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Modification of Solution pH as a Mechanism of Tolerance to Acidity and Aluminium Stress in Selected Cowpea (*Vigna Unguiculata* L. Walp) Cultivar

Sang Janeth, Too J. Emily and Were A. Beatrice

Department of Biological Sciences, School of Science, University of Eldoret, P.O. Box 1125-30100, Eldoret, Kenya

Abstract

Aluminium toxicity is a major constraint to crop production in soils with a pH below 5.5. However, plant species exhibit varied tolerance mechanisms. Nine (9) cowpea cultivars that were coded; UOE-COWPEA-1, UOE-COWPEA-2, UOE-COWPEA-3, UOE-COWPEA-4, UOE-COWPEA-5, KENKUNDE-1, K-80, M-66 and KVU-27-1 were assessed for modification of solution pH and tolerance to acidity and aluminium stress in solution culture. Cowpea seeds were sterilized and pre-germinated in paper-lined trays and the seedlings were transferred to constantly aerated growth trays containing 1/5X Hoagland Nutrient solution with a starting pH of 4.3, supplemented with 0 µM and 185 µM Al. The seedlings' initial root and shoot lengths per cultivar were measured and recorded. pH measurements of the nutrient solutions were recorded daily for seven (7) days without adjusting. The final root and shoot lengths and number of lateral root branches per cultivar and treatment were assessed and recorded. Fresh root and shoot biomass were also measured and recorded. The data collected were subjected to analysis of variance (ANOVA), and the means were compared at significant level of $P \leq 0.05$ and separation of means was done using Tukey's test. All the nine (9) cowpea cultivars progressively increased the pH of the solution culture. The growth of cowpea cultivars at 0 µM Al induced a higher change in pH compared to when grown in 185 µM Al concentration. UOE-COWPEA-4 caused the highest increase in pH from 4.3 to 5.13 while K-80 cultivar induced the least change in pH from 4.21 to 4.58 at 0 µM Al. UOE-COWPEA-5 induced the highest increase in pH when compared to others from 4.03 to 5.06 while K-80 cultivar induced the least increase in pH change from 4.32 to 4.53 when grown in solution culture supplied with 185 µM Al. UOE-COWPEA-4, KVU 27-1, KEN-KUNDE-1 and UOE-COWPEA-2 had higher relative net root length.UOE-COWPEA-3 produced significantly higher number of lateral root branches in low pH without Al compared to the other cultivars. UOE-COWPEA-3 produced a significantly higher number of lateral branches at 185 µM Al. The findings of this study show that cowpea exhibits genotypic variation in tolerance to acidity and aluminium stress. Furthermore, differences in modification of pH varied among the tested cowpea cultivars. It was concluded that acidity and aluminum tolerance were associated with alteration of pH of the solution, suggesting that cowpea adapts to acidity and Al stress by raising the solution pH and through selective root elongation inhibition among the cultivars to alleviate proton and Al rhizotoxicity stress.

Keywords: Acidity, aluminium stress, cowpea cultivars, modification of pH, solution culture

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Correspondence: janosang@gmail.com

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Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important African indigenous leafy vegetable and grain legume cultivated in tropical and subtropical Africa (Adeyemi *et al*., 2020 and Asiwe *et al*., 2020). It is a major staple source of human food for millions of people in under-developed countries in the provision of a health balanced diet and addressing nutritional deficiencies among the resource-constrained people (Da Silva *et al*., 2018). Cowpea green leaves and mature dry grains are rich in proteins, vitamins, macro and micro nutrients, flavonoids, antioxidants, ß-carotene, fatty acids, essential amino acids (lysine and tryptophan), carbohydrates and dietary fibre when compared to cereals (Owade *et al*., 2020). It is also a valuable source of livestock feed (Kebede and Bekeko, 2020) and a dependable commodity that earns income for many small-scale farmers in rural areas through the sale of leaves and grains (Nyagumbo *et al.,* 2020). Cowpea has an ability to fix nitrogen (N) to the soil of about 337 Kg N/ha (Yahaya, 2019) through biological nitrogen fixation (BNF) in a symbiotic association with *Bradyrhizobium spp* (Asiwe and Maimela 2021). Hence, it improves and sustains the fertility of infertile soils (Ajayi *et al*., 2018). Cowpea is a drought tolerant crop and adapts well to soils of a wide range of pH such as infertile acidic soils and low rainfall conditions where many other crops fail to grow (Ddamulira *et al*., 2015).

Despite the cowpea benefits as source of food, its production is limited by abiotic factors such as acidic soils and aluminium toxicity (Bolarinwo *et al.,* 2021). Most African tropical soils like Kenya are infertile and usually acidic characterized with high levels of hydrogen (H), aluminium (Al) and iron (Fe) ions (Keino *et al*., 2015) and deficient in essential nutrients such as phosphorus (P) and nitrogen (N) leading to low production of crops such as cowpea (Gurmessa, 2021). Soils therefore, with a pH below 5.0 have a low pH and termed as acidic characterised with high levels of aluminium.

Acidity therefore, is a major limiting factor to cowpea growth and production on tropical soils. Approximately, 40% of the world's arable land are acidic in many sub tropical and tropical areas (Phukunkamkaew *et al*., 2021) and more than 50% of the world's potentially arable lands (Asfawu *et al.,* 2024). Acidity increases the solubility of aluminium and reduces the availabilty of essential nutrients such as phosphorus which is important for cowpea growth and yield (Ryan, 2018). Acidity associated with aluminium (Al^{3+}) toxicities affects the symbiotic relationship between rhizobia and legume crops including cowpea resulting to reduced nodule formation, development and nitrogen fixation thus limiting plant growth (Sankar *et al.,* 2021).

Aluminium is ranked first among metals and the third most abundant element in the earth's crust (Shetty *et al.,*2021). Aluminium is non-phytotoxic when the soil pH is neutral or slighthly acidic since it exists in the insoluble oxides or aluminosilicate. However, the phytotoxic form occurs mainly on soils with pH values below 5.0, resulting to the release of Al^{3+} (Casierra-Posada *et al*., 2021). The phytotoxic species becomes soluble in the soil as acidity increases and can negatively affect plant growth and development (Casierra-Posada *et al*., 2021 and Wei *et al*., 2024). Aluminium toxicity is a major factor limiting crop productivity on acid soils worlwide including cowpea (Kushwala *et al*., 2017), thus limiting food production. The phytotoxic- aluminium (Al^{3+}) inhibits root elongation in Al-sensitive plants due to its quick inhibition in cell division and cell expansion of root meristems (Phukunkamkaew *et al*., 2021). Consequently, this limits the uptake of water and nutrients (Alemu *et al.,* 2022) leading to poor growth and substantial decrease in yield (Du *et al*., 2020). In the solution culture experiments, A1 toxicity has been shown to inhibit root elongation in crops like wheat, maize, rice and oat which occurs immediately after a few hours on exposure of roots to micromolar concentration of Al^{3+} (Engel *et al.,* 2021 and Abdelgawad *et al*., 2021). Al- toxicity coupled with acidity is a major factor limiting crop productivity, (Gurmessa *et al*., 2021).

