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Effect of Aluminium Stress on Germination, Growth, and Photosynthetic Pigments of *Amaranthus hybridus*

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Abstract

Amaranthus hybridus is utilized as a food plant in various regions across the globe. However, aluminium toxicity poses limitations on crop growth and production. The degree of aluminium tolerance varies among different plant species. This study examines the impact of aluminium stress on the germination, growth, and leaf photosynthetic pigments of *Amaranthus hybridus* seedlings. Seeds of *Amaranthus hybridus* were treated with a sodium hypochlorite solution and placed on dried petri dishes lined with filter papers soaked in 5ml of a nutrient solution containing either 0 mM (distilled water) or varying concentrations (2, 4, 6, 8, and 10 mM) of aluminium toxicity ($AlCl_3 \cdot 6H_2O$), with each treatment replicated three times. Germination was observed over five days. Subsequently, five *Amaranthus hybridus* seeds were planted in five-litre pots filled with soil, receiving daily irrigation with tap water for two weeks. Upon germination, seedlings were thinned to two plants per pot before commencing the treatments. The seedlings were then irrigated daily with nutrient solutions containing either 0 mM (control - tap water) or different concentrations (2, 4, 6, 8, and 10 mM) of aluminium toxicity ($AlCl_3 \cdot 6H_2O$), with triplicate sets in a greenhouse environment. The collected data about growth and photosynthetic pigments were assessed after the experiment and subsequently subjected to analysis of variance. The germination percentage of *Amaranthus hybridus* exhibited a notable reduction due to the stress induced by the aluminium solution compared to the control treatment. Furthermore, the aluminium-induced stress significantly diminished both growth and photosynthetic pigment parameters. These findings from the study underscore the high sensitivity of the *Amaranthus hybridus* species to aluminium toxicity.

Keywords: carotenoids; chlorophyll content; seeds; toxicity; tolerance

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Introduction

Amaranthus hybridus, also known as smooth pigweed, is an annual herbaceous flowering plant reproducing through seeds. It commonly grows as a weed in cultivated fields, gardens, and other disturbed open habitats. Widely used as a food source, its young leaves are considered highly nutritious and are used as vegetables (1). This plant is a green vegetable in Africa, India, Mexico, and the southern USA (2). *Amaranthus hybridus* exhibits growth across various soil types and textures, although soil acidity significantly affects crop production in several regions globally (3). Acidic soils, classified as ultisols or oxisols with a pH of 5.5 or lower, are prevalent in tropical and subtropical areas (4). Aluminium toxicity contributes to nutritional imbalances in upland crops within acidic soils. Aluminum, under acidic conditions, proves toxic to plant roots, hampering their water and nutrient absorption capabilities (5). Notably, soil with low pH levels contains elevated aluminium

concentrations (6). The primary signs of aluminium injury manifest as a uniform reduction in root and shoot elongation (7). These toxic conditions result in physiological and biochemical alterations within plants, leading to root damage and reduced root systems, thereby impeding water and nutrient uptake (8). The extent of aluminium concentration in roots hinges on plant sensitivity differentials (9; 10), with the root apex being particularly sensitive to aluminium toxicity. This toxicity incites reactions like disruptions in cellular redox balance and triggers oxidative stress (11).

Additionally, aluminium interferes with the uptake of macronutrients. Toxicity symptoms in shoots primarily include stunted growth, chlorotic leaf patches, marginal necrosis, cellular leaf modifications, reduced stomatal opening, diminished photosynthesis, and delayed plant maturity (12). Aluminium affects water balance, impairs the photosynthetic apparatus, diminishes chlorophyll and carotenoid content, restricts stomatal opening, and interferes with enzymatic activity by displacing other metal ions (13). Prolonged aluminium stress contributes to chloroplast deformities and oxidative stress (14), causing lowered chlorophyll content and disruption of photosystem II, which subsequently inhibits the electron transport rate of photosynthesis due to aluminium ions (Al^{3+}) in specific plant species (15). Varieties and species display varying degrees of tolerance to aluminium toxicity, with genetic variation for Al tolerance observed in crop plants (16). Al-tolerant species or genotypes are frequently endemic to regions with acidic soils. Cultivars harbouring tolerant genes adapted to acidic soils offer environmentally sustainable solutions for agriculture (17). The soils in western Kenya suffer from high acidity, with pH ranging from 6.5 to less than 4.5, indicating slight to extreme acidity levels (18; 19). Crops cultivated in these soils face potential aluminium toxicity. *Amaranthus hybridus* is a commonly grown vegetable in this region. It is being promoted as a crop in western Kenya; however, its response to aluminium toxicity remains uncertain. This study evaluates the germination, growth, and photosynthetic pigment responses of *Amaranthus hybridus* to aluminium stress.

