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Beta-Carotene Levels Increase with Leaf Maturity of Amaranthus hybridus (L) Grown in Different Soil-Types of Kwale County, Kenya

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Abstract

Poverty and limited accessibility to expensive but rich food sources has been attributed to the prevalence of vitamin A deficiency (VAD) in developing countries. In Kenya where the prevalence stands at 45%, fears of preventable childhood blindness and mortalities arise notably in Kwale County where eye related diseases is in over 2,000 people. It is known that the remedy is consumption of fruits and vegetables as they contain β-carotene, a pro-vitamin A carotenoid. The effect of plant maturity and soil types on vitamin A levels in *Amaranthus hybridus* L leaves grown in Kwale County are reported. For N, P, K, Mg, S and Ca determined in soils, mean levels (µg/g) ranged 0.01 ± 0.01 (P)-20.35 ± 0.25 (Mg). For maturity period reported as days after sowing (DAS), β-carotene levels (mg 100 g-1 DW) ranged from 1.63 ± 0.14 for *Amaranthus hybridus* L leaves grown in Sandy Soil at 25 DAS to 12.74 ± 0.58 in Clay Soil at 75 DAS. β-carotene levels generally increased (Range of increase 6.05 - 243.5%) significantly with maturity ($p < 0.05$) in the different soil types. Growing *Amaranthus hybridus* L. in all soil types in Kwale County is promoted while plant maturity is key in consumption of the same in alleviating VAD in the County.

Keywords: *VAD; β-Carotene; Soil-Types; Amaranthus hybridus L; Plant Maturity*

Introduction

Malnutrition has profound negative effects on economic development and productivity of a country in terms of huge public health financial costs and loss of human resource [1]. In most developing countries, a greater proportion of financial expenditures and about 30% of their yearly GDP are spent on malnourished children. Unfortunately, there is still a risk of losing up to 10% of the life [2]. As a nationwide health problem in Kenya, malnutrition leads to poor health and child development among low-income earning populations being responsible for more than 55% of childhood deaths with its prevalence standing at 35% stunting, 7% wasting and 16% underweight [3]. It has been attributed to poverty with Kenyan statistics showing 56% of the population living below the national destitution line and 47% do not have secure access to enough food that can sufficiently meet their daily dietary requirements [1]. Unavailability of food has been found to worsen the situation and the little available food is of low nutritional value and quality [4]. Kwale County in Kenya experiences cases of malnutrition and in particular, incidences of eye related diseases have been documented for over 2,000 people [4]. This County is sub-divided into three main administrative sub-counties: Matuga, Kinango and Msambweni. The main economic activities in these sub-counties are livestock rearing and crop farming. They have very low nutritional status especially Kinango Sub-County. The predominant forms of malnutrition in this County include stunting, underweight, and acute malnutrition accounting for 35%, 21%, and 6% respectively [5]. Malnutrition also accounts for 53% of infant deaths in this County that has poverty level at 74.9% [5,6].

The dietary sources of vitamin A are the pre-formed vitamin A and pro-vitamin A carotenoids (majorly beta-carotene) with the latter being abundant in yellow and orange fresh fruits and vegetables, and dark-green leafy vegetables [7]. The pre-formed sources of vitamin A are not affordable to majority of people living in developing countries to meet their dietary reference intake (DRI) of vitamin A (6 - 7.5 mg retinol activity equivalent (RAE) β-carotene for an average adult) [8]. Consequently, food fortification and supplementation programmes have been implemented in Kenya to reduce the incidences of VAD but have turned to be costly, and hence their sustainability is challenged. The shift to traditional leafy vegetables has proven not only a cheaper source of this vitamin but also have higher levels. They are also easier to grow noting the levels of beta-carotene in them is affected by genotype, environmental growing conditions, age, postharvest handling and storage [9]. Amaranthus thrives under different climatic conditions, soil types and soil macro-nutrients [10]. For macro-nutrient P, S, Mg, Ca, P, N levels of 0.4 - 5.0, 1.50 - 1.60, 0.6 - 15.0 0.4 - 35.0, 0.1 -5.0 and 2.0 - 4.0 µg/g respectively are recommended [11]. The concentration of Mg^{2+} ions in most soils is always higher than that of K^{+} ions and hence its uptake by the plant roots while a combination of N, P and K is associated with good growth, yield and quality of fruits and vegetables [12,13]. An increase in Ca on one hand has shown decreased carotenoid concentrations in fresh tomatoes while on the other, its insufficient supply would stimulate the synthesis of ethylene and hence biosynthesis of carotenoids (Kays, 1991; De Kreij, 1995; Paiva., *et al*. 1998b). Nevertheless, *Amaranthus hypochondriacus* L. and *Amaranthus cruentus* L. leaves contain between 36.35 - 49.80 and 35.99 - 47.07 mg 100-1 DW of β-carotene respectively [14,15]. It is however unclear whether these levels would be affected should the plant be grown in different soil types and consumed at different stages of maturity.

