Chapter

Carotenoids in Cassava (*Manihot esculenta* Crantz)

Lovina I. Udoh, Josephine U. Agogbua, Eberechi R. Keyagha and Itorobong I. Nkanga

Abstract

Cassava is produced globally and consumed as an important staple in Africa for its calories, but the crop is deficient in micronutrients such as vitamin A. Pro-vitamin A carotenoids including β-carotene are precursors of vitamin A in the human body. Carotenoids are generally associated with colors of fruits and vegetables. Although most cassava varieties have white tuberous roots and generally accepted, naturally; some cassava roots are colored yellow and contain negligible amounts of vitamin A. Several genes have been identified in the carotenoids biosynthesis pathway of plants, but studies show that Phytoene synthase 2 (*PSY2*), lycopene epsilon cyclase, and β-carotene hydroxylase genes have higher expression levels in yellow cassava roots. So far, the *PSY2* gene has been identified as the key gene associated with carotenoids in cassava. Some initiatives are implementing conventional breeding to increase pro-vitamin A carotenoids in cassava roots, and much success has been achieved in this regard. This chapter highlights various prediction tools employed for carotenoid content in fresh cassava roots, including molecular marker-assisted strategies developed to fast-track the conventional breeding for increased carotenoids in cassava.

Keywords: cassava, carotenoids, marker assisted selection, molecular markers, vitamin A, biofortification, phenotyping

1. Introduction

Cassava (*Manihot esculenta* Crantz) is an important crop globally, and in Nigeria, it is consumed as a staple by more than 100 million people every day [1]. Global production of cassava has been given at approximately 278.7 million tons, it was estimated to be 281 million tons and 288.4 million tons in 2015 and 2016, respectively [2]. Global cassava market in 2019 increased by 0.4% to $164.1B, and consumption was peak at $172.1B [3]. Nigeria stands out as largest producer as its progressive cassava pattern increased from 42.5 million tons in 2010 to 61 million tons in 2020; total production area in 2012 was 3.85 million hectares [4]. In Nigeria, the cost of cassava production per hectare is estimated to be 82,055 naira, with a profit of about 123,745 naira. Although, in Africa, 50% of the cassava produced is largely consumed as food after processing; 38% in fresh and/or cooked form; and 12% is utilized for animal feed [5]. The crop is

cultivated mostly by small scale farmers because it outperforms other staple food crops under long-term drought and poor soil conditions [6].

Commonly available white cassava can provide most of the body's daily energy needs, but it does not provide adequate protein, essential micronutrients, and vitamin A. Vitamin A deficiency makes the body susceptible to infection, especially among women and children [7]. It causes illness and eye defects that can lead to partial or complete blindness [7]. Most cultivars of cassava are white or off-white, and the roots of tubers are generally low in carotenoids [8]. Cassava varieties with colored pulp that may be rich in carotenoids are very rarely available and are not well known to the general public. Yellow flesh color of some cassava varieties is associated with the presence of carotenoids [9, 10], and the nutritive importance of carotenoids is attributed to its conversion to vitamin A when consumed. The consumption of tuberous roots of β-carotene-rich cultivars may contribute significantly to addressing vitamin A deficiency in sub-Saharan Africa.

One of the most important micronutrients with deficiency of high public health concern is vitamin A, followed by iron, zinc, and iodine [11]. The generic descriptor for compounds with the qualitative biological activity of retinol is vitamin A. It exists in the form of preformed retinoids that are preserved in animal tissues as pro-vitamin A carotenoids usually gotten from green, yellow, and/or orange plant tissues. A total of two-thirds of dietary vitamin A worldwide and more than 80% in the developing world have been said to come from carotenoids in vegetables [5]. The all-trans-β-carotene is observed to be the most abundant carotenoid in cassava together with isomers such as 9-cis β-carotene, 13 cis-β-carotene, and β-cryptoxanthin [5, 12, 13]. Several carotenoid biosynthesis genes and enzymes such as lycopene epsilon cyclase (*LCYε*), β-carotene hydroxylase (CHYβ), phytoene synthase 1 and 2, lycopene β-cyclase, and phytoene desaturase (*PDS*) have been identified for different plants including cassava [14–16]. Studies by Olayide et al. [13] detected more carotenoids and isomers in the leaves than roots. Phytoene synthase 2 (*PSY2*), *LCYε*, and CHYβ genes were mostly associated with β-carotene content in white and yellow roots, but they generally had higher expression in yellow root cassava [13]. To enhance marker-assisted selection in the conventional breeding to increase carotenoids in cassava roots, six single-nucleotide polymorphisms (SNP) markers were designed on candidate genes and validated on 650 elite cassava accessions of which PSY2_572 explained most of the phenotypic variation (R^2 = 0.75) in root pulp color [12].

Limited access to diets that are rich in vitamin A is known to be the root cause of vitamin A deficiency in Africa and other vitamin A deficiency inflicted regions. Efforts are continually being made to improve the nutritional value of cassava through biofortification, which has led to an improvement of its carotenoid content. These improvements have been successful through the adoption of advanced breeding techniques, which involves the screening of large numbers of genotypes for nutritional quality, agronomic traits, yield traits, etc., in order to select progenies with the best traits for further breeding.

2. Origin and domestication of cassava

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous plant belonging to the Euphorbiaceous family known only in the cultivated form and was first domesticated by the Amerindians of South and Central America [17]. There is archaeological evidence of two major centers of origin for cassava, one in Mexico and Central America and the other in North-eastern Brazil. In sixteenth century, Portuguese navigators

took cassava from Brazil to the western coast of Africa [18] and later to East Africa in eighteenth century through island of Reunion, Madagascar, also Zanzibar as described by Iglesias et al. [18]. It was introduced in India in the nineteenth century. Cassava plantations were set up by the Portuguese, who colonized South American regions by 1500 A.D. They carried cassava from these plantations to other continents [19]; hence, cassava was first introduced to Africa and Asia in the late sixteenth century by the Portuguese travelers. It was initially planted around the Congo River basin from where it moved to West and Central Africa [17, 20]. Nigeria was among the first African countries to receive the crop in the eighteenth century. The cassava crop was perhaps introduced in southern Nigeria by freed slaves who returned from South America through Sao Tome and Fernando Po islands [21].

