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Biofortification of cassava for Africa: the BioCassava Plus program

Richard T. Sayre
Director, BioCassava Plus, Donald Danforth Plant Science Center, St Louis, MO, USA

Correspondence address: Dr Richard T Sayre, BioCassava Plus, Donald Danforth Plant Science Center, 975 N. Warson Rd., St Louis, MO 63132, USA. Email: rsayre@danforthcenter.org

In sub-Saharan Africa starchy root-crop cassava ranks first in total production and second to maize as source of dietary energy. Among the world's staple crops cassava has the lowest protein/energy ratio (7.4 mg protein/cal compared to 26 mg protein/cal for maize) and is deficient in iron, zinc, pro-vitamin A or β-carotene and vitamin E. The BioCassava Plus program, aiming at completion in 2017, seeks in its present phase, to deliver fortification in respect to iron and pro-vitamin A.

Introduction

In many regions of the world over 30% of the population have insufficient calories in their diet to meet their nutritional needs. By 2020, the FAO has estimated that one billion persons will be undernourished or receive insufficient calories in their diet. Nowhere is this problem more severe than in sub-Saharan Africa. In addition to receiving insufficient calories in the diet, many subsistence farmers depend on a single staple crop for most of their calories. Some of these staple crops may not provide a balanced or complete supply of micronutrients and vitamins leading to malnutrition. Globally, vitamin and mineral deficiency affects one third of all people (Micronutrient Initiative, 2004). One mechanism to quantify the impact of malnutrition on human populations is the disability-adjusted life years or DALY. A DALY is the sum of years of life lost due to preventable death and years lost due to disability or disease. Among the micronutrient deficiencies, vitamin A, iron, zinc, and iodine deficiency affect the most persons globally resulting in the loss of over 53 million DALYs per year. Sub-Saharan Africa's share of this total is 55%.

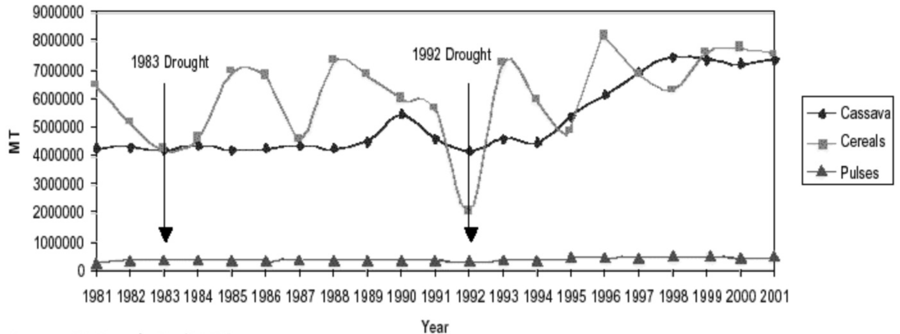
Malnutrition has the greatest impact on the health and development of children under the age of five (Pelletier et al., 1995). It is estimated that malnutrition directly contributes to the death of over one million children per year and contributes to half (5 million) of all childhood (ages 1-5 years) deaths (FAO database). Malnourished children may have impaired immune systems and a reduced ability to fend off disease. Malnutrition also has an impact on growth and development of young children. Significantly, the effects of childhood malnutrition may not be reversed by administration of sufficient nutrients as adults. The permanent physical and mental impairments resulting from childhood malnutrition are an economic burden on the economies of many developing countries associated with reduced physical and mental performance and care for those who are ill.

Acute incidences of under-nourishment and malnutrition are often associated with crop failures. The farming infrastructure in many developing countries is particularly sensitive to disruptions due to a lack of supporting infrastructure to address acute and chronic stresses on agriculture. In sub-Saharan Africa the majority of farmers are subsistence farmers. Subsistence farming is characterized by a heavy reliance on manual labor, small farm sizes, and a lack of fertilizers, irrigation and pesticides. If at any time there is a crop failure or production and harvesting are disrupted, the family may experience an immediate food crisis. These situations are exacerbated at times during the year when reserves are depleted, often just prior to each year's harvest of the major staple crop when reserves are low. During a food crisis, the immediate response is to provide food aid as a short-term solution. As a long-term solution, however, providing food aid at or below local market prices disrupts local economies and may be a disincentive to farm or invest in agriculture. The more sustainable long-term solution to under-nourishment or malnutrition is investment in productive, sustainable agriculture systems that provide greater food security and better nutrition.