Amaranthus hybridus L, a species of the genus *Amaranthus* is one of the African indigenous leafy vegetables grown in Kwale County envisioning it as a solution to minimize the underlying causes of malnutrition (Tang and Lanzillotti, 2005). Soils in Kwale County, Kenya cut across loamy, sandy, and clay soils that were subject to investigation on their support to growing *Amaranthus hybridus* L. Further, the findings are based on the levels of beta-carotene (pro-vitamin A) with maturity of the vegetable grown in the different soil types.

Materials and Methods

Soil Sampling

Traverse method was used to collect the soil samples according to Gachene and Kimura [16]. Four corners of the sampling sites in each of the Sub-Counties were determined and sampling was done diagonally. Ten sub-samples of soils were collected from each sampling site at a depth of 0 - 15 cm and scooped out using soil auger bit. The sub-samples for each type of soil were thoroughly mixed in different gunny bags to form composite samples for clay, sandy and loamy soils.

Reagents and chemicals

The HPLC grade reagents such as n-hexane, acetone, acetonitrile, methanol, and dichloromethane were obtained from Merck, Germany. Ethanol, metaphosphoric acid, acetic acid, potassium di-hydrogen phosphate, hydrochloric acid, magnesium ribbon, anhydrous sodium sulphate, Tetrahydrofuran (THF), Butylated hydroxytoluene (BHT), sodium hexametaphosphate, ammonium sulphate, sodium hydroxide, potassium sulphate, sodium salicylate, sodium citrate, β-carotene standards, sodium tartrate, antimony potassium tartrate, sodium nitroprusside, sodium hypochlorite, ammonium molybdate tartrate, barium chloride, nitric (V) acid, sulphuric (VI) acid, hydrogen peroxide, potassium chloride and sodium acetate were of analytical grade from Sigma (Sigma-Aldrich, Germany). De-ionised distilled water was used in all the experiments.

Instrumentation

The HPLC system used was a Shimadzu (Kyoto, Japan) consisting of a column oven (model CTO-10 AVP), a UV-visible diode-array detector (Waters 2996), a degasser (Model, DGU-14A), an LC pump (model, LC-10 ADVP) and RP-18 column (VP-ODS, 150 mm × 4.6 mm× 5 µm). A 20-µL syringe (Hamilton-Bonaduz, Schweiz) was used for sample injection. Empower programme software was used for processing the chromatographic peaks. The UV-visible spectrophotometer was a CECIL CE 2041 2000 series model for the analysis of beta-carotene, P, N, and S.

