Brainae Journal of Business, Sciences, and Technology
ISSN "2789-374X (print)" "2789-3758 (online)
Volume 11, Issue 2, May 2022
http://www.brainae.org,
info@brainae.org

Application of Biofortification for Good Nutrition: Literature Review

Calixte Ndabumviyubusa, MSc, PhD, Integrated Programs Director, World Vision Niger

Received: 29 May, 2022**; Accepted:** 30 May, 2022**; Published:** 30 May, 2022
DOI: https://doi.org/10.53236/21

Introduction

Bio fortification is a process of increasing the density of vitamins and minerals in a crop through plant breeding, transgenic techniques, or agronomic practices. Bio fortified staple crops, when consumed regularly, will generate measureable improvements in human health and nutrition. This article extends the previously published theoretical framework for bio fortification (Bouis et al., 2011b) and supporting evidence (Saltzman et al., 2013) to discuss delivery experiences and an action-oriented agenda for scaling bio fortification to improve nutrition globally. Delivery experiences are discussed from the perspective of Harvest Plus, which leads a global interdisciplinary alliance of research institutions and implementing agencies in the bio fortification effort. The evidence and building blocks for scale are in place;

Harvest Plus is one component of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH). The CGIAR is a global agriculture research partnership for a food secure future. Its science is carried out by its 15 research centers in collaboration with hundreds of partner organizations. The Harvest Plus program is administered under a joint venture agreement by two of these centers, the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI). The primary role of Harvest Plus is to catalyze, coordinate, and conduct oversight over a complex set of interdisciplinary activities by a large number of partner institutions that lead to reductions in mineral and vitamin deficiencies through bio fortification. Harvest Plus has invested more than $300 million in bio fortification activities during 2003–2016. Principal investors in Harvest Plus currently include the UK Government, Bill & Melinda Gates Foundation, the US Government's Feed the Future initiative, the EU Commission, and donors to A4NH. H.E. Bouis, A. Saltzman with sufficient institutional leadership, bio fortification is poised to reach one billion people by 2030

Comparative advantages

Micronutrient deficiencies afflict more than two billion individuals, or one in three people, globally (FAO et al., 2015). Such deficiencies occur when intake and absorption of vitamins and minerals are too low to sustain good health and development. Over the last 50 years, agricultural research for developing countries has increased production and availability of calorically dense staple crops, but the production of micronutrient-rich non-staples, such as vegetables, pulses and animal products, has not increased in equal measure. Non-staple food prices have increased steadily and substantially, making it more and difficult for the poor to afford dietary quality (Bouis et al., 2011a). In the long-term, increasing the production of micronutrient-rich foods and improving dietary diversity will substantially reduce micronutrient deficiencies. In the near term, consuming bio fortified crops can help address micronutrient deficiencies by increasing the daily adequacy of micronutrient intakes among individuals throughout the lifecycle (Bouis et al., 2011b).

Cost-effectiveness

Ex-post cost-effectiveness data is currently available for orange sweet potato in Uganda, where bio fortification was demonstrated to cost US$15–$20 per Disability Adjusted Life Year (DALY) saved, which the World Bank considers highly cost effective. Results of ex-ante cost-effectiveness studies have shown that for each of the country-crop-micronutrient combinations considered, bio fortification is a cost-effective intervention based on cost per DALY saved, using World Bank standards (Meenakshi et al., 2010). Furthermore, the Copenhagen Consensus ranked interventions for reducing micronutrient deficiencies, including bio fortification, among the highest value-for-money investments for economic development. For every dollar invested in bio fortification, as much as US$17 of benefits may be gained (Hoddinott et al., 2012).

The cost-effectiveness of any given intervention is dependent on the crop, micronutrient, and delivery country. The methodology for determining cost-effectiveness and specific case studies are discussed in greater depth elsewhere.

Nutritional bioavailability and efficacy evidence

Bio fortified crops can improve human nutrition. To develop evidence of nutritional efficacy, nutritionists first measure retention of micronutrients in crops under typical processing, storage, and cooking practices to be sure that sufficient levels of vitamins and minerals will remain in foods that target populations typically eat (for summary results, see De Moura et al. (2015)). Genotypic differences in retention and concentrations of compounds that inhibit or enhance micronutrient bioavailability are considered. Nutritionists also study the degree to which nutrients bred into crops are absorbed, first by using models, then by direct study in humans in controlled experiments. Absorption is a prerequisite to demonstrating that bio fortified crops can improve micronutrient status, but the change in status with long-term intake of bio fortified foods must be measured directly. Therefore, randomized controlled efficacy trials are used to demonstrate the impact of bio fortified crops on micronutrient status and functional indicators of micronutrient status (i.e. visual adaptation to darkness for vitamin A crops, physical activity and cognition tests for iron crops, etc.). Highlights are discussed below, and further detail is summarized in De Moura et al. (2014).

Iron crops

Iron nutrition research has demonstrated the efficacy of bio fortified iron bean and iron pearl millet in improving the nutritional status of target populations. In Rwanda, iron-depleted university women showed a significant increase in hemoglobin and total body iron after consuming bio fortified beans for 4.5 months (Haas et al., 2017). The efficacy of iron pearl millet was evaluated in secondary school children from Maharashtra, India. A significant improvement in serum ferritin and total body iron was observed in iron-deficient adolescent boys and girls after consuming bio fortified pearl millet flat bread twice daily for four months. The prevalence of iron deficiency was reduced significantly in the high-iron bio fortified pearl millet group. Those children who were iron deficient at baseline were significantly (64%) more likely to resolve their deficiency by six months (Saltzman et al., 2013).

Vitamin A crops

Vitamin A bioavailability studies found efficient conversions from provitamin A to retinol, the form of vitamin A used by the body. Efficacy studies demonstrated that increasing provitamin A intake through consuming vitamin A-bio fortified crops results in increased circulating beta-carotene, and has a moderate effect on vitamin A status, as measured by serum retinol. Consumption of orange sweet potato (OSP) can result in a significant increase in vitamin A body stores across age groups. The primary evidence for the effectiveness of bio fortification comes from OSP, assessed through a randomized controlled trial. The OSP intervention reached 24,000 households in Uganda and Mozambique from 2006 to 2009 with adoption rates of OSP greater than 60% above control communities (Hotz et al., 2012a, 2012b). Introduction of OSP in rural Uganda resulted in increased vitamin A intakes among children and women, and improved vitamin A status among children – a decrease in the prevalence of low serum retinol by 9 percentage points. Women who got more vitamin A from OSP also had a lower likelihood of having marginal vitamin A deficiency (Hotz et al., 2012a). Recent research on the health benefits of bio fortified OSP in Mozambique showed that bio fortification can improve child health; consumption of bio fortified orange sweet potato reduced the prevalence and duration of diarrhea in children under five (Jones and de Brauw, 2015).

bio fortified provitamin A maize is an efficacious source of vitamin A when consumed as a staple crop. An efficacy study conducted in Zambia with 5–7-year-old children showed that, after three months of consumption, the total body stores of vitamin A in the children who were in the orange maize group increased significantly compared with those in the control group (Gannon et al., 2014). Consumption of orange maize has been demonstrated to improve total body vitamin A stores as effectively as supplementation, and significantly improve visual function in marginally vitamin A deficient child (Gannon et al., 2014).

