

Characterization and Classification of the Provitamin A carotenoids of Deep Yellow-fleshed Bitter Yam (*Dioscorea dumetorum*) Varieties

Alamu E Oladeji¹, Maziya-Dixon Bussie^{1,*}, Ferede-Menkir Roman², Popoola Ibiroke¹, Asiedu Robert¹, Gondwe Therese¹

¹International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Oyo State, Nigeria

²Santa Monica College, Physical Science Department 1900 Pico Blv, Santa Monica, CA 90094, USA

*Corresponding author: b.dixon@cgiar.org

Abstract Twenty-two genotypes of deep yellow fleshed *D. dumetorum* were analysed for carotenoids using High Performance Liquid Chromatography (HPLC) and for similarities in their provitamin A characteristics using Cluster Analysis (CA). The contribution of each carotenoid to provitamin A content was investigated through correlation and regression analysis. Seven of the genotypes fell within the low provitamin A cluster with values from 2.26 $\mu\text{g g}^{-1}$ to 7.74 $\mu\text{g g}^{-1}$. Twelve genotypes were in the intermediate cluster with values between 8.56 $\mu\text{g g}^{-1}$ and 11.20 $\mu\text{g g}^{-1}$. Three genotypes were within the high cluster with values between 10.13 $\mu\text{g g}^{-1}$ and 14.00 $\mu\text{g g}^{-1}$. Trans- β -carotene-5,8 epoxide was a significant predictor ($\beta_1 = 0.773$, $p < 0.005$) of provitamin A content of the *D. dumetorum* while trans- β -carotene was not a significant predictor ($\beta_2 = 0.593$, $p > 0.05$).

Keywords: bitter yam, deep yellow fleshed, carotenoids, provitamin A, β -carotene

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1. Introduction

One of the most prevailing public health problem in most developing countries is Vitamin A deficiency (VAD) and a leading cause not only of childhood blindness but also morbidity and mortality among pre-school children [1,2,3]. It has been estimated that 26% of the children with VAD reside in Africa and an estimated 250,000-500,000 become blind. Moreover, VAD has been shown to significantly increase the risk of severe illness and death from common childhood infections [2,4]. In addition to contributing to morbidity and mortality in pre-school children, VAD has been implicated in maternal mortality, and poor lactation and pregnancy outcomes [4]. With the condition affecting one in almost every three children nationwide in Nigeria, the prevalence of VAD is classified as severe and a serious public health problem. According to the World Health Organisation (WHO) classification, Nigeria is among the 34 countries with serious problems of VAD-related nutritional blindness [4]. Carotenoids are a group of naturally occurring pigments responsible for the orange, yellow, or red colour of many foods. Some of these pigments have been proven to have provitamin A activity; β -carotene and other carotenoids with β -retinylidene or 3-dehydroretinylidene end-group can be easily bioconverted into retinoids [5]. Hence, they are referred to as precursors of vitamin A or said to have

provitamin A activity. Efforts are made to improve the provitamin A carotenoids content of commonly consumed staples in developing countries to alleviate VAD since the contribution from eggs, milk, and fish is rather low.

Among low income consumers, yam (*Dioscorea* spp.) is considered the next most important dietary staple after cassava. It is a major food not only in West and Central Africa but also in some parts of East Africa, South America, Asia, and the Caribbean [6]. Of the 52 million t of yam produced worldwide in 2007, Nigeria produced 37 million tonnes (71% of the total production [7]). Yam is a good source of carbohydrate as it is composed mainly of starch. It also contains some amount of proteins, lipids, vitamins, and minerals. In Nigeria, yam is consumed boiled, roasted, or fried with oil or sauces [6]. There are five most widely consumed: *D. rotundata* Poir (white yam), *D. alata* (water yam), *D. cayenensis* (yellow yam), *D. bulbifera* (aerial yam), and *D. dumetorum* (bitter yam) [8].