Cowpea is known to have a higher tolerance to Al stress compared to other legumes though, Al toxicity is still a major factor limiting its productivity in acid soils. Different mechanisms of Al tolerance in crops have been established, and studies done have noted that the key to improving crop productivity in the tropics and subtropics is access to acid tolerant genotypes (Abdou Razakou *et al.*, 2013). Tolerance to acidity and high Al levels therefore, varies across plant species and between cultivars of the same species (Ranjan *et al*., 2021) but the exact mechanisms by which certain plants including cowpea tolerate these stresses is still unknown (Liu *et al.,* 2022). Several hypotheses have suggested that acid-tolerant plants exude organic acids anions from their roots such as malate, citrate and oxalate (Zhang *et al.*, 2021) which increases the rhizosphere pH thus reducing the toxic effects of Al^{3+} on the root tip of plants since the availability of Al^{3+} is inhibited at alkaline pH (Shetty *et al*., 2021). Al-tolerant plant species also prevent excess Al ions from entering the root apical cells or detoxify aluminium ions once it has been absorbed (Ranjan *et al*., 2021).

Understanding the mechanism (s) of acidity and aluminium stress tolerance in cowpea would enable targeted breeding for enhanced growth and production of the crop in acidic-aluminium toxic soils. pH modification was favored to other mechanisms since it has a direct impact on Al solubility and nutrients availabilty. The current study was set up to determine if cowpea increases the pH of the growth medium as a mechanism of tolerance to acidity by way of enhanced seedling root and shoot growth.

Materials and Methods

The study was carried out at the University of Eldoret Botany laboratory. Nine cowpea cultivars chosen for the study were; UOE-COWPEA-1, UOE-COWPEA-2, UOE-COWPEA-3, UOE-COWPEA-4, UOE-COWPEA-5, KENKUNDE-1, K-80, M-66 and KVU-27-1 respectively. The attributes of the cultivars are captured in Table 1 below.

Table 1. The description of the cowpea cultivars used in the study

Cultivar	Source	Seed colour	Growth habit	100 Seed weight (g)
UOE-COWPEA-1	Eldoret market	Dark brown	Determinate	12.61
UOE-COWPEA-2	Bumala market-Busia county	Brown with white spots	Indeterminate	12.42
UOE-COWPEA-3	Eldoret market	White with black eyes	Determinate	11.48
Ken-Kunde-1	KALRO	Red brown	Indeterminate	12.18
UOE-COWPEA-4	Sega market-siaya	Black with white eyes	Determinate	7.94
Katumani-80	KALRO	Creamy brown	Determinate	12.32
Machakos-66	KALRO	Creamy brown	Determinate	12.80
UOE-COWPEA-5	Bumala market-Busia	Creamy brown	Determinate	14.49
KVU 27-1	KALRO	Maroon	Determinate	12.42

Seed Sorting, Sterilization and Pre-Germination

Cowpea seeds to be screened were sorted, soaked in soapy sterile distilled water for five (5) minutes and surface sterilized with 0.1% sodium hypochlorite for 8 minutes. Thereafter, the seeds were rinsed eight times with sterilized distilled water to ensure all the traces of chloride were removed. The sterilized seeds of each cultivar were separately placed in between sterilized paper towels moistened with sterile distilled water in labeled petri dishes. The seeds were then pre-germinated in the dark in an incubator set at 26°C for three days.

For each cowpea cultivar, fifteen pre-germinated healthy seedlings with root length between 1.5 cm to 2.5 cm were transferred into a constantly aerated three (3) liters growth trays using an aquarium air pump containing freshly prepared 1/5 Hoagland Nutrient solution adjusted to a pH of 4.3 and supplemented with 0 µM or 185 µM $AlCl_3$. The solution contained KNO_3 (1.2 mM), $Ca(NO_3).4H_2O$ (0.8 mM), $NH_4H_2PO_4$ (0.4 mM), $MgSO_4.7H_2O$ (0.2 mM), and KCl (1.86 mM), H_3BO_3 (0.77 mM), $MnSO_4.H_2O$ (0.169 mM), $ZnSO_4.7H_2O$ (0.288 mM), $CuSO_4.5H_2O$ (0.062 mM), H_2MoO_4 (0.04 mM), and NaFeEDPTA (0.3 mM). Plants were grown in a growth chamber maintained at 28/25^{0}C day/night temperature, 16-h photoperiod using fluorescent tubes, 60% relative humidity and 300 µmol/m^2/s light intensity for the entire experimental period. The experiment was laid out in a completely randomized design and each treatment was replicated three times. The seedlings were acclimatized for twenty-four hours in the growth medium then the initial root and shoot length of each seedling per cultivar and treatment were measured and recorded. pH measurements were taken daily for 6 days without adjustment to determine the effect of genotype on nutrient pH when the cowpea cultivars were grown in the absence or presence of toxic levels of aluminium. Root and shoot lengths were measured after six days using a 30 cm ruler and recorded to evaluate the effect of Al treatment on root and shoot growth of the cowpea cultivars. The data collected were used to calculate growth indices: Net root length (NRL) and Relative Net root length (RNRL).

NRL was calculated as:

$$\mathrm{NRL} = \mathrm{FRL} - \mathrm{IRL} \text{ ....Equation 1}$$

Where FRL is the final root length in both Al treated and control plants and IRL is the initial root length.

RNRL was calculated as:

$$\mathrm{RNRL} = \frac{\mathrm{NRL}_{Al}}{\mathrm{NRL}_{C}} \times 100 \text{ ....Equation 2}$$

Where NRL_{Al} is net root length in Al, and NRL_{C} is net root length in control.

Fresh root and shoot biomass were also recorded at the end of the sixth day prior to drying to constant weight in an oven set at 60°C for 48 hours. The dried samples were cooled in a desiccator and weighed using analytical scale to obtain root dry weight (RDW) and shoot dry weight (SDW). Root-to-shoot ratio (RSR) was calculated as the quotient between RDW and SDW.