Zinc crops

Zinc studies have demonstrated that zinc in bio fortified wheat is bioavailable. Because plasma zinc concentration, the biomarker widely used to estimate zinc status, has limitations in measuring changes in dietary zinc, foundational research to identify and test more sensitive biomarkers is underway. These biomarkers will be tested in the zinc rice and wheat efficacy trial scheduled for 2017. A recent study showed that DNA strand breaks are a sensitive indicator of modest increases in zinc intake, such as the amount of additional zinc that might be delivered by a bio fortified crop (King et al., 2016).

Crop development

Plant breeding can increase nutrient levels in staple crops to target levels required for improving human nutrition, without compromising yield or farmer-preferred agronomic traits. The crop development process entails screening germplasm for available genetic diversity, rebreeding parental genotypes, developing and testing micronutrient-dense germplasm, conducting genetic studies, and developing mole-cular markers to lower the costs and quicken the pace of breeding. After promising lines have been developed, they are tested in several locations across target environments to determine the genotype x environment interaction (GxE) – the influence of the growing environment on micronutrient expression. Robust regional testing enables reduced time-to-market for bio fortified varieties. Early in the conceptual development of bio fortification, a working group of nutritionists, food technologists, and plant breeders established nutritional breeding targets by crop, based on food consumption patterns of target populations, estimated nutrient losses during storage and processing, and nutrient bioavailability (Hotz and McClafferty, 2007).

Transgenic approaches

In crops where the target nutrient does not naturally exist at the required levels in the tens of thousands of varieties in germplasm banks, transgenic plant breeding is a promising approach to produce biofortified crops with the desired nutrient and agronomic traits. For example, transgenic iron and zinc rice has been developed and tested in confined field trials that can provide 30% of the EAR for both nutrients. Golden rice, which contains beta carotene, can provide more than 50% of the EAR for vitamin A. Despite being available as a prototype since early 2000, however, Golden Rice has not been introduced in any country, in large part due to highly risk-averse regulatory approval processes (Wesseler and Zilberman, 2014). Conventional breeding, rather than transgenic breeding, is used in all of the crops released or in the near pipeline for harvest plus programs. Because conventional breeding does not face the same regulatory hurdles and is widely accepted, harvest Plus considers it to be the fastest route to getting more nutritious crops into the hands of farmers and consumers. This article focuses on the evidence developed for conventionally-bred biofortified crops.

Low-cost, high throughput methods

Bio fortification breeding required developing or adapting cost-effective and rapid high throughout analytical techniques for micro-nutrients, as thousands of samples need to be tested for mineral or vitamin content each season. These trait diagnostics include near-infrared spectroscopy (NIRS) and colorimetric methods for carotenoid analysis. For mineral analysis, X-ray fluorescence spectroscopy (XRF) emerged as the method of choice, as it requires minimal pre-analysis preparation and allows for non-destructive analysis (Paltridge et al., 2012).

Vegetative propagated crops

Vegetatively propagated crops – those for which farmers plant stems, tubers or vines rather than seeds – typically have seed systems characterized by small, informal (rather than commercial) actors. Planting materials are perishable, expensive and bulky to transport over long distances, and must be replanted within several days of harvesting. The lack of commercial private sector participa-tion creates both a challenge and an opportunity for producing planting materials of biofortified crops like orange sweet potato (distributed as vines) and provitamin A yellow cassava (distributed as stem cuttings

Cassava in Nigeria and DR Congo

In parallel with strengthening the seed system through both community-based and commercial stem production, awareness of and demand for biofortified crops must be created simultaneously. In the case of provitamin a yellow cassava, extension to farmers was at the forefront of this effort. Initially, free bundles of stems were distributed to farmers, and accompanied by agronomic training and nutrition information. In the following season, farmers who received free stems were required to distribute an equal amount of free stems to two additional farmers, dramatically lowering delivery costs. This promotional strategy was effective in reaching vulnerable populations who typically do not have market access to improved varieties for planting. It also piqued interest and allowed farmers in a low-risk way to test a new product. Many of the farmers who received and planted free stems liked the yellow cassava and are now buying additional stems from commercial traders.

In 2015, Harvest Plus estimated that about 75% of all biofortified harvested roots were consumed on farm, as many households were not yet producing excess from the stem packs they received for trial. Increased commercialization is expected going forward. As farmers began to produce yellow cassava in excess of their household food security needs, harvest Plus and its partners have worked to increase awareness and demand from the food market for biofortified cassava. These efforts include consumer marketing via print, radio, and television media (even feature-length movies), and market development efforts by linking commercial food processing investors to supplies of yellow cassava roots.

Self-pollinated crops

Self-pollinated crops – those which produce seed true to their parent characteristics – can be replanted year after year. While farmers do need to periodically replace their seed to maintain its desirable agronomic traits, the possibility of self-production for seed typically limits private sector investment in producing seed for self-pollinated crops. In many countries, the public sector instead multiplies and distributes self-pollinated seed, and further farmer-to-farmer dissemination is common. Self-pollinated biofortified crops include iron beans, delivered in Rwanda and Democratic Republic of Congo, zinc rice in Bangladesh, and zinc wheat in India and Pakistan. Delivery has progressed most quickly in Rwanda, where initial public sector investments have now spurred private sector interest in meeting growing demand for iron bean seed. Significant delivery has also taken place in Bangladesh, where demand is driven by the zinc rice varieties that have attractive agronomic traits, including a short duration variety that allows for production of a third crop between the wet and dry season rice crops. Delivery of zinc wheat in India and Pakistan is just beginning. In India, zinc wheat is predominantly marketed by the private sector as truthfully labeled seed (TLS), and six private seed companies had incorporated zinc wheat into their product lines. In Pakistan, the first zinc wheat variety was released in 2016, and delivery through public and private sector partners is now underway.