Bitter yam, also known as cluster yam (when the tubers are bunched), three-leaved or trifoliate yam, is an important but underutilized food security crop. It comes in white, pale-yellow, or deep yellow forms. Although reported to be the most nutritious of the commonly consumed species [9], *D. dumetorum* is not consumed as much as *D. rotundata* or *D. alata*, probably owing to its mildly bitter taste which is unacceptable to some consumers and also because the tuber hardens during storage [10]. In terms of cultivation, *D. dumetorum* is high

yielding compared to other species of yam and is easy to harvest [11]. In terms of nutritional quality, the species with a protein content of 9.43-10.3% is substantially higher in protein than *D. alata* (8.2%) and *D. rotundata* (7.6%) [9]. Moreover, the proteins are more balanced than those found in *D. rotundata* and the species is rich in vitamins and minerals. *D. dumetorum* is mostly consumed in Nigeria, Ghana, Cameroon, Guinea, and Mali. Its high nutritional benefit but low consumption in comparison with other yam species makes imperative further research into investigating additional nutritional benefits and also into breeding varieties with improved characteristics.

A study by Ferede et al. [6] identified the potential of deep yellow fleshed *D. dumetorum* in alleviating VAD in developing countries through the breeding of improved genotypes with higher total carotenoids content as well as by identifying and quantifying its unique carotenoids profile. The result of their study shows that the provitamin A carotenoids in these deep yellow genotypes are in fact comparable with those of cassava and yellow maize lines selected for increased concentration of provitamin A. An average content of $8.9 \pm 3.2 \mu\text{g g}^{-1}$, representing 52% of the total content, was reported in a deep yellow fleshed variety of *D. dumetorum*. Selective breeding of genotypes showing a high level of provitamin A will go a long way in improving the nutritional quality of carotenoids in the deep yellow variety. Moreover, although past studies have been focused on identifying and quantifying the carotenoids in *D. dumetorum*, nothing has been done to investigate the relationship between these carotenoids and their contribution to provitamin A activity. This study was aimed at elucidating the relationship existing between the carotenoids contained in deep yellow fleshed varieties for the purpose of breeding for improved varieties and ultimately contributing to alleviating VAD, especially in developing countries where the species is grown and consumed.

2. Materials and Methods

This study used data obtained from the extraction, identification, and quantification of major carotenoids in 22 genotypes of deep yellow fleshed *D. dumetorum* obtained from the research farm of International Institute of Tropical Agriculture, Ibadan, Nigeria by Ferede et al. [6].

Sample Preparation: Samples were prepared according to the method described by Ogunjobi and Ogunwolu [10]. A total of 22 genotypes of deep yellow fleshed tubers were washed with tap water and air-dried, then peeled and rinsed with deionised water. Each tuber was cut longitudinally into four equal parts. Two diagonally opposite parts were taken and combined, then chopped into cubes approximately 1 cm^2 ; these were combined, and mixed. The chopped sample was further divided into four equal parts. Two diagonally opposite sections were collected, mixed, wrapped with aluminium foil, packed in sampling bags, and stored at $-80 \text{ }^\circ\text{C}$. On the day of analysis, samples were allowed to thaw, then weighed, and analysed. Sample preparation was done in the dark room with yellow light to prevent photo-oxidation, isomerisation, and degradation of the carotenoids [6].

Extraction, identification, and quantification of carotenoids: The extraction procedures for yam were

carried out by adapting the HarvestPlus protocol [12] for carotenoids extraction in sweet potato. Total carotenoids for each genotype were determined using the spectrophotometry method, multiplying absorbance at 450 nm by the molar absorption coefficient recommended for a mixture which is 2500. Samples were analysed in duplicate and values presented are means of duplicate determination. Individual carotenoids were determined using HPLC method described by Ferede et al. [6] and Alamu et al. [13]. Waters HPLC system (Water Corporation, Milford, MA) consisting of a guard-column, C30 YMC Carotenoid column (4.6 x 250mm, $3\mu\text{m}$), Waters 626 binary HPLC pump, 717 auto-sampler and a 2996 photodiode array detector (PDA) was used for carotenoids quantification. The system operated with Empower 1 software (Waters Corporation). Solvent A was methanol: water (92:8v/v) with 10mM ammonium acetate; solvent B consisted of 100% methyl tertiary-butyl ether. Gradient elution was performed at 1mL/min with the following conditions: 29 min linear gradient from 83% to 59% A, 6 min linear gradient from 59% to 30% A, 1 min hold at 30% A, 4 min linear gradient from 30% to 83% and a 4 min hold at 83%. β -carotene eluted at approximately 25 min. Chromatograms were generated at 450nm and identification of β -carotene (*cis* and *trans* isomers) a β -cryptoxanthin-5, 6-epoxide (*cis* and *trans* isomers) and β -cryptoxanthin-5, 8-epoxide (*cis* and *trans* isomers) were determined using the external standards method based on the calibration curve from pure standards and with verification of the absorption spectrum and co-elution with available authentic standards purchased from CaroteNature, GmbH (Lupsingen, Switzerland). The provitamin A (PVA) content of the bitter yam was calculated by adding the amount of β -carotene (*cis* and *trans* isomers) to one-half of the amounts of β -cryptoxanthin-5,6-epoxide (*cis* and *trans* isomers) and β -cryptoxanthin-5,8-epoxide (*cis* and *trans* isomers). On the basis of the molecular structure, β -cryptoxanthin-5,6-epoxide and β -cryptoxanthin-5,8-epoxide are considered to have 50% of the provitamin A activity of β -carotene [6]. Thus, the amount of provitamin A activity obtained from β -cryptoxanthin-5, 6-epoxide and β -cryptoxanthin-5,8-epoxide was calculated to be half of the amount obtained from β -carotene.

