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PHYSIOLOGICAL CHARACTERIZATION OF KENYAN SORGHUM LINES FOR TOLERANCE TO ALUMINIUM

Cheprot R. K.

Department of Biological Sciences, University of Eldoret, Eldoret - Kenya

Matonyei T. K.

Department of Biological Sciences, University of Eldoret, Eldoret - Kenya

Maritim K. K.

Department of Biological Sciences, University of Eldoret, Eldoret - Kenya

Were B. A.

Department of Biological Sciences, University of Eldoret, Eldoret - Kenya

Dangasuk O. G.

Department of Biological Sciences, University of Eldoret, Eldoret - Kenya

Onkware A. O.

Department of Biological Sciences, University of Eldoret, Eldoret - Kenya

Gudu S.

Department of Biological Sciences, University of Eldoret, Eldoret – Kenya

ABSTRACT

Eighty nine Kenyan sorghum lines were screened for tolerance to aluminium toxicity in nutrient solution. Relative net root growth; root tip aluminium content and variation in organic acid exudation were used to determine the tolerance or sensitivity of the sorghum lines at 148 µM Al for six days. The lines showed variable reduction in root growth under the Al stress. On the basis of the relative net root growths, three lines were tolerant, nineteen were moderately tolerant and sixty seven were sensitive to the Al stress. The tolerant lines secreted up to five times more citrate compared to sensitive lines under the Al treatment. All the lines secreted extremely low quantities of malate under aluminium stress despite a significant positive regression (R^2 = 0.83) between malate secretion and relative net root growth. There was a negative regression between relative net root growth and root aluminium concentration (R^2 = -0.79) among the selected sorghum lines, and the sensitive lines accumulated up to three times the amount of Al compared to the tolerant lines. The Al tolerant sorghum lines were selected for improved sorghum production in acid soil. The objectives of this study were to (i) identify Al tolerant Kenyan sorghum lines, (ii) investigate tolerance mechanisms employed by Kenyan sorghum lines against Al stress.

Keywords: Aluminium (Al) toxicity, Relative net root growth (RNRG), Organic acids, Malate,

Citrate, Al Tolerance.

1. INTRODUCTION

Aluminium (Al) is one of the most abundant soil mineral elements, oftenly occurs as insoluble alumino-silicates and oxides. The minerals tend to solubilize at pH below 5.5 forming $A1^{3+}$ that is toxic to plants [1]. Acid soils make up approximately 40% of the earth's arable land [2] covering 4 billion hectares [3]. About 13% of Kenya's arable land where sorghum is grown has acid soils [4]. Aluminium toxicity targets root apices causing reduction in root growth [5]; therefore emphasis is often put on response of root apices to Al treatment when determining Al tolerance [6]; [7] reported root growth inhibition by 5% within about half an hour of exposing maize roots to 20 µM Al. The inhibition of root growth could reduce uptake, transport and use of water and nutrients in plants [1].

Sorghum (*Sorghum bicolor* L. Moench) is the world's fifth most important cereal crop after wheat, rice, barley and maize comparing cultivated area and production [8]. Its grain is a staple food for over 500 million people globally. It is ranked second to fifth most important cereal crop in eastern Africa depending on the country [9]. Despite this significant contribution to food security, sorghum has received limited research attention compared to maize, especially in eastern Africa. Studies have also shown less reduction in the root growth of tolerant genotypes of sorghum compared to sensitive genotypes [10]. Little is known about Al tolerance mechanisms among the Kenyan sorghum lines, even though most sorghum cultivation in Kenya is done in acid soils.

Various physiological mechanisms have been postulated to confer Al tolerance to plants but the most common is Al exclusion from growing root tips by organic acid ligands such as malate, citrate, or oxalate secreted into the rhizosphere [2] ;[1]. There are two patterns of organic acid release in plants based on the time taken prior to secretion [11]. First, there is rapid response to A1 treatment [2], in which it is hypothesized that Al^{3+} activates a pre-existing anion channel in the plasma membrane. The second response is characterized by a lag phase between Al exposure and organic acid secretion [2]; [12]; [13]. However, some Al-sensitive plants have been reported to secrete large amounts of organic acids [14]. implying organic acid secretion alone, may not be a tolerance mechanism but is a consequence of biochemical reactions necessary for Al tolerance.