Beans in Rwanda and DR Congo

In Rwanda, harvest plus worked closely with the Rwanda Agriculture Board (RAB) to facilitate production of bean seed through contracted farmers, cooperatives, and small seed companies. From 2011 to 2015, harvest Plus procured about 80% of its certified seed through registered seed farmers under the supervision and certification of RAB, with the remainder being produced through contracts with local seed companies. To increase available seed for the 2015 planting season and beyond, harvest Plus partnered with established local and regional seed companies for seed multiplication, with RAB certifying the biofortified seed. harvest Plus and its partners also proposed a new seed class, “Declared Quality Seed” (DQS) or Certified II seed, first in Rwanda and then in DRC. DQS is produced from certified seed and is priced between certified seed and grain, bridging a price gap for farmers who are inclined to plant recycled grain rather than purchase certified seed. Farmers initially accessed iron bean seed either in small quantities through direct marketing (via established agro dealers or in local markets) or in larger quantities through a payback system that also included cooperatives. By the end of 2014, marketing data showed that an increasing number of farmers were purchasing seed, a trend that is expected to continue. Farmer-to-farmer dissemination is also an important delivery channel; an impact assessment conducted in 2015 found that nearly half of farmers growing iron bean had received their planting material from a person in their social network (Asare-Marfo et al., 2016).

Because the iron trait is invisible and iron beans are not easily distinguished from conventional varieties, the primary approach has been to gain market share for biofortified beans due to their superior agronomic and consumption qualities. Over time, a high percentage of the total national supply of beans is expected to contain the biofortified trait, allowing access to additional iron for much of the population. Harvest Plus and its partners have used a variety of delivery methods, including “swapping” biofortified seed for conventional seed, to ensure a high rate of farmer trial and adoption. Only five years after the first iron bean release, iron beans make up more than 10% of national bean production in Rwanda (Asare-Marfo et al., 2016).

Rice in Bangladesh

At the core of the Bangladesh strategy are rice varieties with attractive agronomic properties and a robust farmer demonstration program. One released zinc rice for the wet season (BRRI dhan 64) is a short duration variety (100 days as compared with 140 days), which allows production of a third crop of lentils or other food between wet and dry season rice crops. Other biofortified zinc rice varieties carry different farmer-preferred agronomic traits, like high height at maturity, which is beneficial for flooded areas in Southern Bangladesh. A robust demonstration program provides farmers a chance to observe these new varieties, as well as training on growing the biofortified rice and the health benefits of zinc. Seed is produced by both the private and the public sector. A private seed association called Seed Net produces truthfully labeled seed alongside the foundation and certified seed produced by government entities. Harvest Plus initially both guarantees a market for a portion of the private sector production and subsidizes the price for any seed that the private sector markets directly to consumers. Free seed is distributed by NGO and government partners in small seed packs, and all free seed recipients agree to pass on the same amount of seed to three neighboring farmers in the subsequent season. As an increasing amount of zinc rice is available on the market, efforts to increase consumer and miller awareness have increased, including outreach via SMS and programs on local television and community radio channels.

Hybrid crops

Hybrid crops – those for which seed must be replaced each year to maintain the same yield and agronomic traits – offer the most potential for private sector commercialization. While utilizing the private sector for delivery may lead to long-term sustainability, the speed of private sector uptake is dependent on their assessment of demand. Therefore, the activities of bio fortification proponents must focus on targeted demand creation for both farmers and consumers.

Maize in Zambia

Because private seed companies dominate the hybrid maize seed market in Zambia, upon release, biofortified hybrid varieties were licensed to companies for commercialization of seed production and distribution. As biofortified maize is scaled up to reach more house-holds in more provinces, the main challenge is to ensure extensive distribution through private networks to outlying areas. Because many rural households purchasing from agro dealers cannot afford to buy large quantities of seed, harvest plus is working with the private seed companies to ensure that large quantities of smaller, affordable pack sizes will be available. Harvest Plus also partners with the Zambia National Farmers Union and government extension services to disseminate information to farmers about the availability of vitamin A maize seed in their local areas. The inclusion of orange maize seed in the Zambian government's Farmer Input Support Program (FISP) has further facilitated access to orange maize, including for vulnerable households. FISP provides at least a 50% subsidy for maize seed and fertilizer to farmers considered economically disadvantaged. The quantity of orange maize seed distributed under FISP grew by 400% Between the first and second year of inclusion in the program. A central element of the delivery strategy is to create awareness and acceptance of orange maize through the use of social marketing campaigns and advertisements placed in public media, including TV, radio, newspapers, and popular music. Educational and awareness-creation activities stimulate consumer demand for orange maize products, while engagement with the private sector helps meet growing consumer demand. To further stimulate cultivation of orange maize, creating markets for surplus production was essential, considering that 20–50% of rural households sell maize after satisfying their own food needs. Harvest Plus therefore links major grain buyers to farmers and offers grain samples to millers and food processors interested in incorporating orange maize in their product lines. The multilateral Ag Results initiative also incentivizes millers to produce and market vitamin A maize product. Strong interest from farmers and food processors encourages increased private sector seed production.

Pearl millet in India

Crop development and delivery in India is implemented through public and private sector partnerships. In crop development, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) supplies parental materials/breeder seeds for next stage seed multiplication. Partners now testing and developing their iron pearl millet varieties for seed sales include 15 private seed companies, 2 public seed companies and 5 public organizations. harvest Plus supports ICRISAT to develop high iron hybrid parental lines and to test hybrids with farmer-preferred traits, including of course high yields. This unique crop development arrangement supports and encourages companies to develop their own biofortified varieties for their target market segments. This approach is expected to more quickly increase the number and range of biofortified varieties available in the years to come.

Agronomic bio fortification of crops to fight hidden hunger in sub-Saharan

Hidden hunger or micronutrient deficiency retards the growth and development of both crops and humans. Soil micronutrient deficiencies limit crop productivity and nutritional quality of foods, which together affect nutrition and human health. Many soils in sub-Saharan Africa are affected by multiple nutrient deficiencies including the macronutrients N, P, K, secondary nutrients S, Ca and Mg, as well as the micronutrients Zn, Fe, Cu, Mn, Mo and B. Soil micronutrient deficiencies are thought to be severe in sub-Saharan Africa, where 75% of the total arable land has serious soil fertility problems. Insufficient micronutrient availability in soils in these regions not only causes low crop productivity, but also poor nutritional quality of the crops and consequently contributes to malnutrition in the human population. Diets in sub-Saharan Africa (especially among resource poor house-holds) are often low in diversity and dominated by staple crops such as maize, rice, cassava, sorghum, millet, banana and sweet potato. Such diets are poor in micronutrients (minerals and vitamins) and conse-quently micronutrient deficiencies are widespread (FAO, 2015). The chronic lack of micronutrients can cause severe but often invisible health problems, especially among women and young children: hence ‘hidden hunger’ Worldwide over 2 billion people suffer from iron (Fe), zinc (Zn) and/or other (multiple) micronutrient deficiencies (WHO, 2016). The problem is most severe in low- and middle income countries, especially in Africa where the estimated risk for micronutrient deficiencies is high for Ca (54% of the continental population), Zn (40%), Se (28%), I (19%) and Fe (5%) (Joy et al., 2014). In sub-Saharan Africa, micronutrient deficiencies are responsible of 1.5–12% of the total Disability Adjusted Life Years (DALYs) (Muthayya et al., 2013). Alarming numbers concern iron deficiency anemia, which affects more than half of the female population in countries such as DR Congo, Ghana, Mali, Senegal, Togo (IFPRI, 2015). Many people suffer from multiple micronutrient deficiencies (Muthayya et al., 2013); for example, in Malawi > 50% of the households are estimated to be at risk of Ca, Zn and/or Se deficiencies (Joy et al., 2015a). Selenium is not essential for plant growth, but contributes to the human diet through uptake by crops from the soil. Even mild to moderate deficiencies of micronutrients can lead to severe human health problems, generally related to sub-optimal metabolic functioning, decreased immunity and consequently increased susceptibility to infections, growth failure, cognitive impairment and, finally, reduced productivity (Tulchinsky,Hidden hunger can be alleviated by direct (nutrition-specific) and indirect (nutrition-sensitive) interventions (Kihara et al., 2013). Direct interventions focus on consumption behavior and include dietary diversification, micronutrient supplementation, modification of food choices and fortification. Nutrition-sensitive interventions address the underlying determinants of malnutrition and include biofortification. Biofortification is the process of increasing the content and/or bioavailability of essential nutrients in crops during plant growth through genetic and agronomic pathways (Bouis et al., 2011).