Statistical Analysis: Analytical data were reported as mean \pm standard deviation of at least duplicate independent extractions of samples. Data analysis was carried out using IBM Statistical Package for Social Sciences (SPSS version 20). Cluster Analysis was carried out to categorise the genotypes based on their carotenoids characteristics; correlation and multiple regression analysis were carried out to determine the relationship between the total carotenoids and provitamin A content as well as the relative contribution of the carotenoids to the provitamin A content.

3. Results and Discussion

3.1. Extraction, Identification, and Quantification of Carotenoids

Total carotenoids (TC) ranged between $5.21 \mu\text{g g}^{-1}$ and $26.61 \mu\text{g g}^{-1}$. A total of seven carotenoids were identified

and characterised: *trans*- β -carotene, *trans*- β -carotene-5,8-epoxide, *trans*- β -cryptoxanthin-5,6-epoxide, *trans*- β -carotene-5,6-epoxide, *cis*- β -carotene, *cis*- β -cryptoxanthin-5,6-epoxide and *cis*- β -carotene-5,6-epoxide (Table 1). Of these seven, the content of *trans*- β -carotene-5,8-epoxide was found to be the highest. On the basis of the molecular structure, β -cryptoxanthin-5,6-epoxide and β -cryptoxanthin-5,8-epoxide are considered to have 50% of the provitamin A activity of β -carotene. Provitamin A content was determined by adding all of the *trans*- β -carotene which is known to have 100% provitamin A activity with half of each of the carotenoids with half structures resembling vitamin A (*trans*- β -carotene-5,8-epoxide, *trans*- β -cryptoxanthin-5,6-epoxide, *trans*- β -carotene-5,6-epoxide, *cis*- β -carotene).

The structure of carotene-5,8-epoxide indicates that it should have only 50% pro-vitamin A activity similar to α -carotene or *cis*- β -carotene as opposed to *trans*- β -carotene. However, it was reported that β -carotene-5,6-epoxide and β -carotene-5,8-epoxide were reported to be more active than *trans* β -carotene in inducing the differentiation of NB4 cells which is critical to be absorbed well by humans [8]. Therefore, it could be assumed that these carotene mono epoxides might have higher than 50% provitamin A activity than what is predicted on the basis of the molecular structure alone [6]. Table 1 contains the total carotenoids (TC) for the 22 deep yellow bitter yam samples, the concentration of each carotenoid, and the provitamin A content.

Table 1. Carotenoids composition (in $\mu\text{g/g}$) of deep yellow-fleshed *D. dumetorum* genotypes