2.1 Taxonomy

Cassava, as it is called in English, is referred to as "manioc" in French, "yuca" in Spanish, and "mandioca" in Portuguese. Cassava comprises about 7200 species. It belongs to the following [22];

Kingdom – Plantae
Subkingdom – Tracheobionta
Super division – Spermatophyta
Division – Magnoliophyta
Class – Magnoliopsida
Subclass – Rosidae
Order – Euphorbiales
Family – Euphorbiaceae
Subfamily – Manihotae
Genus – Manihot
Species – *Manihot esculenta* Crantz

This family is characterized by lactiferous vessels composed of secretory cells [17]. A total of 98 *Manihot* species have been recognized with one species (*Manihotoides pauciflora*) known in the closest related genus [17]. A lot of its characteristics have not been identified in any *Manihot* species, which are its mono-flower inflorescences and leaves borne at the apex of short, condensed stems arising from branch-lets. *M. pauciflora* is suggested to be a possible progenitor of all the Manihot groups. Unfortunately, this species is on the verge of extinction [23], and cassava is the only species that is widely cultivated for food production [23, 24]. The cultivated species may be derived from the wild progenitor *M. flabellifolia* [17].

2.2 Botanical description

Cassava is propagated mainly from stem cuttings, thereby maintaining true-to-type cultivars. Nevertheless, propagation by seed can take place naturally or during plant breeding procedures. When stem cuttings are planted in the moist soil under favorable conditions, they produce sprouts and adventitious roots at the base of the cuttings within a week. If propagated by seeds, it first develops into a tap root system. Cassava leaves are simple; it consists of a lamina and a petiole. Each leaf is subtended by two stipules, about 1 cm long. The petiole is between 5 and 30 cm long and varies from green to purple. The smooth margin of the lamina is palmate or lobed. The

lobes differ in number, ranging from 3 to 9, and are most of the time odd numbers. The lobe's vain color can differ from green to purple. Most cassava varieties grown in Africa have elliptical or lanceolated lobes [17, 25]. The arrangement of cassava leaves on a stem (phyllotaxis) is a 2/5 spiral, meaning that the position of five leaves turns twice spirally around the stem, then the next leaf comes just above the beginning of the other. Their stems are cylindrical and have a diameter, which varies between 2 and 6 cm. Cassava stems usually grow up to 4 m, but some genotypes may grow to only to a height of1 m. The older parts of the stems display prominent knob-like scars, which are leaf scars and their nodes [20, 25]. Cassava is a monoecious plant with male and female flowers located on the same plant. The inflorescences are produced at the reproductive branches [22].

Cassava is propagated from stem cutting or seed. In cassava, the fleshy part is the central portion of the tuberous root. Tuberous roots vary in shape and color, depending on the soil conditions and variety [25]. Cassava grows between 30°N and 30°S in areas where annual rainfall is greater than 500 mm and where mean temperature is greater than 20 °C. However, some cassava varieties grow at 2000 m altitude or in subtropical areas with annual mean temperatures as low as 16 °C. Cassava prefers a sandy or sandy loam soil, but all types of soils, except water logged soils, can be used. Cassava tolerates the high levels of aluminum and manganese often found in tropical soils [26].

3. Carotenoids biosynthesis in plants

Exploitation of the diverse tropical cassava collection for development of high pro-vitamin A cassava cultivars entails understanding and application of knowledge derived from molecular and biochemical studies of carotenoids and their biosynthesis in plants. Carotenoids are naturally occurring organic pigments that are produced by plants and some photosynthetic organisms [27, 28]. They are characterized by their extensive conjugated double bond along their carbon backbone giving them the capability to absorb lights in the range of blue to green range of the visible spectrum [28]. In plants, carotenoids are present mainly as indispensable integral components of the chloroplast, providing multiple services to the photosynthetic machinery participating in the light harvesting process and guarding the photosystems from possible damages by quenching reactive singlet oxygens and radicals created during photooxidation [29–31].

The carotenoid biosynthesis pathway is extensively studied in plants [29–33] and is responsible for the biogenesis of about 600 40-carbon isoprenoid compounds broadly classified as xanthophylls and carotenes. The first reaction dedicated to siphoning substrates to the carotenoid biosynthesis pathway in plants is catalyzed by the enzyme phytoene synthase (*PSY*). In this reaction, two geranylgeranyl pyrophosphate molecules are condensed to produce the first colorless linear carotenoid compound, phytoene. Phytoene is then modified through a series of desaturation and isomerization reactions catalyzed by enzymes including phytoene desaturase (*PDS*) and carotenoid isomerase (*CRTISO*) yielding the red colored carotenoid, lycopene. Lycopene is the forking point in the pathway that leads to two separate downstream branches called α and β branches. In the α branch, carotenoids such as α-carotene and lutein are synthesized, while in the β branch, carotenoids such as β-carotene, β-cryptoxanthin, and zeaxanthin are generated following cyclization of the terminals of the linear structured lycopene. Key enzymes involved in the branched part of the pathway include lycopene epsilon α-cyclase (*LYCε*) and lycopene epsilon β cyclase

(LYCβ) and β-carotene hydroxylase. The *LYCβ* can add β-ionone rings in both ends of lycopene to give β-carotene; while *LYCε* can add ε-ring in one end only to give α-carotene [14, 30]. Hydroxylation at the C-3 position of each ring of β- carotene and α-carotene produces xanthophylls, zeaxanthin and lutein, respectively (**Figure 1**).

Among all carotenoid compounds, only β-carotene has full vitamin A activity due to its doubly ended β-ionone rings, while carotenoids that have single β ring, such as α-carotene and β-cryptoxanthin, have half vitamin A activity of β-carotene [18, 30, 34–36]. Although the mechanism of regulation of the carotenoid biosynthesis is still not fully understood, a lot of progress has been made in this regard [30, 37].

