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Breeding for Enhanced β-Carotene Content in Cassava: Constraints and Accomplishments

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This review presents an overview of the importance, constraints, and prospects on different aspects of cassava (Manihot esculenta crantz) breeding for enhanced micronutrient level, including carotenoids (precursors for provitamin-A) and especially beta-carotene. Early cassava-breeding efforts concentrated on crop yield, dry matter, and disease resistance, which are farmer-preferred traits. However, unacceptably high levels of preventable human diseases caused by malnutrition prompted breeders and nutritionists to screen wild relatives and unimproved germplasms (landraces) to increase micronutrient density in staple crops. The ultimate objective is to reduce diseases caused by micronutrient deficiencies. Nigeria, with 140 million people and the largest producer and consumer of cassava in the world, is characterized by rampant malnutrition and high incidence of nutrient deficiency-related diseases. The tuberous root of cassava is low in micronutrients. It is also well known that vitamin A deficiency is primarily caused by dietary inadequacy that results in progressive eye damage and eventually leads to blindness, especially in children. In addition, affected children suffer from a weakened immune system. Present interventions to eliminate this

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deficiency rely on supplementation and food fortification programs, do not reach all those affected and do not get to the root of the problem, which is an inadequate diet. The development of high micronutrient-content cassava varieties (especially, higher β-carotene and other carotenoids) will contribute to a more sustainable solution of the problem of vitamin A deficiency. A current thrust of research (HarvestPlus initiative) is to determine the genetic potential for increasing the concentrations of bioavailable Fe, Zn, and provitamin A carotenoids in the edible portions of several staple food crops including cassava, rice, wheat, maize, and beans. Currently, the International Institute of Tropical Agriculture (IITA), International Center for Tropical Agriculture (CIAT), and National Root Crops Research Institute (NRCRI), Umudike, are working in collaboration to develop an elite cassava gene pool and to develop varieties that will be released to farmers soon in hope of addressing part of micronutrient malnutrition.

KEYWORDS *cassava, breeding, micronutrient, provitamin A, malnutrition*

INTRODUCTION

Cassava (*Manihot esculenta crantz*) is a major source of calories for roughly two out of five Africans (Ezulike et al. 2006). It is estimated that 50% of Nigeria's population (70 million) eats cassava at least once a day (Njoku & Muoneke 2008). It can be eaten in various forms including boiled roots, fufu, and gari. Gari has become a very popular urban food because of its ease in preparation and storage. However, the use of cassava in Nigeria transcends food as many industrial applications have arisen across time in the areas of starch, glucose, and ethanol production. In other countries of sub-Saharan Africa, the same scenario applies to as many as 30% to 70% (ca 400 million people) of the region's inhabitants (FAO 2005). Africa currently accounts for more than 50% of the world's annual cassava output of 184 million tonnes, with Nigeria being the leading producer in the world followed by Brazil, Thailand, Indonesia, Congo DR, Ghana, Tanzania, Mozambique, Madagascar, and Uganda (Osemeobo 1993; http://www.faoststat.org). However, average cassava yields in Nigeria and other African countries are generally below 20 tons/ha, which is much less compared with the potential yields of 80 tons/ha for the crop (Figure 1).

The major factors limiting cassava productivity in Nigeria include: poor crop- management practices, cultivation of genotypes with low root-yielding

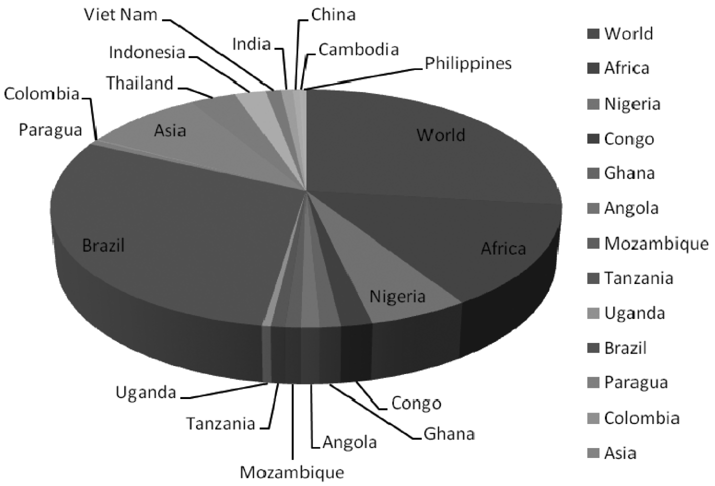


FIGURE 1 Map of cassava production in some selected countries and world (color figure available online).