Response to aluminium toxicity among genotypes has been linked to function of specific genes in maize [15]. In wheat (*Triticum aestivum)*, Alt_{BH} locus known to control 85% of the aluminium tolerance has been mapped onto the long arm of chromosome 4D, [16]. Other genes; *ALMT1* (*TaALMT1)* control Al-induced malate secretion and confers Al tolerance [17]; [18]; [19].

Orthologs of Alt_{BH} gene were mapped on chromosome 4H in barley (designated HvAACT1) [20]; [21], and on chromosome 3 in rice [22] and HvAACT1 gene of the MATE efflux pump family in barley induced citrate exudation in *Hordeum vulgare* [21]. Alt3 gene in rye another ortholog of AltBH is located in chromosome 4 [23] and confers tolerance to Al toxicity. It has been demonstrated that aluminum induces the expression of *ScALMT1* transcripts in the root apices of rye plants causing secretion of citrate and malate into the rhizosphere as a mechanism of Al tolerance [5]. Although Al-activated malate exudation has been reported in wheat, other plant species like maize [15], oat and soybean [24] and sorghum [13] secrete citrate as an Al tolerance mechanism. Studies indicate that Al tolerance in buckwheat and taro depends on root oxalate

release. Some species, such as rye, triticale, and oilseed rape, exhibit an Al-activated exudation of both citrate and malate [25]. Citrate chelates Al^{3+} more strongly than malate, hence more effective at detoxifying Al^{3+} [5].

Studies have shown sorghum line SC283 as Al tolerant and BR007 as Al sensitive and mapped Alt_{SB} gene on chromosome 3. This locus has been shown to be responsible for 80% of the aluminum tolerance phenotype in the sorghum mapping population used by Magalhaes, et al. [25].

Subsequent studies identified the AltSB gene as a member of the multidrug and toxic compound extrusion (MATE) family encoding an aluminum-activated citrate transporter and the main aluminum tolerance locus in sorghum [26]. Alt_{SB} alleles have been shown to cause a marked increase in sorghum aluminum tolerance [10] and improvement of yields in acid soils. Identifying Al tolerant Kenyan sorghum lines could contribute significantly to genetic improvement of the crop through breeding and increase its production in acid soils.

2. MATERIALS AND METHODS

2.1. Sorghum Plant Material

Eighty nine (89) sorghum seed materials were sourced from various parts of Kenya and selfed to purify prior to use. Aluminium(Al) tolerant check, SC283, initially from Tanzanian [27] and BR007 an Al-sensitive check from the Embrapa Maize and Sorghum breeding program [25] and routinely used for reference were included in this study at the Robert Holley Center for Plant and Agriculture, Soil and Nutrition laboratory Cornell University, USA where the study was conducted.

2.2. Root Growth Determination of Tolerance to Aluminium

The eighty nine Kenyan sorghum lines were screened alongside the Al tolerant SC283 and the susceptible BR007 making a total of ninety one accessions. Seeds were treated with a combination of fungicides (Captan 400, Trilex and Allegiance) against both surface and systemic fungi. The seeds were then germinated for three days; six uncontaminated and nearly same length seedlings per cultivar per treatment were selected and transplanted to trays containing eight liters of nutrient solution as prepared by Magnavaca, et al. [28]. Trays were wrapped with black polythene skirts to shield light from weakening the iron chelate. The experiment was set up in a randomized complete block design (RCBD) of three replications. Seminal roots were inserted through a hole at the bottom of polythene cups, supported by black beads and kept under continuous aeration in a growth chamber at 26^0C day and 23^0C night temperatures, a light intensity of 330 µmol photons m^{-2} sec^{-1} and a 14-hour photoperiod.

After 24 hours of acclimatization at pH 4.0, seminal roots were measured and recorded, nutrient solution replaced either with 0 µM Al for control set or with one supplemented with 148 µM Al for the treated set, added as $AlK(SO_4)_2.12H_2O$. Each sorghum line was replicated six times per treatment. After three and six days, the length of seminal roots was measured and recorded.