3.1 Genes associated with carotenoid in cassava

Studies by Iglesias and Chavez et al. [10, 18] reported that relatively few major genes are involved in the determination of carotenoid accumulation in cassava roots. Thus, the trait can be improved to a significant level through the process of selection and recombination. In other crops, genes such as phytoene synthase (PSY), β-carotene hydroxylase, lycopene β, and ε cyclase have been reported to play a role in increasing levels of carotenoids [36, 38]. In cassava, Arango et al. [39] observed three *PSY* genes, one of which was discovered to be associated with stress in the Poaceae homologs. However, the two remaining *PSY* genes contributed differentially to carotenoid accumulation in leaves, roots, and flower parts of cassava. So far, the *PSY* gene has been identified as the key gene associated with carotenoids in cassava [12, 40, 41]. Olayide et al. [13] observed that carotenoid synthesis genes were expressed in both white and yellow cassava roots, but the following genes had higher expression in yellow roots, including phytoene synthase 2, lycopene epsilon cyclase, and β-carotene hydroxylase.

3.2 Breeding for increased carotenoids in cassava roots

Cassava is an highly important diet not only for humans but also in animal diet especially poultry, due to its availability and calories [24, 42, 43]; thus, the need arose

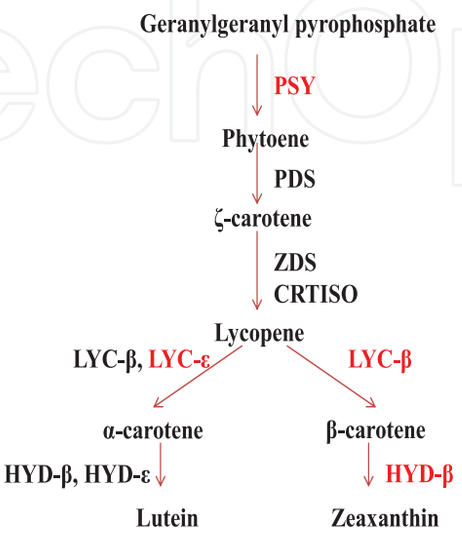

Figure 1.
A simplified diagram of the carotenoid biosynthetic pathway in plants, showing major genes and enzymes involved ***Figure 1****.*

to fortify the crop with micronutrient to improve its nutritional status. Some cassava varieties originally have yellow root color (**Figure 2**) meaning they have negligible amount of pro-vitamin A [18, 23]. Total carotenoid concentration in fresh yellow cassava ranges from 1 to 100 μg/g (fresh weight), primarily as all-trans-β-carotene, and is located in the parenchyma cells, the storage cells of the roots, and isomers such as 9 and 15 cis β-carotene and β-cryptoxanthin have also been detected [5, 18]. Carotenoid concentration is a stable trait and is influenced more by genotype than by its environment. Studies showed that retention of carotenoids differs not only per processing and storage method for a certain variety [10] but also within a variety, and this might be due to the variable distribution of dry weight matter within a root [44]. Retention varies between 10% for heavily processed and roasted cassava granules and 87% for boiling [10, 45].

Genetic improvement for this crop has employed crossing the wild yellow cultivars with elite breeding lines through recurrent selection and recombination [46]. This is accompanied by extensive field evaluation (phenotyping), including observations of disease and pest resistance, plant architecture, flowering ability, and performance in storage root [47]. Recently, rapid cycling recurrent selection was employed, which is able to cut down on the number of breeding cycles [9, 44]. The color of fruits and vegetables is associated with the presence of carotenoids, and the tuber-flesh color of some cassava accessions is yellow [23]. This indicates that naturally in the gene pool there are accessions with negligible amount of carotenoids [48], and this is currently being utilized in breeding. Breeding to biofortify cassava with pro-vitamin A will have a significant positive impact on nutrition and overall health, especially among poorer communities.

3.3 Comparison of carotenoids detected in some root crops

Using the high-performance liquid chromatography (HPLC), carotenoids and isomers have been detected in cassava, yam, and cocoyam, including the all trans-β carotene, 9-cis β carotene, 15-cis β carotene, β-cryptoxanthin (**Table 1**). From **Table 1**, all trans β-carotene was generally higher across cassava, with some accessions having up to 21 μg/g [5]. Also, the maximum value for total carotenoids content (TCC) in cassava as quantified using spectrophotometer is within the same range (12.95–14.8 μg/g). Yam

Figure 2.
Genetic variability of cassava cultivars with respect to carotenoid biosynthesis in storage roots. (A) Deep yellow roots, (B) white roots, (C) cream-colored root, (D) cassava plant, (E) unpeeled cassava roots ***Figure 2****.*

Plant	Trait (μg/g)	Tool	n	Min	Max	SD	Mean	Reference
	βcryp	HPLC	252	0.01	1.93	0.15	0.1	[12]
			4074	0	3.77	0.12	0.14	[5]
Cassava	9cisβc	HPLC	252	0.02	2.77	0.49	0.72	[12]
M. esculenta			4895	0	3.95	0.54	0.99	[5]
	13cisβc	HPLC	252	0.02	2.06	0.31	0.5	[12]
			4920	0	2.24	0.6	1.01	[5]
	Alltransβc	HPLC	252	0.03	6.7	1.17	1.51	[12]
			4952	0	21	3.94	7.28	[5]
	TCC		252	0.07	10.14	1.79	2.73	[12]
			4952	0.11	29	5.08	11.51	[5]
	TCC	Spec	252	0.07	13.34	2.45	3.75	[12]
			35	2.87	12.95		7.99	[9]
			98	0.02	14.8			[15]
Yam	9cisβc	HPLC	1				1.93	[49]
D. cayenensis	13cisβc		1				0.34	[49]
	Alltransβc		1				2.83	[49]
	TCC		1				11.99	[49]
	9cisβc		1				0	[49]
D. dumetorum	13cisβc		1				0	[49]
	Alltransβc		1				0.59	[49]
	TCC		1				3.79	[49]
	9cisβc		1				1.14	[49]
D. bulbifera	13cisβc		1				0.02	[49]
	Alltransβc		1				0.27	[49]
	TCC		1				10.11	[49]
Cocoyam	9cisβc	HPLC	1				1.13	[49]
X. mafa (Scoth)	13cisβc		1				0.91	[49]
	Alltransβc		1				3.88	[49]
	TCC		1				14.87	[49]

Table 1.
Carotenoids from cassava and other tuber crops (fresh weight).

and cocoyam also had higher all trans β-carotene content across the accessions studied. The mean value of this carotenoid was higher in cassava, as stated in Ceballos et al. [5], compared with yam and cocoyam. Mean for TCC quantified by HPLC ranged from 2.73 to 11.51 μg/g. In yam, the TCC varied among the studied genotypes. The variety of cocoyam studied had high TCC (14.79 μg/g) compared with yam (11.99 μg/g).

