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Mitigating Effects of Beta-Carotene on Aluminium Toxicity Induced Stress in *Amaranthus hybridus* L.

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This study explores the role of supplementary β-carotene in mitigating plants of *A. hybridus* exposed to Al toxicity via pre- and post- β-carotene applications. The experiment was conducted by using a 3× 10^{-2} mM aluminium chloride ($AlCl_3$) at pH 4.6 was to induce stress on the plants. The effects of 50 and 200 µM concentrations of β-Carotene on the growth of *Amaranthus hybridus* subjected to Al stress, were evaluated in a hydroponic system. All data collected from growth studies were analysed with one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) was used to separate means at P≤0.05 level of significance. The first noticeable symptoms were prominent inhibition of root growth and results showed that post- β-Carotene treatments (T5 and T6) significantly ameliorated plants from Al stress when compared to pre-β-Carotene treatments (T3 and T4). Higher doses of post-β-Carotene treatment (T6) significantly increased leaf number, plant height, length and number of inflorescence, fresh and dry weights of shoot, root and inflorescence but significantly decreased root length. However, all the treatments (T1, 72, T3, T4, T5, T6 and T7) showed a significant reduction in parameters such as plant height, fresh and dry weights of inflorescence, shoot and root length when compared to the control (T8) except for fresh and dry weights of the root that did not follow the same trend. The present study suggests that plants of *A. hybridus* were susceptible to Al toxicity-induced stress and post-β-Carotene supplementation could significantly ameliorate the stress situation and enhance growth and productivity.

Keywords: Aluminium toxicity, *Amaranthus hybridus*, β-Carotene, Growth and productivity, Hydroponics, Stress amelioration.

INTRODUCTION

Aluminium (Al) toxicity is an important growth-limiting factor for plants in acid soils below pH 5.0 and is responsible for shortages in food production (Blue and Dantzman, 1977; Alak and Adams 1979; De Carvalho et al. 1980; Foy et al. 1992; Seguel et al. 2013; Nunes-Nesi et al. 2014; Soto-Cerda et al. 2015; Rahman and Upadhyaya 2021) and major stress agents that reduce crop productivity and increase food insecurity in the tropics and Sub-Saharan Africa (Kochian 1995; Hede et al. 2001; Rengel et al. 2015). Al is the third most abundant element making up over 8% of the earth's crust (Inostroza-Blancheteau et al. 2012; Bhalerao and Prabhu 2013; Rengel et al. 2015; Schmitt et al. 2016; Jaskowiak et al. 2019).

The general population is exposed to Al from its widespread use in water treatment, food additive, various Al-based pharmaceuticals, toothpaste, antiperspirants, pollutants from electrical power stations, industrial activities and automobile exhaust as well as from Al containers/packaging materials and cooking utensils (Harris et al. 1996; Ma et al. 2001; Pournourmohammadi et al. 2008). Al is solubilized into toxic forms such as hexa aqua aluminium ($[Al(H2O)6]^{3+}$); mononuclear species $[Al(OH)^{2+}]$ or $[Al(OH)^{2+}]$; gibbsite $[Al(OH)_3]$; aluminate $[Al(OH)^{4-}]$; $AlCl_3$ and are generally referred to as Al^{3+} (Kinraide 1990; Abreu et al. 2003; Silva 2012; Schmitt et al. 2016). Soil pH, chemical structure and composition of soluble Al compounds in soil solution as well as the solution's ionic strength have played key roles in Al toxicity (Siecinska and Nosalewicz 2017). Al toxicity affects plants directly and is transferred to humans and animals that consume these plants via the food chain (Fatur et al. 2002; Savvas et al. 2010; Rahman and Upadhyaya 2021).