potential, pests and diseases, poor soil management, declining soil fertility, inadequate use of inputs, and erratic weather conditions, especially in the northern part of Nigeria. Most importantly, areas in Nigeria where cassava is widely cultivated and consumed are characterized by rampant malnutrition because the tuberous roots are low in essential micronutrients such as vitamin A, Fe, and Zn (Table 1). Micronutrient malnutrition among children under five years of age and women in reproductive age is widespread in Nigeria. A U.S. Agency for International Development (USAID) survey showed that a third of Nigerian children under the age of five are deficient in pro-vitamin A (Maziya-Dixon et al. 2006).

Vitamin A deficiency has been identified as a widespread public health problem in 37 countries worldwide, affecting a considerable percentage of the population in northeast Brazil, sub-Saharan Africa, and southeast Asia, where cassava is a staple (Shrimpton 1993). The continued production and consumption of vitamin A-deficient diets results in xerophthalmia, which can range from mild forms of night blindness to ulceration and destruction of the cornea (Shrimpton 1993). A valid strategy to reduce vitamin A deficiency is to enhance the nutritional value of cassava through plant breeding and genetic

TABLE 1 The Burden of Vitamin A Deficiency (VAD) in Nigeria

Target group	Size	Disability adjusted life years
Children < 5	23, 689, 318	891, 280 (33, 600 deaths)
Pregnant women	6, 477, 520	15, 449
Lactating women	3, 637, 821	8, 666

Source: Egesi et al 2010.

transformation (transgenics). The aim of this work was to critically review the importance, constraints, and prospects of cassava breeding for enhanced micronutrient level, including carotenoids (precursors for provitamin-A) and especially beta-carotene.

IMPORTANCE OF CAROTENOIDS

Carotenoids are among the most widespread of all natural pigments and represent one of the largest classes of pigments in nature. The pigments are synthesized in algae, plants, and bacteria (Albrecht et al. 1995), where their colors vary from yellow and orange to red. Carotenoids have a diverse range of functions in plants. In algae and higher plants, carotenoids are essential for photosynthesis as contributors to light harvesting and maintaining the structure and function of photosynthetic complexes (Albrecht et al.1995). Carotenoids quench chlorophyll triplet states, which is essential in preventing chlorophyll from bleaching in high light conditions. Carotenoids are also required for thermal energy dissipation and scavenge reactive oxygen species (Garcia-Asua et al. 1998).

β-carotene, α-carotene, β-cryptoxanthin, lutein, and lycopene are the most common carotenoids found in human plasma. These carotenoids, together with zeaxanthin, have been shown to have health-promoting effects. Structurally, vitamin A (retinol) is essentially one-half of the β-carotene molecule. Consequently, β-carotene has the highest provitaminic potential. It is also the most widespread (Rodriguez-Amaya 2004). The minimum requirement for a carotenoid to have vitamin A activity is an unsubstituted β-ring with an 11-carbon polyene chain. Thus, α-carotene and β-cryptoxanthin exhibit about 50% of the vitamin A activity of β-carotene.

Carotenoids, whether provitamins A or not, have been credited with other beneficial effects on human health; they enhance the immune response and reduce the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract, and macular degeneration. However, other mechanisms have been reported as modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell-to-cell communication, and filtering of blue light.

Pro-vitamin A is essential for optimal growth and differentiation of a number of cells and tissues, excellent sight, intellectual development, and general well-being. Notably during pregnancy and throughout the breastfeeding period, vitamin A has an important role in the healthy development of the fetus and the newborn, with lung development and maturation being particularly important. The German Nutrition Society (DGE) recommends a 40% increase in vitamin A intake for pregnant women and a 90% increase for breastfeeding women. Some years back, Nigeria launched an initiative to promote cassava as a foreign- exchange earner in addition to its key role as the No. 1 staple food crop. The presidential initiative is also promoting cassava as a replacement for imported food items, for example, wheat-flour mills are required by law to blend wheat flour with 10% cassava flour.