Cassava

To address effectively the issue of agricultural development in sub-Saharan Africa it is critical to recognize what are the farmer- and consumer-preferred crops and most importantly the staple crops that provide the majority of calories in the diet. In sub-Saharan Africa, the starchy root-crop cassava ranks first in total productivity and number two behind maize as a source of calories (Dixon et al., 2003). According to the FAO, over 66 million metric tons of cassava were harvested in sub-Saharan Africa in 2003, providing on average 270 calorie per capita per day or about 16% of the total caloric need for an adult (FAO database). Cassava consumption is not equally distributed throughout Africa. The largest per capita consumers are largely in west Africa and include Nigeria, the Democratic Republic of Congo and Ghana.

Figure 1: Total output for crops in five cassava-producing, drought-affected southern African countries from 1981 to 2001.



Source: FAO website (2002)

Cassava was brought to Africa in the 16th century from Brazil by Portuguese sailors and rapidly spread throughout the continent. Cassava can potentially produce 90 tons/hectare/year when grown under ideal conditions. In sub-Saharan Africa yields (10-15 tons/ha/yr) are considerably less, however. Among the world's major crops, cassava ranks fifth in direct human consumption and is an important source of calories for over 800 million persons (FAO database).

Cassava is valued in Africa in part for the food security it provides. Cassava is drought tolerant, grows in poor soils, requires less labor than other crops, and the roots may be banked in the soil for up to three years (Figure 1). In addition, cassava has toxic levels of cyanogenic glycosides in its leaves which deter herbivory. The roots typically have a 20-fold lower cyanogen content than leaves. So-called sweet (low cyanogens varieties) varieties may be consumed directly with little processing but high cyanogen (bitter) varieties must be extensively processed (3-7 days) to remove the cyanogens prior to consumption.

Among the world's staple crops cassava has the lowest protein/energy ratio (7.4 mg protein/cal compared to 26 mg protein/cal for maize) and is deficient in iron, zinc, pro-vitamin A or β-carotene and vitamin E (FAO database). The levels of these nutrients will also vary depending on how the food is prepared (Table 1). Cassava has a number of additional unique constraints as a staple crop. Several viral diseases in Africa including cassava mosaic disease (CMD) and the more recently emerging cassava brown streak (CBS) disease reduce yields up to 30-50%. In addition, once

Figure 2: Cassava leaves, roots, stems and a farm in Malawi. Photo courtesy of Dr. Mark Manary.

Table 1: The nutritional qualities of cassava foods in a 500 gm meal (gfw = g fresh weight; gdw = g dry weight). The recommended minimum daily intake for an adult established by the WHO (Mark Manary). Assumed a 12:1 β-carotene to retinol (vitamin A) conversion ratio.

Cassava meal	**Energy**	**Protein**	**Iron**	**Zinc**	**Vitamin E**	**β-carotene, pro-vitamin A**
Recommended minimum daily (RMD) intake for an adult	1700-2400 (Kcal)	50-80 (g)	18 (mg)	12 (mg)	8 (mg)	11 (mg)
Boiled (500 gfw) (% RMD)	740 (~36%)	5.5 (~9%)	2.0 (11%)	2.0 (17%)	1.0 (13%)	1 (9%)
Dry (500 gdw)	1775 (~87%)	10.5 (~16%)	4.0 (22%)	4.0 (34%)	1.0 (13%)	2 (18%)
Flour (500 gdw)	1710 (~83%)	7.5 (~12%)	4.0 (22%)	3.0 (25%)	1.0 (13%)	0 (0%)
Fresh (500 gfw)	745 (~36%)	6.0 (~9%)	2.0 (11%)	2.0 (17%)	1.0 (13%)	1 (9%)
Roasted (500 gdw)	1360 (~66%)	10 (~15%)	2.5 (13%)	3.0 (25%)	1.0 (13%)	1 (9%)

harvested cassava roots have a very short shelf life (1-2 days) limiting the cultivation area that can be harvested to that amount of root material that can be immediately processed before the roots rot.

Cassava is often called an orphan crop. This designation is applied to those crops for which there has been limited investment in crop improvement either through genetics or agronomic programs. It has been estimated that in Africa cassava has achieved only 10% of its yield potential. Over the last 40 years, development of advanced cassava breeding systems and agronomy has largely been the mandate of three institutions, the International Center for Tropical Agriculture (CIAT) in Cali, Colombia, the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria, and EMBRAPA, the Brazilian agriculture research agency. These groups have generated improved yielding and disease resistant varieties for various regions of the world. Cassava breeding, however, can often be challenging. Many varieties do not flower and those that do flower produce fewer than 200 seeds per plant. Importantly, cassava is not propagated by seed on farms. It is clonally propagated by stem cuttings, that limits plant multiplication by farmers to 10 per plant per year, a multiplication ratio substantially less than seed crops such as maize.