Some of the most noticeable phytotoxic symptoms of Al toxicity include inhibition of root growth (Silva 2012; Kopittke et al. 2015; Rosmaninho et al. 2019), leaf-chlorosis, and stunted plant growth (Gupta et al. 2013; Vasconcelos et al. 2020). The roots exhibit greater signs of cellular damage than the other parts of the plant (Wagatsuma et al. 1987; Rincón and Gonzales 1992; Udengwu and Egedigwe 2014; Kochian et al. 2015).Under Al stress in a nutrient solution, Al-sensitive genotypes were characterized by chlorosis (Udengwu and Egedigwe 2014), decreased Fe concentration in tops, decreased/inhibited Ca and Mg uptake in both shoots and roots (Horst et al. 2010; Bose et al. 2011), a tendency towards the accumulation of P, Al, and Fe in roots and reduced Mn in tops (Foy and Fleming 1982; Gupta et al. 2013). Studies have shown that Al adversely affected several physiological activities, producing severe physiological stress which increased peroxidase activity (Peters et al. 1989; Kochian et al. 2015; Rengel et al. 2015; Rouphael et al. 2016). Al toxicity induces oxidative stress that elicits the production of reactive oxygen species (ROS) (Darkó et al. 2004; Jones et al. 2006; He et al. 2014; Singh et al. 2017) that may damage cellular components if surrounding antioxidant enzymes are suppressed (Darkó et al. 2004; Sharma and Dubey 2007; Bera et al. 2019; Shahnawaz and Sanadhya 2019; Devi et al. 2020). Cytological studies show that Al toxicity results in cell cycle disturbances and a decrease in Mitotic Index (MI) value (Wierzbicka 1988, 1989; Samardakiewicz and Woźny 2005; Nezames et al. 2012) as well as induces C-mitosis, micronuclei, chromosome stickiness, budding nuclei, laggards and chromosome bridges (Aslanturk and Celik, 2005; Zhang et al. 2014). Given the menace of Al toxicity, especially in the sub-Saharan region, current research is now geared towards proffering long-term solutions to mitigate the devastating effects of Al toxicity on crop yield and productivity.

It is pertinent to point out that studies have attributed low pH to be the major cause of stress and the ignition to the production of ROS (Samac and Tesfaye 2003; Inostroza-Blancheteau et al. 2012; Udengwu and Egedigwe 2014; Yang et al. 2015; Rahman and Upadhyaya 2021). Previous studies concerning Al toxicity have focused only on Al being the major cause of stress limiting crop productivity but in reality, low pH sets the stage for Al to become toxic. Al toxicity not only depends on its total concentration but also its chemical forms that wholly depend on low pH (Kochian 1995). Hence, there is a significant correlation between high concentrations of Al3+ in the soil and low pH (Rout et al. 2001; Bojórquez-Quintal et al. 2017; Palani et al. 2018).

There is growing evidence from *in vitro* and *in vivo* laboratory animal studies that β-Carotene can protect phagocytic cells from auto-oxidative damage, enhance T and B lymphocyte proliferating responses, stimulate effector T-cell functions, and enhance macrophage, cytotoxic T- cell and natural killer cell tumoricidal capacities, as well as increase the production of certain interleukins (Bendich 1989; Mueller and Boehm 2011; Esrefoglu et al. 2016). Many of these effects have also been seen with carotenoids lacking provitamin A activity but having the antioxidant and singlet oxygen quenching capacities of β-Carotene, however, reports have it that β-Carotene could be converted into vitamin A, which is essential for normal growth and development (Lemmens et al. 2010; Giuliano et al. 2017)). Since vitamin A is a relatively poor antioxidant and cannot quench singlet oxygen, β-Carotene may have more importance as a nutrient than simply serving as a precursor of vitamin A (Bendich 1989). Carotenoids, including β-Carotene, are very efficient at quenching singlet oxygen (Di Mascio et al. 1989; Baltschun et al. 1997).

Stahl and Sies (2003) have shown that β-Carotene undergo different configurations (either cis or trans isomers) because of their double bonds. Growing observations have also showed that most carotenoids isomerize to their cis-configuration during thermal procession and extraction (Lemmens et al. 2010), storage conditions due to light acids and oxygen (Rao and Rao 2007), thus leading to loss of colour and reduction in biological activities. However, the all-trans isomer may exist predominantly in nature.