On the other hand, increasing the potential of cassava as a source of feeds in the livestock industry and the fast expansion of the domestic livestock sector is anticipated to boost the demand for cassava. The FAO estimated that global cassava utilization as feed remained about 50 million tons in 2002, most of which is concentrated in Latin America and the Caribbean, China in Asia, Nigeria in Africa, and the European Union (EU). The market potential of cassava as food and feed can be enhanced if its vitamin A, zinc, iron, and protein content are improved. The development of high carotene and protein-rich cassava varieties will provide a unique opportunity to increase production and provide highly nutritious animal feed, flour, and chips for the local export markets. The water from processed cassava starch (called *manipueira* in Brazil) from high-carotene (yellow) cassava is very nutritious, serves as a supplementary feed for livestock, and is being used in Latin American countries, especially Brazil (Luiz Carvalho, personal communication).

The importance of high-carotene cassava cannot be over-emphasized. Reports from Morante et al. (2010) and Sanchez et al. (2005) have found that cassava root high in carotene content can reduce or delay the onset of post-harvest physiological deterioration (PPD). It is speculated that the antioxidant properties of carotenoids protect the roots from PPD. They also identified others sources of resistance to PPD that include an interspecific hybird, irradiated materials (where one of the genes involved in PPD was silenced), and spontaneous mutation that was observed as waxy-starch (amylose-free) cassava from a wild species, *M. walkerae,* which is native to the United States. So, there are alternatives available for solving this problem, which will benefit millions of resource-poor farmers across cassava-producing and cassava-consuming countries.

CAROTENOIDS' BIOSYNTHETIC PATHWAY

Carotenoids, like flavonoids, exhibit biological activity of chemo-preventive agents by inhibiting genetic damage, protecting against oxidative damage, increasing metabolic detoxification, restoring tumor-suppressor function, and/or inhibiting oncogene expression and stimulating immune response. Examples of carotenoids include alpha, beta, and zeta carotene, lycopene, phytofluene, phytoene, lutein, zeaxanthin, neoxanthin, viloxanthin, antheraxanthin, and alpha and beta cryptoxanthin. Their polyene structure allows them to absorb light and to quench singlet oxygen-free radicals. This polyene chain, through addition mechanisms, allows the incorporation of free radicals or reactive species, thus slowing their propagation. When this

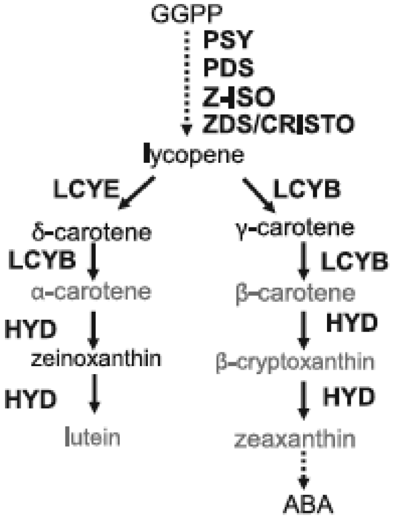


FIGURE 2 Simplified carotenoid biosynthetic pathway in plants (color figure available online).
(Source: Naik et al. 2003)

radical propagation chain is broken, the pigment is destroyed. The antioxidant effectiveness of a carotenoid is determined by the stability of the intermediate formed when a radical is added to the pigment structure. The more stable the intermediate, the more stable the color and the higher the antioxidant activity. However, the sequence for radical-scavenging abilities is canthaxanthin < astaxanthin < echienone < lutein < Zeaxanthin < β-cryptoxanthin < α- carotene < β-carotene < lycopene (Figure 2).

This is a well-studied and characterized pathway (Figure 2). The key alleles controlling beta-carotene have been dissected, hence applied research has a great potential in producing food products with enhanced carotenoid content. The biosynthesis of carotenoids in cassava roots has begun to be studied in detail (Welsch et al. 2010).

Micronutrient Uptake by Plant

Most mineral micronutrients usually are present in the soil in adequate to excess amounts. Deficiency is caused by their presence in an unavailable form rather than by their lack, and a plant can improve its iron or zinc uptake by using strategies to solubilize such nutrients present in the soil. For the most part, plants acquire micronutrients by absorbing them from the soil solution; therefore, the availability of mineral micronutrients to plants is closely related to the solubility of the forms in which they appear. Several environmental factors can affect the solubility of micronutrients. Leached, acid, sandy soils, organic soils, soils that have supported intensive cropping, soils with high pH, and eroded soils all tend to be low in available carotene, iron, and zinc. Uptake efficiency of soil-grown plants may consist of increased capacity to solubilize non-available nutrient forms into forms available to the plant, and/or increased capacity to transport nutrients across the plasma membrane. However, it appears that increased conversion capacity is of greater importance for efficient uptake, especially for nutrients transported to roots by diffusion.

