Aluminium tolerance: a determinant factor to cowpea *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae) productivity

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Abstract. Alternative approach to mitigate the negative consequences of aluminium toxicity on cowpea Vigna *unguiculata* (L.) Walp. (Fabales: Fabaceae) productivity cannot be overemphasized. The effects of aluminium toxicity on some morphological parameters of five cowpea accessions were investigated with the aim of determining the threshold of tolerance for the crop. Five cowpea accessions were collected from the International Institute of Tropical Agriculture (IITA.), Ibadan, Nigeria. The seedlings were raised in perforated plastic pots filled with 10 kg of top soil and treated till maturity with 50 μ m, 100 μ m, 200 μ m of AlCl₃ while those irrigated with tap water served as the control $(0 \mu m)$. Variations were observed among accessions and treatments as plant height was accession dependent in contrast to stem girth, number of branches, root growth and shoot growth. Suppression of root growth among the accessions were attributed to more carbon allocation to the shoot at the expense of shoot growth leading to chlorosis, necrosis and reduced photosynthetic capacity among the accessions. Accession 5 was adjudged the best among the accessions based on the response to aluminium treatment. However, further research on the mechanism of tolerance especially at the molecular level is highly recommended.

Keywords: Tolerance; Productivity; Stress; Aluminium; Toxicity.

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Introduction

Aluminium stress is a major constraint to crop production on acid soils, in view of the fact that 40% of the world's arable land is acidic. Aluminium stress remains a major hurdle for increasing world food production. especially in developing tropical and subtropical regions where increase in food production is needed. Aluminium stress reduces crop yield through root growth inhibition and impairment in nutrient and water uptake (Ma et al., 2001). Restriction of plant growth by excess aluminium could either be due to direct inhibition of nutrient uptake or disturbance of root cell functions. Because root cell function is disrupted, cell elongation and division is impeded thereby root growth is restricted such that ability of plant to explore soil volume for water and nutrient is reduced (Ma et al., 2001).

The exert mechanisms by which certain plants tolerate high levels of aluminium is still debated. Several hypotheses have been suggested, that aluminium tolerant plants either prevent excess aluminium absorption by the roots or detoxify aluminium after it has been absorbed, they have higher rates of root growth, thereby uptake of water and nutrient is greater. They usually contain high level of organic acids that help them chelate and detoxify aluminium within the plant, example of these organic acids are; oxalate, malate and citrate (Riveiro et al., 2001). Several efforts had been made to maximize yield of cowpea, however this had being largely hindered by adverse effect of biotic stresses such leaching and cultivation as poor practices. These effects cause a huge loss due to low yield and failure of the crop to establish in some cases. Alternative approach towards efficient and cost effective means of production of cowpea is therefore very desirable (Riveiro et al., 2011).

Mechanisms of aluminium tolerance are classified as those that

prevent aluminium ions from entering the root apical cells (i.e., apoplastic mechanisms) or that detoxify internal aluminium (i.e., symplastic mechanisms) (Ma, et al., 2001; Kochian et al., 2004). In symplastic mechanisms, aluminium enters the cytoplasm and is detoxified once inside the cell by complexation with organic compounds (Ma et al., 2001). Several compounds can form stable complexes with aluminium inside the cell, including organic acids such as citrate, oxalate, malate (Foy, 1988; Taylor, 1988; Ma and Miyasaka, 1998), and proteins (Suhayda and Haug, 1985). Free Al³⁺ or aluminium complexes with chelating agents can be transported to cell vacuoles, where they are stored without causing toxicity (Kochian et al., 2004).

The present study investigated aluminium the toxicity on the productivity of cowpea Vigna (Fabales: unguiculata (L.) Walp. Fabaceae). This serve to provide information on the relationship between aluminium stress and some aspect of metabolic activities primary of V. unguiculata.

Materials and methods

Plant materials

Seeds of five cowpea accessions (IT96-610, IT97K-568-18, TVU-9256, TVU-4886 and IT98K-555-1) collected from the International Institute of Tropical Agriculture (I.I.T.A), Ibadan, Nigeria were used to raise seedlings in large perforated plastic bowls. The seedlings were transplanted into perforated plastic pots (30 cm diameter and 33 cm depth) filled with 10 kg of top soil at 2 weeks, respectively, after sowing.