| Clones | β -cryptoxanthin-5,6-epoxide | Cis- β -cryptoxanthin-5,6-epoxide | Cis- β -carotene-5,6-epoxide | β -carotene-5,6-epoxide | β -carotene-5,8-epoxide | 9- Cis- β -carotene | Trans β -carotene | TC | PVA |
|---------|------------------------------------|---|------------------------------------|-------------------------------|-------------------------------|---------------------------|-------------------------|-------|-------|
| TDd0416 | 2.97 | 1.19 | 2.24 | 0.89 | 6.69 | 0.24 | 0.62 | 22.2 | 7.74 |
| TDd0510 | 4.18 | 1.77 | 3.07 | 0.76 | 14.83 | 0.51 | 1.44 | 21.53 | 14.00 |
| TDd0514 | 3.06 | 1.33 | 2.16 | 0.29 | 10.45 | 0.44 | 1.27 | 17.34 | 10.13 |
| TDd052 | 2.40 | 1.12 | 2.05 | 0.52 | 6.30 | 0.26 | 0.64 | 19.94 | 6.96 |
| TDd0523 | 3.11 | 1.25 | 2.39 | 0.32 | 9.76 | 0.33 | 0.90 | 19.94 | 9.49 |
| TDd0524 | 3.31 | 1.41 | 2.41 | 0.17 | 12.45 | 0.35 | 0.89 | 19.31 | 10.94 |
| TDd0525 | 2.66 | 1.14 | 1.98 | 0.15 | 9.12 | 0.33 | 0.87 | 16.64 | 8.56 |
| TDd0526 | 3.27 | 1.20 | 2.21 | 0.29 | 11.51 | 0.37 | 0.92 | 19.43 | 10.34 |
| TDd053 | 3.23 | 1.29 | 2.30 | 0.42 | 11.24 | 0.43 | 0.92 | 18.67 | 10.38 |
| TDd055 | 2.24 | 0.94 | 1.99 | 0.60 | 4.40 | 0.15 | 0.54 | 19.85 | 5.70 |
| TDd056 | 2.85 | 1.23 | 2.67 | 0.50 | 11.10 | 0.17 | 0.95 | 19.31 | 10.21 |
| TDd057 | 2.22 | 1.08 | 1.77 | 0.00 | 5.87 | 0.25 | 0.60 | 19.53 | 6.20 |
| TDd058 | 2.99 | 1.29 | 1.87 | 0.88 | 10.43 | 0.38 | 0.99 | 21.57 | 9.91 |
| TDd059 | 3.14 | 1.18 | 2.15 | 0.49 | 11.12 | 0.37 | 0.99 | 19.87 | 10.21 |
| TDd3101 | 0.83 | 0.65 | 0.56 | 0.11 | 2.36 | 0.20 | 0.36 | 5.68 | 2.72 |
| TDd3114 | 1.48 | 1.12 | 1.97 | 0.37 | 8.83 | 0.41 | 0.95 | 19.14 | 8.05 |
| TDd3779 | 0.69 | 0.51 | 0.47 | 0.00 | 2.06 | 0.15 | 0.32 | 5.21 | 2.26 |
| TDd3848 | 4.13 | 1.50 | 0.22 | 1.69 | 14.33 | 0.49 | 1.39 | 20.2 | 12.57 |
| TDd0527 | 3.18 | 1.67 | 2.17 | 0.57 | 11.72 | 0.45 | 1.22 | 26.01 | 11.10 |
| TDd3097 | 0.98 | 0.71 | 0.21 | 1.04 | 3.99 | 0.29 | 0.70 | 6.57 | 4.28 |
| TDd3102 | 3.63 | 1.52 | 1.80 | 0.08 | 12.38 | 0.52 | 1.23 | 27.49 | 11.20 |
| TDd2788 | 4.16 | 1.68 | 3.18 | 0.12 | 14.96 | 0.47 | 1.13 | 26.6 | 13.41 |
| Mean | 2.76 | 1.22 | 1.90 | 0.46 | 9.36 | 0.34 | 0.90 | 18.73 | 8.93 |
| Minimum | 0.69 | 0.51 | 0.21 | 0.00 | 2.06 | 0.15 | 0.32 | 5.21 | 2.26 |
| Maximum | 4.18 | 1.77 | 3.18 | 1.69 | 14.96 | 0.52 | 1.44 | 27.49 | 14.00 |

3.2. Cluster Analysis (CA)

A hierarchical cluster analysis using Furthest's neighbour method on the 22 yam genotypes, produced three clusters (Table 2) where distance between two clusters is represented by the distance between two furthest points [14]. In the first, low provitamin A genotypes were predominant, in the second, there was intermediate provitamin A while in the third there was high provitamin A content. An F-Test was used to investigate the rationale for using each indicator as a predictor of a bitter yam genotype being grouped within a particular cluster while the mean values of each indicator gave a summary of the dissimilarities between the clusters. Three genotypes within the low provitamin A cluster had values from 2.26 $\mu\text{g g}^{-1}$ to 4.28 $\mu\text{g g}^{-1}$ and were characterised by low levels of *trans*- β -carotene from 0.32 $\mu\text{g g}^{-1}$ to 0.70 $\mu\text{g g}^{-1}$ and *trans*- β -carotene-5,8 epoxide from 2.36 $\mu\text{g g}^{-1}$ to 3.99 $\mu\text{g g}^{-1}$. Fourteen genotypes within