The recommendations of the German Nutrition Society (DGE) for the daily intake of vitamin A for children vary between 0.6 mg and 1.1 mg; for adults between 0.8 mg and 1 mg; and for breastfeeding women 1.5 mg. Daily vitamin A intakes are notably insufficient in children and adolescents, in particular when recommended intakes of β-carotene are not being reached. Agriculture has been seen as an instrument to improve public health by using a food-systems approach to deliver more nutritious staple food to the resource poor.

REASONS FOR CONCERN

Malnutrition is a growing problem among low-income earners in developing countries (Maziya-Dixon et al. 2006). More than three billion people currently suffer from micronutrient malnutrition (Graham, Welch, & Bouis 2004). Iron deficiency may affect 3 billion people worldwide (Long, Bänziger, & Smith 2004). It was also estimated that 49% of the world's population is at risk for low zinc intake (Cichy et al. 2005), whereas vitamin A deficiency affects $\geq$ 140 million children under the age of five. These micronutrient deficiencies were concentrated in the semi-arid tropics, particularly in south and southeast Asia and sub-Saharan Africa (Reddy, Ramesh, & Longvah 2005). As the world grapples with a rampaging financial crisis (economic meltdown), it has been disclosed that the world loses about $168 billion (N1 trillion) annually to blindness. An estimated 180 million people are blind across the globe, and about 3.1 million of these live in Nigeria.

A survey conducted in Nigeria from 2004 to 2007 revealed that the highest numbers of blind people were in the northwest geopolitical zone with 320,000 blind people. The northeast had 220,000, whereas 180,000 people were blind in north-central. The southwest had 150,000 blind people. The southeast had 120,000, whereas the south-south Nigeria also had 120,000 blind people (http://www.tribune.com.ng/06102008/news/news4.html). Attempts have been made to alleviate these deficiencies by the use of supplements and food fortification, but these strategies do not reach all those suffering from these deficiencies and have not proven to be sustainable.

Green revolution cropping systems may have led to some unforeseen negative impacts on human nutrition and health. For example, in south Asia, the introduction of high-input modern wheat/rice cropping systems is associated with trends in the growth of Fe- deficiency anemia among poor pre-menopausal women, and negatively correlated to dietary- Fe density.

Data from China, sub-Saharan Africa, South America, middle America/Caribbean and southeast Asia also show the same trends. This has become a major concern for experts and stakeholders in biofortification and nutrition. In Nigeria, one of the most important staple crops is cassava. However, popular cassava varieties grown in Nigeria have very low levels of beta-carotene. Given that cassava constitutes the daily meals of many poor people who cannot afford expensive alternative sources of vitamin-A (or its precursors), bio-fortified cassava with elevated levels of beta-carotene would provide a sustainable approach to alleviating this deficiency.

BREEDING OPTIONS TO ENHANCE BETA-CAROTENE CONTENT IN CASSAVA AND CHALLENGES

Rapid techniques for screening cereals, legumes, and tubers for minerals and careteniods are currently being developed, validated, and implemented at various CGIAR centers and national research institutes.

The availability of genetic variation for micronutrient density is essential for determining the feasibility of achieving meaningful increments through conventional breeding. There is sufficient genetic variation among the wild relatives and landraces. They can be collected and evaluated, and breeders can exploit additive gene effects, transgressive segregation, and heterosis to improve micronutrient density (Iglesias et al. 1997). When the required genetic variation is not available, transgenic approaches can provide additional sources of variation.

In the future, breeding will likely combine both conventional and transgenic approaches (Welsch et al. 2010). Screening objectives entail assaying representative samples of the genetic diversity for micronutrient density available in breeding programs and gene banks. Agronomic and end-use quality features of trait-source genotypes will also be evaluated. So far, only a relatively small portion of the existing genetic diversity for micronutrients, anti-nutrients, and promoters has been assayed.

Micronutrient concentrations are affected by numerous factors, such as macro- and micro-environmental variation, genotype-by-environment (G × E) interaction, and germplasm type. Ranges in micronutrient concentrations reported in the literature reveal significant genetic variation for numerous crops including cassava (Chavez et al. 2005). Maximum micronutrient levels are frequently present in genetically distant sources such as wild relative or landraces. Accessing the genetic variation present in these sources and transferring it to adapted genetic backgrounds usually require pre-breeding and parent development, depending on the extent of "linkage drag," which increases product-development time.