Experimental location and set up

The study was conducted at the screen house of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria (7° 371N latitude, 5° 44' E Longitude, and 100 m above the mean sea level; see Figure 1). The soil was air dried, sieved (in 2 mm sieve) and then 3 kg of the sample was weighed and poured into plastic polythene pots each with holes of approximately 3 mm bored at the bottom to enhance drainage and prevent waterlogging during the course of the experiment. The soil was then treated with different levels of $AlCl_3$ which are 0 μ m (control), 50 μ m, 100 μ m, 200 μ m, respectively. Each treatment was replicated five times with single plant replicate per pot, and were arranged on the screen house bench in a completely randomizes form.



Figure 1. Map of Nigeria showing the study area.

Plant height was measured from the base of the stem to apical bud, using meter rule while stem girth was measured using digital Vernier caliper at 5 cm from the base of the stem. The plants were carefully uprooted after soaking the soil with water to prevent root damage. The roots were washed, counted and their length measured. The leaves, nodes and number of branches were counted. Fresh plant parts were weighed fresh and after drying in an oven at 80 °C to constant weight.

Statistical analysis

All data were statistically analyzed using the statistical package for Social Sciences (SPSS Version 20.0). Statistical means were separated using Tukey Honest Significant Difference (HSD) test at 95% level of significance.

Results and discussion

Suppression of photosynthetic capacity of shoots is also one of the consequences of aluminium toxicity. This

is associated with cellular and ultrastructural modifications in leaves. reduced stomata opening and CO_2 assimilation, reduced chlorophyll concentration, chlorosis and leaf necrosis (Vitorello et al., 2005; Chen, 2006; Miyasaka et al., 2007; Chen et al., 2010). Accessions IT97K-568-18, TVU-9256 and IT98K-555-1 had 100% emergence under the control with respect to other treatments in contrast to TVU-4886 (93.3%) and IT96-610 (80%) as shown in Table 1. Plant biomas was not inhibited by aluminium treatment, with control having the lowest number of biomass in most accession (Table 2). According to Blamey et al. (1998) either Al³⁺ or Al(OH)⁺ are predominantly responsible for decreases in soybean growth. Similarly, Pavan and Bingham (1982) suggested that shoot growth of coffee in nutrient solution was more closely associated with the calculated activity of Al³⁺ than with the activity of other monomers in the shoot environment.

 Table 1. Mean performance of cowpea accessions for emergence as influenced by aluminium treatment.

Accessions/TRT	IT96-610	IT97K-568-	TVII-9256	TVII-4886	IT98K-555-1
	1170 010	18	1.0 /200	110 1000	117011 000 1
0 μm	80.00 ± 20^{b}	100.00 ± 0^{b}	100 ± 0^{b}	93.3±11.55 ^b	100 ± 0^{b}
50 µm	46.67±30.55ª	40±34.64 ^a	66.67±30.55 ^a	53.33±30.55 ^a	86.67±23.09 ^b
100 µm	33.3±41.63ª	53.3±11.55ª	73.3±23.09 ^a	40 ± 20^{a}	86.67±11.55 ^b
200 µm	40±34.64 ^a	53.3±30.55ª	66.67±41.63ª	60 ± 40^{a}	53.3 ± 11.55^{a}
LSD (21.52)	4	4	4	4	4

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P > 0.05.

The effect of aluminium on leaf length varied significantly among the accessions, and was not affected by the treatment with IT98K-551-1 having the lowest terminal leaf length and breadth respectively (Tables 3 and 4). Leaf area represent an integral component of plant growth, hence could be affected by different stresses. A significant decrease in leaf area of sugar cane (*Beta vulgaris* L) in response to salt stress of NaCl has been reported (Jamil et al., 2007). The notable decrease in the number of nodes of IT96-610 and IT97K-568-18 under the control regime (Table 5) as a result of the treatment with increased concentration of Aluminium chloride could be explained by the negative effect of salt on photosynthesis that leads to reduction of plant growth, leaf growth and chlorophyll content (Netondo et al., 2002).