Relative net root growth between Al treated and control sets per line were used to determine line relative root growth as described by Caniato, et al. [10] with slight modification as follows:

$$\mathrm{RNRG} = \frac{\text{frl A} - \text{irl A}}{\text{frl C} - \text{irl C}}$$

Where: RNRG - relative net root growth, frl A - final root length in 148 µM Al, irl A - initial root length in 148 µM Al, frl C- final root length in control and irl C- initial root length in control

2.3. Quantification of Al Accumulation in Root Tips

Two centimeter (cm) root apices were excised after six days of treatment in Al, dried for two days at 40^0C then three root tips per line were weighed on an analytical balance (Mettler Toledo MT-SICS UMX, Switzerland). The samples were heated in 200 µl of 50%/50% mixture of 95% HNO_3 and 70% $HClO_4$ in quartz tubes placed on a dry heating block at 165^{o}C for two hours to dissolve, then dissolved in 10.2 ml of (5%) nitric acid, swirled to solubilize minerals then subjected to Inductively Coupled Argon Plasma Emission Spectroscopy (ICP-AES) (Sciex Model 5000, Perkin Elmer/Sciex, Concord, ON, Canada) as described by Tang, et al. [20]. The ICP-AES reading of Al in 10.2 ml of added acid was computed as:

$$Root\ tip\ Al\ content = \frac{Al\ (\mu g) X 10.2\ ml}{\text{ml}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .(1).$$

$$Root\ tip\ Al\ concentration\ = \frac{\frac{Al\ (\mu g) X 10.2\ ml}{\text{ml}}}{sample\ dry\ weight\ (mg)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .(2).$$

2.4. Determination of Root Organic Acid Exudation in Selected Sorghum Lines

Nine lines (N60, O2, C1, F15, M33, L5, N61, N84 and M49) were selected based on initial screening alongside the checks SC283 and BR007 for determination of Al-induced root citrate exudation. Seeds were germinated for three days, and five seedlings per line per set of treatment were transplanted into the Magnavaca nutrient solution. After 24 hours, the solution was replaced by a similar nutrient solution for control and one supplemented with 148 µM Al for the treated set and kept under continuous aeration for six days. Seedling roots were rinsed in ultrapure water then in 4.3mM $CaCl_2.2H_2O$. The exudates were collected along the whole seminal root for six hours by transferring five seedlings per line per treatment to a 50 ml plastic falcon tube containing 48 ml of 4.3 mM $CaCl_2.2H_2O$, pH 4.5 for control or in 48 ml of 95 µM $AlCl_3.6H_2O$ and 4.3 mM $CaCl_2.2H_2O$, pH 4.5 for the Al treated set, kept in continuous aeration for six hours. The experiment was replicated four times. The solutions were passed through silver resin (add mesh size and manufacturer name) to reduce chloride ions and cation exchange resin (type and manufacturer) to remove Al ions. One milliliter of the processed sample was stored at -20^{o}C awaiting analysis via capillary electrophoresis (CE) (P/ACE MDQ Capillary Electrophoresis System; Beckman Coulter, USA). The capillaries were flushed with 0.1N NaOH for 5 minutes and calibrated with (1, 2, 4 and 8) µM citrate, malate and phosphate standards dissolved in 4.3mM $CaCl_2.2H_2O$ and in 4.3mM $CaCl_2.2H_2O$ with 95 µM $AlCl_3.6H_2O$. For electrophoresis, 40 µl of each sample was passed through a 67-cm capillary (75 µM I.D.) with a constant separation voltage of -28.5 kV at 25°C. The electrolyte used for separation consisted of 0.5 mM dodecyltrimethylammonium bromide, 7.5 mM salicylic acid, and 15 mM tris adjusted to pH 9.5 with NaOH. Peaks were detected with a UV absorbance detector at 230 nm and analysed by the 32 Karat software, version 8.0, Build 825, 1998-2006, Beckman Coulter Inc., USA. Peak identification was based on the basis of migration time, area of peak and corrected values of peak area per time of detection.

