Status of biofortification in tropical root and tuber crops

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Hidden hunger is a form of malnutrition, afflicting one-third of the world's population. It is caused due to the lack of micronutrients, mainly iron, zinc and vitamin A, in the human diet and can lead to mental impairment, poor health, low productivity and even death. It is common in many developing and developed countries. A change in research focus from increased agricultural production of calorie-rich staple crops to nutrient-dense staple crops is crucial to address the above problem. Biofortification is a process of increasing the density of vitamins and minerals in a crop through plant breeding, transgenic or recombinant DNA technology or agronomic practices. Biofortification through breeding has been taken up as a challenge by HarvestPlus for cassava and sweet potato, which has resulted in the release of many biofortified varieties that could fight hidden hunger and ensure food security in many Sub-Saharan African countries. The BioCassavaPlus project adopted transgenic strategies for biofortification in cassava. Transgenic approaches serve as an alternative for biofortification in sweet potatoes. HarvestPlus has not included yam in its biofortification programme, though increasing the provitamin A carotenoid content of yam is much needed. Bioavailability of micronutrients has been thoroughly studied in sweet potatoes. In India, the ICAR-Central Tuber Crop Research Institute (CTCRI), Thiruvananthapuram has been involved in the biofortification of tropical tuber crops and has released many biofortified varieties in sweet potato, cassava and yam. In a collaborative work plan with CIP, ICAR-CTCRI is at present involved in the development of biofortified varieties of sweet potato. The need to release and adopt transgenic biofortified crops is discussed here, as sweet potato is a naturally transgenic crop.

Keywords: Biofortification, hidden hunger, nutrient-smart agriculture, transgenic crops, tubers.

HIDDEN hunger or micronutrient malnutrition, one of the serious global threats affecting a third of the world's population, is caused by deficiencies of iron, zinc, vitamin A and vitamin B9 (folate) in the human diet[1,2]. This is widespread in many developing and developed countries and manifests itself in the form of congenital disabilities, cancer, cardiovascular disease, osteoporosis, neurodegenerative disorders, etc.[3–6]. Vitamin A deficiency (VAD) is widespread in low-income countries in the tropics[7], which has led to the development of biofortification of plant foods with provitamin A carotenoids, resulting in 'golden' crops. Carotenoids such as α-, β- and γ-carotenes and β-cryptoxanthin are converted by the body into vitamin A retinol or provitamin A[8,9], which along with lutein and zeaxanthin is critical for eye health and has antioxidant and potentially chemopreventive effects[10].

To address the above problem, our research focus needs to shift from increased agricultural production of calorie-rich staple crops to nutrition-smart agriculture[11,12]. Biofortification or 'biological fortification' is a process of increasing the density of vitamins and minerals in a crop through plant breeding, transgenic or recombinant DNA technology[13,14] or agronomic practices[15] so that when consumed regularly, they will generate measurable improvement in vitamin and mineral nutritional status.

Biofortification can complement existing measures such as supplementation and industrial food fortification. The advantage of biofortification is its long-term cost-effectiveness and accessibility to underprivileged rural populations to meet the specific nutritional needs of women and children. Biofortification through conventional plant breeding is a quality breeding strategy aimed at increasing the levels of provitamin A, iron and zinc in major food crops to reach 50% of their respective recommended daily allowances (RDAs)[16]. The biofortification process involves screening germplasm for available genetic diversity, pre-breeding parental genotypes, developing and testing germplasm with high micronutrient content, conducting genetic studies, and developing molecular markers to reduce costs and improve breeding. Once micronutrient traits are included in the core breeding objectives of national and international crop improvement programmes, the research expenditure of agricultural research institutions for monitoring and maintenance are negligible. The HarvestPlus programme was launched by the Consultative Group for International Agricultural Research (CGIAR) organizations to breed biofortified staple food crops with a goal to reach 100 million people by 2020 and a billion people by 2030 by boosting three key nutrients – vitamin A, iron and zinc. The programme targeted staple crops like wheat, rice, maize, cassava, pearl millet, beans and sweet potato in Asia and Africa[17].