Data Analysis

Means and standard deviations for each of the traits were calculated based on the replicates for each cultivar and treatment (n=3). A two-way analysis of variance (ANOVA), was performed to assess the effect of cultivar, aluminium concentration treatment and interaction between them and the differences were assessed using the Tukey's test. All statistical analyses were performed using R (version 3.6.3) and a P-value of < 0.05 was considered significant.

Results

The Effect of Cowpea Cultivars Growth on pH of Solution Culture

The cowpea cultivars growth in nutrient solution culture with or without aluminium had a significant effect on pH of nutrient solution culture. The cultivars had a statistically significant effect on pH with P = 0.011 (P<0.05). Generally, all the nine (9) cowpea cultivars that were screened increased the pH of the solution culture as the number of days progressed. The growth of cowpea cultivars at 0 µM Al induced a higher change in pH compared to when grown in 185 µM Al concentration (Figure 1a and b) though there was no statistically significant effect of Al concentration on pH (P<0.05) since P = 0.064. The growth of UOE-COWPEA-4 in the solution culture without Al caused the highest change in pH from 4.3 to 5.13 (increased pH by 0.83) compared to other cultivars while the cultivar K-80 induced the least change in pH from 4.21 to 4.58 (raised pH by 0.37) (Figure 1a). The growth of UOE-COWPEA-5 at 185 µM Al induced the highest change in pH when compared to other cultivars with an increment from 4.03 to 5.06 (raised pH by 1.03). The cultivar K-80 still induced the least pH change from 4.32 to 4.53 (raised pH by 0.21) in solution culture supplemented with 185 µM Al (Figure 1b). The interaction of cultivars and Al concentration had no significant effect on nutrient solution pH since P = 0.94 >0.05.

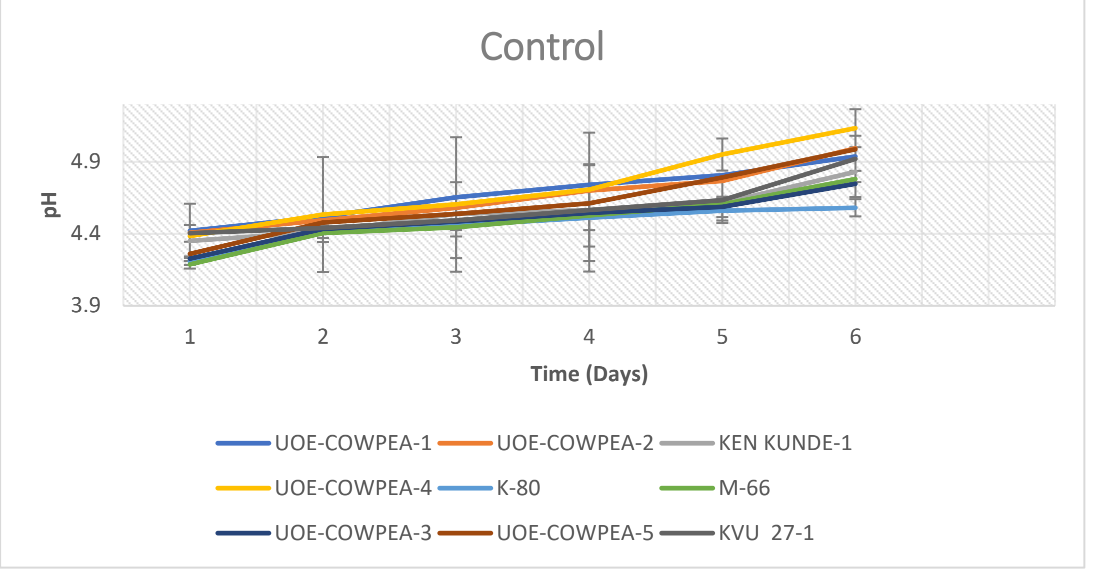


Figure 1a: Changes in solution culture pH as influenced by different cowpea cultivars grown in acidic solution culture without aluminium