Soil to crop

Bioavailability of micronutrients from soil to crop is influenced by many soil factors (i.e. pH, organic matter content, soil aeration and moisture and interactions with other elements) and by the crop variety that, for example, defines the structure and functioning of rooting systems (Alloway, 2009). Some plants can modify the rhizosphere by the excretion of H+ ions or organic acids that enhance micronutrient availability and uptake. Interactions between elements influence the bioavailability for root uptake. Soil phosphorus, for example, can either stimulate root growth and Zn uptake while at the same application of P fertilizer can precipitate already small concentrations of Zn and trigger Zn deficiency (Kihara et al., 2016). Addition of P also appears to induce Zn deficiency through dilution effects and interference with Zn transloca-tion from the roots (Kihara et al., 2016). Soil management with lime or organic manures can alter soil properties such as pH and stimulate micronutrient bioavailability and crop uptake. Symbioses with arbus-cular mycorrhizal fungi (a fungal network acting as an extension of the root system and increasing the volume of soil explored for nutrient uptake) can increase uptake of nutrients that are sparingly soluble in soil, such as P and Zn (Smith and Read, 1997).

Crop to food

Bioavailability from crop to food is influenced by the crop (variety) which defines whether micronutrients are (re-)localized into edible parts of the crop – and by food processing. In rice, Zn and Fe are localized in protein bodies in the outer layer of the grains, which is often removed during processing (dehusking, milling) leaving less Zn and Fe in the consumed rice (Haas et al., 2005). Rice parboiling is an effective method to increase nutrient contents especially when micronutrients are added to the soak water during the parboiling, as the process drives nutrients from the bran and germ layer to the endosperm (Hotz et al., 2015). Other crops like wheat allocate Zn in the consumed part of the grain (endosperm) that remains even after removal of the seed coat and aleurone layer during the process of bread making 2015). Also Se, Fe, Mn and Cu are hardly lost during wheat grain milling and bread production (Lyons et al., 2005) – making wheat more suitable for agronomic biofortification. Food processing generally results in nutrient loss, but it also often reduces the amounts of antinutrients and thus may increase the bioavailability of micronu-trients. For example, soaking of cereals in water can reduce the presence of the antinutrient phytate, enhancing the bioavailability of Fe, Zn and Ca (Hotz and Gibson, 2007).

Food to human

Bioavailability of micronutrients in the food for the human body is influenced by many factors that can be either food or host related (Hotz and Gibson, 2007). Dietary intake is an essential factor, as micronutrient bioavailability depends on the chemical form and amount consumed, the nature of the dietary matrix, as well as interaction between nutrients and/or food components that enhance or inhibit absorption in the gastrointestinal tract (Clemens, 2014). Enhancers like ascorbic acid (available in fruits and vegetables) can increase Fe bioavailability, while polyphenols and especially phytate or phytic acid (with high concentrations in staple grains like wheat) are major inhibitors that form complexes with Fe and Zn and limit uptake in the human body (Clemens, 2014). An individual's health and nutrient status as well as age, sex, ethnicity, genotype, and physiological state also impacts micronutrient bioavailability from foods for uptake into the human body (Clemens, 2014). Absorption of micronutrients is often tightly regulated by the micronutrient status of the individual; for example, Fe and Zn absorption is increased when individuals have Fe or Zn deficiency (Kempen, 2015). Infections and parasites impair micronu-trient absorption and increase the risk for malnutrition, while mal-nutrition itself also makes a person more susceptible for infections and parasites (Kempen, 2015).

Fertilization approaches and agronomic bio fortification

The soils of sub-Saharan Africa are highly diverse, ranging from some of the oldest soils in the world to relatively young volcanic soils in the Great Rift Valley that splits East and Southern Africa and alluvial soils along rivers. Many African soils suffer from multiple micronu-trient deficiencies, due both to their inherent soil properties and to continuous cropping without nutrient replenishment. Current fertiliza-tion programmes in African countries, primarily focus on NPK fertilizers, but many soils are non-responsive to NPK due to (multiple) micronutrient deficiencies. Soil amendment with small amounts of (multiple) micronutrients has been suggested as a sustainable strategy to increase yields and nutritional quality of crops (Haskell, 2004). In the succeeding paragraphs we discuss the impact of different fertilization approaches on agronomic biofortification, as well as the interactions of micronutrients with NPK fertilizers and the importance of Integrated oil Fertility Management (ISFM).

Impact of different fertilization techniques

Effectiveness of mineral fertilizer application on crop performance is influenced by the fertilizer type and application method. The fertilizer formulation largely determines the micronutrient bioavail-ability, as the form of the nutrients and interactions between them can have positive as well as neutral or even negative effects on yields and nutrient use efficiencies (Cakmak et al., 2009). Foliar fertilization with micronutrients often stimulates more nutrient uptake and efficient allocation in the edible plant parts than soil fertilization, especially with cereals and leafy vegetables (Cakmak et al., 2014). The combination of soil and foliar application is often the most effective method (Cakmak, 2010). Foliar pathways are generally more effective in ensuring uptake into the plant because immobiliza-tion in the soil is avoided. The downside of foliar application is that fertilizers can easily be washed off by rain and are more costly and difficult to apply (17. Gannon et al., 2014). Seed priming and seed coating with fertilizers are other strategies for precise micronutrient application, that can stimulate plant development and increase yields, but increased nutritional values of grains are rarely found (Duffner et al., 2014).