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The main challenges faced by HarvestPlus were combining high nutrient density with high yields and high profitability (breeding success), the efficacy of biofortified crops with regard to nutritional status upon adoption, and scaling up the adoption of biofortified crops for a positive health impact on millions of people.

Agronomic biofortification is the enrichment of micronutrients (Fe, Zn, Cu, etc.) in edible plant parts through agronomic management methods such as fertilizers and/or foliar sprays, nanofertilizers, etc.[18].

The strategy of transgenic biofortification aims to increase the micronutrient content of crops by inserting genes from other species to produce transgenic crops, when natural variation in sexually compatible germplasm is insufficient to achieve satisfactory micronutrient levels[19,20]. Although transgenic biofortified crops involve significant development and regulatory costs, they are cost-effective in the long run compared to conventional or complementary approaches[21].

Tropical root and tuber crops such as cassava, sweet potato, yam, taro, elephant foot yam, and other minor tuber crops are a staple food in 29 countries, including tropical Africa, Oceania, and the Caribbean islands, and a secondary staple food in 25 other countries, out of a total of 236 entities of the world[22]. They provide food for about 2.2 billion people in the world and also contribute to animal feed and industry. The CGIAR research centres working on tuber biofortification are International Center for Tropical Agriculture (CIAT) (vitamin A cassava) and International Potato Centre (CIP) (vitamin A orange-fleshed sweet potato) in close collaboration with national programmes and national partners in many African countries, particularly Uganda, Mozambique and Democratic Republic of Congo (DRC).

Cassava

Cassava (*Manihot esculenta* Crantz) is a perennial root crop and a staple food for approximately 600 million people worldwide in developing countries. The starchy roots provide energy and are eaten boiled or processed in different ways for obtaining native or fermented starches, as dried chips, and as meals or pellets for animal feed. Cultivars with <50 mg kg^{-1} fresh weight (FW) cyanogenic glucosides (CGs) in the roots are considered 'sweet' and those above this value as 'bitter'. Other relevant root traits are the percentage of dry matter, amylose, protein and carotenoid. Yellow cassava roots are rich in carotenes[23].

Cassava germplasm is naturally rich in provitamin A (0–19 ppm), proteins (6–8%) in landraces from Central America[24], minerals (iron and zinc) in low concentration (<10 ppm) are used for breeding of biofortified cassava[25,26]. The cultivar 'Amarelinha do Amapá', with *β*-carotene of 27 mg, 100 mg^{-1}, 50-fold higher than other cassava cultivars, is a rich source of *β*-carotene for breeding[27]. To address VAD in Sub-Saharan Africa (SSA)[28], HarvestPlus set 15 μg/g provitamin A carotenoids on FW basis as the breeding target to provide 50% of the mean RDA through normal preparation and consumption habits. In collaboration with International Institute of Tropical Agriculture (IITA), Nigeria, HarvestPlus released six vitamin-A fortified varieties in Nigeria and one in the DRC. In India, research on tropical tuber crops is being done under the aegis of the Indian Council of Agricultural Research (ICAR) at the Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala. Research is underway to release biofortified golden cassava or yellow cassava (17S-325) in India with good culinary quality[29]. Sree Vishakam is a yellow cassava variety released by ICAR-CTCRI in 1977 with carotene 466 IU/100 g in its tubers.