3. RESULTS

3.1. Effect of Al Stress on Root Growth

There was significant variation in root growth among the Kenyan sorghum over the six day period of Al treatment as shown in figure 4 and table 1. In the first three days of Al treatment, root growth inhibition was observed in all the materials including the tolerant check SC283 whose root growth was reduced by over 30%. Based on RNRG analysis for days 0 - 6, tolerant lines seem to have recovered from effects of Al treatment and showed insignificant inhibition whereas susceptible lines showed significant ($p \leq 0.05$) root growth inhibition. The day 3-6 RNRG was very interesting, tolerant lines (O2 and N60) almost doubled their RNRG while the sensitive lines (M49, N84 and N61) had a significant ($p \leq 0.05$) RNRG reduction (Table 1). In general, 60 % of the lines had RNRG of less than 0.20 including the check BR007 with a RNRG of 0.13, 37 % had RNRG of 0.20 - 0.70 including M33 and L5. Interestingly, only 3 % of the lines were tolerant. Lines O2 and F15 developed lateral roots like the tolerant check SC283 (Figure 1) under Al treatment but C1 and N60 did not form lateral roots under the same treatment. None of the sensitive lines including M49, N61 and N84 developed observable lateral roots.

Figure-4. Effect of 148 µM Al on root growth of Al tolerant (O2- Kenyan) and Al tolerant (SC 283- Brazilian) sorghum genotypes in solution culture '0'refers to control treatment and 'Al' refers to treatment with 148 µM Al after six days of growth in nutrient solution.

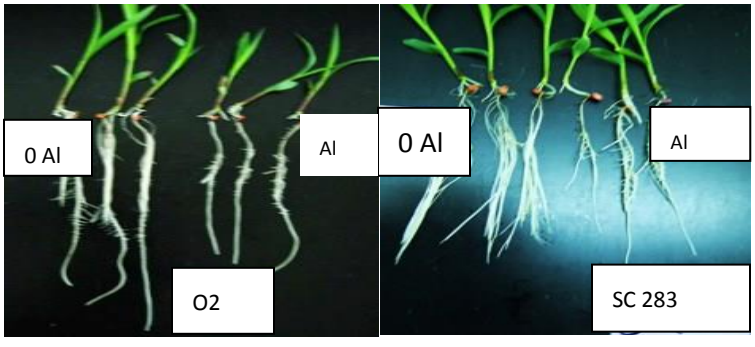


Table-1. Relative net root growths and quantities of Al accumulated by Kenyan sorghum lines

Line	RNRG	Al µg per mg of tissue	Line	RNRG	Al µg per mg of tissue
SC283	1.08±0.13 ^{r}	0.772±0.04	M5	0.15±0.07 $^{a-h}$	1.989±0.04
N60	0.81±0.23^{qr}	0.833±0.15	F16	0.15±0.03 $^{a-h}$	2.137±0.15
O2	0.75±0.08$^{pq r}$	0.757±0.14	J2	0.15±0.03 $^{a-h}$	2.203±0.22
C1	0.73±0.07^{op}	0.921±0.19	C13	0.15±0.04 $^{a-h}$	1.626±0.03
F15	0.69±0.10$^{no p}$	0.827±0.14	M40	0.15±0.03 $^{a-h}$	2.070±0.05
N140b	0.63±0.14$^{mo p}$	1.263± 0.27	N13	0.14±0.05 $^{a-h}$	1.815±0.17
C19	0.57±0.14 $^{l-p}$	1.231±0.22	T54	0.14±0.04 $^{a-h}$	2.916±1.09