Impact in combination with NPK fertilization

Interactions of micronutrients with macronutrients can influence the effectiveness of agronomic biofortification. Good N and P status of plants has a positive effect on root development, shoot transport and re-localization of nutrients from vegetative tissue to the seeds (Gannon et al., 2014). This results in increased micronutrient uptake and concentrations in the edible parts of the crop, as shown in wheat experiments, where high N application increased Zn and Fe concentra-tions in the grain endosperm (the edible part of the grain) (Kutman et al., 2011; Shi et al., 2010). Wheat fertilization with Zn-enriched N and P fertilizer has also been effective to increase wheat grain yields (Cakmak, 2004). As indicated above, addition of P fertilization can also reveal incipient Zn deficiency by precipitation of insoluble Zn phosphate (Gannon et al., 2014).

Impact of integrated soil fertility management

Good soil conditions that enhance micronutrient availability for crop uptake are essential for the success of agronomic biofortification. Not only N and P increase the effectiveness of micronutrient fertiliza-tion, but also other soil chemical, physical and biological characteristics are essential to optimize nutrient use efficiency. A commonly suggested strategy to optimize soil conditions is Integrated Soil Fertility Management which is defined as “a set of soil fertility management practices that necessarily include the use of mineral fertilizer, organic inputs and improved germplasm” (Vanlauwe et al., 2015). The combination of mineral fertilizers and organic inputs is beneficial, because they have complementary functions and enhance mutual effectiveness. Organic resources (plant residues and animal manure) help to sustain soil organic matter with multiple benefits in terms of enhanced soil structure, cation exchange capacity and water holding capacity (Vanlauwe et al., 2015). Furthermore, where organic inputs provide more slow but constant nutrient release, mineral fertilizers offer flexibility in the proper timing, placing and application rate to synchronize nutrient availability with crop demand (Giller et al, 2002). Fertilization with organic matter alone has the potential to increase soil micronutrient content and availability (Giller et al, 2002). Animal manures, for example, are a good source of many micronutrients (Giller et al, 2002). Manzeke et al. (2014) found that Integrated Soil Fertility Management approaches where Zn-enriched fertilizer was applied together with cattle manure and forest leaf litter gave larger increases in maize grain yield and Zn concentration in the grain. Long-term application of organic matter to the soil not only increases total Zn content of the soil but also the proportion of labile Zn, which is the readily available form for plant uptake (Manzeke et al., 2014). However, organic inputs alone are often insufficient to maintain nutrient balances in resource poor farming systems, because of the limited availability of nutrient-rich organic matter (e.g., manures and compost) and overall lack of nutrients in the system. The combined application of organic inputs and mineral micronutrient fertilizers has the potential to alleviate overall micronutrient shortage. Besides, agronomic efficiency of mineral fertilizers is often increased when applied in combination with organic matter (Vanlauwe et al., 2015). Green manures (cover crops that serve as mulch or soil amendment) are also effective to enhance nutrient bioavailability, as was shown in a study on basmati rice in India, where the combined fertilization with green manure and mineral Zn improved yields and grain Zn nutritional quality (Manzeke et al., 2014).

Impact of agronomic biofortification with Se, Zn and Fe on yields and nutritional quality of crops

Agronomic biofortification has so far been most effective with Zn and Se (Cakmak, 2014). Several studies have shown that application of Se-enriched fertilizers can increase grain Se concentrations (in maize and wheat), although yield increases were not observed. One of the most celebrated cases is from Finland, where the nationwide addition of Se to NPK fertilizers (15 mg Se/kg) increased cereal crop Se contents by 15-fold on average. This intervention increased the Se intake of the population to well above nutrition recommendations (Cakmak, 2014). Another experiment on wheat in Australia with the application of Se (4–120 g Se/ha) increased grain Se concentrations progressively up to 133-fold when applied to the soil and up to 20-fold when applied as foliar spray. Other authors observed linear relationships between Se fertilization and maize grain Se concentrations (Chilimba et al., 2012) as well as bioavailable Se in wheat flour and bread (Chilimba et al., 2012).

Most current research and development programmes focus on Zn, as this is a widespread crop yield-limiting factor and one of the most prevalent deficiencies in humans. Evidence is accumulating that Zn fertilization can increase both yields and nutritional quality of crops. Most research has been done in Turkey, where Zn fertilization of various cereals (maize, sorghum, barley, wheat) and dicotyledonous (soybean, safflower, pea, common bean, canola, common vetch) crops showed increased yields and grain Zn concentrations (Cakmak et al., 2010). Field studies in India showed that the use of Zn-enriched urea on rice could increase yields and grain Zn concentrations three-fold (Cakmak, 2009). A review of experiments from ten African countries on the impact of Zn-enriched fertilizers showed that soil Zn application increased the Zn concentration in maize, rice and wheat grains by respectively 23%, 7% and 19% and by 30%, 25% and 63% through foliar application (Joy et al., 2015b). Teff yields in Ethiopia increased with Zn fertilization (Haileselassie et al., 2011). Besides the increased yields and grain Zn concentrations upon Zn-enriched fertilizer application to cereals, another agronomic benefit is that seedlings from seeds with high Zn concentration have better growth performance and resilience against environmental stress, so positive impacts on productivity may be seen in the next cropping generation. Furthermore, Zn fertilization reduces P uptake and the accumulation of phytate in grains, which may increase the Zn bioavailability for humans (Hussain et al., 2013). Iron is the third most studied element, but soil application of Fe-enriched fertilizers is more difficult than with Zn and Se, because Fe is precipitated in insoluble forms in the soil which cannot be absorbed by plants. For example, a greenhouse experiment that compared Zn and Fe application on wheat showed enhanced grain Zn concentrations, while Fe concentrations were not effectively improved (Cakmak et al., 2010). The most effective agronomic practices for the Fe enrichment of crops are through litter fertilization or foliar application of mineral Fe. Foliar application has already showed to increase Fe concentrations in wheat grain and rice grain (Shahzad et al., 2014). However, some studies also showed no response of plants upon foliar Fe application, especially under treatment with inorganic and chelated Fe fertilizers (Shahzad et al., 2014).