Materials and Methods

Germination experiment

Amaranthus hybridus seeds were procured from an agrovet shop in Kisumu town. The germination experiment was conducted in the Department of Botany laboratory at Maseno University. Ten uniform *Amaranthus hybridus* seeds were treated using a sodium hypochlorite solution. Subsequently, these seeds were placed on dried petri dishes, each lined with Whatman No. 1 filter papers. These filter papers were then moistened with 5ml of a nutrient solution containing either 0 mM (control - distilled water) or varying concentrations (2, 4, 6, 8, and 10 mM) of aluminium toxicity ($AlCl_3 \cdot 6H_2O$). Each treatment was replicated three times and arranged in a completely randomized design on a bench. Data regarding the number of germinating seeds each day were meticulously recorded. The germination percentage was subsequently calculated using the following formula:

$$\text{Germination percentage} = \frac{\text{Total seeds germinated}}{\text{Total seeds sown}} x\ 100$$

Growth experiment

Growth experiments were conducted in a greenhouse, maintaining a natural photoperiod with photosynthetic active radiation of 400 µmol m^{-2} s^{-1} and an average temperature of 29°C. Five-litre pots were filled with soil extracted from the Maseno University botanical garden. In order to prevent waterlogging, a total of five holes were perforated at the bottom of each pot. Each of the pots was sown with five seeds of *Amaranthus hybridus.* Over two weeks, watering was carried out each morning using 250 ml of tap water per pot. After this initial period, the seedlings were thinned to retain two plants per pot. Subsequent irrigation involved the daily application of a nutrient solution, wherein concentrations ranged from 0 mM (control - tap water) to varying concentrations (2, 4, 6, 8, and 10 mM) of aluminium toxicity ($AlCl_3 \cdot 6H_2O$) until the nutrient solution started to seep out from the holes at the pot's base (~250 ml), at which point irrigation was stopped. The pH of the nutrient solution was adjusted to the range of 4.1-4.2 using HCl or NaOH. The experimental layout followed a completely randomized design.

Measurement of parameters

Shoot height

Shoot height was measured from the soil level to the highest point of the terminal bud of the seedling using a meter rule.

Number of leaves

The count of fully expanded mature leaves per plant was conducted and recorded every week until the experiment's conclusion.

Leaf area

Leaf length was measured from the tip to the base, and the width was measured at the widest point of the leaf lamina. These measurements were utilized to calculate the leaf area, following the methods outlined by Otusunya *et al.* (20) and Musyimi (21), as indicated below:

$$LA = 0.5\ (LxW)$$

Where:
LA is the leaf area
L is the maximum length of the leaf w
W is the maximum width of the leaf.

Root and shoot fresh weights

After the experiment, the plants were delicately uprooted, with the soil around the roots and some shoots gently removed. The plants were subsequently divided into their shoot and root components, followed by a thorough rinse with deionized water and gentle blotting using tissue paper. The fresh weight of each plant part was then individually measured using an electronic weighing balance (Denver Instrument Model XL – 3100D).

Root and shoot dry weights

Fresh plant parts, including the roots and shoots, were segregated and placed in separate envelopes for drying. The drying process was conducted in an oven at 60°C until a constant dry weight was achieved. Subsequently, the dry weights of the roots and shoots were determined using an electronic weighing balance (Denver Instrument Model XL – 3100D).