M44	0.53±0.12 k-o	2.823±1.83	BR007	0.13±0.02 a-g	1.598±0.02
L5	0.52±0.20 k-o	1.735±0.13	N77	0.13±0.04 a-g	2.404±0.21
J1a	0.50±0.14 k-o	1.283±0.24	N52	0.13±0.01 a-g	1.676±0.22
M33	0.47±0.14 i-n	1.268±0.23	M20	0.13±0.04 a-g	2.072±0.13
I4	0.44±0.12 i-n	0.975±0.09	N85c	0.13±0.02 a-g	2.405±0.03
Q4	0.43±0.17 h-m	1.651±0.29	E8	0.12±0.05 a-g	2.272±0.25
P3	0.41±0.1 g-m	1.569±0.27	N4	0.12±0.05 a-g	2.379±0.34
L6	0.37±0.12 d-m	1.416±0.20	F6	0.12±0.02 a-f	2.019±0.08
T55	0.34±0.06 b-m	1.673±0.35	N81	0.12±0.04 a-f	2.437±0.10
G2	0.34±0.16 b-m	1.349±0.23	N35	0.12±0.04 a-e	1.965±0.02
M46	0.34±0.09 b-m	1.672±0.18	K6	0.11±0.03 a-e	2.414±0.02
M38	0.33±0.09 b-m	1.502±0.13	P1	0.11±0.01 a-e	2.491±0.18
A4	0.32±0.07 a-l	1.102±0.11	N102	0.10±0.04 a-d	2.673±0.23
M45	0.32±0.09 a-l	2.312±0.25	N103	0.10±0.02 a-d	2.299±0.14
Q2	0.31±0.13 a-k	1.730±0.06	N120	0.10±0.01 a-d	2.055±0.25
A3	0.30±0.06 a-k	1.642±0.30	M79f	0.10±0.02 a-d	1.736±0.08
C26	0.30±0.07 a-k	2.254±0.22	M24	0.10±0.06 a-d	2.984±0.06
M3	0.29±0.10 a-k	1.943±0.16	N68	0.10±0.03 a-d	2.393±0.08
N51	0.28±0.10 a-k	0.854±0.05	M11	0.09±0.03 a-d	1.975±0.26
M33a	0.27±0.07 a-j	2.273±0.08	N61	0.09±0.02 a-d	1.996±0.23
P5	0.26±0.13 a-j	1.727±0.01	N88a	0.09±0.03 a-d	1.798±0.06
H3	0.24±0.09 a-j	1.768±0.38	M69	0.09±0.02 a-d	2.539±0.24
M43	0.23±0.08 a-j	1.288±0.06	N24	0.09±0.02 a-d	2.540±0.01
M87	0.22±0.06 a-j	1.938±0.03	N6	0.09±0.01 a-d	1.815±0.58
R3	0.21±0.05 a-j	2.497±0.01	K1	0.09±0.02 abc	2.122±0.11
I19	0.20±0.07 a-j	1.663±0.00	N57	0.09±0.02 abc	1.752±0.04
M17	0.20±0.07 a-j	2.082±0.22	N85	0.09±0.02 abc	2.214±0.14
R1	0.20±0.06 a-	1.778±0.01	M32	0.09±0.01	2.521±0.33

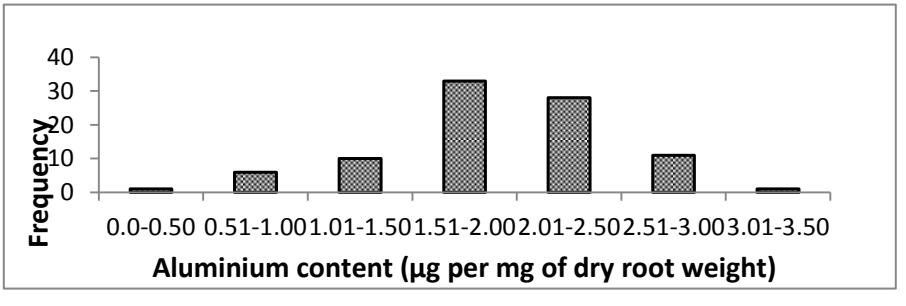
	j			abc	
M42	0.18±0.03 a-i	1.660±0.24	R7	0.08±0.03 abc	2.056±0.01
M37	0.17±0.05 a-h	2.367±0.03	N84	0.08±0.02 abc	1.721±0.05
N157a	0.17±0.07 a-h	1.525±0.01	K8	0.08±0.03 abc	2.597±0.15
T53	0.16±0.02 a-h	2.149±0.15	F8	0.08±0.01 abc	1.899±0.11
Q3	0.16±0.05 a-h	1.915±0.24	K4	0.07±0.02 abc	2.869±0.20
F3	0.16±0.04 a-h	2.484±0.15	L1	0.07±0.01 abc	1.773±0.14
F13	0.16±0.05 a-h	3.015±0.11	M1	0.07±0.01 abc	2.039±0.24
F2c	0.16±0.02 a-h	2.706±0.08	N80	0.06±0.02 abc	1.940±0.04
N119	0.16±0.06 a-h	1.955±0.05	M47	0.06±0.02 ab	1.989±0.04
K12	0.16±0.04 a-h	1.931±0.24	M49	0.05±0.02 a	2.137±0.15
K5e	0.16±0.05 a-h	2.658±0.09			

Lines were grown in [28] nutrient solution at 148 µM of Al treatmet for six days. RNRG and root Al content computed. The codes include the prefix 'MSCR'. Means sharing a prefix are similar whereas those different are $p \leq 0.05$.

