root growth by Al in the nutrient solution is used to quantify Al tolerance in crops. Magnavaca *et al*. (1987) developed an extensively applied protocol that uses basal nutrient solution for screening for Al tolerance. Root length measurement is the most suitable criterion for Al stress is studies in maize and sorghum. It is also suitable for identifying genotypes with superior alleles for Al tolerance (Hede *et al*., 2002).

This study was done to identify new sources of Al tolerance in sorghum and determine the level of variation for tolerance to Al toxicity in the Tanzanian sorghum germplasm.

MATERIALS AND METHODS

Sorghum accessions used in the study. Ninety eight sorghum accessions were collected from sorghum growing areas in Tanzania (Table 1). Five commercial released varieties (Hakika, Macia, Pato, Tegemeo and Wahi) were obtained from Ilonga Research Centre in Morogoro (9^o 4' 0" S and 36^o 51' 0" E) in Tanzania. Sorghum standards for Al tolerance were obtained from International Crops Research Institute for the Semi-Arid (ICRISAT).

The study materials were screened for Al tolerance using nutrient solution as the growth media according to procedure described by Magnavaca *et al*. (1987). Seedlings were subjected to Al treatments of 0 (control), 148, 222 µM supplied as $AlK(SO_4)_2.16H_2O$. Sorghum seeds were surface sterilised in 1% sodium hypochlorite (NaOCl) for 8 minutes and then rinsed through 8 times using sterile distilled water. Seeds were then germinated between moistened sterilized 20 cm x 20 cm Velvex ® paper towels in an incubator at 25 ^{o}C in the dark for 3 days. Initial root length (*irl*) was measured before the seedling were put in growth

TABLE 1. Tanzanian sorghum genotypes studied for tolerance status to aluminium toxicity

S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour
1	MCSR T1 -IBS37#36	Southern Tanzania	Cream white	34	MCSR T26-IBS 585#51	Southern Tanzania	Cream	67	MCSR T52 (Tegemeo)	Ilonga	White
2	MCSR T2-IBS38#37	Southern Tanzania	Brown	35	MCSR T27-IBS 586#52	Southern Tanzania	Brown	68	MCSR T53 (Wahi)	Ilonga	White with maroon specs
3	MCSR T3-IBS40#39	Southern Tanzania	White with brown specs	36	MCSR T28 -E 36-1	Southern Tanzania	Cream with black specs	69	MCSR T54 (Hakika)	Ilonga	White with maroon specs
4	MCSR T4-IBS41#40	Southern Tanzania	White black specs	37	MCSR T29 -WAHI	Southern Tanzania	Cream	70	MCSR T55 (Macia)	Ilonga	White
5	MCSR T5-IBS1#01	Southern Tanzania	cream	38	MCSR T30 -B35	Southern Tanzania	Cream brown	71	MCSR T56	Sumbawanga	White
6	MCSR T6-IBS#2	Southern Tanzania	Cream with brown specs	39	MCSR T31-HAKIKA	Southern Tanzania	Cream	72	MCSR T58	Biharamulo	White
7	MCSR T7-IBS 3#3	Southern Tanzania	Brown	40	MCSR T32 -N 13	Southern Tanzania	Yellow	73	MCSR T63	Tarime	Brown
8	MCSR T8-IBS 4#4	Southern Tanzania	Cream	41	MCSR T33-IBS 42#41	Southern Tanzania	Cream with black specs	74	MCSR T61	Tarime	cream with red specs
9	MCSR T9-IBS 5#5	Southern Tanzania	Cream	42	MCSR T34-IBS 43#42	Southern Tanzania	Brown	75	MCSR T69	Musoma rural	Cream
10	MCSR T10-IBS 36#35	Southern Tanzania	White with brown specs	43	MCSR T35-IBS 44#43	Southern Tanzania	Cream	76	MCSR T64	Ukerewe	Cream

TABLE 1. Contd.

