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CHAPTER 5

PROGRESS UPDATE: CROP DEVELOPMENT OF BIOFORTIFIED STAPLE FOOD CROPS UNDER HARVESTPLUS

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ABSTRACT

Over the past 15 years, biofortification, the process of breeding nutrients into food crops, has gained ample recognition as a cost-effective, complementary, feasible means of delivering micronutrients to populations that may have limited access to diverse diets, supplements, or commercially fortified foods. In 2008, a panel of noted economists that included five Nobel Laureates ranked biofortification fifth among the most cost-effective solutions to address global challenges such as reducing hidden hunger. The 2016 World Food Prize was awarded to biofortification.

Biofortification involves breeding staple food crops to increase their micronutrient content, targeting foods widely consumed by low-income families in Africa, Asia, and Latin America. The focus is on providing sufficient levels of vitamin A, iron, and/or zinc through these crops, based on existing consumption patterns.

HarvestPlus is part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH). HarvestPlus works in partnership with more than 200 scientific and implementation organizations around the world to improve nutrition and public health by developing and promoting biofortified food crops that are rich in vitamins and minerals, and providing global leadership on biofortification evidence and technology.

Crops bred for higher levels of micronutrients using conventional breeding methods have been released in 26 countries in Africa, Asia and Latin America, and are now being grown and eaten by millions of farmers and consumers. This paper reviews crop development progress and varietal release of primary (major) and secondary (regionally important) staple crops, with a focus on progress in Africa.

Key words: Biofortification, Micronutrients, Micronutrient Deficiency, Staple Crops, Breeding, Provitamin A, Iron, Zinc

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HARVESTPLUS BREEDING APPROACH

Early in the conceptual development of the HarvestPlus project, a working group of nutritionists and plant breeders established nutritional breeding targets, based on food consumption patterns of target populations, estimated nutrient losses during storage and processing, and nutrient bioavailability [1]. Breeding targets for biofortified crops were designed to meet the specific dietary needs and consumption patterns of women and children. Targets were set such that for preschool children 4-6 years old and for non-pregnant, non-lactating women of reproductive age, iron-biofortified beans and iron-biofortified pearl millet would provide approximately 60% of the Estimated Average Requirement (EAR) for iron; zinc-biofortified wheat and zinc-biofortified rice would provide 60 to 80% of the EAR for zinc; and provitamin A biofortified maize, cassava and sweet potato would provide at least 50% of provitamin A. Originally established using limited data on consumption patterns, as well as nutrient stability and retention in the biofortified crops [2], the breeding targets were refined to meet the target EARs as more data became available (Table 5.1). The revised assumptions and target levels as well as general methodological approach for setting breeding target levels for HarvestPlus primary staple food crops are presented in Chapter 1.

The HarvestPlus approach to breeding first assesses whether sufficient genetic variation exists in elite or germplasm bank materials for a particular trait of interest. Plant breeders screen existing crop varieties and accessions in global germplasm banks, including both adapted and non-adapted material such as landraces and wild relatives. Initial research indicated that selection of lines with diverse vitamin and mineral profiles could be exploited for genetic improvement [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. When lines with these traits are identified, they are used in early-stage product development and parent building. Intermediate stage product development takes place at CGIAR centers, where breeding materials with improved nutrient content and high agronomic performance, as well as preferred consumer qualities are developed. Final product development takes place at both CGIAR centers and National Agricultural Research Systems (NARS). National research partners may carry out further crosses with locally adapted materials to develop final products that meet specific traits required by local producers and consumers. When promising high-yielding, high-nutrient lines emerge, they are tested across a wide range of environments side-by-side with locally preferred varieties. Participatory variety selection (PVS) involves farmers and/or consumers who compare crop and food preparation performance to select the preferred materials. The best-performing lines are then submitted to national performance trials conducted by governmental institutions prior to release. The breeding process takes six to ten years to complete. As of 2016, HarvestPlus partners have released more than 140 biofortified varieties of 10 crops in 26 countries (Annex 1). All varieties released have undergone official national performance testing and comply with national requirements for release, including agronomic competitiveness or other added value (for example, early maturity or end-use quality traits) compared to existing, non-biofortified varieties.

HarvestPlus has used two strategies to shorten the time to market for biofortified crops: 1) identifying adapted varieties with significant micronutrient content for release and/or dissemination as "fast-track" varieties, while varieties with target micronutrient content are still under development, and 2) deploying multi-location regional trials across a wide range of countries and sites to accelerate release processes by increasing available performance data of elite breeding materials. Regional trials also include already released biofortified varieties and generate data on their regional performance. By substituting temporal-by-spatial environmental variation in large-scale regional genotype-by-environment (GxE) testing, testing steps can be eliminated and time to market shortened by one to two years. In Africa, HarvestPlus is exploring the option to take advantage of regional agreements that harmonize seed regulations of member countries and allow any variety that is tested, approved, and released in one member country to be released simultaneously in other member countries with similar agro-ecologies.

DIAGNOSTIC TOOLS FOR MICRONUTRIENT SCREENING OF MINERALS AND CAROTENOIDS

The availability of adequate diagnostic tools is of key importance for the success of any biofortification breeding effort. Proper technologies and methods are needed for precision analysis of micronutrient content as well as for high-throughput screening of large numbers of samples from breeding trials. Pfeiffer and McClafferty [16] provide a comprehensive overview of analytical methods and diagnostic tools used in HarvestPlus breeding programs, and also discuss other related issues, such as the varying sensitivity requirements depending on the stage of development, contamination (in the case of minerals), effects of milling/polishing, and micronutrient concentration versus content. A detailed review of high-throughput measurement methodologies is presented in Chapter 6.

Minerals (Iron and Zinc)

The gold standard for high-precision mineral analysis of iron and zinc is inductively-coupled plasma (ICP), which is also capable of detecting soil contamination [16, 17]. For high-throughput screening, HarvestPlus and its partners have developed X-ray fluorescence (XRF) spectrometry calibrations and glass standards. This new technology has proven cost- and time-efficient for mineral screening of a wide range of crops including wheat, rice, pearl millet, beans, sorghum, lentil, cowpea, and Irish potato [18, 19, 20]. To date, HarvestPlus has implemented 25 state-of-the-art micronutrient analytical laboratories at nine CGIAR centers, 12 NARS, and four universities, and trained more than 100 laboratory staff in 13 countries in field sampling, sample preparation, equipment calibration, and operation. Thus, the project provides analytical services to partners from the public and private sector in HarvestPlus target countries, enabling high throughput screening of the iron and zinc contents of their genetic material and future product pipeline.

Provitamin A Carotenoids

Similarly, HarvestPlus and its partners have developed analytical methods for provitamin A carotenoid analysis in sweet potato, cassava, maize, and banana. High-performance liquid chromatography (HPLC) is the method of choice for high-precision analysis, due to its ability to reliably separate and quantify individual carotenoids differing in their provitamin A activity. Spectrophotometric methods are less complicated and less costly than HPLC, but cannot distinguish between different carotenoid fractions. In crops where

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β-carotene is the predominant carotenoid, such as sweet potato and cassava, they can be used to measure the amount of total carotenoids as equivalents of β-carotene. However, they tend to overestimate carotenoid content when compared to HPLC due to other compounds also detected [21, 22]. Near-infrared spectrometry (NIRS) has proven a cost- and time-efficient method for high-throughput screening for carotenoid content in sweet potato [23], banana [24, 25], and cassava as well as for other important breeding traits such as dry matter content (DMC) and cyanogenic potential [26, 27, 28]. New portable devices such as iCheck and portable NIRS are being evaluated and show promise for use in the field when transport of fresh cassava roots to the nearest laboratory is a challenge [29].

CROP DEVELOPMENT PROGRESS TO DATE – PRIMARY STAPLES

Provitamin A Orange Sweet Potato

Sweet potato is widely consumed in sub-Saharan Africa. Conventionally bred orange sweet potato (OSP) containing provitamin A was the first biofortified crop developed and released by the International Potato Center (CIP), HarvestPlus, and its partners. Plant breeders have produced several OSP varieties with provitamin A content of 30–100 ppm, exceeding the target level of 32 ppm. The National Crops Resources Research Institute (NaCRRI), with the support of CIP, conducts breeding research in Uganda. The full breeding pipeline consists of both locally developed germplasm and introductions from CIP. The National Agricultural Research Systems (NARS) engage in testing biofortified candidate varieties and providing other technical support to seed systems. As the provitamin A trait is mainstreamed in breeding populations, ongoing OSP breeding focuses on tolerance to biotic and abiotic stress while maintaining/enhancing provitamin A levels.

In Uganda, a HarvestPlus focus country, HarvestPlus coordinates with NaCRRI and CIP to ensure a continuous flow of improved varieties. Two orange-fleshed landrace cultivars named ‘Ejumula’ and ‘SPK004’ (‘Kakamega’), with the full provitamin A target, were released in 2004, and two additional varieties named ‘Vita’ (NASPOT 9 O) and ‘Kabode’ (NASPOT 10 O) were released in 2007 [30, 31]. In 2013, two new OSP cultivars (NASPOT 12 O and NASPOT 13 O) with wide adaptation, high root yield, and high dry matter content were released [32]. Biofortified OSP varieties have been released in more than 15 countries across sub-Saharan Africa, and are also being introduced in many parts of Asia (China, Bangladesh, India) and Latin America.

At the 2016 Annual Sweet Potato Speed Breeders Meeting in Kenya, more than 30 sweet potato breeders working in 14 African countries signed a commitment to mainstreaming beta-carotene into national breeding efforts, striving to ensure that at least 50% of the clones submitted for release are biofortified, orange-fleshed types. A detailed review of sweet potato development and delivery can be found in Chapter 7.

Provitamin A Yellow Cassava

Cassava is a dietary staple in much of tropical Africa, and grows well in poor soils with limited labor requirements. Screening of cassava accessions from the International Center for Tropical Agriculture (CIAT) germplasm collection found a range of 0–19 ppm of provitamin A in existing cassava varieties, exceeding the breeding target of 15 ppm [33, 34]. Studies on GxE interaction for carotenoid content did not find significant changes in the relative ranking of genotypes, and found high (>0.6) heritability of carotenoid content in cassava roots [35, 36, 37, 38]. Rapid-cycling recurrent selection was used to shorten the normal breeding cycle from eight years to two to three years for high carotenoid content [39].