Cassava biofortification

In 1988, the first coordinated effort to bring the tools of modern plant biotechnology to focus on cassava improvement was initiated at CIAT with the holding of the first Cassava Biotechnology Network (CBN) Meeting (Thro et al., 1998). The CBN was the inspiration of Dr. William Roca. It was recognized that given the clonal nature of cassava propagation and the highly heterozygous nature of different cassava cultivars that introduction of transgenes for crop improvement held great potential for cassava improvement. In 1996, the first report of a transgenic cassava was made by Dr. Claude Fauquet's and Dr. Ingo Potrykus' laboratories using transgenes expressing visible marker phenotypes (Li et al., 1996; Schopke et al., 1996). In 2003, the first transgenic cassava plants with improved agronomic traits (reduced cyanogens and elevated starch yields) were reported by Dr. Richard Sayre's lab (Siritunga et al., 2003). The timing of these events was fortuitous. In 2004, the Bill and Melinda Gates Foundation (BMGF) announced the Grand Challenges in Global Health Program (web site). There were 14 Grand Challenges focused largely on eradication of diseases in the developing world. Grand Challenge 9 focused on providing complete nutrition in a staple crop. Four independent programs focusing on the biofortification of rice, banana, sorghum and cassava were funded through the Grand Challenges in Global Health Program. Each of these teams used the tools of modern biotechnology to address the major nutritional constraints for each crop. The BioCassava Plus program funded by the BMGF is the largest coordinated research development and deployment program funded for cassava to date. A critical feature of the Grand Challenges Program was bringing new innovative technologies to solve problems in cassava biology. Over 25 research investigators located on five continents were brought together to develop an integrated research discovery, development and deployment plan (Figure 3).

The overall objectives of the BioCassava Plus Program were to provide the complete minimum daily requirements for protein, iron, zinc, vitamin E and pro-vitamin A in a 500 gm meal for an adult or a 250 gm meal for a child. Significantly, a 500 gm meal provides 80% of the daily caloric requirement for an adult. In addition to the biofortification traits, three important value-added constraints were addressed

Figure 3: The BioCassava Plus Team, 2010. DDPSC, Donald Danforth Plant Science Center; KARI, Kenyan Agricultural Research Institute.

Director
Product Development Manager
Program Oversight Committee

Engineering and Transformation Groups
Richard Sayre, DDPSC
Claude Fauquet, DDPSC
Nigel Taylor, DDPSC
Dan Shachtman, DDPSC
Ed Cahoon, DDPSC
Willi Gruissem, ETH, Zurich
Peng Zhang, Singapore
Joseph Ndunguru, Tanzania

Post-Harvest Physiology Group
John Beeching, Bath Univ, UK
John Fellman, Washington St. Univ.

Plant Breeding and Mapping Groups
Martin Fregene, DDPSC
Hernan Ceballos, CIAT, Colombia

Administration
Shantha Pieris, DDPSC

Biosafety, Regulatory, and IP
Paul Anderson, DDPSC
Jeff Stein, Independent consultant
Scott Shore, independent consultant

Field Trial Assessment
Ivan Ingelbrecht, IITA, Nigeria
Caroline Herron, IITA, Tanzania
Alfred Dixon, IITA, Nigeria
Simon Gichuki, KARI, Kenya
Ada Mbanaso, NRCRI, Nigeria
Dimuth Siritunga, Univ. Puerto Rico
Chiedozie Egesi, NRCRI
Sally Mallowa, KARI

Human Nutrition Assessment
Mark Manary, Washington Univ.
Bussie Maziya-Dixon, IITA, Nigeria

using molecular techniques. These traits include virus susceptibility, toxic levels of cyanogens, and short shelf life. Finding solutions to these constraints were considered important economic drivers for adoption and acceptance of biofortified cassava by farmers and consumers.

Traditional breeding strategies had lead to the development of cassava mosaic disease virus resistance, elevated pro-vitamin A biofortification, and extended shelf life (Hernan Ceballos) but had lead to no improvement in iron, zinc, vitamin E, and protein content or reduced cyanogens levels (Reilly et al., 2003; Siritunga et al., 2007). Furthermore, it was concluded by cassava breeders in the Harvest Plus program, also funded by the BMGF, that there was insufficient genetic variation in cassava to reach the target biofortification objectives for iron (40 ppm dry weight) and zinc (40 ppm dry weight). Thus, transgenic approaches seemingly offered the only solutions for many of the biofortification objectives. Fortunately, cassava is well suited for improvement through transgenic approaches (Takeshima, 2010). As previously stated, cassava is cultivated clonally using genetically uniform material as stem cuttings. In addition, the lack of meiotic cell divisions that occur during the process of sexual reproduction reduces the likelihood of transgene silencing. Finally, cassava is not native to Africa and so the likelihood of transgene flow to wild relatives was minimal.