S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour
11	MCSR T11-IBS 35#34	Southern Tanzania	Cream with brown specs	44	MCSR T36-IBS 11#11	Southern Tanzania	Cream with brown specs	77	MCSR T57	Serengeti	Dark brown
12	MCSR T12-IBS 34#33	Southern Tanzania	White with brown specs	45	MCSR T37-IBS 12#12	Southern Tanzania	Cream with brown specs	78	MCSR T74	Musoma rural	White
13	MCSR T13-IBS 33#32	Southern Tanzania	White	46	MCSR T38-IBS 13#13	Southern Tanzania	Cream brown specs	79	MCSR T94	Igunga	Brown with red specs
14	MCSR T14-IBS 32#31	Southern Tanzania	Brown	47	MCSR T39-IBS 10#10	Southern Tanzania	Cream	80	MCSR T91	Igunga	cream with red specs
15	MCSR T15-IBS 32#31	Southern Tanzania	white with brown specs	48	MCSR T40-IBS 9#9	Southern Tanzania	Cream with brown specs	81	MCSR T105	Nzega	Dark brown
16	MCSR T16-IBS 25	Southern Tanzania	White with brown specs	49	MCSR T41-IBS 8#8	Southern Tanzania	Cream with black specs	82	MCSR T78	Serengeti	White
17	MCSR T17-IBS 24	southern Tanzania	Cream with brown specs	50	MCSR T42-IBS 7	Southern Tanzania	Cream with brown specs	83	MCSR T83	Serengeti	Cream
18	MCSR T18-IBS 23	Southern Tanzania	Cream	51	MCSR T43-IBS 6#6	southern Tanzania	Cream with brown specs	84	MCSR T84	Ukerewe	White
19	MCSR T19-IBS 22#22	Southern Tanzania	Cream brown	52	MCSR T44-IBS 31#30	Southern Tanzania	White with brown specs	85	MCSR T77	Biharamulo	White
20	MCSR T20-IBS 21#21	Southern Tanzania	Cream	53	MCSR T45-IBS 30#29	Southern Tanzania	Cream	86	MCSR T75	Ukerewe	White
21	MCSR T21-IBS 47#46	Southern Tanzania	White brown	54	MCSR T46-IBS 28#28	Southern Tanzania	Brown	87	MCSR T81	Ukerewe	Cream and brown spots

TABLE 1. Contd.

S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour
22	MCSR T22-IBS 436#47	southern Tanzania	Cream	55	MCSR T47-IBS 17#17	Southern Tanzania	Cream with brown specs	88	MCSR T72	Musoma rural	Brown.100% covered with black glumes
23	MCSR T23-IBS 582#48	southern Tanzania	Cream with brown specs	56	MCSR T48-IBS 18#18	southern Tanzania	Brown	89	MCSR T88	Ukerewe	White with few brown spots
24	MCSR T24-IBS 583#49	Southern Tanzania	Cream	57	MCSR T49-IBS 19#19	Southern Tanzania	Cream	90	MCSR T68	Serengeti	Brown
25	MCSR T25-IBS 584#50	Southern Tanzania	Cream with brown specs	58	MCSR T51 (Pato)	Ilonga	White with maroon specs	91	MCSR T100	Igunga	Brown
26	MCSR T82	Tarime	Cream white	59	MCSR T66	Bukoba rural	Brown with blackish spots	92	MCSR T99	Nachingwea	White
27	MCSR T80	Ukerewe	Cream	60	MCSR T65	Ukerewe	White with brown spcs	93	MCSR T101	Igunga	White
28	MCSR T85	Ukerewe	White and brown specs	61	MCSR T70	Ukerewe	White with brown spcs	94	MCSR T97		Cream brown
29	MCSR T87	Biharamulo	White with brown spcs	62	MCSR T73	Mwanza	Brown	95	MCSR T89		Brown
30	MCSR T102	Igunga	Brownish	63	MCSR T71	Bukoba rural	White	96	MCSR T90		White

TABLE 1. Contd.

S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour	S.no	Code	Origin	Seed colour
31	MCSR T59	Biharamulo	Cream white and brown specs	64	MCSR T103	Nachingwea	White	97	MCSR T95		White
32	MCSR T67	Biharamulo	cream with black spots	65	MCSR T60	Musoma rural	Cream with brown spots	98	MCSR T98	Kasulu	White
33	MCSR T76	Ukerewe	white	66	MCSR T79	Sumbawanga	White and brown spots				

plastic cups (2.5 cm x 3.5 cm). Loaded cups were placed on 32.5 cm x 32.5 cm plastic rafts and transferred to trays containing 8-litre nutrient solutions. The seedlings were raised in a growth chamber with continuous aeration of the nutrient solution aeration pump (FIMA® air compressor) for 5 days at a pH of 4.2.