Table 2. Mean performance of cowpea accessions for plant dry weight as influenced by aluminiumtreatment.

Accessions/TRT	IT96-610	IT97K-568-	TVU-9256	TVU-4886	IT98K-555-
		18			1
0 μm	0.51 ± 0.55^{a}	0.61 ± 0.15^{a}	0.53 ± 0.06^{a}	0.70 ± 0.34^{a}	0.31 ± 0.28^{a}
50 µm	0.80 ± 0.08 ab	0.72 ± 0.18^{a}	0.54 ± 0.15^{a}	0.70 ± 0.30^{a}	0.72±0.36 ^b
100 μm	0.53 ± 0.09^{a}	0.78 ± 0.11^{a}	0.51 ± 0.19^{a}	0.86 ± 0.44^{a}	0.50 ± 0.06^{a}
200 μm	0.62 ± 0.28^{a}	0.68 ± 0.17^{a}	0.51 ± 0.21^{a}	0.70 ± 0.30^{a}	0.49 ± 0.25^{a}
LSD (0.217)	1	1	3	3	1

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P>0.05

IT98K-551-1 was having the highest number of node at 200 μ m level of treatment (Table 5). According to Alva et al. (2005), among the Individual aluminium monomers, relative root length of soybean was most highly correlated with calculated activity of Al(0H)²⁺ followed by AlSO₄, A1(0H)⁺, and Al³⁺. They also found through reinterpretation of data from other studies with soybean, subterranean

clover, alfalfa, and sunflower that root growth was most highly correlated with activities of Al(OH)²⁺ or Al(OH)⁺. In the majority of cases, the relationship between root growth and activity of Al³⁺ was relatively poor. This situation is further complicated by the fact that Ca and other cations, as well as pH, influence the expression of aluminium treatment (Cameron et al., 1986).

Table 3. Mean performance of cowpea accessions for terminal leaf length as influenced by aluminium treatment.

Accessions/TRT	IT96-610	IT97K-568-18	TVU-9256	TVU-4886	IT98K-555-
					1
0 μm	10.60±0.31 ^{ab}	9.10±1.13 ^b	9.19±0.48 ^a	10.00±1.22 ^{ab}	8.96±0.72ª
50 μm	8.28 ± 1.05^{a}	7.80 ± 0.62^{a}	9.37 ± 1.12^{a}	8.93±0.79 ^a	10.88 ± 0.75^{b}
100 μm	10.25 ± 2.03^{ab}	9.03 ± 1.00^{ab}	9.35 ± 0.73^{a}	10.19 ± 1.15^{b}	10.79 ± 0.74^{ab}
200 µm	10.63±1.76 ^b	9.07 ± 0.88^{ab}	8.65 ± 0.27^{a}	8.42 ± 0.43^{a}	10.75 ± 2.07^{ab}
LSD (0.897)	3	4	2	3	1

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P > 0.05.

Accessions/TRT	IT96-610	IT97K-568-	TVU-9256	TVU-4886	IT98K-555-
		18			1
0 μm	5.56 ± 0.28^{b}	5.87 ± 0.62^{a}	5.46 ± 0.18^{a}	5.51 ± 0.39^{ab}	4.52 ± 0.18^{a}
50 µm	4.37 ± 0.21^{a}	5.67 ± 0.71^{a}	5.90 ± 0.68^{a}	5.01±0.22 ^b	5.13 ± 0.35^{ab}
100 μm	5.31 ± 0.87^{ab}	6.01 ± 0.27^{a}	5.56 ± 0.30^{a}	5.79 ± 0.84^{b}	4.98 ± 0.15^{ab}
200 µm	5.48 ± 0.71^{ab}	6.11±0.41 ^a	5.79 ± 0.71^{a}	4.51 ± 0.55^{a}	5.16 ± 0.90^{b}
LSD (0.447)	4	2	1	3	1

Table 4. Mean performance of cowpea accessions for terminal leaf breath as influenced by aluminium treatment.

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P > 0.05.

