EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 3 (Special issue): February 2022

Characterization of provitamin status and distribution in commercial grown maize varieties in Tanzania

[1,2,*]MSUNGU S., [3]MUSHONGI A., [1]PAVITHRAVANI B V., [1]MBEGA E R

[1]School of Life Sciences and Bioengineering (LiSBE), Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania
[2]Mwalimu Julius K. Nyerere University of Agriculture and Technology P.O. Box 976, Mara, Tanzania
[3]Tanzania Agricultural Research Institute (TARI) -Ilonga Centre, P.O. Box 33, Kilosa, Morogoro 67409, Tanzania

Corresponding Author: msungus@nm-aist.ac.tz

Abstract

Provitamin A carotenoids are important as precursors for vitamin A, essential for stronger immune system and eyesight. Consumption of a plant-based diet rich in provitamin A such as provitamin A maize is an easy and affordable source of vitamin A. The majority of the commercially grown maize varieties in Tanzania are white whose provitamin A status is uncertain and undocumented. The existing current information suggests that white maize varieties have low provitamin A, an active form of vitamin A, thus putting maize consumers at risk of vitamin A deficiency (VAD). Characterization of provitamin A status in commercial maize varieties grown and consumed in Tanzania is important to provide baseline information required for vitamin A rich-maize improvement programs. Therefore, this study was conducted to map the status of provitamin A in commercial maize varieties to accrue the information needed to improve maize nutrition quality toward controlling VAD in the country. The study involved 14 maize varieties including commercial yellow and white pigmented maize from 3 regions of Tanzania. From maize samples, carotenoid extraction was conducted based on published protocol. The concentration of beta carotene (BC), beta-cryptoxanthin (BCX), alpha-carotene (AC) were determined by an ultraviolet spectrophotometer and provitamin A computed for the individual carotenoids components. The study found that the concentration of provitamin A and associated carotenoids significantly differed (p<0.001) among varieties studied. The mean concentration (in µg/g) for provitamin A and its components in range as provitamin A (2.64-6.50), BC (1.91-4.66), BCX (0.649-1.21), and AC (0.819-1.46). The concentrations of provitamin A in the studied maize varieties were below the recommended levels (15 µg/g). The low concentrations of provitamin A among commercial maize varieties signify the need for introgressions of provitamin A in the adapted maize genotypes for ensured intake of vitamin A.

Keywords: *Beta-carotene; Provitamin A; Carotenoids; Commercial maize varieties; Tanzania*

Received: 28/10/21
Accepted: 13/12/21
Published: 16/02/22

Cite as: *Msungu et al.,(2021)* Characterization of Provitamin A Status and Distribution in Commercial Grown Maize Varieties in Tanzania. *East African Journal of Science, Technology and Innovation 3(Special Issue).*

Introduction

Provitamin A (proVA) are carotenoids which include alpha-carotene, beta-carotene, and beta-cryptoxanthin among others (Faber and Jaarsveld, 2007). They are active form of vitamin A as they are easily converted into retinol units (vitamin A) usable by the human body (Palacios-Rojas *et al.*,2020). Consumption of vitamin A-rich food from either animal sources and/or plants, increase the body's ability to fight vitamin A deficiency (VAD). The VAD is a significant health problem in most Sub-Saharan African (SSA) countries including Tanzania affecting children under five years and women of reproductive ages (Kaur *et al.,* 2016). Vitamin A deficiency is characterized by xerophthalmia (dry eye), night blindness, corneal ulcers, lesions, and Bitot's spot (Berardo *et al.,* 2004; Sukto *et al.,* 2020). It is also among the leading cause of morbidity and mortality cases among Tanzanian children with poor diet diversification situations. The deficiency (VAD) burden has also been exaggerated by the high price of vitamin A-rich animal foods, poor content of provitamin A in commonly grown staples including maize and low purchasing capabilities of many resources' poor rural inhabitants.