Breeding programs for provitamin A cassava are based at CIAT and the International Institute of Tropical Agriculture (IITA). The International Center for Tropical Agriculture (CIAT) generates high-provitamin A sources via rapid cycling in pre-breeding and provides in-vitro clones and seed populations to IITA and the national research programs in two target countries, Nigeria and the Democratic Republic of Congo (DRC), for local adaptive breeding. These national research programs are the Nigerian National Root Crops Research Institute (NRCRI) and the Institut National pour l'Etude et la Recherche Agronomiques (INERA) in the DRC. Participatory rural appraisal and selection is used to identify provitamin A cassava varieties that best meet farmer-preferred traits including high yield, early maturity, tolerance to pests and diseases, dry matter content, poundability, mealiness, sweetness, ease of peeling, marketability, and in-ground storage durability [40]. Genotype-by-environment (GxE) testing is used to verify that varieties proposed for release are widely adapted and stable across different environments [41]. Investments in marker-assisted selection have identified phytoene synthase 2 (PSY2) as one of the major alleles for provitamin A accumulation in cassava roots, and markers are beginning to be tested as a breeding tool, in addition to the ongoing phenotypic selection [42, 43, 44].

Three first-wave provitamin A cassava varieties with 6–8 ppm of provitamin A (about 50% of the target) were released in 2011 in Nigeria. Three second-wave varieties with up to 10 ppm (66% of the target) were released in 2014. A detailed review of activities and experiences with yellow cassava development and delivery in Nigeria can be found in Chapter 9. In the DRC, a variety developed by IITA under HarvestPlus and officially released as I011661 in 2008 contained 7 ppm of provitamin A and is now under multiplication/distribution. National partners have released yellow cassava varieties in Ghana, Malawi, and Sierra Leone, and regional trials are underway for fast-tracking release in other countries in West Africa that have similar agro-ecologies. Trial data are routinely uploaded to Cassavabase (www.cassavabase.org) to inform breeding efforts across Africa.

Crop development research continues to produce varieties with higher carotenoid content and higher dry matter content (DMC). Carotenoid content was found to be inversely correlated with DMC in African cassava clones [38], but not so in Latin American germplasm [38]. The development and introduction to Africa of additional parent materials that combine high carotenoid content and high dry matter content will broaden the genetic base of biofortified germplasm for Africa.

Provitamin A Orange Maize

Maize is the most important cereal crop in sub-Saharan Africa and is also an important staple in Latin America. Initial screening of more than 1,500 maize germplasm accessions found ranges of 0–19 ppm provitamin A in existing maize varieties, exceeding the provitamin A target of 15 ppm [45, 46, 47]. These nutrients were consistently expressed in the maize inbred lines across different growing conditions, and further assessment indicated potential to increase the levels of multiple carotenoids simultaneously [48, 49, 50, 51]. The identification of loci associated with provitamin A carotenoids and the development of DNA markers have led to accelerated genetic gain in breeding for increased provitamin A content. The most important provitamin A enhancing alleles identified to date are lycopene epsilon cyclase (lcyE) and beta-carotene hydroxylase 1 (crtRB1) [51, 52]. Validation experiments showed that the latter alone often doubles, and sometimes triples, the total concentration of provitamin A carotenoid content in maize grain, mainly by increasing the content of beta-carotene [53].

Provitamin A maize breeding programs at the International Maize and Wheat Improvement Center (CIMMYT), IITA, and the Zambia Agriculture Research Institute (ZARI) began in 2007. The breeding pipeline includes materials from the two lead institutions, CIMMYT (tropical mid-altitude) and IITA (tropical lowlands), as well as local germplasm. Both hybrid and open-pollinated (synthetic) biofortified varieties are being developed.

To date, in Africa, more than 40 provitamin A maize synthetics, single-cross hybrids, and three-way hybrids have been released in the DRC, Ghana, Malawi, Mali, Nigeria, Rwanda, Tanzania, Zambia, and Zimbabwe. The first wave of varieties released in 2012/2013 contained 6-8 ppm additional provitamin A (about 50% of the target increment), while second-wave varieties (released in 2015/2016) contain about 10 ppm additional provitamin A (66% of the target increment) [54]. A detailed review of activities and experiences with orange maize development and delivery in Zambia can be found in Chapter 8. Varieties that fully meet the provitamin A target level are being tested in multi-location trials across sub-Saharan Africa and are expected to be released in 2018. All biofortified varieties combine competitive grain yield and consumer preferred end-use quality traits with higher provitamin A content.

In addition to breeding for provitamin A, both CIMMYT and IITA are also breeding for white maize with higher zinc content. Zinc content in maize ranges from 17–42 ppm [55], and elite tropical maize synthetics and hybrids with more than 80% of the target increment (+12 ppm additional zinc) have been identified in breeding programs. Frequently, high zinc maize lines also have high protein content (called Quality Protein Maize, QPM). Zinc maize varieties are being tested in several African and Latin American countries and the first releases are expected in 2017.

Current breeding efforts focus on developing climate smart maize that is higher yielding and tolerant to drought and heat. Additional crop improvement research is underway to develop provitamin A maize with enhanced carotenoid stability, to reduce the rate and pace of carotenoid degradation in storage and end-use [56].

Iron Bean

Common bean is the most common food legume in Latin America and eastern and southern Africa. Bush beans are cultivated in low to mid-altitudes and climbing beans in

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mid- to high-altitude areas. Initial screening found ranges of 30–110 ppm iron (and 25–60 ppm zinc) in cultivated and wild beans from the germplasm collection at CIAT, exceeding the target level of 94 ppm for iron [3, 57]. The highest levels were found in wild and weedy germplasm [58]. High-iron genotypes were used to initiate crosses to combine the high-mineral trait with acceptable grain types and agronomic characteristics. Grain mineral content is influenced by environmental factors such as soil organic matter and precipitation [59, 60]. Genotype-by-environment (GxE) testing is, therefore, used to identify materials with stable mineral accumulation across sites and generations [61].

Biofortified lines are developed by the breeding program at CIAT and are being evaluated for local adaptation by national programs in several East and Southern African countries as well as in South and Central America. The lines are at varying stages in the breeding pipelines in each of these countries. Breeding programs in African target countries Rwanda (Rwanda Agriculture Board—RAB) and the DRC (L'Institut National pour l'Etude et la Recherche Agronomique—INERA) have developed crosses locally and are assuming a greater portion of the selection work. A full breeding pipeline consists of both locally developed germplasm and CIAT introductions.

In Rwanda, four first-wave, fast-track varieties (two bush, two climber) were released in 2010 and five second-wave climbing bean varieties in 2012. In the DRC, five first-wave, fast-track varieties (three bush, two climber) were identified for release and dissemination in 2011 and five second-wave varieties (three bush, two climber) in 2013. Five varieties (two climbers, three bush) were released in Uganda in 2016. In Latin America, high iron bush beans have been released and are being disseminated in eight countries. Released bean varieties contain about 60% of the iron target level (+44 ppm more iron) in the first wave, 80% in the second wave, and 100% in the third wave. In addition, they are resistant to major pests and diseases, have good yield and farmer-preferred end-use quality, and different grain colors and sizes that cover a range of major market classes. New climber and bush bean lines with 90–100% target increment for iron are in advanced line validation trials to identify agronomically competitive third-wave varieties. Crop development activities, strategies and experience-to-date with dissemination of biofortified beans in Rwanda are discussed in more detail in Chapter 10.

In addition to the bean releases in HarvestPlus target countries, several other African countries have released iron beans under the Pan African Bean Research Alliance (PABRA), including Burundi, Kenya, Malawi and Zimbabwe. The International Center for Tropical Agriculture (CIAT) has joined forces with HarvestPlus in an effort to validate the iron levels of these materials under local conditions to determine which varieties meet the threshold of 50% target increment, that is, at least +22 ppm additional iron, compared to locally preferred varieties.

Current breeding efforts focus on developing climate-smart iron beans that are high iron, higher yielding, and tolerant to drought and heat. Additional crop improvement research is underway to combine a Low Phytic Acid (lpa) mutation with the iron trait, which increases the bioavailability of iron when beans are consumed.

Iron Pearl Millet

Pearl millet is a staple dry land cereal for around 90 million people in the arid and semi-arid regions of Africa and Asia. India is the largest producer of pearl millet, and it is also an important crop in West and Central Africa.

The breeding program for iron pearl millet is based at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India. Initial screening of germplasm accessions found ranges of 30–76 ppm iron (and 25–65 ppm zinc) in pearl millet, nearly reaching the full breeding target of 77 ppm (that is, an increment of +30 ppm additional grain iron compared to 47 ppm on average in non-biofortified germplasm). High-iron genotypes were selected to initiate crosses [62, 63]. High correlation between iron and zinc content indicated good prospects for simultaneous selection for the two micronutrients [64, 65, 66]. Both micronutrients are largely under additive genetic control, implying that iron hybrids will require both parental lines to have high iron density [67, 68]. Genotype-by-environment (GxE) testing was used to evaluate the most promising local germplasm and potential parents and verify that mineral accumulation was stable across sites and generations [69].

An iron version of one of the most popular open-pollinated varieties (OPVs), ICTP 8203, was developed by ICRISAT and officially released for Maharashtra state, India, in 2013. Due to its high iron content (exceeding 80% of the iron target increment) and wide adaptation, ICTP 8203-Fe was released and notified under the name “Dhanashakti” in 2014 for cultivation in all pearl millet-growing states of India [70, 71]. Dhanashakti was also included in the Nutri-Farm Pilot Project, initiated by the Government of India, for addressing iron deficiency [72]. Commercial production of Dhanashakti was initiated by Nirmal Seeds Company in 2012 and as of today the variety has been marketed to more than 70,000 farmers, mostly in Maharashtra.

The breeding pipeline at ICRISAT initially included OPV development. However, since approximately 95% of the pearl millet area in India is planted to hybrids, emphasis is now placed on hybrids and hybrid-parent development. The major focus of the breeding program is to develop higher yielding, high-iron hybrids with stable yield and iron performance for the major pearl millet growing areas in India. Major traits include drought tolerance, resistance to downy mildew, and end-use quality traits. Pearl millet biofortification research at ICRISAT is carried out in alliance with HarvestPlus, the All India Coordinated Pearl Millet Improvement Project, six State Agricultural Universities, more than 15 seed companies, and two state seed corporations. To ensure long-term sustainability, HarvestPlus engages seed companies in GxE testing of hybrids and inbred lines developed at ICRISAT, and encourages them to develop their own high iron hybrids for commercialization.

The first high-iron and high-yielding hybrid, ICMH-1201, was developed by ICRISAT and widely tested over 48 field trials during three consecutive years. This hybrid contains +28 ppm additional iron (more than 90% of the target increment) and has 38% higher grain yield than ICTP 8203. The hybrid is suitable for both northern and peninsular India and has been commercialized as Truthfully Labeled Seed (TLS) by Shakti Vardhak Seeds under its brand name Shakti-1201 since 2014 [73]. Several other hybrid varieties are in test marketing with private sector seed companies. Almost all identified iron sources are based on *Iniadi* germplasm (early-maturing, large-seeded landrace materials from Togo, Ghana, Burkina Faso, and Benin) or have a large proportion of *Iniadi* germplasm in their parentage [74].

