Phenotyping Nutritional and Antinutritional Traits

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Dil Thavarajah, Casey R. Johnson, Rebecca McGee, and Pushparajah Thavarajah

Abstract

Evolution of nutrient-rich food systems to calorie-focused production agriculture has created serious agricultural and human health issues: marginalization of traditional agricultural crops, greater dependence of agricultural inputs, and creation of both energy and micronutrient malnutrition. To date more than half of global human populations are suffering numerous health problems associated with excess calories and lack of essential micronutrients. Pulse crops, in particular lentils, are promising crops not only to improve human health but also to reduce agricultural inputs toward greater agricultural sustainability. In this book chapter, human micronutrient malnutrition issues, suggestions to reduce micronutrient deficiencies, promise of pulse crops using lentil as an example, lentil's micronutrient and antinutrient profiles, nutrient analytical procedures, and the needs to shift our thinking from calorie-focused to nutrient-focused approaches are also presented.

Keywords

Micronutrients • Malnutrition • Biofortification • Fe • Zn • Foliates • Methods of analysis

D. Thavarajah (✉)
Vegetable/Pulse Quality and Nutrition, Clemson University, Department of Agricultural & Environmental Sciences, 130 McGinty Court, 270 Poole Agricultural Center, Clemson, SC 29634-0310, USA
e-mail: dthavar@clemson.edu

C.R. Johnson • P. Thavarajah
Food Chemistry and Analysis, Nutrient Interactions, North Dakota State University, IACC 372, 1320 Albrecht Blvd, Fargo, ND 58105, USA

R. McGee
USDA Agriculture Research Service, Grain Legume Genetics and Physiology Research Unit, Johnson Hall, Washington State University, Pullman, WA 99164-6434, USA

15.1 Introduction

Millions of people around the world suffer from appalling nutrition. To combat global malnutrition, novel ways to produce nutritious foods, beyond calorie-focused approaches, are required. Intensive agricultural practices resulting from the "green revolution" increased production of cereal crops, mainly rice (*Oryza sativa* L.), wheat (*Triticum* spp.), and maize (*Zea mays* L.), and have enabled most Asian and African

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populations to get enough calories from their staple foods. However, while cereal staples are a good source of carbohydrates and can satisfy daily caloric requirements, they do not provide daily requirements of protein and a range of micronutrients including iron (Fe), zinc (Zn), iodine (I), vitamins A and C, riboflavin, selenium (Se), copper (Cu), calcium (Ca), folates, and carotenoids. Proteins and micronutrients are not only essential for general well-being; they are also being increasingly recognized as important for human health and disease prevention. Continuing to follow intensive cereal- or "calorie"-only food production practices may further increase the number of malnourished people around the world. Malnourished populations have weak physical and mental growth potential, have reduced work productivities, and continue to suffer from other health problems (Welch and Graham 1999, 2004). The inability to find a solution to micronutrient malnutrition or "hidden hunger" could have irreversible impacts on human health and lives into the future.

During the green revolution, calorie-focused cereal crop production practices replaced a range of traditional food crops, including legumes, tubers, fruits, and vegetables; these are the foods that provided micronutrients to many populations of Southeast Asia and Africa (Welch and Graham 2005). The global population continues to increase, with more than 90 million people to feed each year; global food demands are expected to double by 2050. With limited arable lands, decreasing soil fertility, and declining water resources, the present food systems are already challenged with respect to providing sufficient micronutrients to most global populations. Dependence on animal products for daily nutrients is not an option for most populations in the developing world and is becoming more difficult in most developed countries. Therefore, investigating the potential of traditional food crops may be necessary to provide better nutrition solutions toward improved human health.

Food legumes could be a central part of future sustainable food systems. Development of whole grain legumes and other nutritious crops may address malnutrition epidemics in both developing and developed countries. To this end, lentil (*Lens culinaris Medik*), a cool season food legume, has been a central part of biofortification research efforts due to its superior nutritional profile and short cooking time. This chapter focused on nutritional and antinutritional traits and their phenotyping. Nutritional traits include levels of minerals (e.g., Fe), antinutrients (phytic acid and phenolics), prebiotic carbohydrates, and folates. Lentil is discussed as an illustrative example of pulse crop in the context of recent scientific literature.

15.2 Present Status of Micronutrient Malnutrition

Approximately 40 % of the world's population is currently facing hidden hunger due to diets that are deficient in essential micronutrients. Worldwide, 40 % of women and 50 % of pregnant women are Fe deficient; these are staggering statistics when combined with evidence that approximately 40 % of total childbirth deaths could be prevented by adequate Fe status (Welch and Graham 2005). Severe vitamin A deficiency blinds approximately half a million children in Southeast Asia and Africa each year. Zinc and Se deficiencies reduce the body's ability to fight off malaria, diarrhea, pneumonia, and HIV. Rickets, a forgotten skeletal disorder due to vitamin D or calcium deficiency that results in the inadequate mineralization of the bone, has reemerged as a public health problem in Bangladeshi infants and children. To address these nutritional problems through food systems, the world may need a second revolution: a "greener" revolution to provide not just food but more nutritious foods, including grain legumes. The measureable outcomes would be healthy communities instead of crop production per capita.