With all these drawbacks, most rural dwellers have continued depending on maize reported to possess little amount of provitamin A hence being at high risk of contracting VAD-associated illness (Lividini & Fiedler, 2015; Lockyer *et al.*,2018). The continued consumption of low provitamin A (proVA) maize has made VAD to remain a severe public health issue in Tanzania, with a prevalence rate of 33% among children < 5 years (Oikesh, *et al.,* 2003; TFNC, 2014; Zuma *et al.,* 2018). Various strategies have been taken by the government and other non-governmental organizations (NGOs) to remedy the scourge. However, some strategies such as dosage supplementation and food fortifications are very difficult to implement in rural areas due to limited funds, poor infrastructures, and storage systems. Whereas poor accessibility to fortified food products is another issue as food industries are mainly concentrated in urban areas only. Therefore, efforts are in the pipeline to raise the levels of proVA content on commonly consumed staples such as maize for increased improvement of vitamin A intake among hard-to-reach peripheral communities (Mishra and Singh, 2010).

Maize (*Zea mays* L.) is among the commonly grown and consumed crops in many parts of Tanzania. It is consumed in almost all the three meals of a day as it is a crucial source of calories and proteins (Ranum and Pe, 2014). Maize is also a good source of carotenoids such as provitamin A, a precursor of vitamin A (Wurtzel, 2004). Vitamin A is a micronutrient required in the human body for the improved immune system, better eyesight, strong bones and as ant-diabetic (Obeng-bio *et al.*,2019).

Maize kernel contains provitamin A carotenoids (proVACs) in varying proportions, which have been used by maize breeders to increase the proVA levels to meet, the current breeding target of 15 μg/g dry weight (DW) proVA needed to attain the daily requirement of vitamin A (Pixley *et al.*,2013). Progress in the development of proVA rich maize varieties for combating VAD in Tanzania like other SSA countries is showing up. Two (2) proVA rich maize varieties have been released by Meru Agro seed company (Andersson *et al.,* 2017) which are Meru VAH 517 and Meru VAH 519 (Obeng-bio *et al.,* 2019) (Mugode *et al.*,2014). Since there release in 2016, the level of its provitamin A has not been evaluated and its distribution in the country has not been documented.

Moreover, with the high magnitude of VAD in the country, and the dependency of most people on conventional maize, there comes a need to consider improving the level of provitamin A in adapted maize cultivars by introgression. The information of proVA content on commercially grown maize varieties are highly needed to accelerate the breeding of high proVA maize varieties. The current study was undertaken to map the status of proVA levels in commercial maize verities grown in Tanzania to provide baseline information required for nutrition quality improvement in breeding programs.

Materials and Methods

Study area, sample collection and preparation

The study was conducted in three regions (Mbeya, Morogoro, and Arusha) by collecting 14 commercial maize varieties (Figure 1). The study area had unimodal and bimodal rainfall patterns, where Mbeya has unimodal rain patterns while Arusha and Morogoro have bimodal rain patterns. The sampled areas have an altitude ranging from 431-1935 meters above sea level (masl) with cool and hot months, and a temperature ranging from 11.8 to 29.5^{o}C. Maize samples were collected from agro-dealers and research institutions in the study area. Approximately 100 grains were grounded to pass a sieve of 63 µm sieve for easy provitamin A fractions analysis. The grounded samples were kept in black zipper bags and stored at room temperature to avoid light oxidation before beta-carotene, alpha-carotene, beta-cryptoxanthin determination.