4. Various prediction tools for carotenoids in Cassava

Selection for a trait can be made based on phenotypes or genotype using molecular tools. The physical outlook of organisms, which includes all seen and quantitative characters that can be accessed from the outer part of the plant, is the phenotype. This comprises attributes that provide structural phenotypic information such as counts, dimensions, colors, etc., as well as physiological attributes such as photosynthetic efficiencies, water content, surface properties, etc., resulting from genotype and environmental interactions [50].

Carotenoid phenotyping in cassava is very essential as it measures and quantifies its total carotene content. To ensure optimal quality of breeding programs, there must be an understanding of crop genotype interaction with the environment, and this is expressed by the proceeding genotypes and monitored by phenotyping [51]. As breeding for higher carotenoid levels in cassava advances, selection is a major drawback, as some means of predicting total carotenoid content may be really expensive such as the use of high-performance liquid chromatography.

Further, color intensity in cassava roots has been observed to be closely related to quantity of carotenoids in the roots [10]. While visual selection is useful for separating white from yellow root cassava, it cannot efficiently distinguish the salient differences between yellow roots. Other methods exist to quantify carotenoids or check color intensity such as the use of near-infrared spectroscopy (NIRS) [8], but here, we compare some frequently used phenotyping methods for carotenoids in cassava.

Different instruments employed to predict carotenoids in cassava roots are as itemized below:

4.1 Near-infrared spectroscopy (NIRS)

This technique measures the interrelationship between electromagnetic radiation and the vibrational properties of chemical bonds, which results in the absorption of part of the radiation energy. The visible spectra cover between 380 nm and 780 nm and capture mainly information on pigmentation due to the carotenoids present in the root [52]. NIRS aims to analyze a sample such as to get from it qualitative and quantitative information about its physical and chemical composition. This it does by treating spectra mathematically so as to obtain the relevant information in the spectra, which is connected to the character of interest [44]. Its principle of action involves calibration of the spectrometer in order to develop mathematical models that will connect the standard values to a linear combination of the values of absorbance. NIRS allows the timely screening of many samples and variables and measures samples in different states, i.e., both in solid and liquid forms. When compared with other phenotyping methods, it is a fast and nondestructive alternative for analyzing several constituents simultaneously while requiring minimal to no sample preparation. It is economical and possesses no hazard to the environment [53].

The NIRS provides quality phenotyping method for field-based breeding programs especially where there are no standard laboratories, therefore reducing the need to transport samples from the field while also cutting out the need for sample procession [8, 53]. In NIRS, calibration and data obtained can be shared between spectrometers, thus increasing the chances of developing a network of high-throughput phenotyping technique for screening cassava roots [9].

4.2 Chromameter

The chromameter is a tool for precise and objective assessment of surface color. It can be used to preselect materials for further analysis. It records data output in the form of the L* a* b* color coordinate. This system has been used for different studies pertaining to skin color [8, 54]. The L * corresponds to levels of darkness or lightness between black and white colors. Coordinate a* signifies the balance between red/green, and b* between yellow/blue. This simple technique has equally been used to accurately quantify color intensity and quality in some plant tissues [13]. Sanchez et al. [8] observed that total carotenoid content and color intensity were strongly and positively associated ($R2 = 0.769$, $P < 0.01$), suggesting that the roots of cassava clones with a relatively high total carotenoid content can be selected through a simple visual inspection of the color intensity in the parenchyma. The difference in color of 228 biofortified cassava clones was also analyzed by [55], using the L* a* b* color coordinate system resulting in a high positive correlation between total carotenoids content (TCC) and the variables b* ($r = 0.90$) and chroma ($r = 0.89$). Their results demonstrate that the use of data obtained from this device is an economical, fast, and effective alternative for the development of TCC phenotyping tools with high predictive ability.

4.3 Image-based phenotyping

Digital image analysis allows the extraction of information regarding root color based on the strong correlation that exists between digital and virtual data [55]. Imaging techniques possess high resolutions, which permit the visualization of the sample from several dimensions and generating multiple data. Image-based phenotyping is used to quantify complex plant characters such as growth pattern, photosynthetic abilities, yield, tolerance to biotic and abiotic stress, both in controlled environments and in the open field. Plants imaging aims to measure a character quantitatively through the interaction that takes place between light and the plant such as reflection, absorption, and transmission of sent photons of which all plant cells and tissue possess specific wavelength for light reflection, absorption, and transmission. Since the presence of carotenoid is linked with the intensity of yellow color, it is taken that this type of phenotyping is ideal for the quantification of root carotenoid content. There are different aspects to image-based phenotyping, and they include thermal infrared imaging, imaging spectroscopy, fluorescence imaging, visible imaging, laser imaging, and hyperspectral imaging [55]. The advantages of imaging techniques include the following:

I. It is time saving.

II. Commercially available digital cameras that are easy to handle, transport and open-source software for processing images can be used.

III. It gives room for thorough reexamination of images recorded in cases where doubts arise concerning the phenotyping process.

IV. Calibration of prediction models makes it possible for sample size to be reduced, thus concentrating on samples of greatest interest, thereby reducing cost.