The success of pearl millet biofortification in India suggests that similar achievements could be realized for Western and Central Africa (WCA). The WCA region has the largest area under millets in Africa, of which more than 90% is pearl millet. Studies of pearl millet landraces and other locally adapted materials from Niger and Sudan showed promising ranges of mineral density [75, 76, 77]. The most promising iron pearl millet OPVs are currently being tested on-farm at more than 30 locations across five countries in WCA. Two OPV varieties (GB 8735 and ICTP 8203) have been selected as candidates for fast-tracking in Niger, Ghana and Senegal.

Zinc Wheat

Wheat is one of the most important staples globally, with the highest levels of production occurring in Central and West Asia and North Africa. Compared to a breeding target of 37 ppm zinc, initial screening of more than 3,000 germplasm accessions from CIMMYT's germplasm bank found ranges of 20–115 ppm zinc (and 23–88 ppm iron) in wheat, with the highest levels found in landraces, ancestors and wild relatives [5, 78, 79].

High-zinc tetraploid genotypes were selected to develop synthetic hexaploid wheat and initiate crosses [45, 80]. The high-zinc wheat lines developed by CIMMYT are provided to NARS and agricultural universities in the HarvestPlus target countries India and Pakistan for testing and further local adaptive breeding. The new varieties are 20 to 40% superior in grain Zn concentration (additional +8 to +12 ppm) and are agronomically at par with or superior to the popular wheat cultivars of south Asia, indicating there is no yield trade-off [15, 81]. Resistance to the yellow rust Yr27 is mandatory in germplasm developed under HarvestPlus; resistance to the stem rust race Ug99 was built into zinc wheat as sources became available.

Participatory rural appraisal and multi-environment testing is conducted on-farm and on-station, in collaboration with public and private sector partners, including farmer associations, Agricultural State Universities (ASU) and seed companies. They evaluate the most promising germplasm and verify that varieties proposed for test marketing and commercialization comply with consumer preferred end-use quality attributes and are widely adapted and stable across sites and generations. While variances are associated with environmental effects [10, 82, 83, 84, 85, 86], several elite materials have been identified with high heritability for zinc and iron concentrations across environments [15, 81]. Research efforts continue to identify quantitative trait loci (QTLs) associated with grain zinc content and examine how to increase zinc loading in the grain [80, 87, 88, 89, 90]. Molecular markers associated with grain zinc have been identified and are being validated for further utilization in breeding [91, 92, 93, 94].

First-wave varieties with up to 80% of the zinc target have been released for Asia. In India, two zinc wheat varieties (BHU-3 and BHU-6) are well adapted for the Eastern Gangetic Plains Zone (EGPZ), and have been commercialized as Truthfully Labeled

Seed (TLS) since 2014. The latter of these is particularly sought-after in the market by both farmers and end consumers, due to its extra earliness and red glumes. The varieties are marketed under different brand names (including Akshai, Abhay, Chitra, Sai-3, Sai-6, SS 222, WZ 333, WZ 666, and Zinc Shakthi) by various seed companies [79]. The next wave of varieties is in the pipeline, combing full zinc target levels with varied maturity types (early, medium, and late). Advanced lines are being evaluated across multiple environments in both Asia (Bangladesh, India, Nepal, Pakistan) and Africa (Ethiopia, Kenya, Mexico, South Africa, Sudan, and Zambia).

Zinc Rice

Rice is the most widely consumed staple food, particularly in Asia and West Africa. The zinc breeding target was set at 28 ppm, and initial screening by the International Rice Research Institute (IRRI) found concentrations of 15–58 ppm zinc (and 7.5–24 ppm iron) in unpolished rice grain [7, 95, 96]. Unlike in wheat, zinc in rice grains is spread throughout the endosperm [97, 98, 99, 100]. Hence, estimates of zinc in unmilled rice are reliable indicators of zinc in milled rice; this is not the case for iron, as much of the iron in the aleurone layer is lost during milling [101]. As grain zinc content is influenced by environmental factors such as soil zinc status and temperature [6, 102, 103, 104], GxE testing was used to evaluate the most promising germplasm and verify that mineral accumulation was stable across sites and generations. Positive correlation between iron and zinc allows for simultaneous improvement of both minerals. Quantitative trait loci (QTLs) associated with Zn enhancement in rice have been reported, but none of them with an effect larger than 30% phenotypic variation [105, 106, 107, 108, 109]. Research efforts continue to identify major QTLs associated with grain zinc content and better understand zinc uptake, transport, and remobilization into the grain [110, 111].

Biofortified rice breeding is primarily focused on mega-environments in Asia. Breeding programs at IRRI, the Bangladesh Rice Research Institute (BRRI), and the Indian NARS have developed germplasm in early- to late-development stages and elite line final products. Similarly, zinc rice breeding pipelines were established at CIAT targeted at Latin America for paddy and dryland rice environments. In Latin America, *indica* is the preferred type of rice, while in Asia most rice is of the *japonica* type. Rice hybrids and respective parental inbreds were assessed for zinc; however, zinc hybrid breeding is not currently planned.

In Bangladesh, the first zinc rice Aman (rain-fed) season variety, “BRRI dhan 62,” was released in 2013. It demonstrated up to 92% of the target zinc level and is the shortest duration Aman rice variety ever released. Several additional varieties for both Aman and Boro (irrigated) seasons have been released (one in 2014, two in 2015). The first varieties are being commercialized in India in 2016. Several partners in Africa have expressed interested in testing zinc rice materials, and CIAT and IRRI have assembled nurseries of elite zinc germplasm for evaluation by partners in Western and Central Africa.

SECONDARY STAPLES

In addition to the primary staple crops, HarvestPlus is also working to improve the micronutrient content of five additional crops, which are dominant in local diets depending on the region. These are:

- Zinc and Iron Sorghum: Target countries are India, Mali, and Nigeria
- Zinc and Iron Lentil: Target countries are Bangladesh, India, and Nepal
- Iron Cowpea: Target countries are Brazil and India
- Provitamin A Bananas: Target countries are DRC, Burundi, Rwanda, and Uganda
- Zinc and Iron Irish Potato: Target countries are Rwanda and Ethiopia

These secondary staples are usually consumed in lower quantities than primary staples. Consequently, their contribution to daily micronutrient requirements is also lower. The biofortified varieties currently available can provide roughly up to 30% of iron estimated average requirement (EAR) and up to 40% of zinc (Table 5.2). They are an important complement in daily diets and are frequently consumed together with primary staples such as rice or wheat (in the case of lentil and cowpea in India and Bangladesh) or beans (in the case of Irish potatoes in Rwanda). For example, in Rwanda, iron beans and iron and zinc Irish potatoes, when consumed together, provide up to 100% of iron and up to 60% of zinc EAR for women and children. Complementing the meal with orange sweet potato, yellow cassava, or provitamin A-dense plantains can further add the required vitamin A. This is called the "food basket approach," providing a range of biofortified food crop options suited to local preferences. This approach allows for diversification, both on the plate and in the field. In farmers' fields, different micronutrient-dense crops can be grown in rotation to provide a steady supply of micronutrients throughout the year.

Zinc and Iron Sorghum

Sorghum is a regionally important cereal crop in the semi-arid tropics of Africa, Asia, and Central America. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) screened and assessed more than 2,200 sorghum lines, finding iron concentrations ranging from 20-70 ppm and zinc concentrations of 13-47 ppm [112]. Southern African sorghum cultivars showed ranges of 28-63 ppm for iron and 23-55 ppm for zinc [113].

Iron and zinc density are highly heritable, and are predominantly under additive genetic control [114]. There is no penalty shown in agronomic traits when combined with high iron and zinc concentration [12, 115]. Levels of anti-nutritional factors, like tannin and phytate, were also analyzed for the prospect of breeding high iron and zinc cultivars with lower levels of such compounds [116].

Following initial screening, promising donor parents and hybrids were identified. Guinea landrace cultivars were identified as a novel source of diversity for enhancing micronutrient levels [115]. The development of high-yielding and micronutrient-dense sorghum cultivars adapted to the target environments of India, Mali and Nigeria is ongoing.

As in other iron- and zinc-dense crops, variation of mineral concentrations is associated with environmental effects. Genotype-by-environment (GxE) testing is used to identify elite materials with stable high-mineral content across different environments and crop seasons. Participatory rural appraisal is carried out by testing new varieties on-farm, under both improved and traditional management practices, to ensure that elite materials are accepted by farmers.

Several Indian commercial cultivars and hybrids were found to have high iron and zinc concentrations and could be used for fast-track dissemination [117]. Breeders at ICRISAT have developed new biofortified sorghums that have 50-60% higher grain Zn and Fe concentration than the popular Indian sorghum cultivars (20 ppm Zn and 30 ppm Fe). They are currently under multi-location testing in the All India Coordinated Sorghum Improvement Project (AICSIP) for their release. In Nigeria, popular sorghum cultivars are being assessed for iron and zinc to identify promising candidates for further testing. Biofortified cultivars can provide 13 to 18% of the zinc estimated average requirement (EAR) for children (4-6 years old) and adult women and up to 10% of the iron EAR, when 100 g (children) and 300 g (adult women) of sorghum is consumed on a daily basis.

Zinc and Iron Lentil

Lentil is a regionally important staple and source of protein, particularly in South Asia, the Middle East, and North and East Africa. Screening of more than 1,600 lentil accessions, including breeding lines, landraces and wild relatives, found that mineral content ranged from 23 to 95 ppm for zinc, and 42 to 132 ppm for iron [118, 119, 120, 121, 122].

The International Center for Agricultural Research in the Dry Areas (ICARDA) leads research to biofortify lentils, focusing on Bangladesh, Nepal, and India. Together with national research partners, released varieties were identified in several countries that already had high zinc (51-64 ppm) and good agronomic performance. More than ten such varieties were fast-tracked and are now being disseminated to farmers in Bangladesh, Ethiopia, India, Nepal, and Syria [122, 123].

In parallel to the identification of fast-track varieties, parents with high zinc were identified and have been used in cross-breeding programs at ICARDA, Bangladesh Agricultural Research Institute (BARI), Nepal Agricultural Research Council (NARC), and Indian Agricultural Research Institute (IARI). Particularly high micronutrient levels were found in accessions of a lentil wild relative (*Lens orientalis*), and efforts are underway to transfer this trait into adapted materials via pre-breeding [124]. In addition to high micronutrient content, the new varieties are resistant to major pests and diseases such as rust, wilt and *Stemphylium* blight, have good yield and farmer-preferred end-use qualities (bold seed, red and green cotyledon). Their early maturity makes them ideal for intercropping in traditional “rice-fallow” rotations in India and Bangladesh.