Table 5. Mean performance of cowpea accessions for number of nodes as influenced by aluminiumtreatment.

Accessions/TRT	IT96-610	IT97K-568-18	TVU-9256	TVU-4886	IT98K-555- 1
0 μm	4.16 ± 3.68^{a}	3.66±3.221ª	5.41 ± 0.52^{a}	5.53 ± 0.68^{a}	5.86±0.23 ^a
50 µm	6.83 ± 1.19^{ab}	8.63±1.15 ^b	8.46 ± 1.28^{b}	7.34 ± 2.54^{ab}	7.33±1.41 ^a
100 µm	6.96±0.65 ^b	6.53±1.85 ^b	6.29±0.38 ^a	6.96±1.23ª	7.43 ± 2.18^{ab}
200 µm	4.53 ± 4.31^{a}	5.85 ± 0.40^{ab}	6.60 ± 1.25^{a}	5.76 ± 1.07^{a}	6.68±0.65 ^a
LSD (1.542)	1	1	1	1	1

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P > 0.05.

Shoot elongation when treated with low concentration of salt may induce osmotic adjustment activity in the plants which may improve growth. On the contrary, the observed decrease in plant height in IT98K-551-1 (Table 6) could be due to debilitating effect of salt on photosynthesis, changes in enzymatic activities and decrease in the level of growth hormones, both of which can lead to inhibition of growth (Mazher et al., 2007). Reports of this finding from the number of leaves was corroborated with the results of Welfare et al. (2002) and López-Aguilar et al. (2003) with their study on Phaseolus vulgaris L. and *V. unguiculata*. Their findings revealed that treatment with sodium chloride salt reduced the number of leaves compared with control plants. The decrease in leaf number in IT97K-568-18 and TVU-9256 at 50 µm and 100 µm may be due to accumulation of aluminium chloride in the cellwalls and cytoplasm of the older leaves (Table 7). In addition, their vacuole sap cannot accumulate more salt and thereby decrease the concentration of the intracellular ions (Jamil et al., 2007; Kapour et al., 2010).

Result dry weight of the shoot agreed with the findings of Andriolo et al. (2005), while working on lettuce, they reported that increased concentration of NaCl increased fresh and dry weight of the seedlings (Table 8). The inhibition of shoot elongation is one of the most important and visible effects of toxic concentrations of aluminium in plants (Kochian et al., 2004). The mechanisms Al-induced inhibition of shoot of complex process elongation are a involving physical, anatomical and morphological modifications as well as cell division (Silva, 2012). Complexity in the soil environment increased with aluminium supplement to a greater extent that performance of crop became unpredictable and increasingly variable among the accessions as tolerant to aluminium became more crucial.

Accessions/TRT	IT96-610	IT97K-568-18	TVU-9256	TVU-4886	IT98K-555- 1
0 μm	13.59±1.56ª	12.70 ± 2.24^{ab}	15.44±1.44 ^a	15.23±1.67 ^b	11.01 ± 0.45^{a}
50 µm	12.99 ± 1.12^{a}	9.6±1.65 ^a	15.23 ± 0.52^{a}	12.57 ± 3.26^{a}	12.17 ± 1.22^{b}
100 µm	12.87 ± 1.26^{a}	11.73 ± 1.83^{ab}	15.08 ± 0.80^{a}	12.88 ± 0.77^{a}	11.58 ± 0.13^{a}
200 µm	12.97 ± 1.31^{a}	12.94±1.42 ^b	14.31 ± 1.73^{a}	13.06 ± 1.75^{a}	12.27 ± 2.68^{a}
LSD (1.334)	4	3	4	4	1

Table 6. Mean performance of cowpea accessions for plant height as influenced by aluminium treatment.

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P > 0.05.

Table 7. Mean performance of cowpea accessions for number of leaves as influenced by aluminiumtreatment.