3.2. Root Tip Aluminium Content

The root Al concentration among the eighty nine sorghum lines varied significantly ($p \leq 0.05$) (Figure 2, Table 1)). Sensitive lines accumulated up to three times more Al than the tolerant lines under Al treatment. Lines SC283, O2 and N60 with RNRG > 70% accumulated significantly lower amounts of Al (µg/g dry weight) than sensitive lines M49, N84 and N61 which accumulated more Al (µg/g dry weight) in their root tips. N84, N61 and M49, being Al sensitive, accumulated nearly two times more than the tolerant lines O2, N60 and SC283.

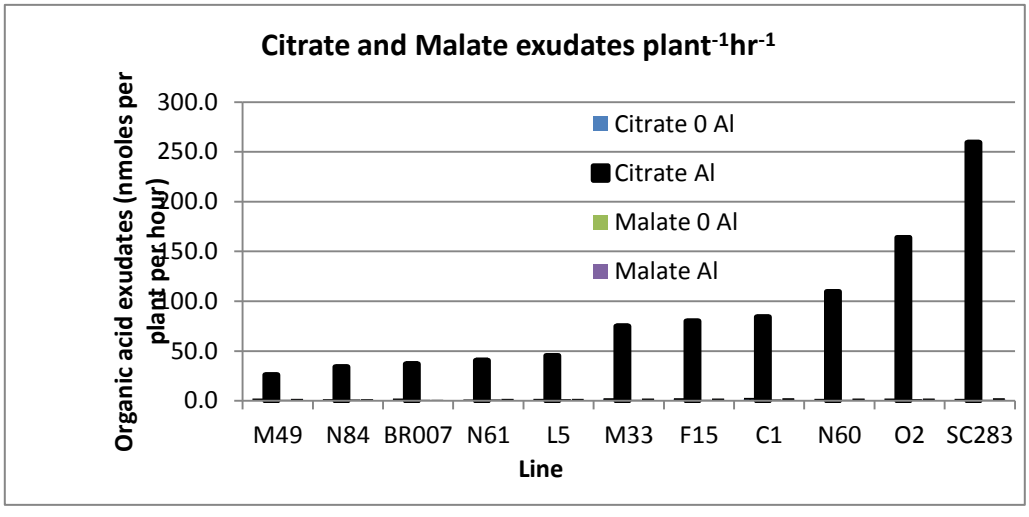
Figure-2. Chart showing the range of Al accumulation among Kenyan sorghum lines based on six days of treatment in 148 µM Al concentration



3.3. Root Organic Acid Exudation

The quantity of organic acid secreted by nine selected sorghum lines under Al treatment varied significantly ($p \leq 0.05$). Lines O2, N60, C1 and the check SC283 secreted the highest amounts of citrate, while M49 and N84 released the least quantities (Figure 3). Citrate exudation strongly regressed positively ($R^2 = 0.70$) with relative net root growth (Figure 4). Malate release by the lines under 148 µM Al treatment varied significantly between susceptible and tolerant sorghum lines (0.9 - 1.5 nmoles plant^{-1}hour^{-1}). The quantity of malate secreted was quite low compared to citrate secretion of between (26.1 and 163.9) nmoles plant^{-1}hour^{-1} by M49 and O2 respectively. Sensitive lines M49 and N61 with 0.9 nmoles plant^{-1}hour^{-1} secreted almost the same quantities of malate as the tolerant cultivars F15 and N60 (1.1 and 1.3 nmoles plant^{-1}hour^{-1}) respectively (Figure 3).

Figure-3. Citrate and malate exudates secretion by nine selected Kenyan sorghum lines collected for six hours after six days of exposure to 148 µM Al stress. BR007 and SC283 were also included as checks.