Mineral accumulation of zinc and iron in lentil seeds is influenced by environmental factors such as temperature, soil type, and fertility status [119, 121, 125, 126, 127]. Multi-location GxE testing is used to identify advanced lines and varieties with stable high-mineral content across different environments and crop seasons. Elite micronutrient-dense lines of red and green lentils were assembled into an international nursery and shared with 14 national programs to select high yielding, high-zinc and -iron materials based on local adaptation. As a result of these efforts, several new biofortified lentil cultivars have been released in Bangladesh (BARImasur-7 in 2012, BARImasur-8 in 2015), Nepal (ILL 7723 in 2013), and India (L4704 in 2013). The biofortified lentil varieties have 50-60% higher grain Zn and Fe concentration than popular local lentil cultivars (45 ppm Zn and 65 ppm Fe). They can provide 23 to 24% of the zinc estimated average requirement (EAR) for children (4-6 years old) and adult women and 15 to 20% of the iron EAR, when 20 g (children) and 40 g (adult women) of lentils are consumed on a daily basis.

Iron Cowpea

Cowpea is a regionally important crop in semi-arid regions of West and Central Africa, as well as India and Brazil. The International Institute of Tropical Agriculture (IITA) maintains the global cowpea germplasm collection with more than 15,000 accessions collected from different countries. They have screened and assessed a representative subsample of more than 2,000 cowpea lines of different origins in replicated screening in Nigeria. Screening activities found that cowpea zinc content ranged from 22-58 ppm and iron content from 34-79 ppm [128]. The genetic variation observed suggests that breeding for higher iron content is feasible.

HarvestPlus' main target countries for iron-dense cowpea are India and Brazil. Local breeding research in India is conducted in collaboration with G.B. Pant University of Agriculture and Technology, Pantnagar. Breeding efforts focused on the introduction and further improvement of photo-insensitive and heat-tolerant "60-day cowpea" varieties developed by IITA, as a *niche crop* for cereal-based "wheat-rice" and "rice-rice" cropping systems. Five early-maturing cowpea varieties with increased iron levels, Pant Lobia-1 to Pant Lobia-5, have been released by the Uttarakhand Government since 2008. They have entered the national seed multiplication system and seed is available to farmers.

Brazil is the second largest producer of cowpea in the world, and most of the production is consumed locally. Biofortification research in Brazil is conducted by the Brazilian Agricultural Research Corporation, EMBRAPA, under the "BioFort" umbrella. Breeding activities have led to the development and release of three cowpea varieties with up to 40% higher iron content.

After India and Brazil, Nigeria is the country with the third-highest per capita consumption of cowpea worldwide (18 kg/capita/year), and plans are in place to introduce elite biofortified cowpea lines from India for testing in Nigeria. They could provide up to 8% of the iron estimated average requirement (EAR) for children (4-6 years old) and adult women, when 20 g (children) and 50 g (adult women) of cowpeas are consumed on a daily basis.

Provitamin A Bananas

Bananas – including dessert and cooking bananas (often referred to as plantains) – are the world's most important fruit crop and a staple food in many tropical countries, particularly in East Africa. Considerable variation for provitamin A exists naturally within the banana genepool [129, 130]. Screening of more than 300 genotypes from the Centre Africain Régional de Recherches sur Bananiers et Plantains (CARBAP, Cameroon), IITA-Uganda, and local collections found that provitamin A carotenoids ranged from 1 to 345 ppm [131, 132]. Orange-fleshed cultivars indigenous to the Pacific region had particularly high provitamin A content, exceeding the target level of 30 ppm [133]. High provitamin A levels were also discovered in several African varieties.

Research programs for provitamin A bananas are based at Bioversity International and IITA. Because breeding is difficult and time-consuming, activities have focused on fast-tracking existing cultivars adapted to relevant conditions in African target countries (Uganda, the DRC, Burundi, and Rwanda) that already had high provitamin A, good agronomic performance and acceptable sensory traits. Several promising cooking and dessert bananas with high (ranging from 25 to more than 70 ppm) provitamin A were identified and are now being deployed to farmers in Burundi, the DRC, and Rwanda along with crop management recommendations. These cultivars can meet more than 100% of the vitamin A EAR for children (4-6 years old) and more than 50% of the EAR for women when 100 g of ripe fruit are consumed.

Zinc and Iron Potato

Potato, also known as Irish potato to distinguish it from the sweet potato, is an important staple and food security crop, particularly in South America, China, and parts of East Africa. The main target countries for biofortified Irish potato are Rwanda and Ethiopia.

The breeding program for zinc and iron potato is based at CIP, and initial screening of germplasm accessions found ranges of 8-25 ppm zinc (dry weight - DW) and 11-30 ppm iron (DW) in existing potato varieties [134, 135]. Levels of vitamin C and phenolic compounds were also assessed, as these affect iron absorption [136]. Heritability of iron and zinc concentrations in potato tubers is moderately high, and no negative correlation was found between micronutrient concentration and important resistance traits [137].

Diploid Andean landrace potatoes with diverse shape, color, and culinary properties were found to have high zinc and iron concentrations, and were used to initiate crosses with disease resistant tetraploid clones developed at CIP. The new biofortified potatoes developed by CIP have tubers with 60-80% higher zinc and iron content than local (14 ppm Zn and 16 ppm Fe) cultivars.

Biofortified potatoes are provided as clones and seed populations to the national research programs in two target countries Rwanda and Ethiopia for testing and further local adaptive breeding: the Rwanda Agricultural Board (RAB) and the Ethiopian Institute for Agriculture Research (EIAR). Participatory rural appraisal in Rwanda led to the selection of the best-performing and farmer-preferred clones for fast-track delivery. The first wave of biofortified potatoes selected for official release in Rwanda in 2017 have resistance to late blight and virus disease, competitive yield and good processing quality. Their tubers have up to 60% higher zinc and iron content than local cultivars, and can meet 12% of the iron EAR for children (4-6 years old) and 20% of the zinc EAR when 200 g of potatoes are consumed.

THE WAY FORWARD

Much progress has been made toward reaching micronutrient density targets for major staple food crops in Africa and Asia. More than 140 biofortified varieties have been released in 26 countries, and these varieties are now being grown and consumed by millions of farming households. HarvestPlus and its partners have developed a strong evidence base for several primary staple crops that biofortification works, further discussed in other chapters of this special issue.

Looking ahead, key investments will help biofortification reach its full potential. First, biofortified traits must become "mainstreamed" in conventional crop development programs. HarvestPlus investments have filled breeding pipelines with high-micronutrient donor parents, pedigree lines, early generation and advanced breeding materials with high yield potential and other desirable traits. To sustain this investment, the public and private sectors must include high micronutrient content as a core trait of breeding programs, by steadily increasing the percentage of micronutrient- dense parental lines.

Second, investment in high-throughput technologies and the development of molecular markers must continue, as marker-assisted selection can greatly accelerate genetic gain. The development of such markers also mutually reinforces mainstreaming; improving the ease, accuracy, and speed of breeding for micronutrient density will lead to more widespread utilization of these technologies in breeding programs.

Finally, more investment in the development of secondary staples will speed the time to market for these regionally important crops. As can be seen in the examples of cowpea and lentil, national investments can lead to the development and release of biofortified varieties with smaller levels of investment from the CGIAR. Given growing national and international interest for pursuing biofortification as a new, complementary intervention to address micronutrient deficiency, it is the hope that a wider array of partners and NARS will come forward to invest in developing the next generation of biofortified crops.

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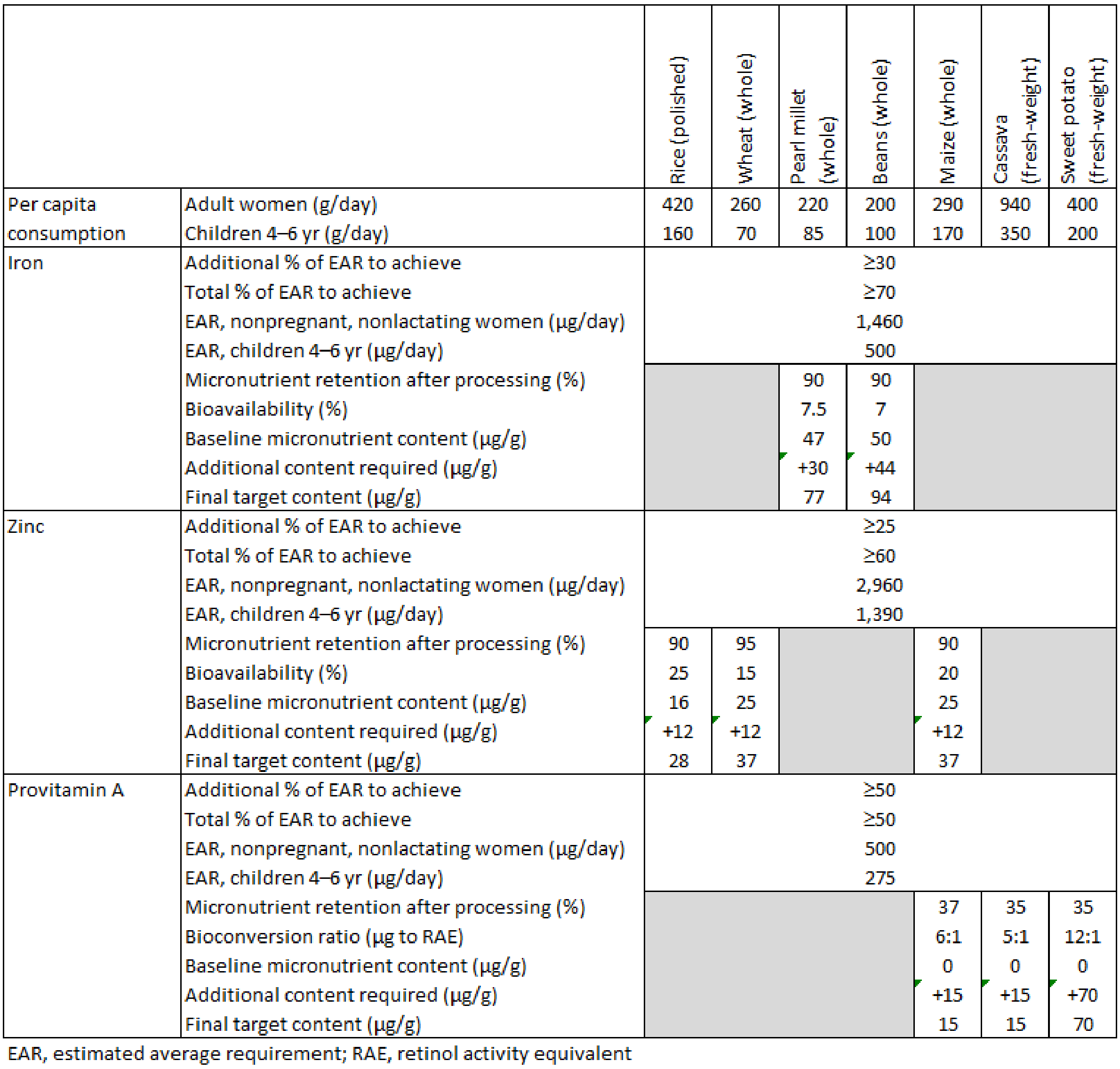
Table 5.1: Information and assumptions used to set revised target levels for micronutrient contents of biofortified primary staple food crops. The targeted micronutrient(s) for each crop are highlighted in 'bold'.