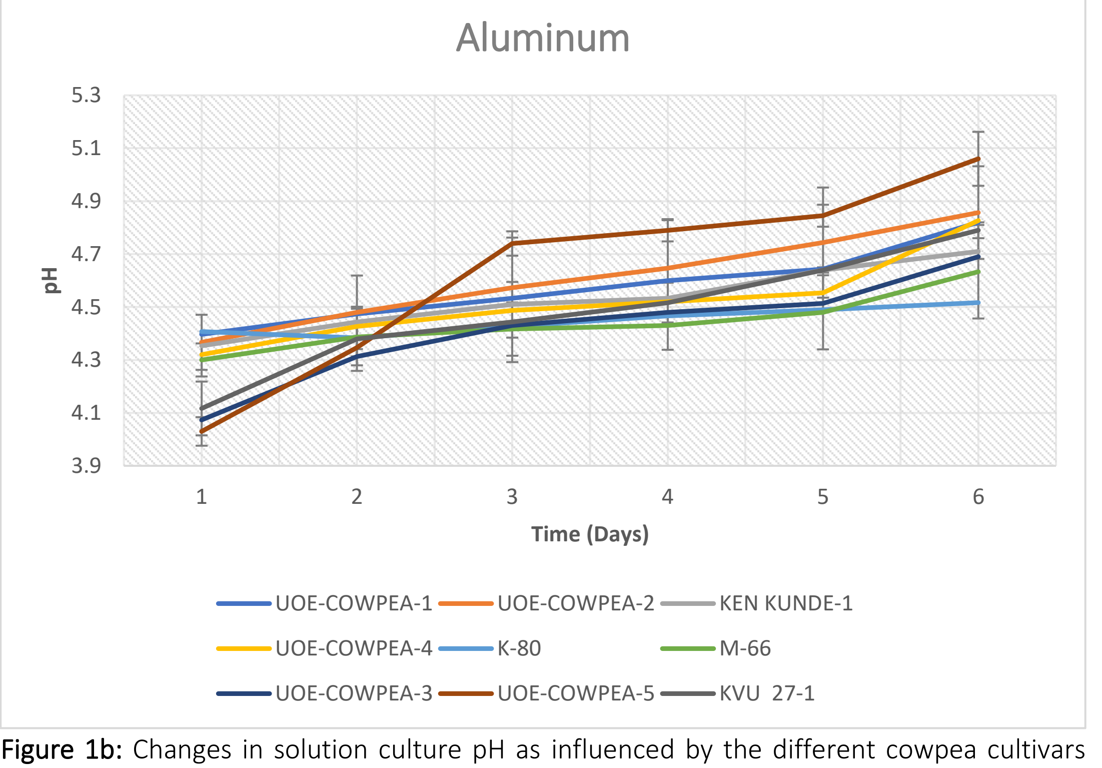
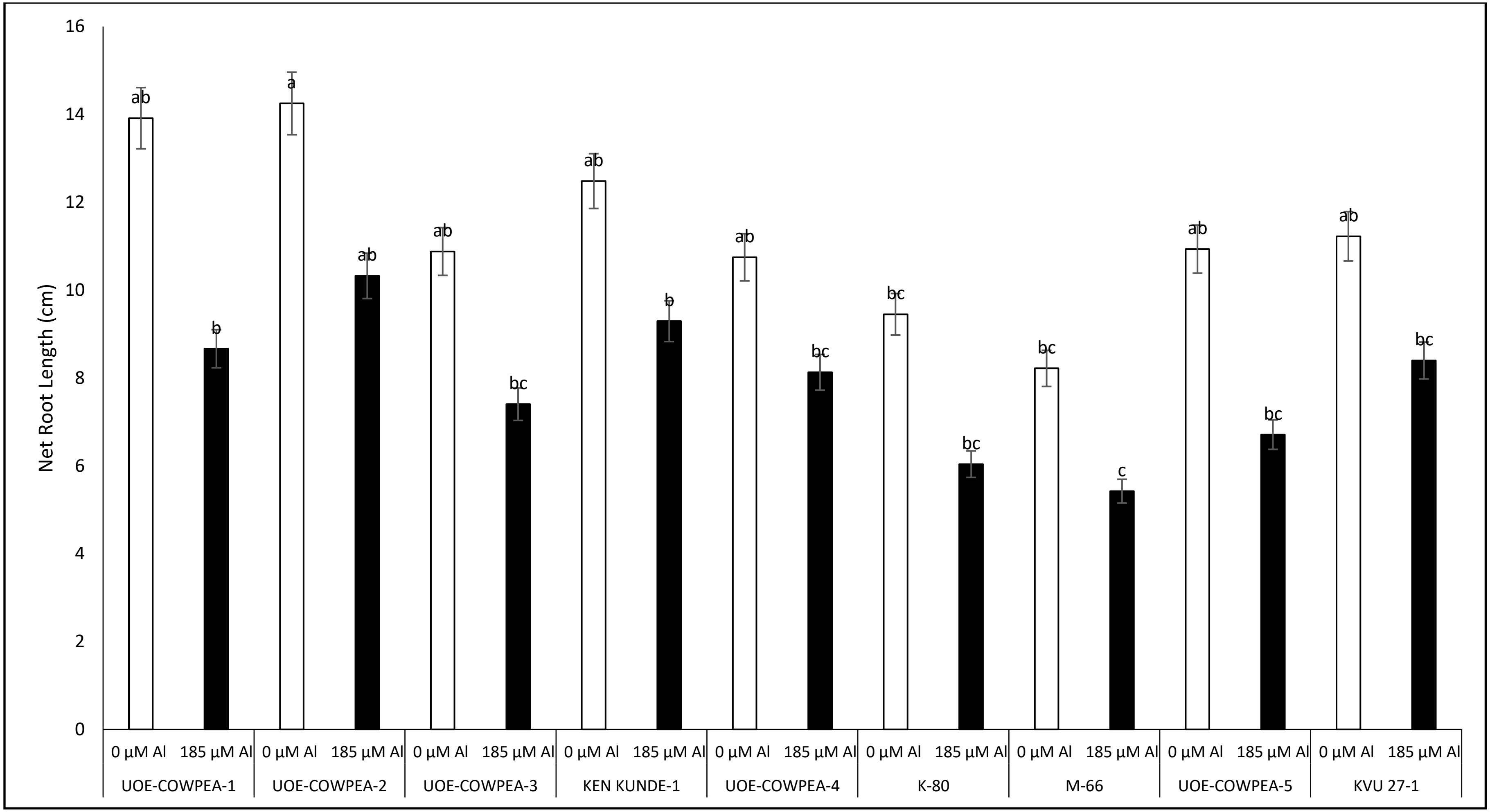


Figure 1b: Changes in solution culture pH as influenced by the different cowpea cultivars grown in acidic solution culture with 185 µM aluminium

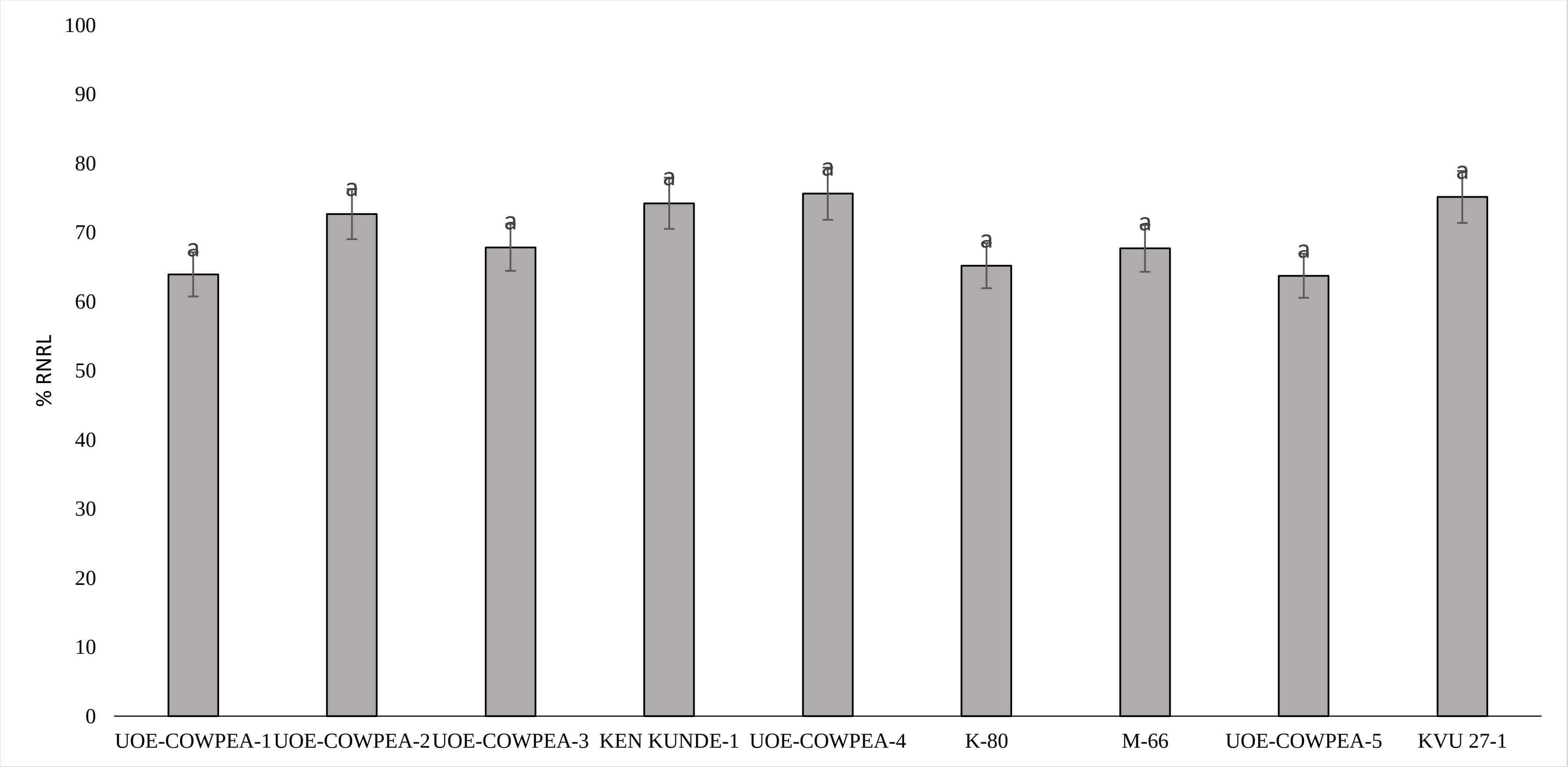
Response of Cowpea Cultivars to Low pH and Al Toxicity in Solution Culture