Photosynthetic pigments contents

Chlorophyll a, b, total chlorophyll, and carotenoid contents were determined following the method outlined by Musyimi *et al.* (22). For this, one gram of *Amaranthus hybridus* leaves was ground in 20 ml of 80% acetone using a mortar and pestle. The resulting mixture was analyzed using a UV-visible spectrophotometer at 664 nm, 647 nm, and 470 nm. Subsequently, the content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids was calculated using the formula provided below:

Chlorophyll a
= 13.19 A664 - 2.57 A647 (mg g^{-1} fresh weight).

Chlorophyll b
= 22.1 A647 - 5.26 A664 (mg g^{-1} fresh weight).

Total chlorophyll
= 7.93 A664 + 19.53 A647 (mg g^{-1} fresh weight).

Carotenoid
= ([1000×A470]–[2.270×Cha]–[81.4×Ch/b]) ÷ 227.

Where; Ch/a = chlorophyll a
Ch/b = chlorophyll b

A664, A647, and A470 absorb at 664, 647 and 470 respectively.

Data Analysis

The data underwent analysis of variance (ANOVA) using the SAS statistical package. Treatment means were distinguished and compared using the Least Significant Difference (LSD) test at a significance level of $P < 0.05$.

Results

Seed germination percentage

The germination percentage of seeds decreased with increasing aluminium concentration compared to the control (Table 1). Significant variations in seed germination percentage were observed among the treatments, except for the 6- and 8-mM aluminium treatment levels.

Table 1. Effect of aluminium toxicity on the seed germination of *Amaranthus hybridus.*

Aluminium Treatment (mM)	Germination (%)
0 (control)	82.00±0.03
2	40.00±0.24
4	17.33±0.27
6	13.33±0.18
8	11.33±0.16
10	6.00 ±0.32
LSD	2.67

Growth Parameters

Shoot height

The shoot height notably decreased as the aluminium concentration increased (Table 2). However, there were no significant differences in

shoot height among the 6-, 8-, and 10-mM aluminium treatment levels.

Number of leaves

The number of leaves decreased as the aluminium concentration increased (Table 2). However, no significant difference was observed in the number of leaves among the treatments of 2, 4, 6, 8, and 10 mM.

Leaf area

The leaf area of *Amaranthus hybridus* decreased with increasing aluminium concentration (Table 2). However, no significant difference in leaf area was observed among the aluminium treatments of 2, 4, 6, 8, and 10 mM.

Table 2: Effect of aluminium toxicity on the number of leaves, leaf area, and shoot height of *Amaranthus hybridus.*

Aluminium treatment (mM)	Shoot height (cm)	Number of leaves	Leaf area ($cm^{2)}$
0 (control)	6.37 ±0.26	8.00±0.09	3.37±0.16
2	3.03±0.44	4.66±0.43	0.70±0.11
4	2.77±0.21	4.33±0.44	0.57±0.13
6	1.87±0.19	4.33±0.44	0.53±0.12
8	1.50±0.18	3.67±0.13	0.37±0.05
10	1.33±0.22	3.33±0.12	0.30±0.10
LSD	0.89	1.033	0.91

Root and shoot weights

The fresh and dry weights of roots and shoots significantly decreased with increasing aluminium concentration (Table 3).

Photosynthetic pigment contents

The content of chlorophyll a, b, total chlorophyll, and carotenoids significantly decreased with increasing aluminium concentration (Table 4).

Table 3: Effect of aluminium toxicity on fresh and dry weights of roots and shoots of *Amaranthus hybridus.*

Aluminium treatment (mM)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
0(control)	0.90±0.12	0.40±0.44	3.20±0.36	1.70±0.33
2	0.60±0.26	0.20±0.23	2.40±0.35	1.20±0.34
4	0.40±0.24	0.10±0.22	2.00±0.26	1.00±0.29
6	0.30±0.27	0.10±0.23	1.80±0.23	0.90±0.23
8	0.20±0.22	0.00±0.00	1.30±0.21	0.60±0.23
10	0.20±0.22	0.00±0.00	1.00±0.16	0.40±0.12
LSD	0.11	0.06	0.32	0.19