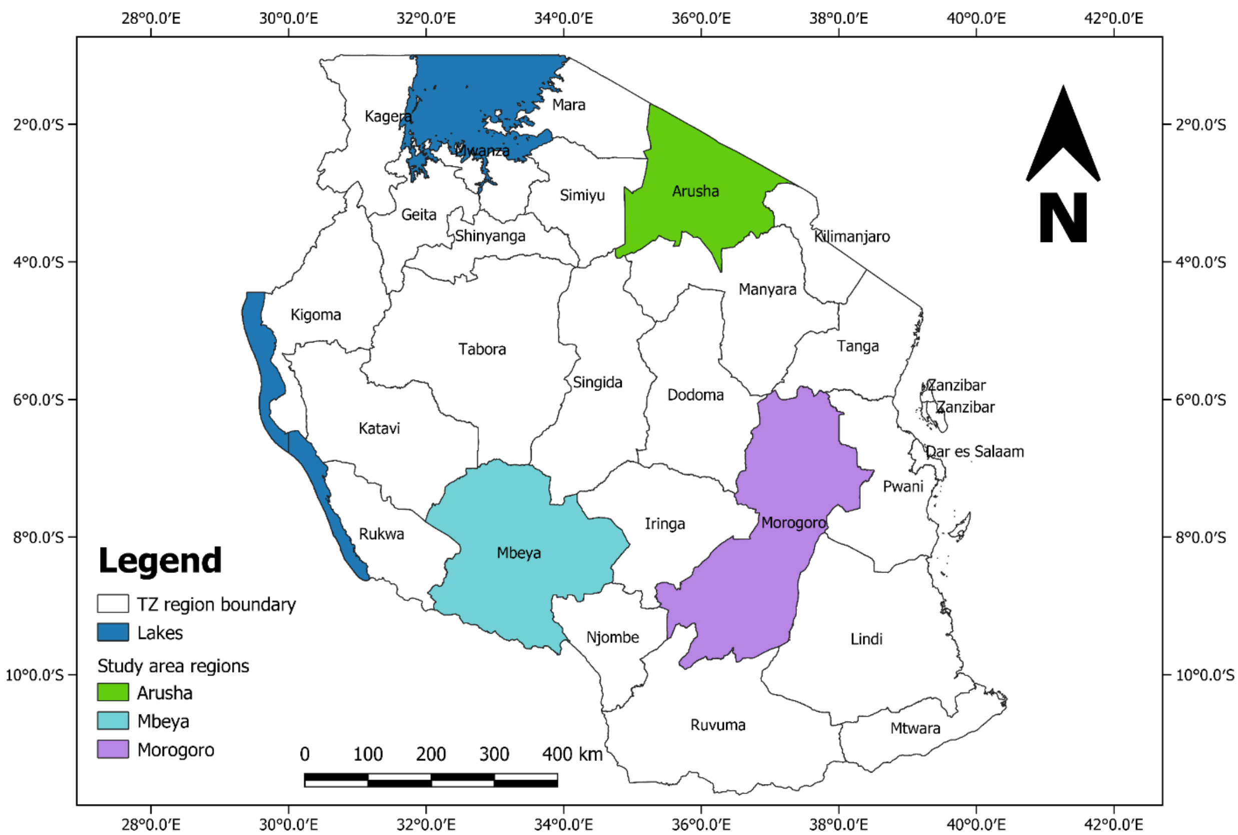

Figure 1. Map of the study area where maize samples were sampled during the 2020/2021 season

Carotenoids extraction and determination

The carotenoids extraction from maize samples were conducted based on a method developed by Kurilich and Juvik (1999) and Weber (1987) with little modification. In summary; approximately 0.6 grams of fine powder of maize sample were weighed into a 50 ml centrifuge (falcon) tube, followed by 6 mL of ethanol with 1% butylated hydroxytoluene (BHT). The mixture was heated in a water bath (85^{o}C) for 3 minutes, followed by the addition of 120 mL of 80% potassium hydroxide (KOH) and heated further for 2 minutes. After 5 minutes of heating, samples were removed from the water bath and vortexed for 20 seconds for sample homogenization. Then samples were returned to the water bath for additional 10 min heating to allow saponification.

After 10 minutes of heating samples were removed from the water bath and three (3) mL of cold deionized distilled water were added followed by 3 mL of hexane. Then upper layers were pipetted, extracted, and pelleted by hexane, 3 mL distilled water was used to the collected upper layer and then centrifuged for 10 minutes at 1200 rpm, where the hexane portions were detached into a fresh tube. The hexane fractions were dried, evaporated, and reconstituted in 200 mL of acetonitrile: methanol: methylene chloride (45:20:35 v/v/v). The reconstituted samples were taken into 96 well microplate readers for provitamin A carotenoids determination in UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All samples were extracted in triplicate. Where proVA was computed based on equation 1 (US Institute of Medicine, 2001).

$$\text{ProVA}\ (\mu g/g) = \text{BC} + \frac{1}{2}\text{BCX} + \frac{1}{2}\text{AC} \qquad (1)$$

Where ProVA is provitamin A, BC= beta carotene, BCX= beta-cryptoxanthin, AC= alpha-carotene, ½ is the proportion contribution of the component to provitamin A carotenoid.