When germinated cowpea seedlings were transplanted to a solution culture with low pH (4.3) with or without Al, the seedlings of all the cultivars continued to grow. Cowpea cultivars differed significantly in net root length elongation regardless of the Al concentration where $F(8,36) = 13.26$, $p < 0.0001$ at ($P<0.05$).



Bars with similar letters are not significantly different at P≤0.05

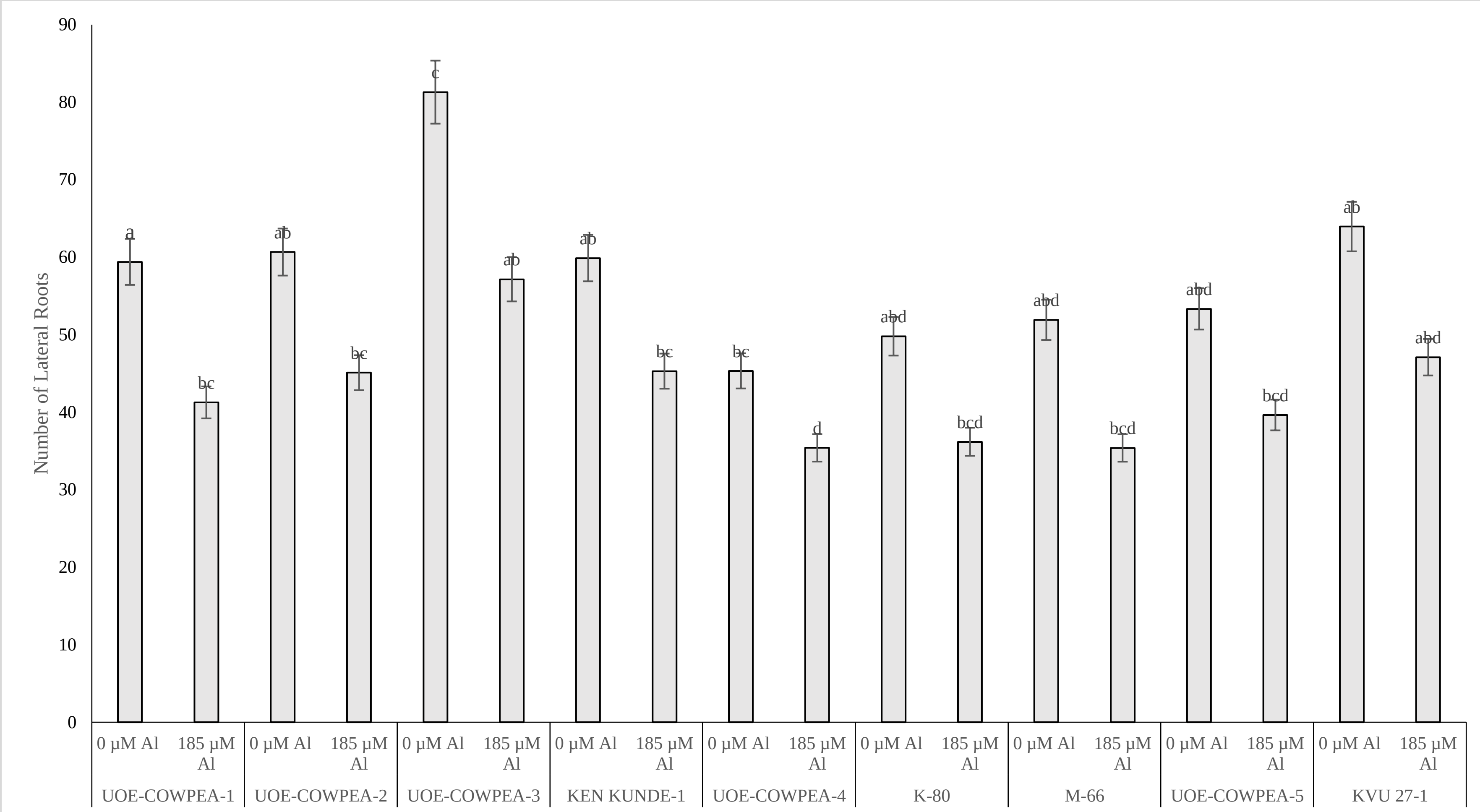
Figure 2a: Effect of low pH and Al toxicity on cowpea net root lengths



%RNRL= Percentage Relative Net Root length

Bars with similar letters are not significantly different at P≤0.05

Figure 2b. Effect of low pH and Al toxicity on cowpea cultivars Relative net root length