Micronutrient malnutrition is defined as a deficiency in one or more vitamins or minerals essential for human health, of which more than 50 have been identified. The three most commonly studied micronutrient deficiencies in the

world are anemia (related to dietary Fe), vitamin A, and iodine (I). Other common micronutrient deficiencies, including zinc (Zn), selenium (Se), and vitamin B_{12}, are equally important for human health (Welch and Graham 2005). The global prevalence of iron-deficiency anemia among children is nearly 47.8 %, and for vitamin A and iodine deficiencies, it is 30.7 and 30.3 %, respectively (WHO 2008). Welch and Graham (2005) argued that this alarmingly increasing micronutrient malnutrition trend is due to the decreased production of micronutrient-rich foods, including pulses (lentils, field peas, chickpea, and common beans) (Welch and Graham 2005). For thousands of years, farmers adopted simpler rotations of high-yielding and more profitable cash crops, i.e., wheat, maize, and rice, with nutritionally rich legume crops to sustain their livelihoods and their societies. However, present-day calorie-centric agriculture is devoid of these traditional or more diverse cropping systems, leading to never-before-seen nutrition transitions that are linked with increased rates of noncommunicable diseases in both developing and developed countries.

Micronutrient deficiencies have been addressed through food fortification and mineral supplementation; however, these efforts have not resulted in reduction of micronutrient epidemics in most parts of the world. Expert recommendations suggest improving human nutritional status through the following efforts: (1) biofortification, i.e., breeding micronutrient-enriched staple food crops through conventional plant breeding and modern biotechnology; (2) diet diversification (includes more pulses, fruits, tubers, small fish, and vegetables); (3) reduction of micronutrient losses from post-harvest and food processing through increased food utilization and accessibility; (4) changing of the combination or mix of food choices to increase micronutrient bioavailability (nutrient absorbance promoters and inhibitors); and (5) recycling of food waste and water (Welch et al. 1997; Bouis and Welch 2010; WHO 2008).

15.3 Biofortification

Past attempts to address hidden hunger have included dietary supplements, food fortification, and, more recently, "biofortification" (breeding crops for increased micronutrient content and bioavailability using conventional breeding and modern biotechnology). Unlike supplementation and fortification, which add ongoing costs to consumers, biofortification offers a unique opportunity to change crop nutritional value within the food system and in ways that have minimum impact on consumer cost. For this reason, biofortification is seen as having great potential as a sustainable, food-based solution to global micronutrient malnutrition (HarvestPlus 2013). Biofortification programs, such as HarvestPlus, BioFORT Brazil, and HarvestPlus-China, have been a tremendous success in Africa, South America, and Asia. These programs collaborate with many scientific institutions, government agencies, and nonprofit agencies around the world. Target crops include rice, wheat, maize, common bean, sweet potato, cassava, and pearl millet for phase one; many other crops are included in phase two. The targeted nutritional traits include Fe, Zn, and provitamin A (Table 15.1). The HarvestPlus program impact pathway has discovery, development, and delivery as its three major phases (Fig. 15.1; Saltzman et al. 2013; Bouis et al. 2011). The discovery phase includes identification of target populations, setting required nutrient targets, validating nutrient targets, and then screening the appropriate germplasm for possible micronutrient traits. The next step is the development of micronutrient-enriched varieties, improving and evaluating these varieties, testing nutritional efficacy, and studying social aspects including farmer adaptation and consumer acceptance. Finally, the new varieties are delivered by release in target countries with concurrent dissemination of information to promote consumption (Fig. 15.1).

The biofortification (nutrient density) and bioavailability of micronutrients are equally important for achieving the optimal micronutrient

Table 15.1 Biofortified crops, target nutrition/agronomic traits, and country-release schedule

Target crop	Nutritional trait	Agronomic trait	Countries	Release schedule
Sweet potato	Provitamin A	Disease/drought/acid soil tolerance	Uganda, Mozambique, Brazil, China	2002–2010
Bean	Fe, Zn	Virus/heat/drought tolerance	Rwanda, DR Congo, Brazil	2008–2012
Pearl millet	Fe, Zn	Mildew/drought/disease tolerance	India	2012
Wheat	Zn	Disease/lodging resistance	India, Pakistan, China, Brazil	2011–2016
Maize	Provitamin A	Disease resistance	Zambia, Nigeria, Brazil, China	2012–2015
Rice	Fe, provitamin A	Disease/pest/cold/submergence	Bangladesh, India, China	2010–2013
Cassava	Provitamin A	Disease tolerance	Nigeria, Kenya	2017
Lentil	Fe, Zn	Disease/heat/drought tolerance	Nepal, Bangladesh, Ethiopia, India	2012
Cowpea	Fe, Zn	Disease	India, Brazil	2008
Banana	Provitamin A, Fe	Unknown	Uganda	2019
Pumpkin	Provitamin A	Unknown	Brazil	2015
Irish potato	Fe	Unknown	Rwanda, Ethiopia	Unknown

Adapted from Saltzman et al. (2013), Miller and Welch (2013), Bouis et al. (2011)