the intermediate provitamin A cluster had values between 5.70 $\mu\text{g g}^{-1}$ and 10.94 $\mu\text{g g}^{-1}$ and were characterised by intermediate levels of *trans*- β -carotene between 0.54 $\mu\text{g g}^{-1}$ and 1.27 $\mu\text{g g}^{-1}$ and *trans*- β -carotene-5,8 epoxide between 4.40 $\mu\text{g g}^{-1}$ and 12.45 $\mu\text{g g}^{-1}$. Lastly, five genotypes within the high provitamin A cluster with values between 11.10 $\mu\text{g g}^{-1}$ and 14.00 $\mu\text{g g}^{-1}$ were characterised by the highest value of *trans*- β -carotene r between 1.13 $\mu\text{g g}^{-1}$ and 1.44 $\mu\text{g g}^{-1}$ and *trans*- β -carotene-5,8 epoxide from 11.72 $\mu\text{g g}^{-1}$ to 14.96 $\mu\text{g g}^{-1}$. A description of the genotypes with their respective provitamin A content as well as *trans*- β -carotene and *trans*- β -carotene-5,8 epoxide contents are presented in Table 1. Cluster observation dendrogram for the carotenoids composition of the yam genotypes is presented in Figure 1. The mean calculations and F-Test results showed that the clusters differ significantly on the basis of the Pro-Vitamin A, Total Carotenoids, *trans*- β -carotene and *trans*- β -carotene-5,8 epoxide as presented in Table 3.

Table 2. *D. dumetorum* clusters based on Provitamin A, *trans*-β-carotene and *trans*-β-carotene-5,8 epoxide contents

| Cluster | Clone | β-Carotene 5,8 epoxide(μg/g) | Trans β-Carotene(μg/g) | ProVit.A (μg/g) | Total carotenoids(μg/g) |
|---------|---------|------------------------------|------------------------|-----------------|-------------------------|
| 1 | TDd3848 | 2.06 | 0.32 | 2.26 | 5.21 |
| | TDd3779 | 2.36 | 0.36 | 2.72 | 5.68 |
| | TDd3114 | 3.99 | 0.70 | 4.28 | 6.57 |
| | TDd3102 | 4.40 | 0.54 | 5.70 | 19.85 |
| | TDd3101 | 5.87 | 0.60 | 6.20 | 19.53 |
| | TDd3097 | 6.30 | 0.64 | 6.96 | 19.94 |
| | TDd2788 | 6.69 | 0.62 | 7.74 | 22.20 |
| | TDd059 | 8.83 | 0.95 | 8.05 | 19.14 |
| | TDd058 | 9.12 | 0.87 | 8.56 | 16.64 |
| | TDd057 | 9.76 | 0.90 | 9.49 | 19.94 |
| | TDd056 | 10.43 | 0.99 | 9.91 | 21.57 |
| 2 | TDd055 | 10.45 | 1.27 | 10.13 | 17.34 |
| | TDd053 | 11.10 | 0.95 | 10.21 | 19.31 |
| | TDd0527 | 11.12 | 0.99 | 10.21 | 19.87 |
| | TDd0526 | 11.24 | 0.92 | 10.38 | 18.67 |
| | TDd0525 | 11.51 | 0.92 | 10.34 | 19.43 |
| | TDd0524 | 11.72 | 1.22 | 11.10 | 26.01 |
| | TDd0523 | 12.38 | 1.23 | 11.20 | 27.49 |
| | TDd052 | 12.45 | 0.89 | 10.94 | 19.31 |
| | TDd0514 | 14.33 | 1.39 | 12.57 | 20.20 |
| | TDd0510 | 14.83 | 1.44 | 14.00 | 21.53 |
| 3 | TDd0416 | 14.96 | 1.13 | 13.41 | 26.60 |