Temperature and light were maintained at 26 ^{o}C and 550 µmol photons per square metre per second, respectively. Final root length (*frl*) was measured from the root tip to the base on the 5th day after transfer to nutrient solution. The net root length (nrl) was used to group sorghum into tolerant and sensitive phenotypic classes.

Data were subjected to analysis of variance and means separated by Least Significant Difference at 5% probability level using SAS Version 8 (SAS, 2002).

RESULTS

Overall, final root length and net root length differed significantly ($P<0.05$) with Al concentration (Table 2). The highest root reduction was observed at 222 µM Al treatment and plants grown in this (highest) Al concentration had stunted roots with blackish tips, typical symptoms of Al on the meristematic region. This treatment was too severe even for the cultivars that appeared to tolerate the stress imposed by 148 µM Al.

Genotypic differences in Al tolerance among the screened sorghum germplasm was very clear from the fact that the root growth of the genotypes screened in the solution culture varied. Based on net root growth (*nrl)*, MCSR T33 had *nrl* of 1.94 and was above the Al tolerant standard check. The standard check, ISCR 110 had *nrl* of 1.70 cm. Three sorghum genotypes, MCSR T69, T53 and

TABLE 2. Root growth means across treatments of sorghum accessions studied

Al treatment (mM)	Initial root length (cm)	Final root length (cm)	Net root length
0	3.29	7.49	4.20
148	3.33	5.38	2.04
222	3.28	3.78	1.50
LSD(0.05)	0.07	0.09	0.08

TABLE 3. Net root lengths, relative root lengths and Al tolerance status of some genotypes screened for Al tolerance

Genotype	Net root length 0 µM (cm)	Net root length 148 µM (cm)	Aluminium tolerance status	Genotype	Net root length 0 µM (cm)	Net root length 148 µM (cm)	Aluminium tolerance status
T33	4.10	1.94*	T	T66	1.58	0.49	S
T53	5.60	1.64*	MT	T85	3.79	0.47	S
ICSR 110	2.50	1.70*	T	T21	1.44	0.46	S
T69	4.21	1.32*	MT	T54	3.26	0.44	S
T11	5.04	1.19*	MT	T42	4.45	0.44	S
T76	3.17	0.96*	S	T7	2.53	0.44	S
T30	5.33	0.95*	S	T65	4.79	0.44	S
T31	3.91	0.93*	S	T103	4.13	0.42	S
T45	2.44	0.92	S	T52	3.36	0.42	S
T59	4.96	0.92	S	T90	4.26	0.39	S
T3	3.31	0.91	S	T79	3.78	0.39	S
T51	5.28	0.90	S	T78	2.77	0.38	S
T56	1.06	0.89	S	T84	3.86	0.37	S
T41	4.09	0.87	S	T27	0.41	0.36	S
T97	2.71	0.84	S	T102	3.90	0.36	S
T70	1.45	0.83	S	T4	4.27	0.35	S
T19	2.91	0.83	S	T36	3.03	0.35	S
T5	0.81	0.81	S	T68	2.60	0.35	S
T38	2.59	0.81	S	T16	3.87	0.32	S
T75	0.55	0.79	S	T28	3.09	0.32	S
T61	0.72	0.79	S	T13	1.00	0.32	S
T96	3.25	0.77	S	T29	3.44	0.30	S
T18	4.44	0.74	S	T17	2.98	0.30	S
T43	1.76	0.72	S	T82	1.63	0.30	S
T35	0.49	0.72	S	T73	2.85	0.27	S
T87	3.01	0.71	S	T10	1.29	0.27	S
T25	2.09	0.71	S	T77	0.07	0.24	S
T94	1.79	0.69	S	T62	2.16	0.23	S
T93	1.10	0.69	S	T89	4.04	0.21	S
T8	3.09	0.67	S	T39	3.73	0.20	S
T64	2.48	0.67	S	T72	0.79	0.20	S
T74	0.94	0.66	S	T63	3.01	0.19	S
T100	3.79	0.64	S	T37	0.65	0.19	S
T34	3.15	0.60	S	T15	1.44	0.19	S
T55	4.11	0.60	S	T92	1.13	0.19	S
T91	2.19	0.60	S	T67	4.32	0.18	S
T81	2.76	0.57	S	T47	4.78	0.18	S
T12	1.86	0.57	S	T80	4.14	0.16	S
T14	0.95	0.57	S	T26	0.73	0.15	S
T9	2.81	0.53	S	T40	0.71	0.15	S
T46	2.98	0.52	S	T106	2.34	0.14	S
T23	2.80	0.52	S	T71	3.42	0.12	S
T22	1.32	0.52	S	T24	2.47	0.09	S
T49	3.44	0.51	S	T1	4.97	0.08	S
T57	4.12	0.51	S	T58	1.39	0.03	S
T98	2.55	0.50	S	T60	0.97	0.01	S