Impact of agronomic bio fortification on nutrition and human health status

The only known case that clearly showed a direct effect of agronomic bio fortification on human micronutrient status comes from Finland, where nationwide agronomic Se bio fortification was practiced since 1985 (Alfthan et al., 2015). This programme resulted in significantly increased cereal grain Se concentrations, which in turn led to increased human and animal Se intake and significantly decreased Se deficiencies among the population. The average dietary intake doubled from 0.04 mg Se/day/10 MJ in 1985 to 0.08 mg Se/ day/10 MJ in 2014, which is above nutrition recommendations leading to an average human plasma Se concentration of 1.4 μmol/L and reflecting an optimal Se status (Alfthan et al., 2015). This long-term intervention showed that Se (sodium selenate) supplementation of fertilizers was a safe and effective method to increase Se intake of humans as well as animals. Interestingly, foods of animal origin accounted for over 70% of the human daily Se intake, indicating that interventions in sub-Saharan Africa would require dietary changes next to agronomic Se bio fortification in order to achieve similar results as in Finland. We are unaware of other studies that similarly quantified the direct impact of agronomic bio fortification on dietary intake of micronutrients on human health. Even though it is shown that agronomic bio fortification has the potential to increase micronutrient contents in crops, literature connecting these enhanced concentrations to micronutrient bioavailability, dietary intake and human health are scarce (Joy et al., 2014). Such studies do exist on genetically bioforti-fied crops, such as in the case of the increased Fe status of Filipino women who consumed Fe-biofortified rice (Haas et al., 2005), of Rwandan iron repleted university women consuming iron biofortified beans (Haas et al., 2016). Modelled estimations have been made on the potential of agronomic bio fortification using agronomic and dietary data. For example, Chilimba et al. (2012) calculated that application of about 5 g Se per ha to all maize crops in Malawi could increase the average dietary intake with 0.04 mg Se/day, considering a maize-based diet. Joy et al. (2015b) modelled the potential of Zn-enriched fertilizers to alleviate human dietary Zn deficiency, focusing on ten African countries where Zn supply is low and agronomic bio fortification has potential through fertilizer subsidy programmes (Burkina Faso, Ethiopia, Ghana, Kenya, Malawi, Mali, Nigeria, Senegal, Tanzania and Zambia). Based on data from other studies on Zn concentrations in maize, rice and wheat.

Impact of agronomic bio fortification on the environment

Application of micronutrient-enriched fertilizers is considered to have minimal negative environmental impact. Most micronutrients are not susceptible to leaching because they are strongly bound in the soil. The downside is that these elements accumulate over time and cause toxicity if large amounts are applied repeatedly. Selenium is the only micronutrient that can be lost by volatilization from the soil in gaseous form (Malagoli et al., 2015). To optimize nutrient use efficiency and minimize risks for toxicity, fertilization practices should include precise application strategies. The 4 R strategy aims to optimize precise application by fertilization of the “Right source and Right amount at the Right place and Right time” (Malagoli et al., 2015). Studies such as that of Wang et al. (2013) investigating optimal fertilizer application rates are essential to increase production efficiency while minimizing environmental pollution and toxicity. When micronutrient demand and supply are well-matched, there should be no negative environmental effects. In fact, crop health improves when micronutrient deficiencies in the crop are alleviated. The improved general crop health enhances growth and nutrient uptake efficiency, as well as resilience against pests and diseases, what may reduce the need for pesticides and herbicides (Dimpka and Bindraban, 2015). Mineral resources are mined for manufacture of micronutrient fertilizers, causing concern of natural resources and environmental pollution. Further concerns have been raised about the limited global availability of micronutrient rocks that may be exhausted in future (Dimpka and Bindraban, 2015). Although it is difficult to predict future supply and demand, these concerns emphasize the importance to increase nutrient use efficiency and nutrient recycling of micronutrients.

Agronomic bio fortification compared with other interventions

The question remains whether agronomic bio fortification is an effective, feasible and sustainable approach to alleviate micronutrient deficiencies; especially in comparison with other intervention strategies such as genetic bio fortification, food fortification, supplementation and dietary diversification. Studies that compare the relative benefits of different interventions on nutrition are hardly available, and economic analyses available did not consider agronomic bio fortification. Among the other interventions, genetic bio fortification is more cost effective than food fortification, supplementation or dietary diversification in the long run, because it requires only one period of (breeding) investments (Ma et al., 2008). Agronomic bio fortification is often considered as a short-term solution to increase micronutrient availability and mainly to complement genetic biofortification (breeding), which is seen as a more sustainable approach (Ma et al., 2008). Cakmak et al. (2010) argued that breeding is the only agricultural intervention to improve nutritional contents of staple crops in low-income countries, because fertilizers are not accessible and affordable for resource poor farmers. The CGIAR bio fortification programme HarvestPlus, (http://www.harvestplus.org), suggests that dietary diversification is the most sustainable solution, yet diverse foods are often not affordable for those at greatest risk. Bouis and Welch (2010) argue that supplementation and diet diversification programmes work best in centralized urban areas, whereas agronomic bio fortification is the best approach to reach rural populations. Even though currently food fortification and supplementation are the most commonly used strategies to alleviate micronutrient deficiencies among humans, bio fortification (agronomic and/or genetic) is considered to have more potential in the long-term because it seems more cost-effective, and practical (Bouis and Welch, 2010).

Potentials and constraints for implementation in sub-Saharan Africa

Multiple factors play a role in the potential of agronomic biofortification to be implemented in sub-Saharan agricultural systems and to eradicate micronutrient deficiencies among the undernourished population. Mineral micronutrient fertilizer use is currently limited in African countries due to general issues of cost and supply, the lack of information on micronutrient problems, a reliable fertilizer recommendation system, and the poor availability of micronutrient fertilizers. Weak infrastructure causes high prices, while investments are not always profitable for rural farmers when market accessibility or storage capacity are limited (Sanchez and Swaminathan, 2005). Nevertheless there is intense current interest in expansion of fertilizer use in sub-Saharan Africa among fertilizer companies and new fertilizer blending plants are under construction in several countries (Sanchez and Swaminathan, 2005).

Especially in regions with limited access to micronutrient fertilizers, integrated soil fertility management practices are the most realistic approach to alleviate micronutrient deficiencies (Cakmak and Hoffland, 2012). There are many low-cost, locally available and environmentally sustainable technologies that smallholder farmers can use to create fertile soil conditions using an integrated approach (Kerr et al., 2012). An example is micro-dosing: a strategy of fertilizer application in small quantities and close to the seed or plant. The precise targeting for the roots minimizes nutrient losses as well as fertilizer costs (Thilakarathna and Raizada, 2015). However, nutrient management can be a challenge for farmers (especially smallholders) who face obstacles such as limited availability of organic and mineral resources, high investment costs, extra labour requirements and environmental stress from drought, extreme rainfall, pests and crop diseases (Giller, 2002). Development of the bio-physical, eco-nomic, social and political environment is necessary to facilitate proper technologies, allocation of resources and food processing systems. A key issue is the commercialization of smallholder agriculture to create markets for the extra production, because otherwise investments in (extra) mineral fertilizer are not economically feasible (Giller, 2002). In this regard, Kempen et al. (2015) engaged in initial analyses of spatial patterns of limiting soil micronutrients along with crop responses to micronutrient to identify where and what combination of nutrients are required (see also http://africasoils.net). Such information can guide agri-business and policymakers to target their interventions. Mapping of micronutrient deficiencies in order to provide field-specific fertilization recommendations, remains a challenge. Furthermore, knowledge and tools should be accessible and affordable for farmers in rural African regions. Soil test kits have been developed to assess soil fertility, but such tests are not sensitive or accurate enough to detect micronutrient deficiencies. The scientific world generally has more trust in models that can derive nutrient management recommendations on the basis of soil, climate and land-use characteristics. Along with recommendations for mineral fertilizers, ISFM recommendations could be provided to ensure highest effectiveness and nutrient use efficiency. The African Soil Health Consortium (ASHC) works towards this goal (http://africasoilhealth. cabi.org). Next, new fertilizer products and management practices need to be matched with local socio-cultural environments in order to enhance adoption (Kempen et al.,2015).