4.4 iCheck Carotene

This is a portable device consisting of two components, namely the measuring unit (iCheck™ Carotene) and the disposable reagent vial (iEx™) where the reaction is performed. The disposable reagent vial contains 2 mL of a mixture of reagents, which is needed for carrying out the reaction. The iCheck Carotene is very portable weighing about 250 g with dimensions (200 mm x 104 mm x 40 mm) making it easily transportable. It uses rechargeable batteries, which can be used to take up to about 400 measurements, which saves automatically and can be retrieved at will as a text file with the use of a USB cable. The iCheck Carotene is a rapid screening method, which is cost-effective, user-friendly, simple, and inexpensive. It does not require highly skilled and specialized personnel for its operation, neither does it need an expensive laboratory setup with equipment and specified chemicals; therefore, it is suitable for the quantification of a large number of samples within a short period of time with accurate results especially where there are no labs available, and there is a large number of cassava genotypes to be screened [56].

4.5 High-performance liquid chromatography (HPLC)

This is an advanced form of liquid chromatography, which is used in the separation, identification, and quantification of components in a mixture of molecules encountered in chemical and biological systems. It is associated with high reproducibility, ease of selection, manipulation, and high rate of recovery [57]. Its working principle involves a solution of the sample being injected into a column of a porous material (stationary phase) while a liquid (mobile phase) is pumped at high pressure into the column. The sample separates based on the differences in the rates of migration through the column, which results from the partitioning of the sample between the stationary and the mobile phase [57, 58].

In cassava phenotyping, HPLC is used in the separation and quantification of individual carotenoids, which are different in their provitamin A activity. Although it has high reproducibility, its analysis is expensive, costing 50–70 US dollars per sample with very low throughput. It is time-consuming, labor-intensive, and requires a highly sophisticated laboratory setup with highly skilled personnel and strictly adhered quality control regiment [57].

4.6 Ultraviolet–visible (UV–vis) spectrophotometer

The UV–Visible Spectrophotometer is a type of spectrophotometer, principle of which is based on the absorption of ultraviolet light or visible light by chemical compounds, and this results in the production of distinct spectra. It is a device that precisely measures electromagnetic energy at specific wavelengths of lights. UV–visible spectrophotometer uses light over the ultraviolet range of (185–400 nm) and visible range (400–700 nm) of the electromagnetic radiation spectrum. Carotenoids concentration, for example, is determined spectrophotometrically by measuring the absorbance (also referred to as optical density) of the extract at various wavelengths. The absorption spectrum of β-carotene (carotenoids) peaks between 450 and 475 nm. UV spectrophotometer has been mostly used to quantify carotenoids in cassava and other plants. Jaramillo et al. observed that spectrophotometer reading gave a higher quantity of total carotenoids content (30.0 μg/g) compared with the use of iCheck devise (24.7 μg/g). Other authors have also quantified carotenoids in cassava using the

spectrophotometer [5, 12, 15, 57]. The major throwback with the use of this instrument is that it is cumbersome and time-consuming with low throughput especially when dealing with large breeding populations.

5. Marker-assisted selection of carotenoid-rich cassava

Over the years, conventional breeding has been augmented by various innovative molecular marker-aided techniques. Genetic differences that exist between individual species and organisms represent a genetic marker. Generally, they do not represent the target genes themselves but act as "signposts" or "landmarks" representing DNA along chromosomes. The first marker technologies involved the use of biochemical markers such as isozymes and allozymes. These gave way to the first-generation DNA markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR). Advances in sequencing technology enhanced the use of DNA-sequencing based markers such as SSR and SNP, giving rise to automated high-throughput genotyping [59]. For a genetic marker to be useful, the marker locus has to show experimentally detectable variation among individuals [15, 60]. The variation can be due to single-nucleotide polymorphisms or deletions/insertions or major chromosomal changes. Molecular genetic markers can be used to study the diversity of the observable variation at population or species level [59]. They can also be used to map genomes, identify regions of the genome controlling a trait, and follow a segment of interest of the genome in a plant breeding scheme [59–61].

Molecular markers are usually utilized in a breeding program to facilitate and speed up the selection process as such, carotenoids are boosted through marker-assisted selection (MAS) on target genes [62]. Some of the applications of molecular markers such as RFLP, AFLP, RAPD, SSR in cassava include taxonomical studies, understanding the phylogenetic relationships in the genus, confirmation of ploidy, genetic diversity assessment, and genetic mapping studies in cassava [59], making MAS a reality for application in breeding programs [63]. SSRs have also been used to select for carotenoids in cassava [64]. The reduced cost of the new technologies increases the discovery and utilization of new set of molecular markers that is amenable for the high-throughput genotyping [65].

Recently, single-nucleotide polymorphism (SNP) markers are increasingly being used for genotyping to study gene function. SNPs work as molecular markers that help locate genes associated with a trait and are used for genotype sequencing. SNPs may play a direct role in a trait and affect gene function if they occur within a gene or in a regulatory coding region and thus serve as molecular markers. These markers can be applied in the following: genetic architecture detection, association studies, conservation genetics, genetic diversity, and are fast becoming the marker system of choice in marker assisted plant breeding programs. Some genotyping methods that can specifically genotype an SNP affecting a trait in a collection of population include the use of KASP (competitive allele-specific polymerase chain reaction (PCR) markers, especially for a small number of SNPs [65, 66]. It utilizes a unique form of competitive allele-specific PCR combined with a novel, homogeneous, fluorescence-based reporting system for the identification and measurement of genetic variation occurring at the nucleotide level to detect single-nucleotide polymorphisms (SNPs) or inserts and deletions (InDels) [36, 38]. KASP chemistry provides a versatile choice that can be applied to small- and large-scale projects. It is suitable for use on a variety of equipment platforms and provides flexibility in terms of the number of SNPs and

Marker	MAF	Het	PIC	Trait	*p* value	Marker R^2
PSY2_572	0.81	0.16	0.26	Color chart	3.26×10^{-198}	0.75
				b*	7.38×10^{-199}	0.78
				TC SPEC	1.95×10^{-20}	0.62
				TBC	3.96×10^{-18}	0.57
PSY2_549	0.76	0.25	0.3	Color chart	3.64×10^{-146}	0.63
				b*	7.77×10^{-120}	0.59
				TC SPEC	1.08×10^{-19}	0.59
				TBC	5.58×10^{-17}	0.54
lcyE_1066	0.73	0.32	0.32	TBC	9.63×10^{-04}	0.13
				TC SPEC	0.00322	0.11
				Color chart	0.00262	0.02
				b*	0.01152	0.01
lcyE_1294	0.98	0.04	0.04	Color chart	3.93×10^{-06}	0.03
				b*	1.94×10^{-07}	0.04
lcyE_1015	0.96	0.05	0.07	Color chart	1.36×10^{-04}	0.03
				b*	8.86×10^{-06}	0.04
lcyE_829	0.82	0.19	0.21	Color chart	0.01377	0.01