		Rice (polished)	Wheat (whole)	Pearl millet (whole)	Beans (whole)	Maize (whole)	Cassava (fresh-weight)	Sweet potato (fresh-weight)
Per capita consumption	Adult women (g/day)	420	260	220	200	290	940	400
	Children 4–6 yr (g/day)	160	70	85	100	170	350	200
Iron	Additional % of EAR to achieve	≥30						
	Total % of EAR to achieve	≥70						
	EAR, nonpregnant, nonlactating women (µg/day)	1,460						
	EAR, children 4–6 yr (µg/day)	500						
	Micronutrient retention after processing (%)			90	90			
	Bioavailability (%)			7.5	7			
	Baseline micronutrient content (µg/g)			47	50			
	Additional content required (µg/g)			+30	+44			
	Final target content (µg/g)			77	94			
Zinc	Additional % of EAR to achieve	≥25						
	Total % of EAR to achieve	≥60						
	EAR, nonpregnant, nonlactating women (µg/day)	2,960						
	EAR, children 4–6 yr (µg/day)	1,390						
	Micronutrient retention after processing (%)	90	95			90		
	Bioavailability (%)	25	15			20		
	Baseline micronutrient content (µg/g)	16	25			25		
	Additional content required (µg/g)	+12	+12			+12		
	Final target content (µg/g)	28	37			37		
Provitamin A	Additional % of EAR to achieve	≥50						
	Total % of EAR to achieve	≥50						
	EAR, nonpregnant, nonlactating women (µg/day)	500						
	EAR, children 4–6 yr (µg/day)	275						
	Micronutrient retention after processing (%)					37	35	35
	Bioconversion ratio (µg to RAE)					6:1	5:1	12:1
	Baseline micronutrient content (µg/g)					0	0	0
	Additional content required (µg/g)					+15	+15	+70
	Final target content (µg/g)					15	15	70

EAR, estimated average requirement; RAE, retinol activity equivalent

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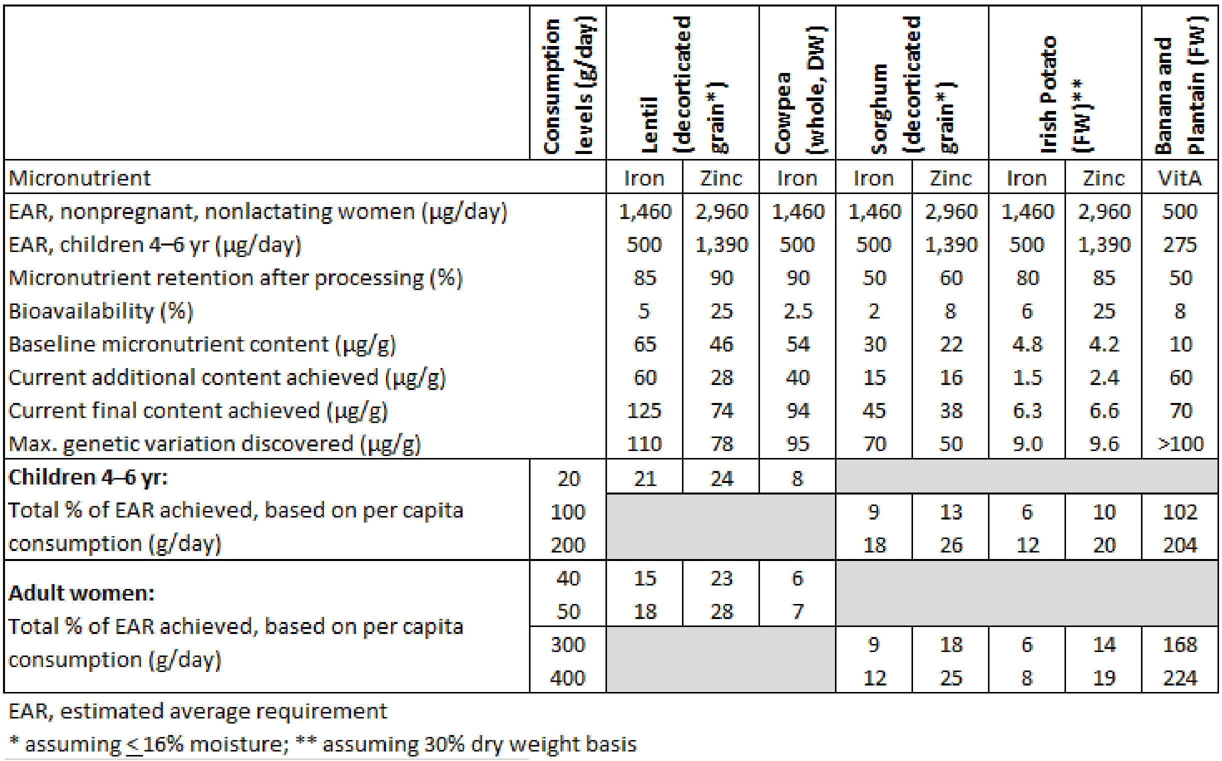
Table 5.2: Information and assumptions used to calculate the contribution of biofortified secondary staple food crops to estimated average requirements (EAR) for iron, zinc and provitamin A (HarvestPlus, unpublished data).



	Consumption levels (g/day)	Lentil (decorticated grain*)		Cowpea (whole, DW)	Sorghum (decorticated grain*)		Irish Potato (FW)**		Banana and Plantain (FW)
Micronutrient		Iron	Zinc	Iron	Iron	Zinc	Iron	Zinc	VitA
EAR, nonpregnant, nonlactating women (µg/day)		1,460	2,960	1,460	1,460	2,960	1,460	2,960	500
EAR, children 4–6 yr (µg/day)		500	1,390	500	500	1,390	500	1,390	275
Micronutrient retention after processing (%)		85	90	90	50	60	80	85	50
Bioavailability (%)		5	25	2.5	2	8	6	25	8
Baseline micronutrient content (µg/g)		65	46	54	30	22	4.8	4.2	10
Current additional content achieved (µg/g)		60	28	40	15	16	1.5	2.4	60
Current final content achieved (µg/g)		125	74	94	45	38	6.3	6.6	70
Max. genetic variation discovered (µg/g)		110	78	95	70	50	9.0	9.6	>100
Children 4–6 yr: Total % of EAR achieved, based on per capita consumption (g/day)	20	21	24	8					
	100				9	13	6	10	102
	200				18	26	12	20	204
Adult women: Total % of EAR achieved, based on per capita consumption (g/day)	40	15	23	6					
	50	18	28	7					
	300				9	18	6	14	168
	400				12	25	8	19	224

EAR, estimated average requirement

* assuming < 16% moisture; ** assuming 30% dry weight basis

REFERENCES

1. **Hotz C and B McClafferty** From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food Nutr. Bull.* 2007; **28** (2 – Suppl. 1): S271-S279.

2. **Bouis HE, Hotz C, McClafferty B, Meenakshi JV and WH Pfeiffer** Biofortification: A new tool to reduce micronutrient malnutrition. *Food Nutr. Bull.* 2011; **32** (Supplement 1): 31S-40S.

3. **Beebe S, Gonzalez AV and J Rengifo** Research on trace minerals in the common bean. *Food Nutr. Bull.* 2000; **21(4):** 387-391.

4. **Maziya-Dixon B, Kling JG, Menkir A and A Dixon** Genetic variation in total carotene, iron, and zinc contents of maize and cassava genotypes. *Food Nutr. Bull.* 2000; **21(4):** 419-422.

5. **Monasterio I and RD Graham** Breeding for trace minerals in wheat. *Food Nutr. Bull.* 2000; **21(4):** 392-396.

6. **Gregorio GB** Progress in breeding for trace minerals in staple crops. *J. Nutr.* 2002; **132(3):** 500S–502S.

7. **Jiang SL, Wu JG, Thang NB, Feng Y, Yang XE and CH Shi** Genotypic variation of mineral elements contents in rice (*Oryza sativa* L.). *Eur. Food Res. Technol.* 2008; **228(1):** 115-122.

8. **Menkir A** Genetic variation for grain mineral content in tropical-adapted maize inbred lines. *Food Chem.* 2008; **110(2):** 454-464.

9. **Menkir A, Liu W, White WS, Maziya-Dixon B and T Rocheford** Carotenoid diversity in tropical-adapted yellow maize inbred lines. *Food Chem.* 2008; **109(3):** 521-529.

10. **Gomez-Becerra HF, Erdem H, Yazici A, Tutus Y, Torun B, Ozturk L and I Cakmak** Grain concentrations of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. *J. Cereal Sci.* 2010a; **52(3):** 342-349.

11. **Talukder ZI, Anderson E, Miklas PN, Blair MW, Osorno J, Dilawari M and KG Hossain** Genetic diversity and selection of genotypes to enhance Zn and Fe content in common bean. *Can. J. Plant Sci.* 2010; **90(1):** 49-60.

12. **Ashok Kumar A, Reddy BVS, Ramaiah B, Sahrawat KL and WH Pfeiffer** Genetic variability and character association for grain iron and zinc contents in sorghum germplasm accessions and commercial cultivars. *Eur. J. Plant Sci. Biotechnol.* 2012; **6(Special Issue 1):** 66-70.

13. **Dwivedi SL, Sahrawat KL, Rai KN, Blair MW, Andersson MS and W Pfeiffer** Nutritionally enhanced staple food crops. *Plant Breed. Rev.* 2012; **36:** 169-291.

DOI: 10.18697/ajfand.78.HarvestPlus05

14. **Fageria NK, Moraes MF, Ferreira EPB and AM Knupp** Biofortification of trace elements in food crops for human health. *Commun. Soil Sci. Plant.* 2012; **43(3):** 556–570.

15. **Velu G, Singh RP, Huerta-Espino J, Peña RJ, Arun B, Mahendru-Singh A, Mujahid MY, Sohu VS, Mavi GS, Crossa J, Alvarado G, Joshi AK and WH Pfeiffer** Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crop Res.* 2012; 137: 261-267.

16. **Pfeiffer WH and B McClafferty** HarvestPlus: Breeding crops for better nutrition. *Crop Sci.* 2007; **47(Suppl. 3):** S88-S105.