The potential for rapid increase in carotene content in cassava roots through cycles of recurrent selection is possible. Previous efforts were able to increase the concentration from 0.42 mg/100 g of fresh roots in the base population to 1.34 mg/100 g after two cycles of selection and recombination (Jos et al. 1990; Sanchez et al. 2005). However, there is scanty information on genetic variability for beta-carotene. Researchers have given various reasons for the lack of information. Hershey and Ocampo (1989) and Iglesias et al. (1997) suggested that the inheritance of root color, and therefore carotene content, was complex. Although major genes dominate the transport and accumulation of carotene in the roots, the quantitative variability observed within root color classes suggested that a number of genes with smaller effects are involved in the accumulation process. Therefore, there is good scope of achieving maximum levels of expression through recurrent selection (Jos et al. 1990). Thus, there is a need to screen the available germplasm to determine its variability for beta-carotene and other quality traits.

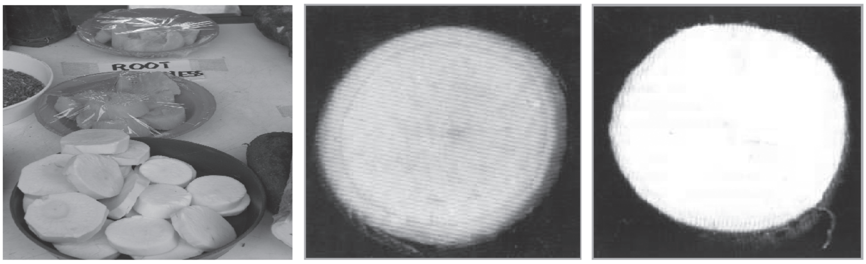
β-carotene enrichment does not reduce yield potential (Graham, Welch, & Bouis 2002; Chavez et al. 2005). Moreover, high-carotenoid content may offer the advantage of extended shelf life of the roots (Morante et al. 2010). The National Root Crops Research Institute (NRCRI) in Umudike, Nigeria, has been involved in activities aimed at developing varieties that will meet the needs of poor farmers who depend on cassava for their livelihoods. NRCRI is a collaborating institute in the Harvest-Plus Challenge Programme, a consortium of stakeholders involved in mitigating micronutrient deficiency globally since 2003. A threshold of 15 μg beta-carotene per gram of fresh root has been set as a goal for cassava from the nutritional standpoint.

The NRCRI, in close collaboration with International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, and the International Center for Tropical Agriculture (CIAT) in Colombia, has taken giant initiatives in the development of biofortified cassava. Advances have been made in enhancing the levels of beta-carotene in adapted varieties. Cassava with high beta-carotene content tends to have yellow fresh roots and positive correlation between root color and carotene content has been reported (CIAT 2004 2008). In 2010, CIAT reported the identification of four genotypes with more than 15 μg β-carotene (H. Ceballos, personal communication).

CONCLUSION

Four main approaches have been used by breeders and nutritionists to prevent micronutrient deficiencies: dietary diversification, food fortification, supplementation, and biofortification. The later three approaches are relatively cost-effective, but have failed to completely eradicate the problem for a variety of reasons (West 2003). Biofortification is a new approach that relies on conventional plant breeding and modern biotechnologies to increase the micronutrient density of staple crops. It holds great promise for improving the nutritional status and health of poor populations in both rural and urban areas of developing countries like Nigeria. Plant breeding to increase micronutrient density gained legitimacy when deficiencies in micronutrients

Cassava Plants Cassava roots Cassava processed products



Assorted cassava root colors Yellow root cassava White root cassava

FIGURE 3 Cassava plants and root products available to farmers in Nigeria (color figure available online).

such as Fe, I, Zn, and vitamins were recognized as an issue of overwhelming global public health significance and one of the major development challenges of the 21st century. Good nutrition is a fundamental component of medical care, and an individually designed nutritional program can boost immune function, increase the efficiency of other medical treatments, improve energy levels, as well as enhance a general quality of life. Scientific reports support the view that increased beta-carotene intake from staple food significantly increases the total white blood cell count, thereby improving the survival rate in HIV-positive people. Nigeria, with over 140 million people and the largest producer and consumer of cassava, should not lag behind in this new global trend. Moreover, yellow processed cassava (Gari) has a higher price premium than white or intermediate color gari by 30%–50% in most cassava markets in Nigeria (Figure 3).

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