Though evidence for carotenoids roles' in animals and humans are ubiquitous, studies about the supplementation of plants with β-Carotene are scarce. However, lycopene had ameliorating effects on chromosome aberrations in *Allium cepa* (Aslanturk and Celik 2005); while a higher concentration of lycopene alleviated and bolstered plants of *A. hybridus* from the devastating effects of Al-induced stress (Udengwu and Egedigwe 2014). It is well known that animals cannot synthesize carotenoids because they lack chromoplasts and thus depend on plants for the nutritious and protective values of carotenoids. However, Moran and Jarvik (2010) showed that some aphids manufacture toluene *de novo*.

A. hybridus, being a staple vegetable eaten in the tropics, is sensitive to low concentrations of Al and such low concentrations subjected the same plant to stress and reduced growth and productivity (Udengwu and Egedigwe, 2014). In another study, Osaki et al. (1997) classified *A. hybridus* and some other tropical plants under the Al-sensitive group. Despite the intrinsic antioxidants inherent in plants, some plants, including staple crops, may not be capable of synthesizing high levels of antioxidants during stress situations and thus cause devastating damages to plant growth and yield. Excessive reduction in crop yield and productivity have consequently led to food insecurity in sub-Saharan regions. The current study, therefore, explores the role of supplementary β-carotene in mitigating plants of *A. hybridus* exposed to Al toxicity via pre- and post- β-carotene applications.

MATERIALS AND METHODS

Soil Analysis

Topsoil for raising nursery plants was collected from the Botanic Garden, University of Nigeria Nsukka. The soil sample was air-dried and analysed in the Soil Science Analytical Laboratory in the Department of Soil Science, University of Nigeria Nsukka. The soil was analysed using the standard method of the Association of Official Analytical Chemists (2005).

β-Carotene extraction and purification

Extraction and purification of β-Carotene werecarried out following the methods of Udengwu and Egedigwe (2014). Fresh and matured carrots weighing 15kg were purchased from Nsukka local market. β-Carotene crystals were got following the methods of Yaping et al. (2002). Extracted β-Carotene was protected from light and stored at 2^{o}C to avoid transformation to inactive isomers.

Determination of antioxidant activity

Adapting the methods of Udengwu and Egedigwe (2014), thiobarbituric acid (TBA) value was used to determine the antioxidant activity of β-Carotene. All readings were taken thrice and antioxidant activities were calculated as follows using the method of Hanachi and Sh (2009).

$$\% \text{ Antioxidant Activity} = \frac{ABcontrol - ABsample}{ABcontrol} \times 100$$

Where AB_{sample} = Absorbance of sample and $AB_{control}$ = Absorbance of control

High-performance liquid chromatography analysis (HPLC)

The percentage (%) purity of the extracted β-Carotene was determined using the methods of Udengwu and Egedigwe (2014). HPLC was done in the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka, while UV spectrometry was determined using a UV-visible spectrophotometer. Readings of β-Carotene standard in ethanol were taken to confirm the peak absorbance of extracted β-Carotene.

Stock preparations

Fresh 1M stock solutions of β-Carotene, $AlCl_3$, and full Hoagland's nutrient were prepared daily using the methods of Udengwu and Egedigwe (2014). They were stored at 4^{o}C in the refrigerator before use.

β-Carotenestock solution:

One gram of β-Carotene was mixed in 10 mL of ethanol before the addition of 990mL of distilled water. A 1% alcohol dilution of β-Carotene was used in this study following the protocol of Fiskesjo (1981); who showed that 1% of alcohol dilutions of lipophilic solutes were not toxic to *Allium*roots.

$AlCl_3$ stock solution (1 Molar):

This was prepared by dissolving 133.5g of AlCl3 in little distilled water and the final volume made up to 1000mL.The pH of the solution was buffered to 4.6.

Al treatment concentration:

Al treatment concentration of 3 × 10^{-2}mM was achieved through serial dilution and pH 4.6 was through adjustments with H_2SO_4.

Hoagland's nutrient solution:

This was prepared using the formulation of Hoagland and Arnon (1950 revised).

Determination of actual Al concentration in solution

Actual Al in solution was determined using Shull (1960) modified Aluminon method for aluminium determination.