**MAF–major allele frequency, Het–heterozygosity, PIC–polymorphic information content, Chromameter b*, PSY2–Phytoene synthase2 gene, lcyE–Lycopene epsilon cylase gene, Pulpcol–pulp-color score, TC SPEC–total carotenoid by spectrophotometer, TC–iCheck total carotenoid by iCheck Fluoro, TBC–Total β-carotene.*
*Source: **Table 2** [12].*

Table 2.
Summary results of validated SNP markers on cassava breeding collection.

the number of samples able to be analyzed. To facilitate the selection of carotenoid-rich cassava genotypes, six KASP SNP markers were designed on candidate genes and validated on 650 elite cassava accessions of which PSY2_572 explained most of the phenotypic variation (R^2 = 0.75) in root pulp color (**Table 2**) [12].

Most recent advances in next-generation sequencing technologies have enabled the use of genome-wide SNP markers for genomic selection. The genomic selection tool is believed to significantly increase the efficiency of breeding by increasing the speed and accuracy of selection in a breeding program by predicting the genetic value of individuals at an early selection stage [67]. Genomic selection models have also been implemented by [68], to fast-track the improvement of provitamin A carotenoids in cassava using a total of 23,431 single-nucleotide polymorphic markers.

6. Conclusion

Cassava (*Manihot esculenta* Crantz) is produced globally and a food security crop for many households in sub-Saharan Africa. Commonly available white cassava can provide most of the body's daily energy needs, but it is deficient in vitamin A and some essential micronutrients such as iron and zinc. Vitamin A deficiency makes the body susceptible to infection. Most widely consumed cultivars of cassava are white

or off-white, and the roots are generally low in carotenoids [10]. Although recently, some yellow pulp-colored varieties associated with the presence of carotenoids are being propagated, and it is gradually gaining public acceptance. The nutritive importance of carotenoids is attributed to its conversion to vitamin A when consumed. Numerous genes have been identified in the carotenoids biosynthesis pathway of plants, but studies show that phytoene synthase 2 (*PSY2*), lycopene epsilon cyclase, and β-carotene hydroxylase genes have higher expression levels in yellow cassava roots. So far, the *PSY2* gene has been identified as the key gene associated with increased carotenoids in cassava and has also been tested for its efficiency in breeding [12, 41].

One bottleneck associated with the breeding for increased carotenoids in cassava storage roots is the phenotyping as large populations need to be subjected to selection. The most highly reproducible tool in predicting carotenoids is the high-performance liquid chromatography (HPLC), but its analysis is expensive, costing 50–70 US dollars per sample with very low throughput [57]. Thus, other easy-to-use devices have been accessed for use in phenotyping carotenoids in cassava such as the near-infrared spectroscopy, Chromameter, iCheck Carotene device. These devices have been observed to have high correlation with HPLC, for instance, total β-carotene as quantified by HPLC had high correlation ($r = 0.75$) with total carotenoids quantified using the iCheck device [12].

Also, molecular markers tools such as simple sequence repeats, single-nucleotide polymorphisms and even genomic selection [12, 64, 68] have been employed to speed up the breeding for increased carotenoids in cassava roots.

Acknowledgements

We acknowledge the support of Mr. A. I. Udoh.

Conflict of interest

No conflict of interest.

Notes/thanks/other declarations

Thanks to all coauthors for contributing to the success of this work.

Appendices and Nomenclature

NIRS	Near-infrared spectroscopy
HPLC	High-performance liquid chromatography
TCC	Total carotenoid content
PSY2	Phytoene synthase 2
LCYE	Lycopene epsilon cyclase
MAS	Marker-assisted selection
SSRs	Simple sequence repeats

Author details

Lovina I. Udoh[1*], Josephine U. Agogbua[2], Eberechi R. Keyagha[3] and Itorobong I. Nkanga[1]

1 Faculty of Biological Science, Department of Botany, Akwa Ibom State University, Ikot-Akpaden, Akwa Ibom State, Nigeria

2 Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria

3 Department of Crop Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria

*Address all correspondence to: lovinaudoh@aksu.edu.ng

References

[1] Onyemauwa CS. Analysis of Household Consumption of Cassava Products in Ohaozara, Ebonyi State, Southeast Nigeria. Research. 2010;**2**:1-6

[2] FAO: Agriculture: Why cassava. FAO. 2009. Available from: http://www.fao.org/ag/agp/agpc/gcds/index_en.html. [Accessed 24 September 2016]

[3] Global Cassava Market Is Expected to Successfully Resist the COVID Pandemic. Global Trade Magazine. Available from: https://www.globaltrademag.com/global-cassava-market-is-expected-to-successfully-resist-the-covid-pandemic/. [Accessed 17 April 2022]

[4] World Food and Agriculture – Statistical Yearbook 2021. World Food Agric – Stat Yearb 2021. Epub ahead of print 28 October 2021. DOI: 10.4060/CB4477EN

[5] Ceballos H, Davrieux F, Talsma EF, et al. Carotenoids in Cassava Roots. Carotenoids. Epub ahead of print 14 June 2017. DOI: 10.5772/INTECHOPEN.68279.