Table 3. Mean pro-vitamin A, total carotenoids, *trans* β-carotene and β-carotene 5,8 epoxide of the *D. dumetorum* genotype clusters

| Cluster | Provitamin A(μg/g) | | Total carotenoids(μg/g) | | Trans β-carotene(μg/g) | | β-carotene 5,8 epoxide(μg/g) | |
|---------|--------------------|------|-------------------------|------|------------------------|------|------------------------------|------|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 1 | 3.09 | 0.96 | 5.82 | 0.66 | 0.46 | 0.19 | 2.81 | 0.94 |
| 2 | 9.01 | 1.89 | 19.51 | 1.42 | 0.88 | 0.24 | 9.36 | 2.61 |
| 3 | 12.55 | 0.96 | 24.82 | 2.93 | 1.28 | 0.17 | 13.71 | 1.71 |

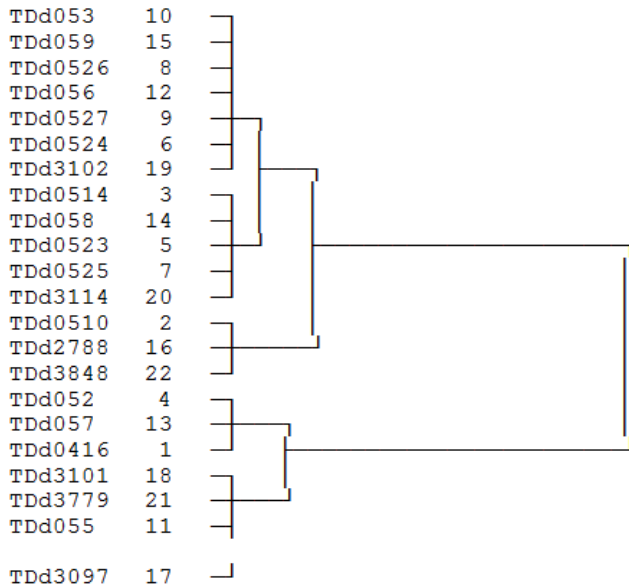


Figure 1. Cluster observation dendrogram for the carotenoids composition of the twenty-two *D. dumetorum* genotypes

3.3. Correlation Analysis

Correlation analysis was carried out using Pearson's Correlation to determine the relationship among the total carotenoids of the genotype clusters, the provitamin A content, the predominant carotenoid, and the carotenoids with vitamin A activity (Table 4). At low levels of provitamin A and total carotenoids (Cluster 1), a very strong positive correlation was reported between the provitamin A content of the genotypes, total carotenoids,

trans-β-carotene, and *trans*-β-carotene-5, 8. At the intermediate level (Cluster 2), a weak negative correlation was reported between total carotenoids and provitamin A but a very strong positive correlation between *trans*-β-carotene and provitamin A as well as between *trans*-β-carotene-5,8 and provitamin A. Lastly, for Cluster 3 (high provitamin A level), a strong negative correlation was reported between total carotenoids and provitamin A but a weak positive correlation between *trans*-β-carotene and provitamin A and a strong positive correlation between *trans*-β-carotene-5,8 and provitamin A. At the low level of total carotenoids and provitamin A 1 (Cluster 1) provitamin A accounted for 43 to 65% of the total carotenoids; 50% of *trans*-β-carotene-5,8 epoxide (considering 50% vitamin A activity) accounted for 43 to 46% of the provitamin A while 100% of *trans*-β-carotene contributed between 13 to 16%. At the intermediate level (Cluster 2), 29 to 57% of the total carotenoids and 50% of *trans*-β-carotene-5, 8 epoxide contributed 39 to 57% of the provitamin A while *trans*-β-carotene contributed 8 to 12%. Lastly, at high provitamin A level (Cluster 3), provitamin A accounted for 43 to 65% of the total carotenoids and 53 to 56% of this was contributed by of half of the *trans*-β-carotene-5,8 epoxide contained in the genotypes while *trans*-β-carotene contributed 8 to 11%. A multiple regression analysis was carried out to investigate the relative contribution of the dominant carotenoids in the deep yellow-fleshed *D. dumetorum* yam genotypes (*trans*-β-carotene-5, 8 epoxide) and *trans*-β-carotene which is the known carotenoids with 100% vitamin A activity to the provitamin A content of the yam genotypes. The regression model is specified below:

$$PVA = \beta_0 + \beta_1X_1 + \beta_2X_2 + E$$

Where:

PVA = Provitamin A Content

β_0 = intercept

β_1 = Slope (coefficient) trans- β -carotene-5, 8 epoxide

X1 = trans- β -carotene-5, 8 epoxide

β_2 = Slope (coefficient) trans- β -carotene

X2 = trans- β -carotene

E = error term.