Growing Amaranthus hybridus

Viable *Amaranthus* seeds were obtained from the *Amaranthus* germplasm maintained in the Botanic Garden, University of Nigeria, Nsukka. Seeds maintained in the germplasm were acquired from National Horticultural Research Institute (NIHORT), Ibadan Nigeria. The exact methods of Udengwu and Egedigwe (2014) were used to grow *Amaranthus* plants. Plants were arranged using a randomized complete block design (RCBD) in the screen house of the Botanic garden. Five plants per treatment, replicated three times, were used to monitor the growth of plants. Plants were allowed to stabilize in full strength Hogland's nutrient solution for 10 d before the 8 treatments were applied (Table 1).

All plants received treatments for 21 days. Using a non-continuous flow system, all treatments were renewed daily to ensure adequate nutrient supply and uniformity of treatment. When treatment application elapsed, the experiment was terminated. Fresh and dry weights of shoots, roots and inflorescences, as well as other growth parameters such as the numbers of leaves and inflorescences, plant height, root length, length of inflorescence, were evaluated and recorded.

Statistical analysis

Data collected from growth studies were analysed with one-way analysis of variance (ANOVA). The Least Significant Difference (LSD) was used to separate means at P≤0.05 level of significance. SPSS v23, Microsoft Excel 2016 and Gen-Stat packages were used for computation, data analysis and graphics.

Table 1: Details of the 8 Treatments given to experimental plants

S/N	Type of Treatment	Abbreviation	Symbol	Details
1.	β-carotene (50μM)	bc_1	T1	*Amaranthus* plants (AP) were grown in Hoagland's nutrient solution (HNS) and 50μM bc_1 for 21d
2.	β-carotene (200μM)	bc_2	T2	AP was grown in HNS and 200μM bc_2 for 21d
3.	Pre-β-carotene (50μM)	(bc_1-Al)	T3	AP was grown in HNS and 50μM $bc_1$for 72 h before transfer into HNS and 3 x 10^{-2} mM Al for 18 d.
4.	Pre-β-carotene (200μM)	(bc_2-Al)	T4	AP was grown in HNS and 200μM bc_2 for 72 h before transfer into HNS and 3 x 10^{-2} mM Al for 18 d.
5.	Post-β-carotene (50μM)	(Al-bc_1)	T5	AP was grown in HNS and 3 x 10^{-2}mM Al for 18 d before transfer into 50μM bc_1 for 72 h.
6.	Post-β-carotene (200μM)	(Al-bc_2)	T6	AP was grown in HNS and 3 x 10^{-2}mM Al for 18 d before transfer into 200μM bc_2 for 72 h.
7.	Aluminium (3 x 10^{-2} mM)	Al	T7	AP grown into HNS and 3 x 10^{-2} mM for 21 d
8.	Control	Ctrl	C	AP grown into HNS for 21 d

RESULTS AND DISCUSSION

Soil analysis

The result of soil analysis was the same as the results of Udengwu and Egedigwe (2014). Results showed no presence of Al (Table 2). The results of soil analysis used in this study are the same as the results of Udengwu and Egedigwe (2014). It follows that Al was completely absent in the soil used and that aluminium chloride ($AlCl_3$) is the only source of Al used in this study.

Table 2: Physical and chemical composition of the topsoil in the Botanic Garden, University of Nigeria, Nsukka, used for seed germination and seedling production (Udengwu and Egedigwe 2014)

Parameters	Values
pH (H_2O)	6.2
pH (KCl)	5.2
Fine soil (%)	29.0
Silt (%)	5.0
Clay (%)	27.0
Coarse soil (%)	39.0
Organic matter(%)	5.8
Organic Carbon (%)	3.4
Total Nitrogen(%)	0.2
Available P (ppm)	37.4
Exchangeable cations (mg/100g)	31.2
Calcium (mg/100g)	8.8
Magnesium (mg/100g)	15.2
Sodium (mg/100g)	0.5
Potassium (mg/100g)	0.1
Hydrogen ion (mg/100g)	3.4

Vegetative and Reproductive

Before treatment applications, *Amaranthus* plants stabilized and were acclimatized in full strength Hoagland's nutrient solution (Plate 1).