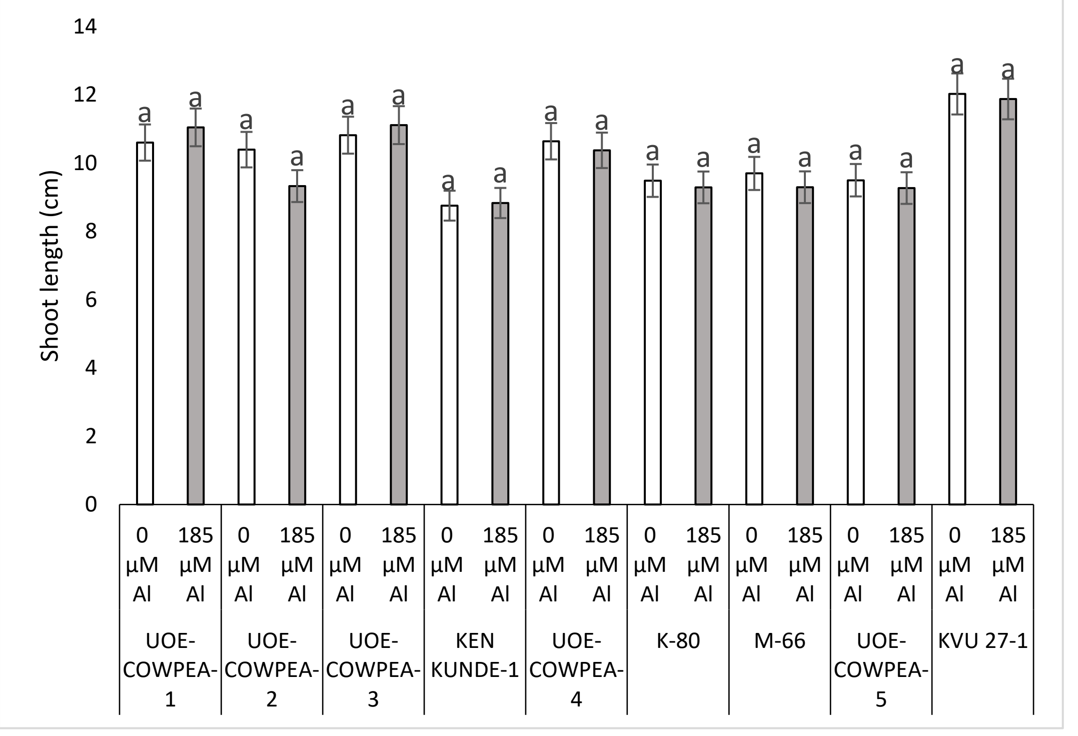


Bars with similar letters are not significantly different at P≤0.05

Figure 2c. Effect of low pH and Al toxicity on number of lateral roots on cowpea cultivars

A significant difference was observed in the net root lengths of cowpea cultivars at 0 µM and 185 µM Al (Figure 2a). The cowpea cultivars, UOE-COWPEA-2 (14.2 cm) and UOE-COWPEA-1 (13.9 cm) had significantly higher net root lengths while M-66 (8.2 cm) had the least at 0 µM. Al concentrations significantly affected ($P<0.05$) net root lengths on the cowpea cultivars screened where $F(1,36) = 121.83$, $p < 0.0001$.. At 185 µM Al, a significant difference was observed among the cultivars. UOE-COWPEA-2 (10.3 cm) had the highest net root length whereas M-66 (5.4 cm) had the least. The interaction of cowpea cultivars and Al concentration had no significant effect ($P<0.05$) on net root length. There was no significant difference observed among the cowpea cultivars on relative net root length (RNRL) response to the aluminium treatment (Figure 2b). UOE-COWPEA-4, KVU 27-1, KEN KUNDE-1 and UOE-COWPEA-2 (75.6 %, 75.1%, 74.2%, and 72.7%) cultivars had higher RNRL whereas UOE-COWPEA-1 and UOE-COWPEA-5 cultivar (63.9%) and 63.7%) had the least (Figure 2b). owpea cultivars differed significantly ($P< 0.05$) in their ability to produce lateral roots. Some cultivars inherently formed more lateral roots than others, regardless of aluminium stress since $F(8,36) = 13.43$, $p = 9.16E-09$ (< 0.05). UOE-COWPEA-3 cultivar recorded the highest number of lateral roots (81) while UOE-COWPEA-4 cultivar (45) recorded the least. At 185 µM Al, the number of lateral roots were significantly affected ($P< 0.05$) by aluminium concentration and a significant difference was observed among the cultivars where $F(1,36) = 100.50$, $p = 5.81E-12$ (< 0.05). UOE-COWPEA-3 cultivar (57) still recorded a higher number of lateral roots while UOE-COWPEA-4 and M-66 cultivars (35) recorded the least (Figure 2c). The interaction of cultivar and Al concentration had no significant effect on number of lateral roots where $F(8,36) = 0.673$, $p = 0.712$ (> 0.05). The effect of Al concentration on lateral root formation was similar across the cowpea cultivars. No particular cowpea cultivar showed a unique tolerance or sensitivity pattern to Al concentration.

Cowpea differed significantly in shoot length with $F(8,36) = 15.67$, $p = 1.23E-09$ (< 0.05). Some cultivars produced much taller shoots while others were shorter, showing genetic differences in growth potential in cowpea cultivars. KVU 27-1 cultivar (12.0 cm) had longer shoot length while Ken-Kunde-1 cultivar (8.8 cm) had the least at 0 µM Al. Aluminium concentration did not significantly affect shoot length. Cultivars grew to similar shoot lengths regardless of exposure to 185 µM Al since $F(1,36) = 0.79$, $P = 0.381$ (> 0.05). Though, the values recorded at 185 µM Al were lower than that of 0 µM Al. KVU 27-1 cultivar (11.9 cm) recorded a higher shoot length while Ken- Kunde-1 cultivar (8.8 cm) recorded the least (Figure 3).