Table 4: Effect of aluminium toxicity on photosynthetic pigments (Chlorophyll a, Chlorophyll b, Total chlorophyll, and Carotenoids) content in *Amaranthus hybridus.*

Aluminium treatment (mM)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoids content (mg/g)
0 (control)	12.64±0.12	5.91±0.33	18.57±0.18	2.20±0.44
2	4.82±0.18	2.92±0.29	8.01±0.13	1.07±0.32
4	4.39±0.26	2.25±0.24	6.64±0.31	0.96±0.37
6	4.21±0.26	1.70±0.12	5.90±0.26	0.93±0.15
8	3.91±0.22	0.97±0.11	4.89±0.12	0.87±0.14
10	3.55±0.27	0.90±1.10	4.45±0.13	0.84±0.19
LSD	1.98	0.24	2.16	0.21

Discussion

Aluminum (Al) toxicity is a complex disorder affecting plant growth and development, often resembling a deficiency in essential nutrients (23). Different plants exhibit varying degrees of tolerance to aluminium stress, with genetic and physiological tolerance mechanisms differing across species. This study shows that aluminium treatments significantly diminished germination, growth, and photosynthetic pigment parameters in *Amaranthus hybridus*. The germination percentage of seeds decreased with increasing aluminium concentration. It is known that aluminium can interfere with germination percentage and average germination time in certain plant species (24; 25). Many plant species' seeds demonstrate reduced germination rates when exposed to various abiotic stresses (26).

The growth outcomes indicated that the plants' shoot height, leaf count, leaf area, fresh weight, and dry weight declined with increasing aluminium chloride concentration. These findings align with the results reported by Sevugaperumal (27). The primary mechanism behind the growth reduction in *Amaranthus hybridus* could be cell elongation or cell division inhibition. It is plausible that aluminium-induced water stress in the plants hinders overall plant growth. Studies on root growth parameters, such as cell division and elongation, have shown decreased mitotic activity in root tips of numerous crop species when exposed to toxic levels of aluminium (28). Aluminium toxicity is known to impede root growth.

Moreover, the content of chlorophylls and carotenoids also exhibited a decrease. Similar results were observed in the leaves of *C. grandis* and *C. sinensis* (29), along with studies by Neogy *et al.* (30) and Cheng *et al.* (31) in citrus leaves. The reduction in chlorophyll a and b with increasing aluminium concentration in the leaves could be attributed to inhibition of aminolaevulinic acid dehydratase activity (32). These findings correlate with prior studies that reported biomass reduction, photosynthetic performance alterations, and chlorophyll content changes (33, 34, 35). Diminished chlorophyll concentrations could indirectly contribute to reduced photosynthesis, leading to growth decline. Furthermore, decreased carotenoid content with increasing aluminium chloride concentration might negatively influence plant photosynthesis. Carotenoids play a vital role in safeguarding the photosynthetic apparatus against the detrimental effects of light and oxygen (36; 37).

The symptoms of toxicity manifested by the plants are contingent upon factors like aluminium ion quantity, organic matter amount and form, and the plant's genotype (38). In this study, aluminium substantially reduced shoot height, leaf count, leaf area, and shoot and root biomass. This decline in growth might directly relate to reduced photosynthetic activity. Similar outcomes were obtained by Chang *et al.* (39) in tobacco plants treated with a combination of Al and Fe. Aluminium could hinder nutrient uptake and transport, reducing plant growth (40).

This study's outcomes highlight *Amaranthus hybridus'* high susceptibility to aluminium toxicity. Notably, the highest aluminium concentrations (8 and 10 mM) exhibited reduced seed germination, growth, and leaf photosynthetic pigments. Further investigations are necessary to elucidate the molecular and physiological mechanisms underlying this species' response to aluminium.

Conclusions

The germination, growth, and photosynthetic pigments of *Amaranthus hybridus* were notably diminished due to aluminium-induced stress. These reductions exhibited an upward trend with increasing levels of aluminium stress. The outcomes of this study distinctly indicate the high sensitivity of the *Amaranthus hybridus* species to aluminium toxicity. Further investigations should delve into evaluating the physiological responses of this species to aluminium toxicity.

Conflict of interest

None declared.

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