Accessions/TRT	IT96-610	IT97K-568-	TVU-9256	TVU-4886	IT98K-555-
		18			1
0 μm	9.75±1.08 ^b	6.89±0.73 ^b	7.85 ± 0.87^{a}	8.23±1.53 ^a	7.20 ± 0.80^{a}
50 µm	8.75 ± 1.08^{a}	5.33 ± 0.57^{a}	6.90±2.35 ^a	7.55 ± 1.67^{a}	9.40 ± 0.52^{ab}
100 µm	8.58 ± 2.12^{a}	6.67 ± 1.33^{ab}	8.22±0.38 ^b	8.44 ± 1.38^{a}	9.74 ± 0.06^{b}
200 µm	9.67 ± 2.30^{ab}	8.22±0.69 ^b	8.15 ± 1.12^{ab}	8.24±0.21 ^a	9.41 ± 1.42^{ab}
LSD (1.063)	4	3	2	2	1

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P>0.05

Reduction in root dry weight was significantly affected with increase in aluminium toxicity. Root dry weight of TVU-4886 (0.70) at 200µm relative to the control in contrast to IT98K-551-1 (0.49) which was reported to be the lowest (Table 9). The present result confirmed that root biomass was significantly affected compared to other physiological parameters. Decrease in shoot biomass among the various accessions possibly indicates an inverse relationship between aluminium toxicity and biomass production. Findings from this study is in agreement with Gururaja Rao et al. (2005), that root growth was significantly affected by salinity levels than shoot growth. The biomass accumulation in TVU-4886 could therefore indicate optimal acquisition and uptake of nutrient for efficient metabolic activities (Ologundudu et al., 2012).

Table 8. Mean performance of cowpea accessions for shoot dry weight as influenced by aluminiumtreatment.

Accessions/TRT	IT96-610	IT97K-568-	TVU-9256	TVU-4886	IT98K-555-
		18			1
0 μm	3.34±2.91 ^a	4.55±0.54 ^b	4.26±0.25 ^a	5.07 ± 0.68^{a}	4.92±0.17 ^b
50 µm	4.54±0.21 ^b	3.93 ± 0.45^{a}	5.00 ± 0.35^{ab}	5.27±0.51ª	4.30 ± 0.19^{a}
100 µm	4.26 ± 0.58^{ab}	3.63 ± 0.16^{a}	5.02±0.35 ^b	4.93±0.13 ^a	4.50 ± 0.22^{a}
200 µm	4.42 ± 0.61^{ab}	3.98 ± 0.30^{a}	4.85 ± 0.11^{a}	5.18 ± 0.37^{a}	4.71 ± 0.19^{a}
LSD (0.619)	1	4	1	2	4

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P>0.05

Accessions/TRT	IT96-610	IT97K-568-	TVU-9256	TVU-4886	IT98K-555-
		18			1
0 μm	1.75 ± 1.52^{a}	2.66 ± 2.52^{a}	2.39±0.35 ^a	2.75 ± 0.66^{a}	2.71±0.66 ^a
50 µm	3.76 ± 0.25^{ab}	3.00 ± 0.87^{a}	2.91 ± 0.14^{a}	2.99 ± 0.57^{a}	2.73 ± 0.23^{a}
100 μm	3.41 ± 1.37^{b}	2.83 ± 0.76^{a}	3.13 ± 0.63^{a}	3.16 ± 1.04^{a}	3.66±0.31 ^b
200 µm	2.00 ± 1.73^{a}	2.55 ± 0.50^{a}	2.95 ± 0.73^{a}	2.99 ± 0.87^{a}	3.99 ± 0.57^{ab}
LSD (0.822)	1	2	1	1	1

Table 9. Mean performance of cowpea accessions for root dry weight as influenced by aluminiumtreatment.

Each value is a mean of 5 replicates. For each parameter, means with the same letter(s) in superscript on the same column are not significantly different at P > 0.05.

Conclusion

The biomass accumulation in TVU-4886 could therefore indicate optimal acquisition and uptake of nutrients for effective metabolic activities. TVU-4886 appears to dictate the growth pattern of other accessions based on its performance under aluminium treatment. Such physiological and biochemical changes exhibited are important strategies to adapt to Al-toxic environment. For further study, biochemical mechanism of aluminium tolerance in acid soils can be explored.

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Conflict of interests

The authors declare that there are no conflicts of interest.

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