[6] Esuma W, Herselman L, Labuschagne MT, et al. Genome-wide association mapping of provitamin A carotenoid content in cassava. Euphytica. Epub ahead of print 9 September 2016. DOI: 10.1007/s10681-016-1772-5

[7] Saltzman A, Birol E, Bouis HE, et al. Biofortification: Progress toward a more nourishing future. Global Food Security. 2013;**2**:9-17

[8] Sánchez T, Ceballos H, Dufour D, et al. Prediction of carotenoids, cyanide and dry matter contents in fresh cassava root using NIRS and Hunter color techniques. Food Chemistry. 2014;**151**:444-451

[9] Ortiz D, Sánchez T, Morante N, et al. Sampling strategies for proper quantification of carotenoid content in cassava breeding. Journal of Plant Breeding and Crop Science. 2011;**3**:14-23

[10] Chávez AL, Sánchez T, Jaramillo G, et al. Variation of quality traits in cassava roots evaluated in landraces and improved clones. Euphytica. 2005;**143**:125-133

[11] Owens BF, Gore MA, Magallanes-Lundback M, et al. A foundation for provitamin a biofortification of maize: Genome-wide association and genomic prediction models of carotenoid levels. Genetics. 2014;**198**:1699-1716

[12] Udoh LI, Gedil M, Parkes EY, et al. Candidate gene sequencing and validation of SNP markers linked to carotenoid content in cassava (Manihot esculenta Crantz). Mol Breed; 37. Epub ahead of print 2017. DOI: 10.1007/s11032-017-0718-5

[13] Olayide P, Large A, Stridh L, et al. Gene Expression and Metabolite Profiling of Thirteen Nigerian Cassava Landraces to Elucidate Starch and Carotenoid Composition. Agronomy. 2020;**10**:424

[14] Carvalho LJ, Agustini MA, Anderson JV, et al. Natural variation in expression of genes associated with carotenoid biosynthesis and accumulation in cassava (Manihot esculenta Crantz) storage root. BMC Plant Biology. 2016;**16**:133

[15] Luo X, Tomlins KI, Carvalho LJCB, et al. The analysis of candidate genes and loci involved with carotenoid metabolism in cassava (Manihot esculenta Crantz)

using SLAF-seq. Acta Physiologiae Plantarum. 2018;**40**:1-11

[16] Nisar N, Li L, Lu S, et al. Carotenoid Metabolism in Plants. Molecular Plant. 2015;**8**:68-82

[17] Isendahl C. The Domestication and Early Spread of Manioc (Manihot Esculenta Crantz): A Brief Synthesis. Latin American Antiquity. 2011;**22**:452-468

[18] Iglesias C, Mayer J, Chavez L, et al. Genetic potential and stability of carotene content in cassava roots. Euphytica. 1997;**94**:367-373

[19] Manu-Aduening JA, Lamboll RI, Ampong Mensah G, et al. Development of superior cassava cultivars in Ghana by farmers and scientists: The process adopted, outcomes and contributions and changed roles of different stakeholders. Euphytica. 2006;**150**:47-61

[20] Olsen KM, Schaal BA. Evidence on the origin of cassava: Phylogeography of Manihot esculenta. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**:5586

[21] The spread of cassava (manioc) in Igboland, south-east Nigeria: a reappraisal of the evidence on JSTOR, Available from: https://www.jstor.org/stable/23317131. [Accessed 29 March 2022]

[22] Rival L, McKey D. Domestication and diversity in manioc (Manihot esculenta Crantz ssp. esculenta, Euphorbiaceae). Current Anthropology. 2008;**49**:1119-1128

[23] Nassar NMA, Junior OP, Sousa MV, et al. Improving carotenoids and amino-acids in cassava. Recent Patents on Food, Nutrition & Agriculture. 2009;**1**:32-38

[24] Cock JH. Cassava: A Basic Energy Source in the Tropics. Science (80-). 1982;**218**:755-762

[25] Alves AAC. Cassava botany and physiology. Cassava: Biology, Production and Utilization. 2002:67-89

[26] Costa AG, Da L, Souza S, et al. Characterization of soils cultivated with cassava under different managements. Brazilian Journal of Agricultural and Environmental Engineering Caracterização de solos cultivados com mandioca sob diferentes manejos. 2021:764-771

[27] Beyene G, Solomon FR, Chauhan RD, et al. Provitamin A biofortification of cassava enhances shelf life but reduces dry matter content of storage roots due to altered carbon partitioning into starch. Plant Biotechnology Journal;**16**. Epub ahead of print 2018. DOI: 10.1111/pbi.12862

[28] Cazzonelli CI. Goldacre Review: Carotenoids in nature: insights from plants and beyond. Functional Plant Biology. 2011;**38**:833

[29] Tracewell CA, Vrettos JS, Bautista JA, et al. Carotenoid Photooxidation in Photosystem II. Archives of Biochemistry and Biophysics. 2001;**385**:61-69

[30] Sun T, Rao S, Zhou X, et al. Plant carotenoids: recent advances and future perspectives. Molecular Horticulture. 2022;**2**:1-21

[31] Bang H, Yi G, Kim S, et al. Watermelon lycopene β-cyclase: promoter characterization leads to the development of a PCR marker for allelic selection. Euphytica. 2014;**200**:363-378

[32] Giuliano G. Plant carotenoids: genomics meets multi-gene engineering.

Current Opinion in Plant Biology. 2014;**19**:111-117

[33] Udoh LI, Gedil M, Parkes EY. Candidate gene sequencing and validation of SNP markers linked to carotenoid content in cassava (Manihot esculenta Crantz). Epub ahead of print 2017. DOI: 10.1007/s11032-017-0718-5

[34] Giuliano G. Provitamin A biofortification of crop plants: a gold rush with many miners. Current Opinion in Biotechnology. 2017;**44**:169-180

[35] Li F, Murillo C, Wurtzel ET. Maize Y9 Encodes a Product Essential for 15-cis-ζ-Carotene Isomerization. Plant Physiology. 2007;**144**:1181-1189

[36] Harjes CE, Rocheford TR, Bai L, et al. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science. 2008;**319**:330-333

[37] Cunningham FX, Gantt E. Genes and enzymes of carotenoid biosynthesis in plants. Annual Review of Plant Physiology and Plant Molecular Biology. 1998;**49**:557-583

[38] Yan J, Kandianis CB, Harjes CE, et al. Rare genetic variation at Zea mays crtRB1 increases β-carotene in maize grain. Nature Genetics. 2010;**42**:322-327

[39] Arango J, Wüst F, Beyer P, et al. Characterization of phytoene synthases from cassava and their involvement in abiotic stress-mediated responses. Planta. 2010;**232**:1251-1262

[40] Welsch R, Arango J, Bär C, et al. Provitamin A accumulation in cassava (Manihot esculenta) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. Plant Cell. 2010;**22**:3348-3356

[41] Ige A, Olasanmi B, Bauchet GJ, et al. Validation of SNP markers for marker-assisted selection of genotypes with increased carotenoid and dry matter contents in cassava. bioRxiv Prepr. 2022:1-23

[42] Morgan NK, Choct M. Cassava: Nutrient composition and nutritive value in poultry diets. Animal Nutrition. 2016;**2**:253-261

[43] Tonukari NJ. Cassava and the future of starch. Electronic Journal of Biotechnology 2004; 7: 0-0.