By the fourth year of the project (2009) it was evident that molecular solutions had been identified that met the target objectives for iron biofortification (Sayre lab); pro-vitamin A and vitamin E biofortification (Cahoon lab); protein biofortification (Fauquet and Sayre labs); extended shelf life (Cahoon, Fregene and Sayre labs); virus resistance (Fauquet, Gruissem and Zhang labs) and reduced cyanogens (Sayre lab). Only the zinc biofortification transgenic plants had significant secondary phenotypes (zinc deficient leaves) that impaired yield. As of 2010, over 1200 transgenic plants were in field trials in Mayaguez, Puerto Rico (Dr. Siritunga). In addition to quantifying the primary trait performance characteristics, the field trials focused on assessing the effects of transgene expression on crop yield and plant performance. The decision to stage the primary field trials in Puerto Rico was made to insure that uncertainties regarding approval of field trials in Nigeria or Kenya would not delay the program and importantly would provide supporting data for field trial applications made by our African partners in Nigeria and Kenya. As a measure of the success of the program, the first application for transgenic crop field trials in Nigeria was approved in 2009 for a pro-vitamin A biofortified cassava at the National Root Crops Research Institute (NRCRI) in Umidike, Nigeria (Mbanaso, Egesi, Fregene, Cahoon). Subsequently, iron biofortified plants were also approved for field trials in Nigeria (Mbanaso, Egesi, Fregene, Sayre).

Recognizing the fact that substantial progress was being made, the BMGF provided supplemental funding in 2008, to accelerate the translational aspects of the program and to bring transgenic crops to Africa at an accelerated pace. In addition, biosafety studies (Dr. Paul Anderson), human nutrition studies (Dr. Manary, Dr. Maziya-Dixon, Dr. Egesi and Ms. Malowa) and *ex ante* economic analyses (Dr. Jack Fiedler and Dr. George Norton) were initiated. The results of these studies indicated that the benefit cost ratio (dollars health imports/dollars intervention development, production and delivery costs) of a biofortified cassava with the pro-vitamin A trait

(23) was nearly twice as high as vitamin A intervention using oral vitamin delivery. The cost for each DALY saved by consumption of cassava biofortified with only pro-vitamin A was estimated to be $58, while a protein, iron and vitamin A biofortified plant would have a cost of $17/DALY saved. These costs are well below the annual income of Nigerians, the standard by which an intervention is considered economically justifiable. The supplemental funding also supported efforts to identify the farmer-preferred cultivars in Nigeria and Kenya and to develop transformation systems for these cultivars (Mbanaso, Maya-Dixon, Manary, Egesi, Mallowa, Gichuki, Fregene, Taylor and Sayre labs). The selection of the farmer-preferred cassava cultivars was made by engaging Nigerian and Kenyan agronomists and cassava breeders. Plants were subsequently collected and sent to the enabling technologies group (Taylor and Sayre labs) to develop robust genetic transformation systems. Biofortification of farmer-preferred cultivars is currently underway and is expected to increase the likelihood of adoption and acceptance of biofortified cassava by farmers and consumers.

Importantly, the supplemental funding also supported the training of African scientists in labs doing cassava transformation, phenotypic analyses and conducting confined field trials. BioCassava Plus's long-term objectives are to engage African scientists in the construction of biofortified cassava in Africa using farmer-preferred cultivars. African ownership and engagement in the project is critical for adoption, acceptance and longer term development of improved cassava.

At this juncture, BioCassava Plus is entering phase II. Phase II will focus largely on product improvement and delivery. Our objectives will include trait stacking (pro-vitamin A and iron), human nutritional efficacy testing of biofortified cassava, and passing regulatory approval in Nigeria and Kenya. Dr. Martin Fregene will be directing BioCassava Plus phase II. Our current target is for regulatory approval and dissemination of our first products to farmers by 2017. These improved varieties will be distributed to farmers for humanitarian purposes either free or at a small cost to cover their propagation and dissemination. Ultimately, only follow up, post ante, impact analysis studies will determine the effectiveness of cassava biofortification on the health and development of children and subsistence farmers in sub-Saharan Africa.

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