17. **Yasmin Z, Paltridge N, Graham R, Huynh BL and J Stangoulis** Measuring genotypic variation in wheat seed iron first requires stringent protocols to minimize soil iron contamination. *Crop Sci.* 2014; **54(1):** 255-264.

18. **Paltridge NG, Milham PJ, Ortiz-Monasterio JI, Velu G, Yasmin Z, Palmer LJ, Guild GE and JCR Stangoulis** Energy-dispersive X-ray fluorescence spectrometry as a tool for zinc, iron and selenium analysis in whole grain wheat. *Plant Soil.* 2012a; **361(1-2):** 261-269.

19. **Paltridge NG, Palmer LJ, Milham PJ, Guild GE and JCR Stangoulis** Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant Soil.* 2012b; **361(1-2):** 251-260.

20. **Guild GE and JCR Stangoulis** Non-Matrix matched glass disk calibration standards improve XRF micronutrient analysis of wheat grain across five laboratories in India. *Front. Plant Sci.* 2016; **7:** 784. doi: 10.3389/fpls.2016.00784

21. **Rodriguez-Amaya DB and M Kimura** HarvestPlus Handbook for Carotenoid Analysis. HarvestPlus Technical Monograph Series 2, 2004.

22. **Kimura M, Kobori CN, Rodriguez-Amaya DB and P Nestel** Screening and HPLC methods for carotenoids in sweet potato, cassava and maize for plant breeding trials. *Food Chem.* 2007; **100(4):** 1734-1746.

23. **zum Felde T, Burgos G, Espinoza J, Eyzaguirre R, Porras E and W Grüneberg** Screening for β-carotene, iron, zinc, starch, individual sugars and protein in sweetpotato germplasm by Near-Infrared Reflectance Spectroscopy (NIRS). **In:** 15th International Symposium of the International Society for Tropical Root Crops (ISTRC). Roots and tubers for sustainable development and food security: Issues and strategies. November 2-6 2009, Lima, Peru. 2009: 27-33. https://assets.publishing.service.gov.uk/media/57a09de8ed915d622c001c55/s8_zumfelde.pdf Accessed December 2016.

24. **Davey MW, Saeys W, Hof E, Ramon H, Swennen RL and J Keulemans** Application of Visible and Near-Infrared Reflectance Spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. *J. Agric. Food Chem.* 2009a; **57(5):** 1742-1751.

25. **Davey MW, Mellidou I and W Keulemans** Considerations to prevent the breakdown and loss of fruit carotenoids during extraction and analysis in *Musa*. *J. Chromatogr.* 2009b; **1216(30):** 5759-5762.

26. **Sanchez T, Ceballos H, Dufour D, Ortiz D, Morante N, Calle F, zum Felde T and F Davrieux** Prediction of carotenoids, cyanide and dry matter contents in fresh cassava root using NIRS and Hunter color techniques. *Food Chem.* 2014; **151:** 444-451.

27. **Belalcazar J, Dufour D, Andersson MS, Pizarro M, Luna J, Londoño L, Morante N, Jaramillo AM, Pino L, Becerra López-Lavalle LA, Davrieux F, Talsma EF and H Ceballos** High-throughput phenotyping and improvements in breeding cassava for increased carotenoids in the roots. *Crop Sci.* **(in press)** doi: 10.2135/cropsci2015.11.0701.

28. **Davrieux F, Dufour D, Dardenne P, Belalcazar J, Pizarro M, Luna C, Londoño L, Jaramillo A, Sanchez T, Morante N, Calle F, Becerra Lopez-Lavalle LA and H Ceballos** LOCAL regression algorithm improves near infrared spectroscopy predictions when the target constituent evolves in breeding populations. *J. Near Infrared Spec.* 2016; **24:** 109-117.

29. **Islam KMS and FJ Schweigert** Comparison of three spectrophotometric methods for analysis of egg yolk carotenoids. *Food Chem.* 2015; **172:** 233-237.

30. **Mwanga ROM, Odongo B, Niringiye C, Alajo A, Abidin PE, Kapinga R, Tumwegamire S, Lemaga B, Nsumba J and EE Carey** Release of two orange-fleshed sweetpotato cultivars, ‘SPK004’ (‘Kakamega’) and ‘Ejumula’, in Uganda. *HortScience.* 2007; **42(7):** 1728–1730.

31. **Mwanga ROM, Odongo B, Niringiye C, Alajo A, Kigozi B, Makumbi R, Lugwana E, Namukula J, Mpembe I, Kapinga R, Lemaga B, Nsumba J, Tumwegamire S and CG Yencho** NASPOT 7’, ‘NASPOT 8’, ‘NASPOT 9 O’, ‘NASPOT 10 O’, and ‘Dimbuka-Bukulula’ Sweetpotato. *HortScience.* 2009; **44(3):** 828-832.

32. **Mwanga ROM, Kyalo G, Ssemakula GN, Niringiye C, Yada B, Otema MA, Namakula J, Alajo A, Kigozi B, Makumbi RNM, Ball AM, Grüneberg WJ, Low JW and CG Yencho** NASPOT 12 O’ and ‘NASPOT 13 O’ Sweetpotato. *HortScience.* 2016; **51(3):** 291-295.

33. **Chavez AL, Sanchez T, Jaramillo G, Bedoya JM, Echeverry J, Bolaños EA, Ceballos H and CA Iglesias** Variation of quality traits in cassava roots evaluated in landraces and improved clones. *Euphytica.* 2005; **143(1-2):** 125-133.

34. **Ceballos H, Kulakow P and C Hershey** Cassava breeding: current status, bottlenecks and the potential of biotechnology tools. *Trop. Plant Biol.* 2012; **5(1):** 73-87.

35. **Ssemakula GN and AGO Dixon** Genotype x environment interaction, stability, and agronomic performance of carotenoid-rich cassava clones. *Sci. Res. Essays.* 2007; **2(9):** 390-399.

36. **Ssemakula G, Dixon AGO and B Maziya-Dixon** Stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava (*Manihot esculenta* Crantz). *J. Trop. Agric.* 2007; **45(1-2):** 14-20.

37. **Morillo-CY, Sanchez T, Morante N, Chávez AL, Morillo-C AC, Bolaños A and H Ceballos** Preliminary study of inheritance of the carotenoids content in roots from cassava (*Manihot esculenta* Crantz) segregating populations. *Acta Agron.* 2012; **61(3):** 253-264.

38. **Njoku DN, Gracen VE, Offei SK, Asante IK, Egesi CN, Kulakow P and H Ceballos** Parent-offspring regression analysis for total carotenoids and some agronomic traits in cassava. *Euphytica.* 2015; **206(3):** 657-666.

39. **Ceballos H, Morante N, Sanchez T, Ortiz D, Aragon I, Chavez AL, Pizarro M, Calle F and D Dufour** Rapid cycling recurrent selection for increased carotenoids content in cassava roots. *Crop Sci.* 2013; **53(6):** 2342-2351.

40. **Njoku DN, Egesi CN, Gracen VE, Offei SK, Asante IK and EY Danquah** Identification of Pro-vitamin A Cassava (*Manihot esculenta* Crantz) Varieties for adaptation and adoption through participatory research. *J. Crop Improvement.* 2014; **28(3):** 361-376.

41. **Maroya NG, Kulakow P, Dixon AGO, Maziya-Dixon B and MA Bakare** Genotype x Environment interaction of carotene content of yellow-fleshed cassava genotypes in Nigeria. *J. Life Sci.* 2012; **6:** 595-601.

42. **Welsch R, Arango J, Bar C, Salazar B, Al-Babili S, Beltran J, Chavarriaga P, Ceballos H, Tohme J and P Beyer** Provitamin A accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *Plant Cell.* 2010; **22(10):** 3348-3356.

43. **Ferguson M, Rabbi I, Kim DJ, Gedil M, Lopez-Lavalle LAB and E Okogbenin** Molecular markers and their application to cassava breeding: Past, present and future. *Trop. Plant Biol.* 2011; **5(1):** 95-109.

44. **Rabbi I, Hamblin M, Gedil M, Kulakow P, Ferguson M, Ikpan AS, Ly D and JL Jannink** Genetic Mapping using genotyping-by-sequencing in the clonally-propagated cassava. *Crop Sci.* 2014; **54(4):** 1384–1396.

45. **Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R and RJ Peña** Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *J. Cereal Sci.* 2007; **46(3):** 293-307.

46. **Harjes CE, Rocheford T, Ling B, Brutnell TP, Kandianis CB, Sowinksi SG, Vallabheni SR, Williams M, Wurtzel ET, Yan J and ES Buckler** Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science.* 2008; **319(5861):** 330-333.

47. **Menkir A, Rocheford T, Maziya-Dixon B and S Tanumihardjo** Exploiting natural variation in exotic germplasm for increasing provitamin-A carotenoids in tropical maize. *Euphytica.* 2015; **205(1):** 203-217.

48. **Menkir A and B Maziya-Dixon** Influence of genotype and environment on β-carotene content of tropical yellow-endosperm maize genotypes. *Maydica.* 2004; **49:** 313-318.

49. **Dhliwayo T, Palacios-Rojas N, Crossa J and KV Pixley** Effects of S1 recurrent selection for provitamin A carotenoid content for three open-pollinated maize cultivars. *Crop Sci.* 2014; **54(6):** 2449-2460.

50. **Menkir A, Gedil M, Tanumihardjo S, Adepoju A and B Bossey** Carotenoid accumulation and agronomic performance of maize hybrids involving parental combinations from different marker-based groups. *Food Chem.* 2014; **148:** 131-137.

51. **Suwarno WB, Pixley KV, Palacios-Rojas N, Kaeppler SM and R Babu** Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Sci.* 2014; **54(1):** 14-24.

52. **Yan J, Kandianis CB, Harjes CE, Bai L, Kim EH, Yang X, Skinner DJ, Fu Z, Mitchell S, Li Q, Fernandez MGS, Zaharieva M, Babu R, Fu Y, Palacios N, Li J, DellaPenna D, Brutnell T, Buckler ES, Warburton ML and T Rocheford** Rare genetic variation at *Zea mays* crtRB1 increases beta-carotene in maize grain. *Nat. Genet.* 2010; **42(4):** 322-327.

53. **Babu R, Palacios-Rojas N, Gao S, Yan J and K Pixley** Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on provitamin A concentrations for 26 tropical maize populations. *Theor. Appl. Genet.* 2013; **126(2):** 389-399.

54. **Pixley K, Palacios N, Babu R, Mutale R, Surles R and E Simpungwe** Biofortification of maize with provitamin A carotenoids. **In:** SA Tanumihardjo (Ed.). Carotenoids and Human Health. Humana Press 2013: 271-292.

55. **Queiroz VAV, Guimarães PEO, Queiroz LR, Guedes EO, Vasconcelos VDB, Guimarães LJ, Ribeiro PEA and RE Schaffert** Iron and zinc availability in maize lines. *Cienc. Tecnol. Aliment.* 2011; **31:** 33.