Plate 1: Growth of Amaranthus hybridus plants in hydroponics

The first noticeable symptoms observed three days after Al treatment applications, which was the same as the results of Udengwu and Egedigwe (2014), were prominent inhibition of root growth (Plate 2),

Plate 2: Roots of Al toxicity stressed normal plant enlarged. DMLR=dense mesh of lateral roots

Yellowing and wilting of leaves and overall stunting plant growth (Plate3)

Plate 3: Aluminium toxicity stressed plant WL=Wilting of leaves; DR=Damaged roots

as compared to the control plants (Plate 4).

Plate 4; Control Amaranthus plant NL=Normal leaves; NR=normal roots

Plants were grown in Hoagland's nutrient solution to eliminate uncertainties of roots absorbing unknown amounts of different nutrient elements and other toxic metals if grown in soil. There is also an added advantage of the ease in controlling the pH. Using transparent bottles allowed for full monitoring of roots during growth and unwanted cutting of root tips if grown in soil. Despite these advantages, it is important to mention that the growth of *Amaranthus* plants in nutrient solution will not be as robust as when grown in soil because *A. hybridus* is not an aquatic plant.

The results of this study showed that increasing concentrations of post-β-Carotene treatments, Al-bc_2 (T6), significantly increased plant height compared to increasing concentrations of pre-β-carotene treatments (T3 and T4). However, all the treatments showed a significant reduction in plant height when compared with control plants (Table 3).

At the end of 21 days, Al-bc_2 (T6) also significantly yielded higher number of leaves in comparison with other treatments except control (Ctrl). Only T6, a post-β-carotene treatment, significantly yielded more leaves than that observed in pre-β-Carotene treatments, bc_1-Al (T3) and bc_2-Al (T4). More so, Al treatment (Al) (T7) significantly reduced the number of leaves by 44.1% in comparison with the control (Table 3).

In similar manner, the length of inflorescences across all treatments differed significantly from T6 except the control. T6 yielded the highest number of inflorescence and differed significantly from other treatments while T7 significantly reduced the number of inflorescences. Treatment 2 and 3 significantly reduced the number of inflorescences by 88.6% and 95.2% respectively as compared to the control. Post-β-Carotene treatments (T5 and T6) yielded a higher number of inflorescences when compared to pre-β-Carotene treatments (T3 and T4) (Table 3).

Table 3: Effects of treatments on the plant height, number of leaves, length of inflorescence and umber of inflorescence

Treatments	Plant Height	Number of leaves	Length of inflorescence	Number of inflorescences
bc_1-Al	20.87 ± 1.51^{d}	11.33 ± 1.12^{cd}	1.57 ± 0.58^{d}	0.67 ± 0.23^{e}
bc_2-Al	21.74 ± 2.11^{d}	11.60 ± 1.61^{cd}	2.49 ± 0.61^{cd}	3.53 ± 1.40^{de}
Al-bc_1	25.83 ± 1.44^{cd}	16.53 ± 2.21^{bc}	3.45 ± 0.97^{bcd}	5.60 ± 1.41^{cd}
Al-bc_2	37.53 ± 3.85^{b}	26.00 ± 3.71^{a}	8.37 ± 0.90^{a}	15.13 ± 2.44^{a}
bc_1	24.33 ± 3.38^{cd}	14.07 ± 2.45^{bcd}	2.79 ± 1.11^{cd}	4.87 ± 2.00^{cde}
bc_2	22.23 ± 3.14^{cd}	9.40 ± 1.29^{d}	1.73 ± 0.54^{d}	1.60 ± 0.77^{de}
Al	28.60 ± 1.61^{c}	17.27 ± 1.86^{b}	5.54 ± 0.88^{b}	9.40 ± 1.25^{bc}
Ctrl	45.07 ± 1.55^{a}	30.87 ± 2.48^{a}	8.79 ± 1.61^{a}	14.13 ± 2.64^{ab}
LSD	**6.998**	**6.256**	**2.688**	**4.756**

*significant means are presented with different superscripts alphabet along each vertical arrays

In comparison with the study of Udengwu and Egedigwe (2014), the findings of this study still showed that Al, at a low concentration of 3 × 10^{-2} mM and low pH of 4.6, significantly reduced both vegetative and reproductive growths of *A. hybridus* (Inineoma). This conforms to the findings of other studies that Al toxicity may not affect plants except under low pH conditions (Moore 1974; Dickson 1978). In essence, Udengwu and Egedigwe (2014) grouped *A. hybridus* as an Al-sensitive plant in the tropics. Tamás et al. (2006) reported that *Hordeum vulgare* (barley) roots represented only 20% of the control when plants were subjected to 10mM Al for 12 h.