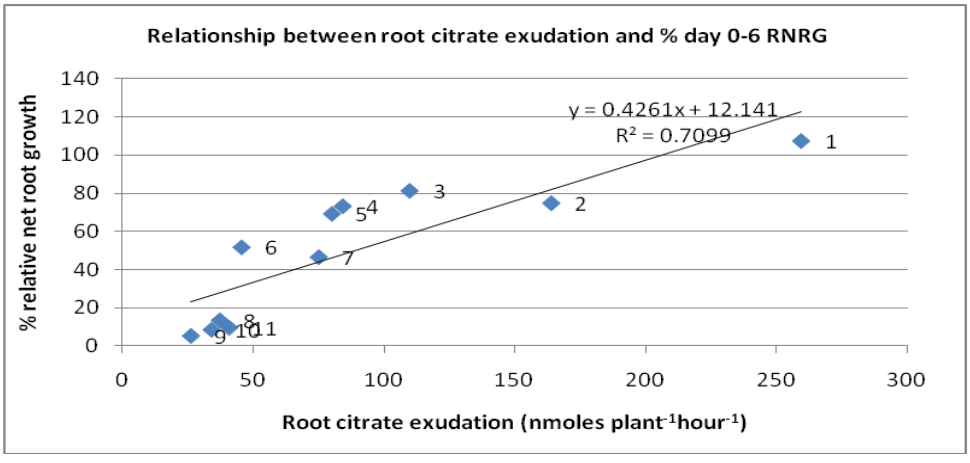


The lines released extremely low amounts of malate ($\leq$ 1.5 nmoles plant^{-1}hour^{-1} malate) at 148 µM Al treatment. Citrate exudation under 148 µM Al treatment was significantly high commensurate with the tolerance index of the lines.

3.4. Relationship between Al-Induced Citrate Exudation and Relative Root Growth

There was significant negative regression ($R^2 = 0.71$) between root citrate secretion and relative net root growth among the sorghum lines (Figure 4). SC283 and O2 secreted higher quantities of citrate and accumulated the least quantities of Al. Susceptible lines M49 and N84 released very low amounts of citrate but accumulated large quantities of Al in their root tips. About seventy one percent of variation in tolerance to Al could be explained by exudation of citrate by sorghum root tips.

Figure-4. Relationship between root citrate secretion and % relative net root growth. The codes (1-11) represent: 1-SC 283, 2-O2, 3-N60, 4-C1, 5-F15, 6-L5, 7-M33, 8-BR007, 9-M49, 10-N84 and 11-N61. A strong positive regression (R^2 = 0.71) was observed between citrate secretion and % relative net root growth.



4. DISCUSSION

In this study, the first three days (day 0-3) of treatment in 148 µM Al reduced root growth in all sorghum lines including the tolerant check SC283. This suggested that in the first 3 days, all the lines have nearly a similar response to Al injury prior to the onset of the tolerance mechanism as reported by Magalhaes [13]. Unlike in the first three days, there was variation in RNRG among sorghum lines tested for six days of treatment. The lines N60, O2, C1 and F15 including the tolerant check SC283, exhibited little inhibition and were therefore tolerant. Based on day 3-6 relative net root growths, the tolerant lines O2 and N60 nearly doubled their day 0-6 relative net root growths unlike the susceptible lines that suffered further decrease in root growth. This indicated presence of an Al induced resistance mechanism in these lines which is not present in the sensitive lines M49, N61and N84. Studies by Magalhaes [13] reported that in sorghum, Al-activated root citrate exudation correlates closely with Al tolerance. When the roots of an Al-tolerant near-isogenic line of sorghum were exposed to a moderate level of Al for a period of six days, significant increase in Al tolerance was realized after two days of Al exposure such that root growth was inhibited by about 40%, whereas by day six in Al, there was no inhibition of root growth. Over the same time period, the Al-activated root citrate exudation actually exhibited a slight decrease, suggesting that another process was induced to facilitate this increase in Al tolerance.

Considering root growth based on day 3-6 of treatment period, tolerant lines had higher RNRGs than that based on the day 0-6 period. This revealed that induction of tolerance in tolerant sorghum lines take about three days to fully respond to Al stress. Aluminium tolerance genes in sorghum seem to have a lag phase that take at least three days to be activated, and during which period the response is induced. Unlike tolerant lines, intermediate and sensitive lines realized greater inhibition between day three and day six indicating absence of effective tolerance mechanism or presence of an alternative tolerance mechanism yet to be established. The lines O2

and F15 developed lateral roots under treatment further boosting their tolerance reaction. Al tolerant sorghum lines N60 and C1 lacked the lateral roots. This suggested that various tolerance mechanisms contributed to Al tolerance among sorghum lines [5].