Conclusion

Scaling will require building new and expanding existing partner-ships, maintaining engagement, and increasing partner capacity. More than 100 Harvest Plus delivery partners have trained thousands of extension staff on agronomic practices and nutrition messages for bio fortification, and developed technical packages for partners to use in delivery programming. Going forward, harvest Plus will add new and diverse partners, including food processing companies and retailers, UN agencies, regional organizations, and innovative financing mechanisms and development banks. To reach one billion people by 2030, however, bio fortification must move beyond harvest Plus. Policymakers must give higher priority to the role of agriculture to improve health. National governments and multilateral institutions must ensure that bio fortification is included on the nutrition agenda. Public and private sector breeding partners must mainstream the biofortified trait across their product lines. Food processors and other actors along the value chain must include biofortified crops in their products. Only through a collaborative effort that reaches across the value chain will bio fortification become business as usual, and the vision of reaching one billion become a reality.

The effectiveness of agronomic bio fortification largely depends on the bioavailability of micronutrients throughout the entire pathway from soil to plant, food and into the human body. Enhanced micro-nutrient uptake by crops is observed when the micronutrient-enriched fertilizer is applied to the soil in combination with NPK and organic fertilizers - highlighting the importance of integrated soil fertility management. The application of micronutrient-enriched fertilizers should have no serious negative environmental effect when used at appropriate rates and generally has agronomic benefits as it improves soil fertility and crop health. Agronomic bio fortification can be effective in increasing yields and nutritional quality for certain crop-micronutrient combinations; especially Zn and Se on wheat and maize, whereas Fe has shown little potential to date. Studies that link micronutrient fertilizer application to improved human health are scarce, especially for sub-Saharan Africa, which hampers definite conclusions about the efficacy and effectiveness of agronomic bio fortification to alleviate micronutrient deficiencies among humans. We recommend to set up experiments and pilot-scale fertilization programmes in sub-Saharan Africa, to further explore this knowledge gap of the direct link between micronutrient-enriched fertilizer application to crops and the dietary micronutrient intake and uptake in the human bodies of consumers. Concerning the wide-scale implementation in sub-Saharan Africa, it is clear that multiple technical and socio-economic development steps are required to make micronutrient-enriched fertilizers more accessible and affordable for farmers.

References

Alfthan, G., Eurola, M., Ekholm, P., Venäläinen E.R, Root T, Korkalainen, K., Hartikainen, H., Salminen, P., Hietaniemi, V., Aspila P., Aro, A. (2015). Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. J Trace Elem Med Biol. 31:142-7.

Alloway, B.J. (2009). Soil factors associated with zinc deficiency in crops and humans. Environ Geochem Health. 31(5):537-48.

Asare-Marfo D, Herrington C, Birachi E., et al. (2016). Assessing the Adoption of High Iron Bean Varieties and Their Impact on Iron Intakes and Other Livelihood Outcomes in Rwanda. Main Survey Report. International Food Policy Research Institute, Harvest plus, Washington, DC.

Bouis, H.E., Hotz, C., McClafferty, B., et al. (2011). Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr. Bull. 32, 31–40.

Bouis, H.E., Welch, R.M. (2010). Biofortification – a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci. 50, 20–32.

Cakmak, I. (2014). Agronomic biofortification. Conference brief #8, In: Proceedings of the 2nd Global Conference on Biofortification: Getting Nutritious Foods to People, Rwanda, 2014.

Cakmak, I., Hoffland, E. (2012). Zinc for the improvement of crop production and human health. Plant Soil. 361:1–2.

Cakmak, I. (2009). Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. J. Trace Elem. Med. Biol. 23 (4), 281–289.

Cakmak, I., Pfeiffer, W.H., McClafferty, B. (2010). Biofortification of durum wheat with zinc and iron. Cereal Chem. 87 (1), 10–20.

Chilimba, A.D.C., Young, S.D., Black, C.R., Meacham, M.C., Lammel, J., Broadly, M.R., (2012). Agronomic biofortification of maize with selenium (Se) in Malawi. Field Crops Res. 125:118–128.

Clemens, S. (2014). Zn and Fe biofortification: the right chemical environment for human bioavailability. Plant Sci. 225:52–57.

Dimpka, C.O., Bindraban, P. (2015). Fortification on micronutrients for efficient agronomic production. Agron. Sustain. Dev. 36:1–26.

Duffner, A., Hoffland, E., Stomph, T.J., Melse-Boonstra, A., Bindraban, P.S. (2014). Eliminating zinc deficiency in rice-based systems. VFRC Report 2014/2. Virtual Fertilizer Research Center, Washington, D.C.

FAO, IFAD, WFP, 2015. The State of Food Insecurity in the World 2015. FAO, Rome.

Finkelstein, J., Mehta, S., Udipi, S., et al. (2015). A randomized trial of iron-biofortified pearl millet in school children in India. J Nutr. 145(7):1576-81

Gannon, B., Kaliwile, C., Arscott, S., et al. (2014). Biofortified orange maize is as efficacious as a vitamin A supplement in Zambian children even in the presence of high liver reserves of vitamin A: a community-based, randomized placebo-controlled trial. Am. J. Clin. Nutr. 100 (6), (1541-50).

Giller, P. S., & O'Donovan, G. (2002). Biodiversity and Ecosystem Function: Do Species Matter? Biology and Environment: Proceedings of the Royal Irish Academy, 102B(3), 129–139.

Haas, A.L, et al. (2005) Thi20, a remarkable enzyme from Saccharomyces cerevisiae with dual thiamin biosynthetic and degradation activities. Bioorg Chem 33(4):338-44

Haas, J., Luna, S.V., Lung'aho, M.G., Ngabo, F., Wenger, M., Murray-Kolb, L., Beebe, S., Gahutu, J., Egli, I. (2016). Consuming iron biofortified beans significantly improved iron status in Rwandan women after 18 weeks. J Nutr. 146(8):1586-92

Haileselassie, B., Stomph, T.-J., & Hoffland, E. (2011). Teff (Eragrostis tef) production constraints on Vertisols in Ethiopia: farmers' perceptions and evaluation of low soil zinc as yield-limiting factor. Soil Science and Plant Nutrition, 57(4), 587–596.