Responses of plants to all treatments significantly reduced the fresh weight of inflorescence **(**FWI) in comparison with control (Fig 1).

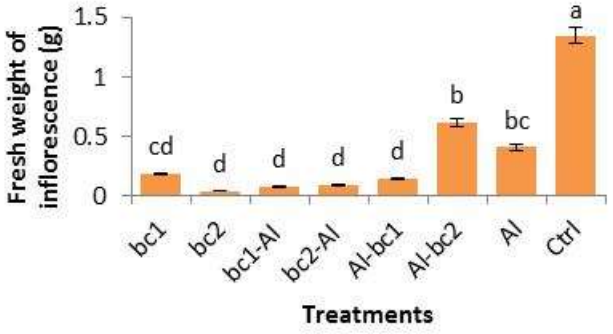

Figure 1: Effects of treatments on fresh weight of inflorescence (FWI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

There were no significant differences in FWI between pre-β-Carotene and post-β-Carotene treatments, except T6. Having a similar trend with the results got in LOI and NOI, T2 and T3 significantly reduced FWI by 86.5% and 97% respectively (Fig 2). The responses of plants to T6 and T7 did not differ significantly from each other and the control but differed significantly from other treatments (Fig 2).

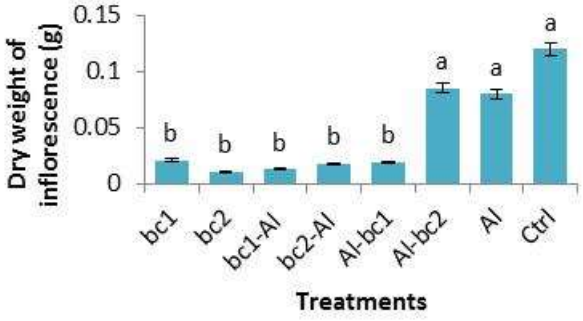

Figure 2: Effects of treatments on the dry weight of inflorescence (DWI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

The fresh weight of shoot decreased with increasing pre-β-carotene concentrations. however, increasing post-β-Carotene concentrations were directly proportional to a significant gain in fresh weight of shoot. All treatments significantly reduced FWS as compared to the control except T6 (Fig. 3).

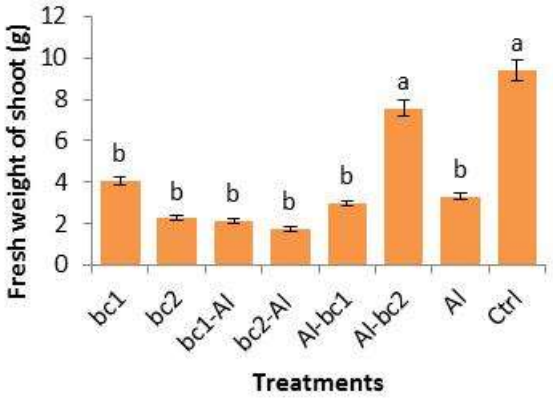

Figure 3: Effects of treatments on fresh weight of shoot (FWI). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

Dry weight of shoot (DWS) as presented in Fig. 4 shows that decreasing pre-β-carotene concentrations increased DWS while increasing post-β-carotene concentrations significantly increased DWS. Only T6 differed significantly from other treatments except for the control. Though not significant, responses of plants to T1 and T2 yielded higher DWS compared to T3, T4, T5 and T7.