Generally, several parameters like, Al induced root growth inhibition [20]; [13]; [10], reduced rooting depth and root branching [29], inhibition of lateral root development [30] and seminal root elongation [7] are some of the direct means of estimating Al toxicity. The analytical parameters used are usually expressed as relative values using results from controls not exposed to Al as references [10]. Various authors have used relative root growth to determine crop tolerance to Al stress, like wheat [20]; barley [31] and sorghum [10]; [13]. In all such cases, low values of relative root growth to imply susceptibility whereas high relative net root growth to indicate Al tolerance**.**

The apparently Al tolerant Kenyan sorghum lines accumulated less than a microgram of Al per gram of dry root in their two centimeter long root apices. Tolerant lines O2, N60, F15 and C1 accumulated lower quantities of Al compared to sensitive lines M49, N84 and N61. Although M49 was the most susceptible, N84 accumulated slightly more Al suggesting contribution of other factors. Similarly, Al-sensitive maize lines were reported to accumulate higher quantities of Al in their root tips than Al-resistant lines [32]; [14]. Injuries on root apices by Al as it accumulates has been studied using haematoxylin staining and inductively coupled argon plasma emission spectroscopy (ICP-AES) on wheat cultivar Atlass 66 and near isogenic lines [20].

The sorghum lines displayed a significant negative regression linking tolerance and root tip Al content from day 3-6. This findings confirmed the report by Caniato, et al. [10] which revealed that growth of tolerant near isogenic sorghum lines were least inhibited by Al. The correlation coefficients implicate Al exclusion as a resistance mechanism among Kenyan sorghum lines. The lack of Al activated mechanisms in sensitive lines led to their very low tolerance from day three to six, because their tolerance depended on constitutive gene expression mechanisms which became exhausted shortly after exposure to Al, thereby accumulating more Al. This suggests that Al exclusion is an effective tolerance mechanism in sorghum.

In this study, Al tolerant Kenyan sorghum lines O2 and N60 released three to five times more citrate than the sensitive lines (M49 and N84) under Al treatment. Citrate release in sorghum therefore contributed immensely to root Al exclusion among the tolerant lines of sorghum. The quantities of malate secreted were too little to counter the injurious effects of Al toxicity. Worse still, Al-sensitive N61and M49 produced (0.9 nmoles $plant^{-1}hour^{-1}$) nearly as much as the tolerant lines C1, N60 and F15 (1.1 - 1.3 nmoles $plant^{-1}hour^{-1}$) under Al-treatment. Thus, malate release seems not to play a role a mechanism in Al tolerance in sorghum but only a genotypic phenomenon since even at control conditions there was varying secretion among the lines.

The tolerance mechanism based on Al-activated exudation of organic acid anions from root apices is well documented [2]; [33]; [5]; [18]. Evidence showed that availability and function of organic acid transporters determines Al-activated organic acid exudation from roots [33]. In wheat, studies showed that Al-resistant genotypes exuded more malate than the sensitive genotypes, and accumulated significantly less Al in their root apices than the Al-sensitive genotypes [18]. Studies have revealed a positive correlation between citrate secretion and Al resistance in 21 barley varieties [31].

Apparently, the Kenyan sorghum lines could be employing both exclusion and internal tolerance mechanisms in ameliorating Al toxicity. Tolerant lines accumulated less Al implying more Al was prevented from accessing the root tips. Some lines like M33 and L5 showed moderate tolerance, moderate Al accumulation and moderate citrate release. This might indicate non-inducible tolerance with the response resulting from constitutive expression at the AltSB locus. The Al exclusion and tolerance varied among lines thus enabling characterization.

5. CONCLUSIONS

There is variability for tolerance to Al toxicity among Kenyan sorghum lines useful in the breeding programs. Lines O2, N60, C1 and F15 were tolerant and M49, N61 and N84 were among the susceptible. Al tolerant Kenyan sorghum lines seems to employ exclusion mechanisms in response to Al toxicity by secreting citrate to chelate Al eventually accumulating less Al in their root tips. Aluminium tolerance among Kenyan sorghum lines was genetically determined and could be conferred through *Sorghum bicolor* Multi-drug and Toxic Extrusion compound (SbMATE) gene activity inducing citrate exudation. Therefore, it would be possible to transfer tolerance to aluminium sensitive lines with high yield potential. This would enable sorghum cultivation in agroecologies where Al toxicity is a major problem.

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