* significant at $P<0.05$; T =tolerant; MT = medium tolerant; S = sensitive to Al toxicity. - Scale for classification (*nrl 148 µM*): T > 1.70cm; MT 1.5- 1.69 cm; S <1.5 cm - ICSR 110 was used as standard check from ICRISAT for Al tolerance

MCSR T 11 were closer to the standard check. On the basis of the same parameter (*nrl*), sorghum genotypes were grouped into three different classes that is tolerant, medium tolerant and sensitive (Table 3).

DISCUSSION

Although sorghum root growth was impaired by the presence of Al in the nutrient solution, there was differential response of genotypes to Al stress (Table 2). Normally, the root is the plant organ most affected by Al toxicity, and more specifically the root tip is considered to be the main site for Al toxicity (Archambault *et al*., 1997). As a result, root elongation is considered to be the most sensitive parameter under short-term exposure to Al and, therefore, may represent the whole-plant reaction to Al. The inhibition of root elongation seems to explain the retardation in plant growth through reduced nutrient and water uptake, consequently resulting in poor yield. The variability in Al tolerance has previously been noted in sorghum (Magalhaes *et al*., 2006), barley (Tamas *et al*., 2006) and maize (Ligeyo, 2007). This experiment based on net root length to discriminate the genotypes into respective tolerance groups.

The distinct difference in root growths at different levels of aluminium concentration in the nutrient solution indicates that after exposing sorghum roots to aluminum treatments for 5 days, the nutrients uptake by the seedlings was limited due to effect of aluminum on the tips. Root tips are directly involved in nutrients and water absorption by plants. The tolerant genotypes showed little effect of aluminium across the treatments and had better growth.

It was also found that 148 µM Al concentration was sufficient to discriminate tolerant Tanzanian sorghum genotypes from sensitive ones. However, Al concentration at 222 µM was too high and this classified tolerant genotypes into sensitive. Majority of the Tanzanian genotypes screened in this study were sensitive to Al stress. Majority of sorghum growing areas are reported to have soils with pH ranging from 4.5 to 5.5 (MARI, 2006). Therefore, cultivation of the broad germplasm largely aluminium sensitive could be one of the contributing factors to low sorghum production. This justifies the need to breed and select for sorghum cultivar(s) tolerant to Al stress.

Several experiments for selection of genotypes tolerant to Al in the nutrient solution have been successfully conducted in sorghum (Furlani and Clark, 1981; Giaveno *et al.*, 2001). Galvez and Clark (1991) demonstrated that two sorghum genotypes maintained their relative differences to Al toxicity tolerance independently whether they were grown separately or in the same nutrient solution. According to Magalhaes *et al.* (2006), genetic variation for Al tolerance in plants has allowed the development of cultivars that are high yielding on acidic, Al toxic soils.

Only one accession MCSR T33 of the sorghum genotypes screened for Al tolerance was classified as tolerant. This genotype had relatively higher net root growth in aluminium treatment as compared to the standard check (Table 2). MCSR T33 was collected from the southern Tanzania. The most sensitive genotype, MCSR T60 was collected from Musoma rural in the Mara region of Tanzania. Three genotypes were in medium tolerant class, while the remaining genotypes were sensitive to Al toxicity. The medium tolerant genotype T53 (*Wahi)* is at the same time a Striga tolerant variety (Mbwaga, 2006) which make it a suitable candidate to be included in breeding programmes for developing a multiple stress varieties of sorghum.

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