[44] Ceballos H, Morante N, Sanchez T, et al. Rapid cycling recurrent selection for increased carotenoids content in cassava roots. Crop Science. 2013;**53**:2342-2351

[45] Taleon V, Sumbu D, Muzhingi T, et al. Carotenoids retention in biofortified yellow cassava processed with traditional African methods. Journal of the Science of Food and Agriculture. 2019;**99**:1434-1441

[46] Ssemakula G, Dixon AGO, Maziya-Dixon B. Stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava (Manihot esculenta Crantz). Journal of Tropical Agriculture. 2007;**45**:14-20

[47] Okechukwu RU, AGO D. Genetic Gains from 30 Years of Cassava Breeding in Nigeria for Storage Root Yield and Disease Resistance in Elite Cassava Genotypes. Journal of Crop Improvement. 2008;**22**:181-208

[48] Bradbury JH et al. Chemistry of Tropical Root Crops: Significance for Nutrition and Agriculture in the Pacific. Australian Centre for International Agricultural Research Canberra; 1988

[49] Ukom AN, Ojimelukwe PC, Ezeama CF, et al. Proximate composition

and carotenoid profile of yams (Dioscorea spp.) and cocoyam (Xanthosoma maffa (Scoth)) root tubers from Nigeria. Department of Food Science and Technology, Michael Okpara University of Agriculture; 2014. pp. 1-10

[50] Mackay TFC. Question & Answer Q & A: Genetic analysis of quantitative traits. Journal of Biology:8

[51] Alamu EO, Nuwamanya E, Cornet D, et al. Near-infrared spectroscopy applications for high-throughput phenotyping for cassava and yam: A review. International Journal of Food Science and Technology. 2021;**56**:1491

[52] Pellicer A, del C Bravo M. Near-infrared spectroscopy: A methodology-focused review. Seminars in Fetal & Neonatal Medicine. 2011;**16**:42-49

[53] Abincha W, Ikeogu UN, Kawuki R, et al. Portable spectroscopy calibration with inexpensive and simple sampling reference alternatives for dry matter and total carotenoid contents in cassava roots. Applied Sciences. 2021;**11**:1-11

[54] Abincha W, Kawuki R, Ikeogu U, et al. Comparison of Near-infrared Spectroscopy with other options for total carotenoids content phenotyping in fresh cassava roots. Journal of the Science of Food and Agriculture. 2020;**4**:55-60

[55] de Carvalho RRB, Cortes DFM, e Sousa MB, et al. Image-based phenotyping of cassava roots for diversity studies and carotenoids prediction. PLoS One. 2022;**17**:e0263326

[56] Maroya NG, Kulakow PA, Parkes EY, et al. Quantification of total carotene content of yellow root cassava genotypes using iCheck TM Carotene in comparison with the standard spectrophotometer method Contact. 2006;**57881**

[57] Jaramillo AM, Londoño LF, Orozco JC, et al. A comparison study of five different methods to measure carotenoids in biofortified yellow cassava (Manihot esculenta). PLoS One:13. Epub ahead of print 1 December 2018. DOI: 10.1371/journal.pone.0209702

[58] Belalcazar J, Dufour D, Andersson MS, et al. High-throughput phenotyping and improvements in breeding cassava for increased carotenoids in the roots. Crop Science. 2016;**56**:2916-2925

[59] Ferguson M, Rabbi I, Kim D-J, et al. Molecular Markers and Their Application to Cassava Breeding: Past, Present and Future. Tropical Plant Biology. 2012;**5**:95-109

[60] Okogbenin E, Porto MCM, Egesi C, et al. Marker-Assisted Introgression of Resistance to Cassava Mosaic Disease into Latin American Germplasm for the Genetic Improvement of Cassava in Africa. Crop Science. 2007;**47**:1895

[61] Bernardo AN, Bowden RL, Rouse MN, et al. Validation of molecular markers for new stem rust resistance genes in U.S. hard winter wheat. Crop Science. 2013;**53**:755-764

[62] Tanksley SD, Orton TJ. Isozyme in Plant Genetics and Breeding.

[63] Chen X, Sullivan PF. Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. The Pharmacogenomics Journal. 2003;**3**:77-96

[64] Njoku DN, Gracen VE, Offei SK, et al. Parent-offspring regression analysis for total carotenoids and some agronomic traits in cassava. Euphytica. 2015;**206**:657-666

[65] Yang S, Yu W, Wei X, et al. An extended KASP-SNP resource for

molecular breeding in Chinese cabbage (Brassica rapa L. ssp. pekinensis). PLoS One. 2020;**15**:1-17

[66] Zhao S, Li A, Li C, et al. Development and application of KASP marker for high throughput detection of AhFAD2 mutation in peanut. Electronic Journal of Biotechnology. 2017;**25**:9-12

[67] Crossa J, Pérez P, Hickey J, et al. Genomic prediction in CIMMYT maize and wheat breeding programs. Heredity (Edinb):112. Epub ahead of print 2014. DOI: 10.1038/hdy.2013.16

[68] Esuma W, Ozimati A, Kulakow P, et al. Effectiveness of genomic selection for improving provitamin A carotenoid content and associated traits in cassava. G3 Genes|Genomes|Genetics:11. Epub ahead of print 6 September 2021. DOI: 10.1093/G3JOURNAL/JKAB160