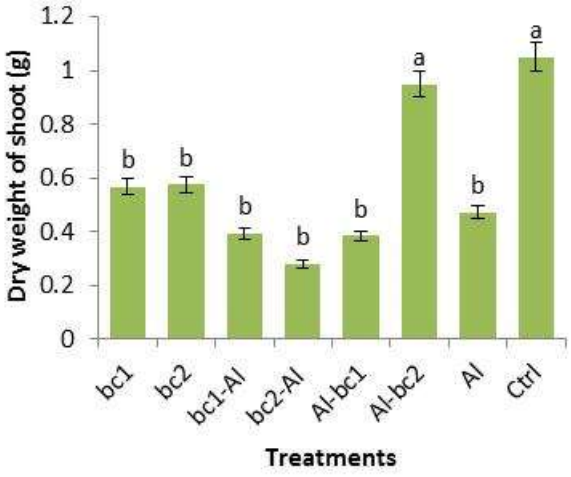

Figure 4: Effects of treatments on the dry weight of shoot (DWS). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

The root length of control plants differed significantly from the rest of the treatments. The response of plants to

bc_1 (T1) had significantly longer roots which differs significantly from plants responses to T2, T4, T5 T6, T7(Fig. 5).

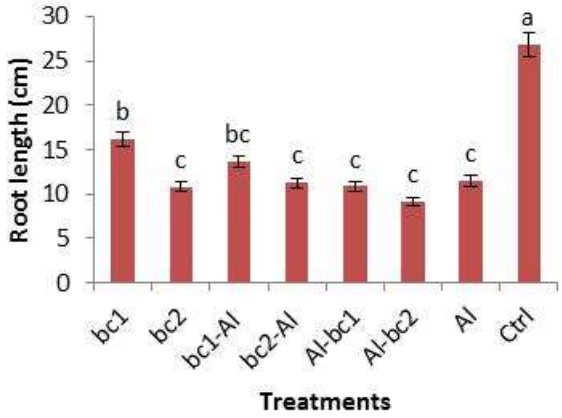

Figure 5: Effects of treatments on root length (ROL). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

Decreasing β-carotene concentrations across treatments were directly proportional to root length. Though there were no significant differences. Increasing concentrations of pre-β-carotene treatments did not significantly increase fresh weight of root (FWR) except in post-β-carotene gain in concentration. There was no significant reduction by T7 in comparison with control. However, the responses of plants to T1and T6increased FWR by 20% and 68.2% respectively (Fig. 6).

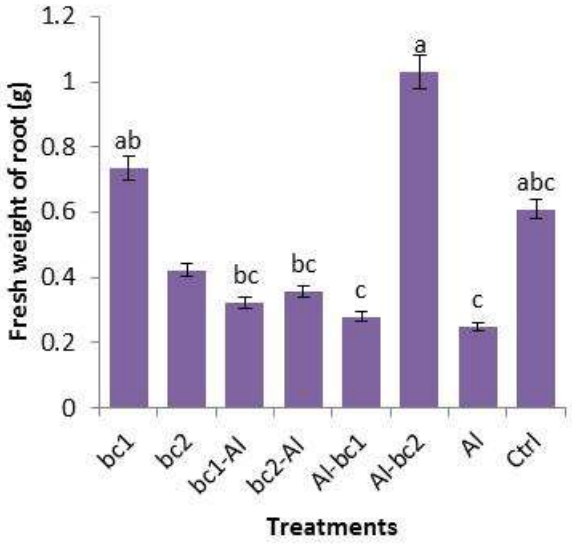

Figure 6: Effects of treatments on fresh weight of root (FWR). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

The responses of plants to T3 and T5 recorded the highest and least dry weight of root (DWR) respectively compared to control (Fig.7).