The main challenge in cassava breeding is the combination of high *β*-carotene and starch content as these traits are negatively correlated with a 50–60% decrease in dry matter in the maximum carotenoid-accumulating storage roots of different cultivars[30,31]. Similarly, combining multiple trait genes through conventional breeding is difficult in cassava due to high heterozygosity. Therefore, 'stacking' beneficial trait genes through a biotechnology approach was selected as a component of the BioCassava Plus (BC+) programme by the Grand Challenges in Global Health initiative[32,33]. The target was 40 μg/g provitamin A carotenoids on dry weight (DW) basis in the storage roots to meet 100% RDA of vitamin A by a two-year-old staple cassava consumer, and increasing minerals zinc and iron along with reduced cyanogen content, delayed postharvest deterioration and development of virus-resistant varieties through adoption and acceptance of biofortified cassava using a multidisciplinary approach[13,33,34]. In this context, cassava enriched with provitamin A was developed according to the principle of 'Golden Rice'[35]. Co-expression of the *crtB* gene and a gene for 1-deoxy-D-xylulose 5-phosphate synthase (DXS) from *Arabidopsis* under the control of separate patatin promoters resulted in maximum carotenoid accumulation of 40–60 μg/g DW in cassava storage roots compared with a nontransformed control, whereas expression of a bacterial *crtB* gene for phytoene synthase (PSY) alone with a potato patatin type-1 promoter resulted in a carotenoid concentration of ~25 μg/g DW in the roots[32]. Overexpression of a PSY transgene increased the carotenoid level (6.7 μg/g DW)[36]. Expression of the cauliflower mutant orange (*Or*) gene resulted in very low carotenoid levels (3–4 μg/g DW) in cassava[37]. A 85–90% of the accumulated carotenoids are the nutritionally active carotenoids called all-trans-*β*-carotene. Storage roots enriched with *β*-carotene showed delayed onset of postharvest physiological decline[31], a major constraint in the use of cassava products.

Overexpression of the *Arabidopsis thaliana* vacuolar iron transporter VIT1 resulted in a three- to sevenfold increase in iron content in transgenic storage roots[38]. Co-expression of a mutant *A. thaliana* iron transporter (IRT1) and *A. thaliana* ferritin (FER1) in transformed plants accumulated 7–18 times higher iron levels and 3–10 times higher zinc levels than non-transgenic controls[39]. Retention and bioaccessibility studies showed that processed tubers of IRT1+FER1 plants could provide 40–50% of EAR for iron and 60–70% of EAR for zinc in one- to six-yr-old children and non-lactating, non-pregnant West African women.

Overexpression of an *Arabidopsis* ZIP plasma membrane zinc transporter resulted in a two- to tenfold increase in tuber root zinc content[40] and reduced zinc concentration in the leaves of transformed plants[41]. Various transgenic strategies have been utilized to address protein deficiency in cassava by targeting linamarase to the vacuole using a vacuolar sequence from barley and transforming an artificial storage protein gene (*ASP132*)[42]. Many transgenic biofortified cassava varieties are under field trials in the BC+ program in African countries[42].

Sweet potato

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the world's most important resilient functional food crops[43] with great potential to reduce the Global Hunger Index[25], particularly in SSA, parts of Asia and the Pacific Islands[44]. Its potential as a food crop and carbohydrate source is widely documented[45]. Orange-fleshed sweet potato (OFSP) cultivars rich in β-carotene and vitamin C, as well as fibre, iron, potassium and protein[46], and purple-fleshed sweet potato rich in anthocyanins are highly valued[47,48].

The enormous genetic diversity existing in sweet potatoes due to heterozygosity and hexaploidy ($2n = 6x = 90$) and cross-pollinated nature is an important factor for its improvement as this is the primary source of the specific genes for the desired genetic gains[49]. Its genetic diversity has been harnessed through breeding for biofortification with targeted nutritional quality attributes, virus resistance and climate resilience. Breeding sweet potatoes is challenging due to its genetic complexity, and marker-assisted breeding tools are needed to facilitate crop improvement. Quantitative trait loci (QTL) for DM, starch and β-carotene content were identified in a hexaploid sweet potato mapping population of 240 hybrid clones derived from a cross between a white-fleshed African landrace with high DM content ('Tanzania') and a low-DM OFSP cultivar popular in the United States ('Beauregard'). Strong correlations were observed between starch and DM content ($r > 0.8$, $P < 0.0001$) in storage roots, whereas moderate correlations ($r = -0.6$, $P < 0.0001$) were observed for β-carotene and starch content. In both parental maps, the analysis revealed the presence of eight QTLs for β-carotene content[50] and transgressive segregation[51], suggesting that further improvement of this trait is possible. Several QTL regression models developed for the segregation of alleles in each parent suggest 17–35% variation in β-carotene content.