Bars with similar letters are not significantly different at P≤0.05

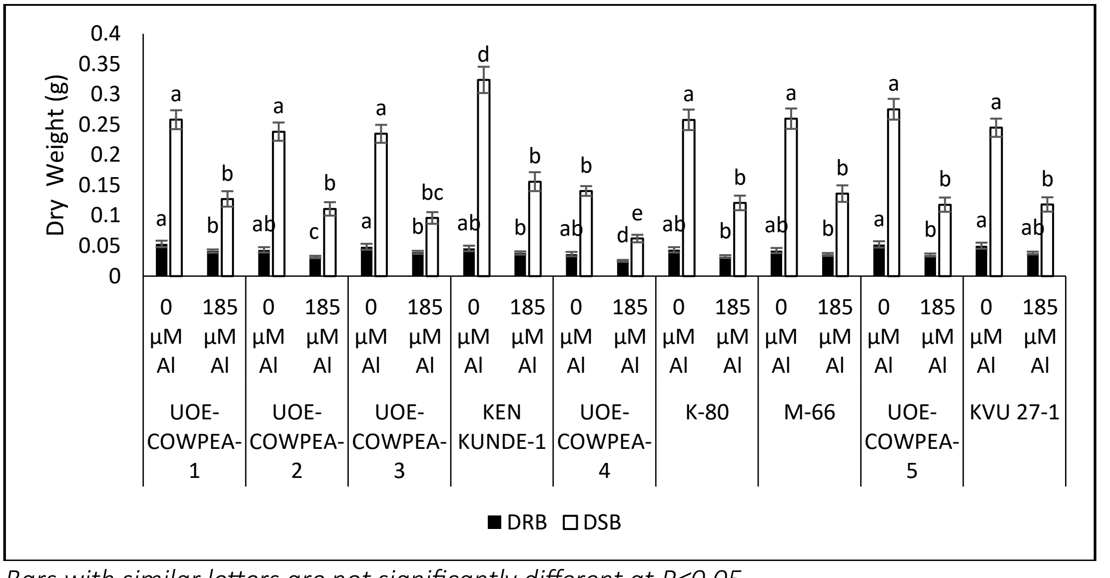
Figure 3. Effect of low pH and Al toxicity on cowpea cultivars shoot length

Effects of Low pH and Al Toxicity on cowpea Plant Biomass

There was no significant differences among the cowpea cultivars in dry root weight where $F(8,36) = 0.24$, $P = 0.981$ (> 0.05). Cowpea root mass production was broadly similar in all the cultivars. Aluminium concentration did not significantly affect dry root weight with $F(1,36) = 1.56$, $P = 0.220$ (> 0.05). The interaction (Aluminium × Cultivar) was not significant since $F (8,36) = 0.036$, $P= 0.9999$ (> 0.05). The effect of aluminium concentration on dry root weight was consistent across all cultivars. None of the cowpea cultivars showed a distinct tolerance or sensitivity in root dry mass production.

The root dry weight was significantly lower in Al treated cultivars relative to control .UOE-COWPEA-1 and UOE-COWPEA-5 (0.053g and 0.052g) recorded a higher root dry weight at 0 µM Al while UOE-COWPEA-4 (0.034g) recorded the least. At 185 µM Al, UOE-COWPEA-1 (0.048 g) had higher root dry weight and UOE-COWPEA-4 (0.025g) still had the least (Figure 4). Shoot dry weight did not differ significantly among the cultivars where $F (8,36) = 0.498$, $P = 0.850$ (> 0.05) and treatments with $F (1,36) = 3.25$, $P= 0.0796$ (> 0.05). The interaction (Aluminium × Cultivar) on shoot dry weight was not significant where $F(8,36) = 0.035$, $p = 0.99998$ (> 0.05). The effect of aluminium concentration on dry shoot biomass was consistent across all cowpea cultivars. KEN KUNDE-1 cultivar (0.324 g) recorded a higher shoot dry weight and UOE-COWPEA-4 (0.141 g) recorded the least at 0 µM Al. At 185 µM Al, M-66 cultivar (0.136 g) recorded higher shoot dry weight and the least UOE-COWPEA-4 (0.062 g).

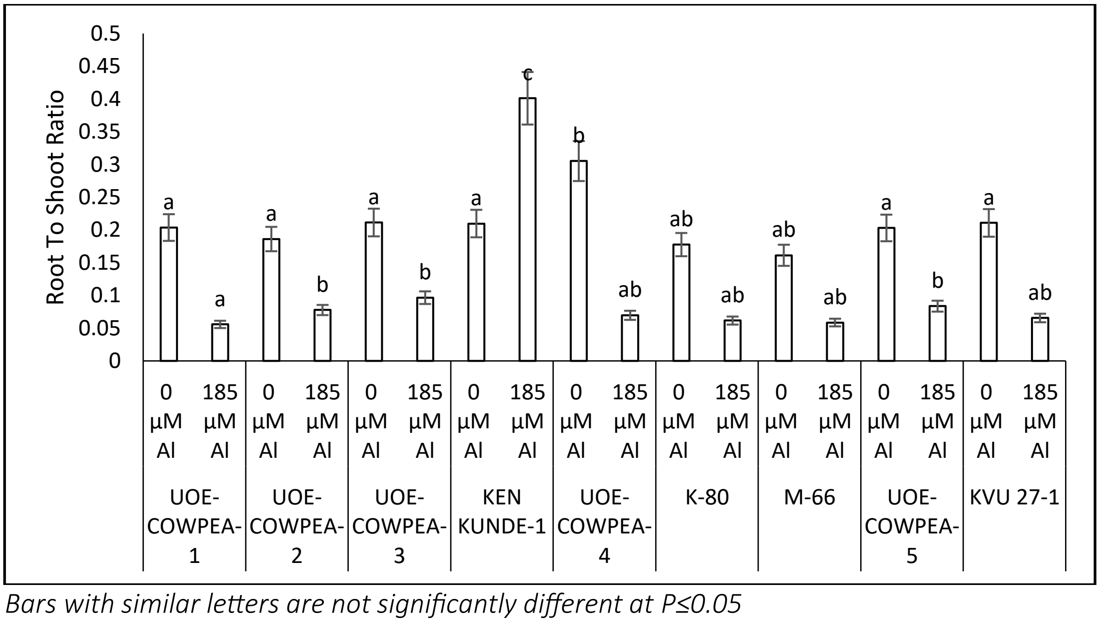
Cowpea cultivars differed significantly in their root-to-shoot ratio with $F(8,36) = 3.05$, $p = 0.010$ (< 0.05). Some cultivars allocated more biomass to roots relative to shoots, while others allocated less. Treatments had no significant effect on the root-to-shoot ratio with F(1,36) = 0.249, p = 0.621 (> 0.05). Higher root-to-shoot ratio was observed at 0 µM Al than at 185 µM Al except for KEN-KUNDE-1 cultivar. At 0 µM Al, UOE-COWPEA-4 (0.4) had higher root to shoot ratio while M-66 cultivar (0.03) had the least, and at 185 µM Al KEN-KUNDE-1 cultivar (0.4) had higher root to shoot ratio while UOE-COWPEA-1 and M-66 cultivars(0.05) had the least (Figure 4b). The interaction (Aluminium × Variety) had no significant effect on the root-to-shoot ratio where F(8,36) = 1.30, p = 0.274 (> 0.05). The effect of treatments on the root-to-shoot ratio was similar across all cowpea cultivars.