56. **Ortiz D, Rocheford T and MG Ferruzzi** Influence of temperature and humidity on the stability of carotenoids in biofortified maize (*Zea mays* L.) genotypes during controlled post-harvest storage. *J. Agric. Food Chem.* 2016; **64(13):** 2727-2736.

57. **Islam FMA, Basford KE, Jara C, Redden RJ and SE Beebe** Seed compositional and disease resistance differences among gene pools in cultivated common bean. *Genet. Res. Crop Evol.* 2002; **49(3):** 285-293.

58. **Guzman-Maldonado SH, Acosta-Gallegos J and O Paredes-Lopez** Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L.). *J. Sci. Food Agric.* 2000; **80(13):** 1874-1881.

59. **Beebe S** Common bean breeding in the Tropics. *Plant Breed. Rev.* 2012; **36:** 357-426.

60. **Hossain KG, Islam N, Jacob D, Ghavami F, Tucker M, Kowalski T, Leilani A and J Zacharias** Interdependence of genotype and growing site on seed mineral compositions in common bean. *Asian J. Plant Sci.* 2013; **12:** 11-20.

61. **Blair MW, Monserrate F, Beebe SE, Restrepo J and J Ortube** Registration of high mineral common bean germplasm lines NUA35 and NUA56 from the red-mottled seed class. *J. Plant Regist.* 2010; **4(1):** 55-59.

62. **Velu G, Rai KN, Muralidharan V, Kulkarni VN, Longvah T and TS Reveendran** Prospects of breeding biofortified pearl millet with high grain iron and zinc content. *Plant Breed.* 2007; **126(2):** 182-185.

63. **Gupta SK, Velu G, Rai KN and K Sumalini** Association of grain iron and zinc content with grain yield and other traits in pearl millet [*Pennisetum glaucum* (L.) R.Br.]. *Crop Improvement.* 2009; **36(2):** 4-7.

64. **Velu G, Bhattacharjee R, Rai KN, Sahrawat KL and T Longvah** A simple and rapid screening method for grain zinc content in pearl millet. *J. SAT Agric. Res.* 2008a; **6**.

65. **Velu G, Rai KN, Sahrawat KL and K Sumalini** Variability for grain iron and zinc contents in pearl millet hybrids. *J. SAT Agric. Res.* 2008b; **6**.

66. **Rai KN, Yadav OP, Rajpurohit BS, Patil HT, Govindaraj M, Khairwal IS and AS Rao** Breeding pearl millet cultivars for high iron density with zinc density as an associated trait. *J. SAT Agric. Res.* 2013; **11**.

67. **Velu G, Rai K, Muralidharan V, Longvah T and J Crossa** Gene effects and heterosis for grain iron and zinc density in pearl millet (Pennisetum glaucum (L.) R. Br). *Euphytica.* 2011; **180(2):** 251-259.

68. **Govindaraj M and KN Rai** Breeding biofortified pearl millet cultivars with high iron density. *Indian Farm.* 2016; **65(12):** 53-55.

69. **Rai KN, Govindaraj M and AS Rao** Genetic enhancement of grain iron and zinc content in pearl millet. *Qual. Assur. Saf. Crops.* 2012; **4(3):** 119-125.

70. **Rai KN, Patil HT, Yadav OP, Govindaraj M, Khairwal IS, Cherian B, Rajpurohit BS, Rao AS, Shivade H and MP Kulkarni** Notification of crop varieties and registration of germplasm: Pearl millet variety ‘Dhanashakti’. *Indian J. Genet. Plant Breed.* 2014a; **74(3):** 405-406.

71. **Rai KN, Patil HT, Yadav OP, Govindaraj M, Khairwal IS, Cherian B, Rajpurohit BS, Rao AS and MP Kulkarni** Dhanashakti - a high-iron pearl millet variety. *Indian Farm.* 2014b; **64(7):** 32-34.

72. **Purushottam Singh SK and R Uddeen** Nutri-farms for mitigating malnutrition in India, Chapter 34. **In:** Singh U, Praharaj CS, Singh SS and NP Singh (Eds.). Biofortification of Food Crops. Springer: India. 2016: 461-477.

73. **Govindaraj M, Rai KN and P Shanmugasundaram** Intra-population genetic variance for grain iron and zinc contents and agronomic traits in pearl millet. *Crop J.* 2016; **4(1):** 48-54.

74. **Rai KN, Velu G, Govindaraj M, Upadhyaya HD, Rao AS, Shivade H and KN Reddy** Iniadi pearl millet germplasm as a valuable genetic resource for high grain iron and zinc densities. *Plant Genet. Resour. C.* 2015; **13(1):** 75-82.

75. **Bashir EMA, Ali AM, Ali AM, Ismail MI, Parzies, HK and BIG Haussmann** Patterns of pearl millet genotype-by-environment interaction for yield performance and grain iron (Fe) and zinc (Zn) concentrations in Sudan. *Field Crops Res.* 2014a; **166:** 82-91.

76. **Bashir EMA, Ali AM, Ali AM, Melchinger AE, Parzies HK and BIG Haussmann** Characterization of Sudanese pearl millet germplasm for agro-morphological traits and grain nutritional values. *Plant Genet. Resour. C.* 2014b; **12(1):** 35-47.

77. **Pucher A, Hogh-Jensen H, Gondah J, Hash CT and BIG Haussmann** Micronutrient density and stability in West African pearl millet - potential for biofortification. *Crop Sci.* 2014; **54(4):** 1709-1720.

78. **Cakmak I, Ozkan H, Braun HJ, Welch RM and V Romheld** Zinc and iron concentrations in seeds of wild, primitive, and modern wheats. *Food Nutr. Bull.* 2000; **21(4):** 401-403.

79. **Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, Cakmak I and Y Saranga** Genetic diversity of grain nutrients in wild emmer wheat: potential for wheat improvement. *Ann. Bot.* 2010; **105(7):** 1211-1220.

80. **Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y and RP Singh** Biofortification strategies to increase grain zinc and iron concentrations in wheat. *J. Cereal Sci.* 2014; **59(3):** 365-372.

81. **Velu G, Singh R, Balasubramaniam A, Mishra VK, Chand R, Tiwari C, Joshi A, Virk P, Cherian B and W Pfeiffer** Reaching out to farmers with high zinc wheat varieties through public-private partnerships – an experience from Eastern-Gangetic plains of India. *Adv. Food Technol. Nutr. Sci.* 2015; **1(3):** 73-75.

82. **Oury FX, Leenhardt F, Emesy CR, Chanliaud E, Duperrier B, Balfourier F and G Charmet** Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. *Eur. J. Agron.* 2006; **25(2):** 177-185.

83. **Morgounov AI, Gomez-Becerra HF, Abugalieva AI, Djunusova M, Yessimbekova M, Muminjanov H, Zelenskiy Y, Ozturk L and I Cakmak** Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica.* 2007; **155(1-2):** 193-203.

84. **Gomez-Becerra HF, Abugalieva A, Morgounov A, Abdullayev K, Bekenova L, Yessimbekova M, Sereda G, Shpigun S, Tsygankov V, Zelenskiy Y, Peña R and I Cakmak** Phenotypic correlations, G × E interactions and broad sense heritability analysis of grain and flour quality characteristics in high latitude spring bread wheats from Kazakhstan and Siberia. *Euphytica.* 2010b; **171(1):** 23-38.

85. **Joshi AK, Crossa J, Arun B, Chand R, Trethowan R, Vargas M and I Ortiz-Monasterio** Genotype x environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. *Field Crops Res.* 2010; **116(3):** 268-277.

86. **Gopalareddy K, Singh AM, Ahlawat AK, Singh GP and JP Jaiswal** Genotype-environment interaction for grain iron and zinc concentration in recombinant inbred lines of a bread wheat (*Triticum aestivum* L.) cross. *Indian J. Genet. Plant Breed.* 2015; **75(3):** 307-313.

87. **Tiwari VK, Rawat N, Chhuneja P, Neelam K, Aggarwal R, Randhawa GS, Dhaliwal HS, Keller B and K Singh** Mapping of quantitative trait loci for grain iron and zinc concentration in diploid a genome wheat. *J. Hered.* 2009; **100(6):** 771-776.

88. **Xu YF, An DG, Liu DC, Zhang AM, Xu HX and B Li** Molecular mapping of QTLs for grain zinc, iron and protein concentration of wheat across two environments. *Field Crops Res.* 2012; **138:** 57-62.

89. **Hao Y, Velu G, Peña RJ, Singh S and RP Singh** Genetic loci associated with high grain zinc concentration and pleiotropic effect on kernel weight in wheat (*Triticum aestivum* L.). *Mol. Breed.* 2014; **34(4):** 1893-1902.

90. **Srinivasa J, Arun B, Mishra VK, Singh GP, Velu G, Babu R, Vasistha NK and AK Joshi** Zinc and iron concentration QTL mapped in a *Triticum spelta* × *T. aestivum* cross. *Theor. Appl. Genet.* 2014; **127(7):** 1643-1651.

91. **Crespo-Herrera LA, Velu G and RP Singh** Quantitative trait loci mapping reveals pleiotropic effect for grain iron and zinc concentrations in wheat. *Ann. Appl. Biol.* 2016; **169(1):** 27-35.

92. **Tiwari C, Wallwork H, Balasubramaniam A, Mishra VK, Velu G, Stangoulis J, Kumar U and AK Joshi** Molecular mapping of quantitative trait loci for zinc, iron and protein content in the grains of hexaploid wheat. *Euphytica.* 2016; **207(3):** 563-570.

93. **Velu G, Crossa J, Singh RP, Hao Y, Dreisigacker S, Perez-Rodriguez P, Joshi AK, Chatrath R, Gupta V, Balasubramaniam A, Tiwari C, Mishra VK, Singh Sohu V and G Singh Mavi** Genomic prediction for grain zinc and iron concentrations in spring wheat. *Theor. Appl. Genet.* 2016a; **129(8):** 1595-1605.

94. **Velu G, Tutus Y, Gomez-Becerra HF, Hao Y, Demir L, Kara R, Crespo-Herrera LA, Orhan S, Yazici A, Singh RP and I Cakmak** QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. *Plant Soil.* 2016b; doi: 10.1007/s11104-016-3025-8.

95. **Graham RD, Senadhira D, Beebe S, Iglesias C and I Monasterio** Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Res.* 1999; **60(1-2):** 57-80.

96. **Jiang SL, Wu JG, Feng Y, Yang XE and CH Shi** Correlation analysis of mineral element contents and quality traits in milled rice (*Oryza sativa* L.). *J. Agric. Food Chem.* 2007; **55(23):** 9608-9613.

97. **Cakmak I** Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil.* 2008; **302(1-2):** 1-17.