As part of the breeding strategy, several OFSP cultivars were bred with a provitamin A content of 30–100 ppm, which is higher than the target value of 32 ppm set by HarvestPlus and confirmed by HPLC and spectrophotometric studies. Since the provitamin A trait is already prevalent in breeding populations, ongoing breeding of OFSP is focused on tolerance to biotic and abiotic stresses while maintaining or increasing provitamin A content. According to HarvestPlus[52,53], conventionally bred provitamin A-biofortified OFSP is considered the first biofortified crop that can be grown on a large scale. Sweet potato is not biofortified with iron and zinc, but OFSP can contribute about 20%, 20%, 25% and 50% to the RDA of iron, zinc, calcium and magnesium respectively, when the crop is used as a staple food[54]. Sweet potato can be considered biofortified when the target levels for vitamin A reach 50% of the RDA and are 60 and 40 ppm for iron and zinc respectively[16]. Doubling the iron and zinc contents in sweet potato roots is possible with fewer breeding cycles[18]. Leaves also contain iron and zinc[55,56], the bioavailability of which is unknown.

The four strategies of the CIP and National Agricultural Research System (NARS) affiliated with the host countries for efficient breeding of sweet potatoes were as follows: more recombination and parenting, accelerated breeding and better allocation of breeding resources, more controlled cross-breeding than polycross breeding, and heterosis-exploiting breeding programmes and use of molecular tools. As early as 2003, NARS had established collaborations and exchanged seeds obtained in crossing blocks for strategic improvement of OFSP populations under the HarvestPlus programme, which was continued by Sweetpotato Action for Security and Health in Africa (SASHA) in 2009. This first step towards decentralized OFSP breeding is the plan adopted today for South and Southeast Asia[18]. As a result, 56 sweet potato varieties, including 15 OFSP varieties, were released by HarvestPlus and CIP between 1994 and 2003, and 89 varieties, including 62 OFSP varieties, were released by SSA in 12 countries between 2004 and 2013, with six varieties released in Uganda and three in Zambia. Studies on OFSP consumption in Uganda and Mozambique showed measurable improvement in vitamin A levels in children, for which the coordinated team effort of Jan Low, Maria Andrade, Robert Mwanga and H. E. Bouis was rewarded with the 2016 World Food Prize. Similarly, researchers have identified several sweet potato genotypes with no or only trace amounts of β-amylase in their storage roots, which can be used for processing and as a staple food[57]. Thus, conventionally bred, biofortified crops from HarvestPlus (including OFSP and cassava) have helped alleviate VAD in an estimated 10 million people, with a significant impact on food security in Sub-Saharan Africa.

Agronomic biofortification of β-carotene in OFSP has been observed through irrigation and chemical fertilizer treatment[58]. Gene modifications serve as an alternative method of increasing carotene, lutein and total carotenoids and other micronutrients in sweet potatoes by down-regulation or up-regulation of key regulatory enzymes. Down-regulation of β-carotene hydroxylase (CHY-beta), a key enzyme in the beta–beta branch of carotenoid biosynthesis, increased beta-carotene and total carotenoids in transgenic cultured cells of sweet potato[59]. Overexpression of IbOr-Ins in the

Table 1. Biofortified varieties of sweet potato released by ICAR-CTCRI and AICRP-TC for cultivation across India