Bars with similar letters are not significantly different at P≤0.05

Figure 4a: The effect of low pH at 0 um AL on cowpea cultivar root and shoot biomass (g).

DRB= Dry Root Weight, DSB= Dry Shoot Weight



Bars with similar letters are not significantly different at P≤0.05

Figure 4b: The effect of low pH and aluminium toxicity on cowpea cultivar dry root to shoot ratio

Discussion

Nutrient solution pH changes

All the nine cowpea cultivars screened for Al tolerance induced a change in pH of the nutrient solution culture. There was a clear trend for pH increase of the nutrient solution in 0µM Al and 185 µM Al in all the cultivars with increase in duration (number of days) of culture. UOE-COWPEA-5 cultivar raised the pH higher after exposure to 185 µM Al as compared to others. Aluminum tolerant cowpea cultivars and Al sensitive cowpea cultivars increased the growth medium pH. The increased pH in the nutrient solution culture is attributed to the fact that Al-tolerant cowpea cultivars increased the pH of the solution through modification of toxic Al^{3+} to non-toxic forms hence reducing the solubility of Al^{3+} which enhanced the secretion of organic acid exudates that further modified the nutrient solution pH and thus, reduce Al solubility and toxicity. pH modification is an effective strategy against Al toxicity and favoured to other mechanisms of tolerance since it directly affects the availability of Al and nutrients in the soil. The increased pH- induced decreased the level of Al in roots, stems, and leaves and Al uptake per root DW which might be responsible for the elevated pH- induced alleviation of cowpea Al- toxicity. Yang *et al*., (2019) attributed such an increase in pH to the relative higher Al-detoxification capability of some genotypes when compared to those that cause smaller changes in pH of the growth medium. The response of cowpea cultivars to acidity and aluminium toxicity among cowpea cultivars is in agreement with the findings of Kidd and Proctor (2001), who showed that plant species adapt differently to H^+ and Al^{3+} toxicity as a result of the difference in the nature of soil parent materials and where the species originated. Pinheiro de Carvalho *et al*., (2003) also noted that there were significant differences among cowpea genotypes on rhizosphere pH modification upon exposure to 100 µM and 200 µM Al.

All the cowpea cultivars showed varied responses to growth rates (root and shoot elongation rates and number of lateral root branches) and plant biomass at 0 µM and 185µMAl. The results of the present study revealed that there is cultivar difference in growth rates and plant biomass among the cowpea cultivars proofing that it is cultivar dependent. Cowpea cultivars root growth was significantly inhibited after exposure to 185µM Al. This is attributed to the fact that Al toxicity stress inhibits root elongation in plants by limiting cell expansion and cell division, thereby inhibiting plant growth. The substantial cultivar difference in RNRL, with UOE-COWPEA-4, KVU 27-1 and Ken-Kunde-1 performing better than UOE-COWPEA-5 highlights their tolerance to Al stress. Cowpea cultivars with a higher relative net root length were classified as tolerant to low pH and aluminium toxicity while those with lower RNRL were classisied as sensitive. The results of the study are in line with the report of (Aguilera *et al*., 2016) who reported that RRL was strongly and negatively correlated with soil exchangeable Al and used to differentiate between sensitive and tolerant wheat cultivars, with sensitive cultivars exhibiting the lowest RRL. Negusse *et al*., (2022) also demonstrated that RRL was significantly affected by varying Al rates in chickpea and was a reliable criterion for distinguishing tolerant from sensitive varieties.

Higher concentration of Al also decreased the number of lateral roots which would decrease the ion absorption area of the root system. Kochian *et al*., (2024) reported on the detrimental effects of Al toxicity on crop growth, including rapid inhibition of root elongation, and water leading to reduced yields. The inhibition may be attributed to excess Al binding tightly to the cell walls of plant root cells, resulting in decreased cell wall turgidity impacting root development, (Singh *et al.,* 2017).

There was no significant effect of aluminium concentration on cowpea cultivar shoot elongation. Giannakoula *et al*., (2008) and Giongo and Bohnen (2011) reported that in the presence of Al^{3+}, Ca, P and Mg is precipitated in the root apoplast, reducing Al translocation to the aerial parts of the plant, resulting in little effect of Al concentrations on shoot elongation. Through the mechanism, Ca, P and Mg nutrients inhibit Al effects in the root system, favoring seedling growth and greater accumulation in the root. Mattiello *et al.,* (2008) also observed similar results while studying root growth and Ca, P and Al absorption in coffee plants, which they concluded that the accumulation of Al in the root system and restriction of its transport to the shoots are important factors in relation to plants tolerance to aluminum, providing evidence that the Al element can be accumulated in the roots, preventing its toxicity from reaching other plant parts (Grifferty and Barrington, 2000).

The results of this experiment confirm that aluminium concentration significantly reduced plant biomass. Aluminium concentration had a significant effect on dry root and shoot biomass in all the cultivars. The reduced root biomass could be associated with damage to root cell wall and plasma membrane impairing nutrient uptake in cowpea cultivars. Hayes *et al*., (2020) performed a hydroponic-based study on lettuca sativa grown under $AlCl_3$ toxicity and reported that $AlCl_3$ reduced dry root biomass by 22.3% and 9.96% respectively. Qu *et al*., (2020) also reported that Al toxicity reduced root length, diameter, volume and overall plant biomass by hindering protein biosynthesis and reducing carbohydrate content in Al stressed *Camellia oleifera Abel*. Other researchers also observed that Al ions interact with absorption, translocation, allocation, and metabolic activity of nutrients such as Ca, N, K, Mg, P, Mn, Fe, Cu and B (Ren *et al*., 2022; Tscuchiya *et al.*, 2021; Xia *et al*., 2020).

Conclusion

Cowpea's ability to modify the pH of its growth medium is a significant mechanism for tolerating acidity and aluminum stress. The genotypic variation in cowpea's ability to increase the solution pH was directly correlated with its tolerance to aluminum stress, as evidenced by modification of solution pH, better maintenance of root elongation, lateral root formation, and overall plant biomass in UOE-COWPEA-5, UOE-COWPEA-1 and UOE-COWPEA-2 cultivars. Under aluminum stress (185 µM Al), cowpea cultivars like UOE-COWPEA-5 exhibited the most significant increase in solution pH, and these cultivars generally showed superior growth characteristics compared to less-effective cultivars like K-80.

Recommendation

Future research should focus on identifying the specific genes and physiological processes, such as organic acid exudation, responsible for this pH modification to further enhance breeding efforts for cowpea production in acid-prone regions.

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