Quality Assurance**:** to ensure the reliability of the obtained data values, reagent blanks and solutions with known concentrations were used as a check to monitor carotenoids concentration determination quality. Doubled distilled were used to wash all glassware used in the study to avoid any possible contamination.

Statistical data analysis

Various statistical methods and software (Jamovi 1.2.25 and IBM SPSS Statistics 24 programs (IBM: Chicago, IL, USA)) were applied to analyze carotenoids among commercial maize varieties on the studied parameters. One-way ANOVA and Tukey post hoc tests ($P < 0.05$) were used to determine the statistical difference between maize varieties and agricultural zones. The study site map was developed by QGIS 3.10.7 software.

Results

Carotenoids status and distribution

This study is part of the large research project and presented here is the preliminary results that are sought to be necessary for early sharing to the scientific community. The results revealed that the concentration of proVA carotenoids varied significantly ($p<0.05$) among maize varieties (Table 1). The concentrations in µg/g varied from 0.26 to 13.9 for BC, 0.360 to 2.86 (BCX), and 0.13 to 6.64 (AC). The mean concentration varied widely among carotenoids where BC had the highest mean concentration (2.98 µg/g) and AC recorded the lowest mean concentration (1.39 µg/g) (Table 1).

Table 1. The summary description of carotenoids concentrations (µg/g) in studied maize genotypes

	BC	**BCX**	**AC**	**ProVA**
Mean	2.89	0.85	1.05	3.84
Standard deviation	2.17	0.39	0.96	2.76
Minimum	0.56	0.40	0.31	0.92
Maximum	6.50	1.50	2.00	8.25

The results indicate that carotenoid composition and content, differ suggestively ($p<0.001$) among maize varieties. The BC, the mean concentration varied from 1.94 (CWSV) to 4.66 µg/g commercial yellow varieties (CYSV). The BCX, the mean concentration varied from 0.649 (CWSV) to 1.21 µg/g commercial yellow varieties (CYSV), The AC, the mean concentration varied from 0.819 (CWSV) to 1.46 µg/g commercial yellow varieties (CYSV) and The ProVA, the mean concentration varied from 2.64 (CWSV) to 6.00 µg/g commercial yellow varieties (CYSV) The same trend was observed in BCX, AC, and proVA (Table 2 and Figure 2).

The analysis of carotenoids individuals varied significantly among varieties ($p<0.05$) (Table 3). While UH5350 and CKDHLWE recorded higher concentration (2.88 µg/g) of BC, the TMV1 recorded low BC (0.56 µg/g). The same trend was observed in other carotenoid fractions (Table 3). On other hand, the yellow seeded commercial maize varieties (CYSV) recorded a slightly high concentration of carotenoids fractions. The Meru VAH519 recorded a high BC concentration (6.5 µg/g) and YM31END had a low concentration (3.24 µg/g) of BC among commercial yellow varieties. The study indicates that yellow maize verities had high carotenoids fraction compared to white maize, for example, 2.88 µg/g the high values of BC in white maize was lower than the lowest value recorded in yellow maize (3.24 µg/g). The similar of high carotenoid fractions in yellow maize varieties than white were observed in the rest of carotenoids (BCX and AC) (Table 2). Provitamin A in commercial white seeded varieties (CWSV) varied between 0.92 (TMV1) and 3.97 µg/g (UH5350) with a mean concentration of 2.64 µg/g. This show that the amount of proVA in the most preferred white maize is quite low and inadequate to meet human requirements of 15 µg/g proposed by Harvest Plus. The results indicate that provitamin A in the CYSV ranged from 4.13 (YM31-END) to 8.25 µg/g (Meru VAH519) with a mean of 6.00 µg/g. In CYSV, the results show that they have at least high levels of proVA compared to CWSV, however, they are far below 15 µg/g, the level suggested by Harvest Plus.