98. **Takahashi M, Nozoye T, Kitajima B, Fukuda N, Hokura N and Y Terada** *In vivo* analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X-ray fluorescence imaging of Fe, Zn, Mn and Cu. *Plant Soil.* 2009; **325:** 39-51.

99. **Ishimaru Y, Bashir K and NK Nishizawa** Zn uptake and translocation in rice plants. *Rice.* 2011; **4(1):** 21-27.

100. **Ajiboye B, Cakmak I, Paterson D, de Jonge MD, Howard DL, Stacey SP, Torun AA, Aydin N and MJ McLaughlin** X-ray fluorescence microscopy of zinc localization in wheat grains biofortified through foliar zinc applications at different growth stages under field conditions. *Plant Soil.* 2015; **392(1-2):** 357-370.

101. **Sison MEGQ, Gregorio GB and MS Mendioro** The effect of different milling times on grain iron content and grain physical parameters associated with milling of eight genotypes of rice (*Oryza sativa*). *Philipp. J. Sci.* 2006; **135(1):** 9-17.

102. **Wissuwa M, Ismail AM and RD Graham** Rice grain Zn concentrations as affected by genotype, native soil Zn availability, and Zn fertilization. *Plant Soil.* 2008; **306(1):** 37-48.

103. **Impa SM, Morete MJ, Ismail AM, Schulin S and SE Johnson-Beebout** Zn uptake, translocation, and grain Zn loading in rice (*Oryza sativa* L.) genotypes selected for Zn deficiency tolerance and high grain Zn. *J. Exp. Bot.* 2013; **64(10):** 2739-2751.

104. **Nakandalage N, Nicolas M, Norton RM, Hirotsu N, Milham PJ and S Seneweera** Improving Rice zinc biofortification success rates through genetic and crop management approaches in a changing environment. *Front. Plant Sci.* 2016; **7:** 764.

105. **Stangoulis JCR, Huynh BL, Welch RM, Choi EY and RD Graham** Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica.* 2007; **154(3):** 289-294.

106. **Banerjee S, Sharma DJ, Verulkar SB and G Chandel** Use of in silico and semi quantitative RT-PCR approaches to develop nutrient rich rice (*Oryza sativa* L.). *Indian J. Biotechnol.* 2010; **9(2):** 203-212.

107. **Anuradha K, Agarwal S, Venkateswara Rao Y, Rao KV, Viraktamath BC and N Sarla** Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. *Gene.* 2012; **508(2):** 233-240.

108. **Neelamraju S, MallikarjunaSwamy BP, Kaladhar K, Anuradha K, VenkateshwarRao Y and AK Batchu** Increasing iron and zinc in rice grains using deep water rices and wild species – identifying genomic segments and candidate genes. *Qual. Assur. Saf. Crops.* 2012; **4(3):** 138-138.

109. **Norton GJ, Douglas A, Lahner B, Yakubova E, Guerinot ML, Pinson SRM, Tarpley L, Eizenga GC, McGrath SP, Zhao FJ, Islam MR, Islam S, Duan G, Zhu Y, Salt DE, Meharg AA and AH Price** Genome wide association mapping of grain arsenic, copper, molybdenum and zinc in rice (*Oryza sativa* L.) grown at four international field sites. *PLoS ONE.* 2014; **9(2):** e89685. doi:10.1371/journal.pone.0089685

110. **Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK and D Sanders** Zinc biofortification of cereals: problems and solutions. *Trends Plant Sci.* 2008; **13:** 464-473.

111. **Stomph TJ, Jiang W and PC Struik** Zinc biofortification of cereals: rice differs from wheat and barley. *Trends Plant Sci.* 2009; **14(3):** 123-124.

112. **Ashok Kumar A, Reddy Belum VS, Ramaiah B, Sanjana Reddy P, Sahrawat KL and HD Upadhyaya** Genetic variability and plant character association of grain Fe and Zn in selected core collection accessions of sorghum germplasm and breeding lines. *J. SAT Agric. Res.* 2009: **7**.

113. **Ng'uni D, Geleta M, Hofvander P, Fatih M and T Bryngelsson** Comparative genetic diversity and nutritional quality variation among some important Southern African sorghum accessions [*Sorghum bicolor* (L.) Moench]. *Aust. J. Crop Sci.* 201; **6(1):** 56-64.

114. **Ashok Kumar A, Reddy Belum VS, Ramaiah B, Sahrawat KL and WH Pfeiffer** Gene effects and heterosis for grain iron and zinc concentration in sorghum [*Sorghum bicolor* (L.) Moench]. *Field Crops Res.* 2013a; **146:** 86-95.

115. **Ashok Kumar A, Anuradha K, Ramaiah B, Frederick H, Rattunde W, Virk P, Pfeiffer WH and S Grando** Recent advances in sorghum biofortification research. *Plant Breed. Rev.* 2015; **39:** 89-124.

116. **Reddy BVS, Ramesh S and T Longvah** Prospects of breeding for micronutrients and b-Carotene-dense sorghums. *SAT eJournal.* 2005; **1(1):** 1-4.

117. **Ashok Kumar A, Reddy BVS and B Ramaiah** Biofortification for combating micronutrient malnutrition: Identification of commercial sorghum cultivars with high grain iron and zinc concentrations. *Indian J. Dryland Agric. Res.* 2013b; **28(1):** 89-94.

118. **Baum M, Hamweih A, Furman B, Sarker A and W Erskine** Biodiversity, genetic enhancement and molecular approaches in lentils. **In:** Sharma AK and A Sharma (Eds.). Plant Genome, Biodiversity and Evolution, Vol. 1. CRC Press: Boca Raton, FL, USA. 2008: 135-152.

119. **Thavarajah D, Thavarajah P, Sarker A and A Vandenberg** Lentils (*Lens culinaris* Medikus subsp. *culinaris*): a whole food for increased iron and zinc intake. *J. Agric. Food Chem.* 2009; **57(12):** 5413-5419.

120. **Karaköy T, Halil E, Baloch FS, Toklu F, Eker S, Kilian B and H Ozkan** Diversity of macro- and micronutrients in the seeds of lentil landraces. *Scientific World J.* 2012; Article ID 710412: 1-9.

121. **Kumar H, Dikshit HK, Singh AM, Singh D, Kumari J, Singh A and D Kumar** Characterization of elite lentil genotypes for seed iron and zinc concentration and genotype x environment interaction studies. *Indian J. Genet. Plant Breed.* 2013; **73(2):** 169-176.

122. **Kumar J, Gupta DS, Kumar S, Gupta S and NP Singh** Current knowledge on genetic biofortification in lentil. *J. Agric. Food Chem.* 2016; **64(33):** 6383-6396.

123. **ICARDA** Combating micronutrient malnutrition with biofortified lentil. Science Matter, Research Brief 1. 2015. Available from: https://www.icarda.org/publications-resources/science-matters Accessed December 2016.

124. **Sarker A, Kumar S, Kumar J, Dikshit HK, Alam J and N Ghimire** Breeding pulses for nutritional quality with emphasis on biofortification. **In:** Abstract Book of International Conference on Pulses, Marrakesh, Morocco, April 18-20, 2016. p. 51. Available online: http://www.icarda.org/sites/default/files/u158/ICP2016-Conference-abstract-book.pdf Accessed December 2016.

125. **Thavarajah D, Thavarajah P, Wejesuriya A, Rutzke M, Glahn RP, Combs GF and A Vandenberg** The potential of lentil (*Lens culinaris* L.) as a whole food for increased selenium, iron, and zinc intake: Preliminary results from a 3 year study. *Euphytica.* 2011; **180(1):** 123-128.

126. **DellaValle DM, Thavarajah D, Thavarajah P, Vendenberg A and RP Glahn** Lentil (*Lens culinaris* L) as a candidate crop for iron biofortification: is there genetic potential for iron bioavailability? *Field Crops Res.* 2013; **144:** 119-125.

127. **Kumar H, Dikshit HK, Singh A, Jain N, Kumari J, Singh AM, Singh D, Sarker A and KV Prabhu** Characterization of grain iron and zinc in lentil (*Lens culinaris* Medikus *culinaris*) and analysis of their genetic diversity using SSR markers. *Aust. J. Crop Sci.* 2014; **8(7):** 1005-1012.

128. **Boukar O, Massawe F, Muranaka S, Franco J, Maziya-Dixon B, Singh B and C Fatokun** Evaluation of cowpea germplasm lines for protein and mineral concentrations in grains. *Plant Genet. Res.* 2011; **9(4):** 515-522.

129. **Englberger L, Darnton-Hill I, Coyne T, Fitzgerald MH and GC Marks** Carotenoid-rich bananas: A potential food source for alleviating vitamin A deficiency. *Food Nutr. Bull.* 2003a; **24(4):** 303-318.

130. **Englberger L, Aalbersberg W, Ravi P, Bonnin E, Marks GC, Fitzgerald MH and J Elymore** Further analyses on Micronesian banana, taro, breadfruit and other foods for provitamin A carotenoids and minerals. *J. Food Comp. Anal.* 2003b; **16(2):** 219-236.

131. **Davey MW, van den Bergh I, Markham R, Swennen R and J Keulemans** Genetic variability in *Musa* fruit provitamin A carotenoids, lutein and mineral micronutrient contents. *Food Chem.* 2009c; **115(3):** 806-813.

132. **Fungo R and M Pillay** β-Carotene content of selected banana genotypes from Uganda. *Afr. J. Biotechnol.* 2011; **10(28):** 5423-5430.

133. **Englberger L, Schierle J, Aalbersberg W, Hofmann P, Humphries J, Huang A, Lorens A, Levendusky A, Daniells J, Marks GC and MH Fitzgerald** Carotenoid and vitamin content of Karat and other Micronesian banana cultivars. *Int. J. Food Sci. Nutr.* 2006; **27(5-6):** 399-418.

134. **Burgos G, Amoros W, Morote M, Stangoulis JCR and M Bonierbale** Iron and zinc concentration of native Andean potato cultivars from a human nutrition perspective. *J. Food Sci. Agric.* 2007; **87(4):** 668-675.

135. **Bonierbale M, Burgos Zapata G, zum Felde T and P Sosa** Nutritional composition of potatoes. *Cah. Nutr. Diet.* 2010; **45(6 Suppl. 1):** S28-S36.

136. **Burgos G, Auqui S, Amoros W, Salas E and M Bonierbale** Ascorbic acid concentration of native Andean potato varieties as affected by environment, cooking and storage. *J. Food Comp. Anal.* 2008; **22(6):** 533-538.

137. **Paget M, Amoros W, Salas E, Eyzaguirre R, Alspach P, Apiolaza L, Noble A and M Bonierbale** Genetic evaluation of micronutrient traits in diploid potato from a base population of Andean landrace cultivars. *Crop Sci.* 2014; **54(5):** 1949-1959.