Digestion on soil samples for macro-nutrient analysis

The digestion of soil samples for analysis of N, P, K, Ca, S and Mg and was done according to Kimbrough., *et al* (1989). A mass of 0.5 kg of each composite soil sample was oven dried to 70°C for four hours. The soil samples were then ground and placed in a dry clean 125 mL conical flask. A 4 mL aliquot of concentrated sulphuric (VI) acid was added and the flask swirled to ensure that the entire sample was wet. The contents were heated on an electric hot plate for 10 minutes to a temperature of 90°C. The conical flask was then removed, allowed to cool and 10 drops of 30% hydrogen peroxide were added, four drops at a time to avoid rapid and vigorous reaction. The flask was then swirled keeping the contents at the bottom of the flask to avoid excessive heating. The flask was again allowed to cool and six drops of hydrogen peroxide added carefully and reheated, cooled and this was repeated until the solution turned colourless. The digested sample was then transferred into 100 mL volumetric flask and topped up to 100 mL mark. It was then transferred into a clean labelled plastic container. Quantification of P, N and S were performed using UV-visible spectrophotometry, Ca and Mg were measured using FAAS while flame photometry was employed for K determination.

Experimental plant pots

Equal amounts of each soil type were placed in nine experimental plastic pots and labelled accordingly as Clay-Kinango (CSK), Clay-Msambweni (CSMS), Clay-Matuga (CSMT), Loamy-Kinango (LSK), Loamy-Msambweni (LSMS), Loamy-Matuga (LSMT), Sandy-Kinango (SSK), Sandy-Msambweni (SSMS) and Sandy-Matuga (SSMT). Certified seeds of *A. hybridus* L. were then sown in the different soil samples in the nine plastic pots at a spacing of 13 cm between plants and 1.2 cm deep. The plants were thinned after every three weeks so as to ensure that only five plants remained per plastic pot. Weeding was done carefully when the plants were 2 weeks old using a piece of stick to avoid root disturbance. This was then repeated once per week until the 75th day. The harvesting of leaves for analysis of β-carotene was done at 25 days after sowing (DAS), 50 DAS, and 75 DAS.

Extraction and quantification of β-carotene

The method by Rodriguez-Amaya and Kimura (2004) and Hena., *et al*. [17] were adopted with minor modifications.The green leaves were trimmed, cut into small pieces and then blended for 2 minutes using an electric blender and 10g of the sample were homogenized with 100 mL acetone and 20g of sodium sulphate as desiccant and BHT) (0.1% in acetone) as antioxidant. The mixture was then filtered and the sample extracted 3 times to completely remove the green colour. It was then concentrated before carrying out saponification. This procedure was done in triplicates.

Fresh standard solution of beta-carotene was prepared by weighing 25 mg of the standard into a 100 mL volumetric flask and topped up the solution to the mark with stabilized tetrahydrofuran (THF). Aliquots of 0, 1.0, 2.0, 4.0, 6.0 8.0 and 10.0 mL were measured and brought to volume in separate 50 mL volumetric flasks. The standard solutions were used to obtain the calibration curve for quantification.

The HPLC analysis of β-carotene was done by injecting 20 µL of sample solution and standards into the system and detecting at 450 nm. The mobile phase consisted of methanol, dichloromethane, and water in the ratio 79: 18: 3. Analysis was done isocratically at a flow rate of 1.0 L/min. β-carotene was identified by comparing its retention time with that of the standard while quantification was done using the calibration curve of peak areas versus concentrations of standard in parts per million. The retention time of β-carotene was 33 minutes. Analysis of ascorbic acid was done by determining the absorbances of extracted samples and the standards using a UV-visible spectrophotometer at 451.5 nm against blank solutions.

Data Analysis

One-Way ANOVA at 95% significance level using IBM SPSS, version 21 assuming that there were significant differences among means when the statistical comparison gave $p < 0.05$ was used for the analysis. Student Newman Keuls (SNK) test was used to compare the mean levels of the plant macro-nutrients in soil samples and β-carotene in the leaves of *A. hybridus* L. grown in the nine soil samples at 25, 50 and 75 DAS. Whenever a significant difference existed, the means were compared at $p = 0.05$ significance level which accounted for errors.

Results and Discussion

The mean ± SE levels of the soil macro-nutrients (µg/g) and levels of β-carotene (mg 100-1 DW) are given in tables 1 and 2 respectively.