Table 2. The mean concentration (µg/g) for carotenoid components and provitamin A among studied commercial maize varieties

Variety	Sample ID	BC	BCX	AC	ProVA
Commercial white (CWSV)	UH5350	2.88±0.15ijk	0.83±0.21ijk	1.01 ±1.12pqrstu	3.95 ±0.24mn
	Staha (LT8)	2.73±0.12klmno	0.8±1.12ijklm	1.25±0.12 ijklmn	3.76 ±0.12pqr
	STUKA M-1	2.39±r0.12stu	0.74 ±0.12nopqr	1.12 ±0.14mnopq	3.32 ±0.12uv
	CKD HL WE	2.88±1.21ijk	0.83± 0.21ijkl	2.43 ±0.21c	4.54±0.21gh
	HSG 5410	2.28±0.18stuv	0.72 ±0.18opqrs	0.39 ±0.13GHI	2.84±0.21 y
	Staha Ilonga	1.42±0.11EF	0.56±0.11BCD	0.65±0.15BCDE	2.02±0.11FG
	WE 3113	1.1±0.21HIJ	0.5±0.21EFGH	0.68±0.21ABCD	1.69±1.21HI
	UH 6303	0.91±0.54KL	0.46±0.54GHI	0.34±0.25HI	1.31±0.13KL
	TMV1	0.56±0.15N	0.4±0.15IJ	0.31±0.31HI	0.92±0.10MN
Grand Mean		**1.65±0.83**	**0.60±0.16**	**0.69±0.39**	**2.29±1.05**
Commercial Yellow (CYSV)	Meru VAH 517	5.08±0.44b	1.08±41d	1.17 ±0.12klmnop	5.22 ±0.11e
	CP 808	3.87±0.54e	1.21±0.25c	1.72 ±0.16gh	5.98 ±0.25d
	Meru VAH519	6.5±0.16a	1.5 ±0.16a	2 ±0.11e	8.25 ±0.11b
	YM31END	3.24±0.12fg	1.24 ±1.43b	1.94 ±0.14ef	6.59 ±0.03c
	CP 201	4.61±0.25c	1.02±0.54ef	1.1±0.41nopqr	5.03±0.54f
Grand Mean		**5.02±1.00**	**1.20±0.19**	**1.69±0.37**	**6.46±1.23**
Overall Mean		**3.335±0.915**	**0.9±0.175**	**1.005±0.38**	**4.375±1.14**

Mean values in the column followed by the same letter are not statistically significantly different at p<0.05 level of significance.