Result shows that trans- β -carotene-5, 8 epoxide was a significant predictor ($\beta_1 = 0.773$, $p < 0.005$) of provitamin A content of the *D. dumetorum* while trans- β -carotene

was not a significant predictor ($\beta_2 = 0.593$, $p > 0.05$). The overall model fit (R^2) was 0.98. The provitamin A can be predicted from trans- β -carotene and trans- β -carotene-5, 8 epoxide by the model below:

$$\text{PVA} = 1.15 + 0.773X_1 + 0.593X_2.$$

This implies that each 1.15 $\mu\text{g g}^{-1}$ increase in provitamin A is accounted for by 0.77 $\mu\text{g g}^{-1}$ increase in trans- β -carotene-5, 8 epoxide and 0.59 $\mu\text{g g}^{-1}$ increase in trans- β -carotene.

Table 4. Correlation Coefficients of Provitamin A (PVA), Total Carotenoids, Trans β -carotene and β -carotene 5, 8 epoxide

| | PVA | TC | Trans β -carotene | β -carotene 5,8 epoxide |
|-------------------------------|--------|-------|-------------------------|-------------------------------|
| PVA | 1 | | | |
| Total Carotenoids | .530* | 1 | | |
| Trans β -carotene | .850** | .455* | 1 | |
| β -carotene 5,8 epoxide | .993** | .520* | .824** | 1 |

* Correlation significant at 0.05 level

** Correlation significant at 0.001 level.

4. Discussion

The cluster analysis provided a means of classifying the genotypes on the basis of their provitamin A content (PVA), total carotenoids, carotenoids with vitamin A activity, and the predominant carotenoid. Light shades of yellow in tubers indicate a high correlation between provitamin A content and total carotenoids as well as trans- β -carotene and trans- β -carotene-5,8 epoxide; at a low level, total carotenoids in *D. dumetorum* are almost exclusively provitamin A. This result is further reinforced by the relative proportion of provitamin A to total carotenoids in Cluster 1. It can be inferred that, at a low level of total carotenoids, *D. dumetorum* exhibits provitamin A activity similar to that reported by Hagenimana et al. [15]. for orange-fleshed sweet potato. Previous study by Sánchez [16] established a strong positive correlation between total carotenoids and the colour intensity of cassava roots. Hence genotypes with intermediate to high total carotenoid content would have been expected to have a higher proportion of provitamin A. However the results of this study showed a negative correlation between total carotenoids and provitamin A for genotypes in Clusters 2 and 3. For instance, TDd 0055 contained up to 20 $\mu\text{g g}^{-1}$ total carotenoids, only 28% of this is provitamin A and only 9.5% of this provitamin A was contributed by trans- β -carotene while 39% was contributed by trans- β -carotene-5,8. This is an indication that other carotenoids with little or no provitamin A activity may have been responsible for the deep yellow colour. This result is contrary to those of several authors on the carotenoid profile of other root crops such as sweet potato where β -carotene was strongly correlated with total carotenoids at all total concentration levels Hagenimana et al. [15]. Hence, selecting genotypes for breeding through simple visual inspection as suggested by Sánchez et al. [16] for cassava may not yield selection of better quality varieties of *D. dumetorum* in terms of pro-vitamin A activity. This implies that *D. dumetorum* has a unique carotenoid profile which makes breeding for its better nutrition in terms of provitamin A a worthy task. Although the proportion of provitamin A in the deep

yellow genotypes may be lower in total carotenoid content, breeding for deeper yellow color may not be an outright waste of effort. Considering that the genotypes in Clusters 2 and 3 contain more provitamin A than those in Cluster 1 which has a ratio of more provitamin A to total carotenoid, this shows the potential of both the light yellow and deep yellow genotypes to contribute to the alleviation of VAD in developing countries

5. Conclusion

However, taking into consideration the relative abundance and bioavailability of trans- β -carotene-5,8 epoxide in *D. dumetorum*, genotypes showing high level of trans- β -carotene-5,8 epoxide could play an important role in alleviating vitamin A deficiency among vulnerable groups of *D. dumetorum* consuming communities of developing countries. Also, genotypes showing higher provitamin A content could be given due attention for further improvement through selective breeding. Results will be useful for breeders for breeding for higher content of provitamin carotenoids and especially for nutritionists to properly educate the consumers in developing countries on the nutritional benefits of *D. dumetorum* yam specie.

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