	(Mean ± SE) in µg/g of macro-nutrients;					
Soil-type	**N**	**P**	**K**	**Mg**	**Ca**	**S**
CSK	0.71 ± 0.01^{c}	0.03 ± 0.01^{a}	8.41 ± 0.03^{c}	4.13 ± 0.47^{a}	0.11 ± 0.07^{a}	3.36 ± 0.00^{c}
CSMS	0.59 ± 0.02^{a}	1.04 ± 0.02^{b}	1.97 ± 0.01^{a}	20.35 ± 0.25^{c}	0.04 ± 0.06^{a}	2.46 ± 0.00^{b}
CSMT	0.65 ± 0.01^{b}	1.02 ± 0.00^{b}	7.18 ± 0.01^{b}	13.64 ± 0.41^{b}	0.24 ± 0.11^{a}	2.30 ± 0.01^{a}
p-value	< 0.001	< 0.001	< 0.001	< 0.001	0.374	< 0.001
LSK	0.76 ± 0.00^{b}	0.01 ± 0.02^{a}	7.90 ± 0.02^{c}	19.98 ± 0.34^{c}	1.96 ± 0.17^{a}	3.22 ± 0.00^{a}
LSMS	0.87 ± 0.03^{c}	0.01 ± 0.01^{a}	1.87 ± 0.01^{b}	8.24 ± 0.19^{b}	2.03 ± 0.11^{b}	3.43 ± 0.01^{b}
LSMT	0.58 ± 0.01^{a}	0.09 ± 0.01^{b}	0.11 ± 0.01^{a}	0.41 ± 0.09^{a}	3.81 ± 0.20^{c}	5.93 ± 0.01^{c}
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SSK	0.61 ± 0.01^{b}	0.04 ± 0.01^{b}	1.99 ± 0.01^{c}	14.11 ± 0.09^{c}	3.54 ± 0.17^{a}	3.00 ± 0.00^{b}
SSMS	0.93 ± 0.01^{c}	0.02 ± 0.01^{a}	0.04 ± 0.02^{b}	1.71 ± 0.32^{a}	3.48 ± 0.07^{a}	4.24 ± 0.01^{c}
SSMT	0.59 ± 0.03^{a}	0.06 ± 0.00^{c}	0.04 ± 0.01^{a}	2.92 ± 0.09^{b}	3.35 ± 0.17^{a}	2.79 ± 0.00^{a}
p-value	< 0.001	< 0.001	< 0.001	< 0.001	0.648	< 0.001

Table 1*: Mean levels of macro-nutrients in different types of soils from different Sub- Counties of Kwale County, Kenya (µg/g, n= 3).*

Mean values followed by the same small letter within the same column for the same soils do not differ significantly from one another (One-way ANOVA, SNK-test, α =0.05).

Abbreviations: CSK: Clay Soil-Kinango Sub-County; CSMS: Clay Soil-Msambweni Sub-County; CSMT: Clay Soil-Matuga Sub-County; LSK: Loamy Soil-Kinango Sub-County; LSMS: Loamy Soil-Msambweni Sub-County; LSMT: Loamy Soil-Matuga Sub-County; SSK: Sandy Soil-Kinango Sub-County; SSMS: Sandy Soil-Msambweni Sub-County; SSMT: Sandy Soil-Matuga Sub-County