Haskell, M.J., Jamil, K.M., Hassan, F., et al. (2004). Daily consumption of Indian spinach (Basella alba) or sweet potatoes has a positive effect on total-body vitamin A stores in

Bangladeshi men. Am. J. Clin. Nutr. 80, (705-14).

Hoddinott, J., Rosegrant, M., Torero, M. (2012). Investments to reduce hunger and undernutrition. International Food Policy Research Institute Report

Hotz, C., McClafferty, B. (2007). From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. Food Nutr. Bull. 28 (2), (S-271–279).

Hotz, C., Loechl, C., de Brauw, A., et al. (2012a). A large-scale intervention to introduce orange sweet potato in rural Mozambique increases vitamin A intake among children and women. Br. J. Nutr. 108, 163–176.

Hotz, C., Loechl, C., Lubowa, A., et al. (2012b). Introduction of B-carotene-rich orange sweet potato in rural Uganda results in increased vitamin A intakes among children and women and improved vitamin A status among children. J. Nutr. 142, 1871–1880.

Hussain, A. I., Anwar, F., Chatha, S. A. S., Latif, S., Sherazi, S. T. H., Ahmad, A., … Sarker, S. D. (2013). Chemical composition and bioactivity studies of the essential oils from two Thymus species from the Pakistani flora. LWT - Food Science and Technology. 50(1), 185–192.

Jones, K., de Brauw, A. (2015). Using agriculture to improve child health: promoting orange sweet potatoes reduces diarrhea. World Dev. 74:15–24.

Joy, E. J., Ander, E. L., Young, S. D., Black, C. R., Watts, M. J., Chilimba, A. D., Chilima, B., Siyame, E. W., Kalimbira, A. A., Hurst, R., Fairweather-Tait, S. J., Stein, A. J., Gibson, R. S., White, P. J., & Broadley, M. R. (2014). Dietary mineral supplies in Africa. Physiologia plantarum, 151(3), 208–229.

Kempen, B., Vereijken, P.F.G., Keizer, L.P.C., Ruipérez González, M., Bindraban, P., Wendt, J. (2015). VFRC preliminary evaluation of the feasibility of using geospatial information to refine soil fertility recommendations. VFRC Report 2015/6. Virtual Fertilizer Research Center, Washington, D.C.

Kerr, R.B. (2012). Lessons from the old green revolution for the new: social, environmental and nutritional issues for agricultural change in. Afr. Prog. Dev. Stud. 12 (2 & 3), 213–229.

Kihara, J., Nziguheba, G., Zingore, S., et al. (2016). Understanding variability in crop response to fertilizer and amendments in sub-Saharan Africa. Agric Ecosyst Environ. 229:1-12.

King, J., Brown, K., Gibson, R., Krebs, N., Lowe, N., Siekmann, J., Raiten, D. (2016). Biomarkers of nutrition for development – zinc review. J. Nutr. 146(4): 858S–885S

Lyons, G.H., Genc, Y., Stangoulis, J., Palmer, L.T., Graham, R.D. (2005a). Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. Biol. Trace Elem. Res. 103, 155–168.

Ma, G., Jin, Y., Li, Y., Zhai, F., Kok, F.J., Jacobson, E., Yang, X. (2008). Iron and zinc deficiencies in China: what is feasible and cost-effective strategy? Public Health Nutr. 11 (6), 632–638.

Malagoli, M., Schiavon, M., dall'Acqua, S., Pilon-Smits, E. a.H. (2015). Effects of selenium biofortification on crop nutritional quality. Front. Plant Sci. 6:1–5.15.

Sifakis, S., Pharmakides, G (2000) Ann NY Acad Sci. 900:125–136. 16.

Manzeke, G. M., Mtambanengwe, F., Nezomba, H., & Mapfumo, P. (2014). Zinc fertilization influence on maize productivity and grain nutritional quality under integrated soil fertility management in Zimbabwe. Field Crops Research. 166: 128–136.

Meenakshi, J.V., Johnson, N., Manyong, V., De Groote, H., Javelosa, J., Yanggen, D., Naher, F., et al. (2010). How cost-effective is biofortification in combating micronutrient malnutrition? An ex-ante assessment. World Dev. 38 (1), 64–75

Moura, F., Palmer, A., Finkelstein, J., et al. (2014). Is bio fortified staple food crops improving vitamin A and iron status in women and children? New evidence from efficacy trials. Adv. Nutr. 5:568–570

Moura, F., Miloff, A., Boy, E., 2015. Retention of provitamin A carotenoid in staple crops targeted for biofortification in Africa: cassava, maize, and sweet potato. Crit. Rev. Food Sci. Nutr. 55 (9), (1246-69).

Paltridge, N. G., Palmer, L. J., Milham, P. J., Guild, G. E., & Stangoulis, J. C. R. (2012). Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. Plant and Soil. 361(1-2), 251–260.

Saltzman, A., Birol, E., Bouis, H. E., Boy, E., De Moura, F. F., Islam, Y., & Pfeiffer, W. H. (2013). Biofortification: Progress toward a more nourishing future. Global Food Security. 2(1), 9–17.

Sanchez, P.A., Swaminathan, MS. (2005). Hunger in Africa: the link between unhealthy people and unhealthy soils. Lancet. 365(9457):442-4.

Shahzad, Z., Rouached, H., Rakha, A. (2014). Combating Mineral Malnutrition through Iron and Zinc Biofortification of Cereals. Comprehensive Reviews in Food Science and Food Safety, 13(3), 329–346.

Smith, S.E., Read, D.J. (1997) Mycorrhizal Symbiosis. 2nd Edition, Academic Press, London.

Thilakarathna, M., Raizada, M. (2015). A Review of Nutrient Management Studies Involving Finger Millet in the Semi-Arid Tropics of Asia and Africa. Agronomy. 5(3), 262–290.

Wang, W., Ciais, P., Nemani, R. R., Canadell, J. G., Piao, S., Sitch, S., Myneni, R. B. (2013). Variations in atmospheric CO2 growth rates coupled with tropical temperature. Proceedings of the National Academy of Sciences. 110(32), 13061–13066.

Wesseler, J., & Zilberman, D. (2014). The economic power of the Golden Rice opposition. Environment and Development Economics. 19(6), 724-742.

World Health Organization and Food and Agriculture Organization of the United Nations Guidelines on food fortification with micronutrients. Archived December 26, 2016, at the Wayback Machine. 2006

Vanlauwe, B., Descheemaeker, K., Giller, K. E., Huising, J., Merckx, R., Nziguheba, G., Wendt, J., and Zingore, S. (2015). Integrated soil fertility management in sub-Saharan Africa: unravelling local adaptation, SOIL. 1: 491–508