Variety	Duration (days)	Key characteristics	Average yield (t/ha)	Dry matter (%)/carotene (mg/100 g FW)	Year of release
Gouri (hybrid)	110–120	Moisture stress-tolerant	18–25	22–27/4.5–5.5	1998
Sree Vardhini (selection)	100–105		20–25	18–20/2.5	1987
Sree Rethna (hybrid)	90–105		20–22	30–32/3	1996
Sree Kanaka (hybrid)	75–85	Early maturity	10–15	28/8.8–10.0	2004
Bhu Sona (clonal selection from exotic source)	105–110	Processing industry, cooking quality	20–24	27–28/11.5–12.5	2017
Bhu Krishna (clonal selection from exotic source)	110–120	Purple-fleshed tubers, sweet potato weevil/salt stress-tolerant	18–22	Anthocyanin content of 90 mg/100 g of fresh tuber and starch content of 18% dry weight basis	2017
Bhu Ja (clonal selection from CIP origin)	100–110	Salt stress-tolerant	20–22	23–25/5.5	2017
Bhu Kanthi (clonal selection from CIP origin)	105–110	Weevil, mid-season drought and salt stress-tolerant	22–24	25–26.6/6.5	2017
Indira Madhur (clonal selection from CIP origin)	110–120		21.62	26/4.6	2004
Kamala Sundari (selection)	110–120	Adaptable for river beds	21	26.47/8.2	2008
Indira Narangi (clonal selection)	90–100	Tolerant to weevil	23.79	25.17/5.72	2011
CO-5 (CIP440038; clonal selection from CIP)	135	Tolerant to weevil	25–35	18.27/2	2013

anthocyanin-rich, purple-fleshed cultivar Sinzami increased carotenoid content (up to seven-fold) in the storage roots[60]. Overexpression of IbMYB1, an important regulator of anthocyanin biosynthesis in the storage roots, increased the antioxidant capacity of OFSP[47]. Down-regulation of LCY-ε (ref. 61) and overexpression of the lycopene-β-cyclase gene (*IbLCYB2*)[62] significantly increased carotenoid content in transgenic sweet potato and tolerance to high temperatures (47°C) in sweet potato and other plants[63].

IbMADS10, a MADS-box gene cloned from sweet potato and *MYB* genes, are associated with anthocyanin accumulation[64,65]. The *IbARF5* gene from sweet potato line HVB-3 increased carotenoid content and improved tolerance to salt and drought in transgenic *Arabidopsis*[66].

ICAR-CTCRI has so far released 42 varieties of sweet potato through multilocation trials under the All-India Coordinated Research Project on Tuber Crops (AICRP-TC), which included hybrids from controlled crosses, polycrosses and selections from germplasm and seedlings from exotic collections which consisted of biofortified varieties as well (Table 1).

In collaboration with CIP, ICAR-CTCRI evaluated hybrid seeds of the Jewel population from 225 crosses during 2009, of which 40 advanced high-carotene clones were selected for use in further breeding programmes. These hybrids were tested in the uplands and lowlands with the CTCRI, β-carotene cultivar 'Sree Kanaka' (8.8 mg/100 g FW) as a control for storage root yield, β-carotene (10–15 mg/100 g FW) and DM (18.5–29.2%). At both locations, DM content was negatively associated with carotene content[67]. In another study, a population consisting of 200 F1 progeny from a cross of S-1 (white-fleshed) and ST-14 (orange-fleshed) was evaluated for DM, starch and β-carotene content[68]. Thirty percent of the progeny had a DM of 35–40%. Starch content of >30% was found in only 9% of the progeny. The β-carotene content ranged from 0.02 to 11.03 mg/100 g FW. Orange flesh colour was predominant in the progeny, followed by light orange flesh (28%), medium orange (22%) and deep orange (4%).