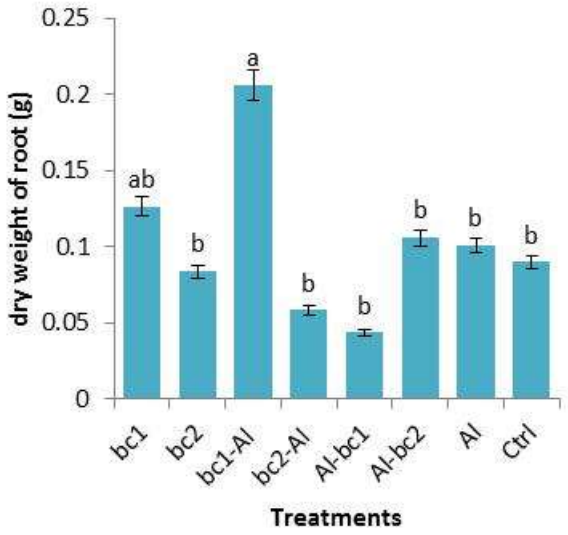

Figure 7: Effects of treatments on the dry weight of root (DWR). *Bars bearing different letters differ significantly (LSD ≤ 0.05)

T7, T6, T1 and T3 treatments increased DWR by 13.5%, 18.9%, 41.8% and 131.3% respectively. Increasing β-Carotene concentrations increased DWR except in post-β-carotene increment.

The response of T7 in comparison with T6 significantly reduced growth for NOL, PLH, LOI, NOI, FWS, FWR, and DWS but was non-significant for DWR and DWI. Udengwu and Egedigwe (2014) attributed the decrease in growth to prior treatment of *A. hybridus* plants to Al for 21 d at 4.6 pH and higher H^+ concentration of 2.5 × 10^{-5} mol L^{-1} before transfer to lycopene solutions for only 72 h, at a pH of 5.8 and lower H^+ concentration of 1.5 × 10^{-6} mol L^{-1}.Interestingly, Al-stressed plants grown in T5 and T6 recovered significantly for NOL, PLH, LOI, NOI, FWS, FWR and DWS, but non-significantly for FWI, DWR and DWI. β-carotene ameliorated Al-stressed plants in this study than lycopene reported by Udengwu and Egedigwe (2014). It could then be deduced that β-carotene possesses a higher antioxidant power in combating Al-related stress, though both lycopene and β-Carotene contain the same number of conjugated double bonds, as well as β-carotene being a derivative of lycopene. Several studies have attributed β-carotene's anti-oxidative activity to its high number of conjugated double bonds (Woodall et al. 1997; Muller and Boehm, 2011). Miller et al. (1996) reported that the properties underlying the activities of carotenoids towards free radicals and their scavenging effects related particularly to their abilities to donate electrons or hydrogen atoms and their relative propensities to undergo oxidation.

CONCLUSION

The present study has shown that under Al stress, *A. hybridus*, 'Inineoma' plants suffer deteriorating effects if not protected or mitigated with increased concentration of β-carotene. An interesting aspect of this work was that plants' fight against Al stress was bolstered by supplemented β-carotene. Higher doses of post-β-carotene treatment ameliorated Al stress more than other treatments. This is to say that β-carotene is not only a precursor of vitamin A but may possess strong anti-oxidative properties against Al toxicity in *A. hybridus*. Independent β-carotene treatments without Al stress that did not significantly affect growth suggested that plants may not require extra antioxidant supplementation unless subjected to Al stress as obtained in pre-and-post-β-carotene treatments. From the results, it was evident that plants of *A. hybrids* were not tolerant to Al stress thus, an Al-sensitive plant. This, in turn, could decrease biomass productivity. This study deduces that Al may have interfered with nutrient uptake as well as accumulated in the leaves. Since *A. hybridus* is a staple vegetable consumed in the Sub-Saharan region, primary concerns should be to check the transfer of high Al ions to consumers via the food chain. Further studies are needed to explore whether the functional groups on the β-ionone ring of β-carotene are responsible for the extra antioxidant activity of the carotenoid compared to lycopene. More so, the identification and characterization of specific post-β-carotene Al-induced stress genes and their gene products as well as comparing these candidate genes/gene products with other Al-stress related genes/gene products deposited in global databases, will be a promising approach to future research in combating the menace of Al toxicity in Sub-Saharan Africa.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

EUO and UOS designed the experiment, NIE and MCJ prepared the treatments and growth media, JOV sourced for the seeds, OH, ITC, OGO, OVC, NEG, BEA and ALN performed the experiment and collected data, OEO conducted data analysis, EUO, UDC, ICN OVC and OEO wrote and reviewed the manuscript. All authors read and approved the final version.

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