	Mean content of β - carotene (mg 100 g^{-1} DW) ± SE at;				
Soil type*	**25 DAS (n = 3)**	**50 DAS (n = 3)**	**75 DAS (n = 3)**	**p-value**	**% change**
CSK	5.72 ± 0.02^{Aa}	7.00 ± 0.05^{Ba}	7.33 ± 0.05^{Ab}	0.043	28.15
SSK	5.74 ± 0.03^{Ba}	8.83 ± 0.40^{Cb}	9.89 ± 0.15^{Bc}	< 0.001	72.30
LSK	4.43 ± 0.04^{Aa}	5.30 ± 0.02^{Ab}	5.94 ± 0.04^{Ac}	< 0.001	34.09
***p*-value**	< 0.001	< 0.001	< 0.001		
CSMS	11.47 ± 0.21^{Ca}	11.60 ± 0.06^{Ba}	12.74 ± 0.58^{Cb}	< 0.001	11.07
SSMS	8.14 ± 0.30^{Ca}	5.58 ± 0.04^{Bb}	11.29 ± 0.08^{Ca}	0.421	38.70
LSMS	5.54 ± 0.02^{Ba}	5.71 ± 0.11^{Ba}	6.99 ± 0.09^{Bb}	< 0.001	26.17
***p*-value**	0.315	< 0.001	0.001		
CSMT	8.92 ± 0.03^{Bb}	5.01 ± 0.03^{Aa}	9.46 ± 0.18^{Ba}	< 0.001	6.05
SSMT	1.63 ± 0.14^{Aa}	3.87 ± 0.0^{1A}b	5.60 ± 1.08^{Ac}	0.004	243.56
LSMT	8.90 ± 0.89^{Ca}	10.39 ± 0.06^{Cb}	11.18 ± 0.12^{Cc}	0.001	25.62
***p*-value**	< 0.001	0.001	0.001		

Table 2: *Mean contents of β-carotene with A. hybridus L. leaf maturity grown in different soils of Sub-Counties of Kwale County (mg 100 g^{-1} DW, n = 3).*

**Key as stated in table 1. Mean values followed by the same capital letter (s) within the same column do not differ significantly and mean values followed by the same small letter (s) within the same row do not differ significantly from one another (One-Way ANOVA, SNK-test, α=0.05).*

The macronutrients Ca, P, N, K, S and Mg in the soils ranged between 0.04 - 3.81, 0.01 - 1.04, 0.58 - 0.93, 0.04 - 8.41, 2.30 - 5.93 and 0.41 - 20.35 µg/g respectively. The levels of K, S and Mg were higher than the recommended levels contrary to those of Ca, P and N [11]. This can be attributed to lower rates of soil mineralization and mobilization as Ca^{2+} ions are always released during these processes while positive correlations are reported for N and P [18,19]. Further, the high levels of Mg can be ascribed to lower levels of protons [12,20]. The levels of macro-nutrients differed significantly within and between the Sub-Counties ($p < 0.05$).

The levels (mg 100 g^{-1} DW) of beta-carotene in the vegetable ranged between 5.72 - 11.47, 5.01 - 11.60 and 7.33 - 12.74 at 25, 50 and 75 days after sowing (DAS) respectively in clay soils. In sandy soils, the ranges were 1.63 - 8.14, 3.87 - 8.83 and 5.60 - 11.29 at 25, 50 and 75 DAS respectively while in loamy soils levels ranged between 4.43 - 8.90, 5.30 - 10.39 and 5.94 - 11.18 at 25, 50 and 75 DAS respectively. The amounts were generally found sufficient to provide RDA for an adult [8,15]. Higher amounts have been reported in *Amaranthus hypochondriacus* and *Amaranthus cruentus* leaves [15]. Statistics showed that the levels of the carotenoid in the different soil types in the different Sub-Counties of Kwale County generally varied significantly between soils and as well for the different maturity periods ($p < 0.05$). The levels of the macro-nutrients in the soil in the sub-counties (Table 1) would be a strong determinant for this [13]. As a result, increase in K levels enhance concentration of β-carotene in vegetable leaves since the macro-nutrient speeds up the rate of acetic thiokinase activity for the catalysis of condensation of acetyl CoA units which form the initial steps in the biosynthesis of carotenoids [21].

The percentage (%) increase in β-carotene levels between the 25^{th} and 75^{th} days of growth ranged from 6.05% (CSMT) to 243.5% (SSMT) majorly attributed to the levels of Ca (Table 1) although increased rate of carotenogenesis with plant age cannot be underestimated [14,15,22-24].

Conclusion

All the soil types; loamy, sandy and clay in Kwale County amidst their Ca, P, N, K, S and Mg macro-nutrient levels had ability to support the growth of *A. hybridus* L whose beta-carotene levels increased within the 75 DAS. The levels of beta-carotene were sufficient to provide the recommended dietary requirement of vitamin A projecting a remedy for reducing its deficiency in the County.

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