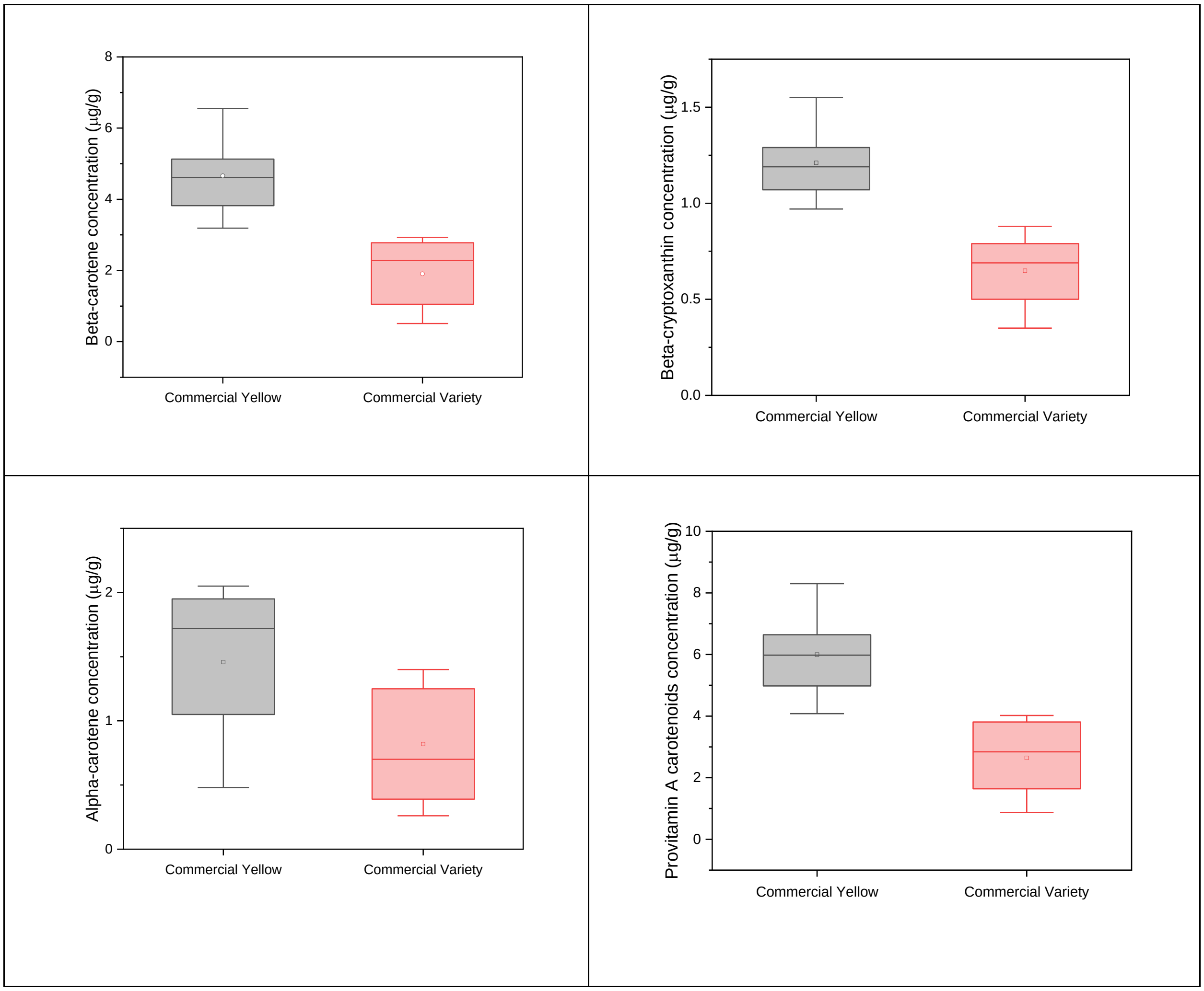

Figure 2. Box plots, presenting the concentrations of (a) β-carotene, (b) a-carotene, (c) β-cryptoxanthin and (d) provitamin A in studied commercial maize varieties

Beta-carotene, Beta-cryptoxanthin and alpha-carotene

The study found that proVA in commercially yellow maize varieties were significantly higher ($p<0.001$) than in the white seeded maize, where the yellow seeded maize had a 3.06 times higher concentration of beta carotene than white seeded maize. The mean concentration of BCX in maize genotypes varied significantly ($p<0.05$) where an appreciable amount of BCX was observed in CYSV than in CWSV and (Table 4). These results depict that the mean concentration for beta-cryptoxanthin was higher in yellow maize genotypes. The CWSV, the most grown had significantly low levels of BCX. The alpha-carotene as the important component of proVA carotenoids and vitamin A was also determined in studied maize genotypes. The results display that the mean values for AC were significantly variable ($p<0.001$) among genotypes, where it was observed that the CYSV had a 3.66 times higher concentration of AC than the white seeded maize varieties.

Spatial distribution of provitamin A carotenoid in Tanzania

The study found that the mean concentrations for carotenoid components across the agro-ecological zones had significant differences ($p<0.001$). The results indicate that the mean concentration of BC ranged from 1.85 µg/g (EZ) to 2.91 µg/g (NHZ) and for BCX, the mean concentration ranged from 0.637 µg/g (EZ) to

0.814 μg/g (NHZ) (Table 4). The results also recorded the mean concentration of AC from 0.922 μg/g (SHZ) to 1.39 μg/g (NHZ). The proVA mean concentration across the agro-ecological zone varied from 2.64 μg/g (EZ) to 3.97 μg/g (NHZ). The study found that provitamin A content and its components carotenoids (BC, BCX and AC) were significantly higher in maize varieties from the Northern highland zone (NHZ) than those from the EZ and SHZ (Table 3).