A collaborative work plan between ICAR and CIP was initiated in 2018 at ICAR-CTCRI to develop biofortified (β-carotene/anthocyanin-rich) sweet potato varieties with high DM along with adaptation and tolerance to abiotic stress. The evaluation of 20,000 hybrid seeds from 996 controlled crosses revealed variability of flesh colour with total carotenoids between 2 and 20 mg/100 g FW and a combination of both β-carotene and anthocyanin in the storage roots of the progeny. Promising hybrids are being selected and multiplied clonally for evaluation in different locations across India through AICRP-TC.

Yam/aroids

Since VAD is widespread in yam-growing regions, increasing the provitamin A carotenoid content of yam has been cited as an important nutritional enhancer[69]. However, the biofortification of *Dioscorea* is still in the early stages and not included in the HarvestPlus programme. Breeding strategies for *Dioscorea* biofortification are currently hampered by the lack of genetic resources and the literature on the biochemical composition and diversity of the global germplasm collection[70]. β-Carotene and xanthophyll esters are the carotenoids present in yam, although the β-carotene content (96.3–326 μg/100 g DW) is low compared to that in many plant foods[20,71,72]. Although the yellow yam *D. cayennensis* has higher carotenoid content[73,74], the white yam *D. rotundata* is preferred by farmers and consumers. *Dioscorea dumetorum* has similar vitamin A activity to improved cassava genotypes[75]. Some accessions of *D. dumetorum* accumulate more β-carotene epoxides that are comparable in terms of provitamin A with the mutant yellow cassava cultivar and transgenic golden potato. Therefore, the stability of these epoxides needs to be studied to determine their true provitamin A activity in humans[76].

Some *Dioscorea alata* accessions from the Philippines and Sri Lanka[77], and *Dioscorea trifida* L. from South America[78] have an attractive purplish colour. Anthocyanins in *D. alata* are cyanidin-based[79]. Sree Neelima is a recently released anthocyanin-rich variety of *D. alata* from ICAR-CTCRI, which is a selection having an average yield of 33 t/ha with good cooking and nutritional quality. Biofortification of taro (*Colocasia esculenta*) has not been studied much, except for the work of Champagne *et al.*[80].

Nutritional bioavailability

The main concerns with the use of biofortified crops for human consumption are the preservation of micronutrients in the plants during processing and storage to ensure that adequate amounts of vitamins and minerals remain in the food consumed by the target population and bioavailability, both of which depend on genotype[81]. OFSP can lead to a significant increase in the storage of vitamin A in the body in all age groups[82], as shown by studies in Mozambique[83]. The vitamin A equivalence factor was determined to be 13 : 1 for β-carotene in sweet potato[84]. The retention of β-carotene from orange sweet potatoes after cooking was about 80% of the original concentration[85]. The *in vitro* bioavailability or absorption of iron at the intestinal level was 47–62% in sweet potatoes compared to 6–24% in pearl millet, various bean varieties and rice, which may be attributed to the relatively low phytate and high ascorbic acid content in sweet potato[86].

Biofortified provitamin A cassava (yellow cassava) also showed significant improvements in vitamin A status in 5–13-yr-old children in eastern Kenya, as measured by both serum retinol and β-carotene, compared to normal cassava[87].

Conclusion

Sustainable biofortification strategies, in combination with dietary diversification and nutrition education, hold great potential for eliminating micronutrient malnutrition and improving our living standards. It is welcome that under the auspices of CIP and its partners, nearly seven million farm households, mostly in Africa, now grow and consume OFSP (https://www.cipotato.org). Through their combined efforts, biofortified crops have now been released in 40 countries worldwide, which can also be used to overcome hidden hunger in India. The fact that the most commonly grown and consumed crop species such as sweet potato, yam and banana are naturally transformed by T-DNA of *Agrobacterium*[88,89] should be taken into consideration while developing regulatory frameworks for the release of biofortified transgenic crops to improve human welfare and sustainability. It is the responsibility of scientists to disseminate the peruse facts, and convince the public and policymakers about transgenic crops.

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