Table 3. The summary description of carotenoid components in maize genotypes across three Tanzanian agro-ecological zones (n=14)

Parameter	Agro-ecological zone	Mean (μg/g)	SD	SE
BC	Eastern zone (EZ)	1.854	0.939	0.1916
	Northern highlands (NHZ)	2.91	1.338	0.223
	Southern highlands (SHZ)	2.079	0.923	0.1967
BCX	Eastern zone (EZ)	0.637	0.173	0.0354
	Northern highlands (NHZ)	0.814	0.235	0.0392
	Southern highlands (SHZ)	0.696	0.203	0.0433
AC	Eastern zone (EZ)	0.943	0.594	0.1213
	Northern highlands (NHZ)	1.393	0.638	0.1063
	Southern highlands (SHZ)	0.922	0.501	0.1067
ProVA	Eastern zone (EZ)	2.644	1.255	0.2562
	Northern highlands (NHZ)	3.974	1.679	0.2798
	Southern highlands (SHZ)	2.888	1.161	0.2476

Discussion

Provitamin A carotenoids are important to human nutrition and health. Understanding carotenoid contents in consumed maize for increased development, availability the and delivery varieties with much higher provitamin A carotenoids maximize the health benefits to consumers of maize. The study analyzed fourteen commercial maize varieties to determine β-carotene (BC), β-cryptoxanthin (BCX), and α-carotene (AC) contents. The variation in concentration of proVA carotenoids among maize varieties in the current study are in line with those reported by Muzhingi et al. (2016) and Sukto et al. (2020). The significant differences among maize genotypes in provitamin A content observed by this study, indicates the presence of variability in accumulating provitamin among maize varieties grown and consumed in Tanzania. The mean concentration varied widely among provitamin A carotenoid where BC had the highest mean concentration (2.98 μg/g) and AC recorded the lowest mean concentration (1.39 μg/g). TMV1, the popular maize in Tanzania had inadequate BC (0.56 μg/g). Poor content of provitamin A in the most grown and consumed white maize varieties calls for intentional measures that has to be taken by breeders to enhance proVA carotenoids in the adapted varieties. Some white maize genotypes examined showed high proVACs concentrations, for instance UH5350 and CKDHLWE depicted promising amount of BC. These maize genotypes could effectively be employed in breeding for high provitamin A maize aiming for 15 μg/g of retinol equivalents adapted to Tanzanian environment that are also acceptable by the consumers. The high carotenoid content in yellow seeded varieties (CYSV) with comparable tendencies of BC, BCX, AC, and proVA has also been documented by Lux et al (2021). Among the commercial yellow maize genotype, the Meru VAH519 had high levels of proVA, however, they are far below the level suggested by Harvest Plus (15 μg/g). Of interesting, the yellow seeded maize genotypes found with high proVA might be the good source of provitamin A improvement or breeding through introgression. The low levels of proVA among varieties of maize commonly consumed requires intentional effort by breeders to augment the content of these carotenoids needed to alleviate vitamin A deficiency.

The poor concentrations of proVA in most consumed maize undermines the supply of provitamin A carotenoid that is important in combating vitamin A deficiency among vulnerable populations.

The significant differences for mean concentration of proVA and its components carotenoids (BC, BCX and AC) in maize varieties from the Northern highland zone (NHZ) than from other zones (EZ and SHZ) might have been attributed by some factors including soil discrepancies. Generally, the observed variability of carotenoids concentration in different commercial maize varieties across agro-ecological zones assume great importance in food and nutritional security and play a significant role in alleviating VAD in the country. Also, the variation highlights that potentially carotenoids concentration in maize varieties might be influenced by agro-environmental factors which affect accumulation and production of provitamin A carotenoid in maize grains in different agricultural zones.

Conclusion and recommendation

Among the commercial maize varieties studied showed a variation in provitamin A carotenoid, where common white seeded maize genotypes which are highly grown and consumed in Tanzania had very low levels of carotenoids and provitamin A thus posing a huge health risk to maize consumers. On other hand, the spatial distribution of carotenoid content in maize genotypes has been observed to vary among agro-ecological zones where the northern highland zone is observed to have high provitamin A carotenoid, which might be influenced by climatic and agro-environmental conditions. Generally, yellow/orange maize genotypes are the potential to be included in the nutrition improvement program of maize breeding for sustainable maize production and improved health of consumers. More research might be needed including different landraces from different agro-ecological zones to characterize their provitamin A carotenoids.

Acknowledgement

Authors thanks all community leaders and local government officers in the study area for their cooperation and support throughout the study

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