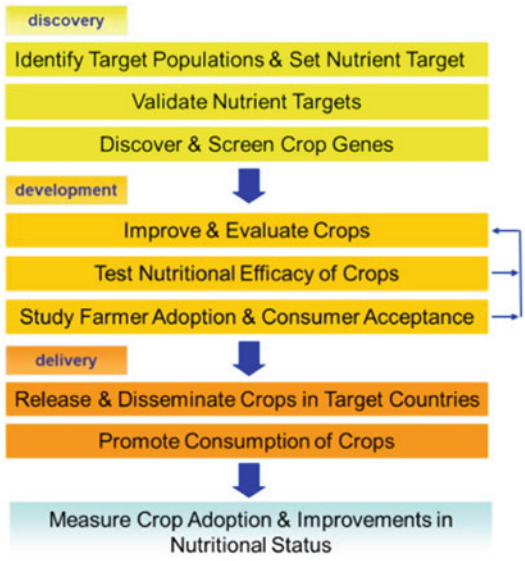

Fig. 15.1 HarvestPlus impact pathway (Adapted with permission from Saltzman et al. (2013))

status of a diet to combat global hidden hunger. Nutrient density is a measure of nutrients in a food per calorie or per unit weight. In contrast, bioavailability is the proportion of an ingested nutrient that is absorbed and utilized for essential metabolic functions. Therefore, bioavailability is a far more important concept with respect to achieving biofortification program goals (Welch 2002; Miller and Welch 2013). The bioavailability of a mineral micronutrient is governed by many factors, including the host, digestive environments, and the presence of mineral absorption promoters and inhibitors in a food. Absorption promoters, such as ascorbic acid, carotenoids, prebiotic carbohydrates, certain fibers, sulfur amino acids, and meat factors, increase Fe absorption in the human digestive system; phytic acid and polyphenols in plant-based food are the major inhibitory factors of Fe and Zn bioavailability (Table 15.2).

15.4 Nutritional Traits for Phenotyping

15.4.1 Fe Bioavailability in Lentils

As presented in Table 15.2, determining the total amount of Fe in seeds is not enough to predict the impact of consumption with respect to meeting human dietary requirements. The bioavailable Fe is the only true measure of the nutritional impact of any Fe-rich food crop. Measurement of human

Table 15.2 Micronutrient bioavailability promoters and inhibitory substances in foods

Nutrient	Promoter substances	Antinutrients (inhibitors)
1. Fe and Zn	1. Organic acids (ascorbic acid, malate, citrate, fumarate)	1. Phytic acid
	2. Amino acids (methionine, cysteine, histidine, lysine)	2. Polyphenols (kaempferol, gallic acid, chlorogenic acid)
	3. Beta-carotene	3. Tannins
	4. Meat factors	4. Certain fibers
	5. Prebiotic carbohydrates (fructooligosaccharides, galactooligosaccharides, sugar alcohols, raffinose family oligosaccharides)	5. Heavy metals
	6. Some phenolic acids-quercetin and ferulic acid	
	7. Hemoglobin (only for Fe)	
	8. Long-chain fatty acids (palmitate; only for Zn)	
2. Se	9. I	
3. Vitamin A	10. Beta-carotene	
	11. Fats and lipids	

Adapted from Welch and Graham (2005), Johnson et al. (2013a)

bioavailability of Fe from plant-based foods is a complex process because numerous factors influence the final Fe bioavailability in the gut: (1) crop genetic selection and production practices; (2) other meal composition; (3) individual characteristics; (4) processing, cooking, and preparation of foods; and (5) individual ingestion, absorption, and utilization of bioavailable Fe. As a result of this complexity, the data obtained from bioavailability models are unclear (Welch and Graham 2005; House 1999; Van Campen and Glahn 1999). Human efficacy trials are the most appropriate way to test for true Fe bioavailability; however, it is impractical to test the bioavailability of Fe of thousands of genotypes that can be generated from breeding programs.

Lentil breeding and nutritional quality programs in the USA and ICARDA together developed a robust model to select appropriate lentil germplasm for Fe biofortification based on food matrix factors. First, Fe bioavailability in lentil germplasm is screened based on food matrix factors, including Fe bioavailability promoters (ascorbic acid, prebiotic carbohydrates, phytoferritin, and carotenoids) and inhibitors (phytic acid, kaempferol, gallic acid, and chlorogenic acid). Once appropriate breeding efforts are carried out, based on the phenotyping data and nutritional quality, Fe bioavailability studies are conducted using an in vitro Caco-2 cell model for selected advanced breeding lines only. Finally, these selected varieties are used in human trials to test the true Fe bioavailability of lentils.

Phenotyping of lentil for true Fe bioavailability is an expensive process, as bioavailability is governed by many food matrix factors. Available data on lentil Fe biofortification are based on the total Fe concentration and the direct measurement of Fe bioavailability using Caco-2 cell culture models (Table 15.3; Thavarajah et al. 2009a; Boum et al. 2008; Della Valle et al. 2013a, b) in the seeds and do not consider food matrix factors or regular household cooking methods that can govern the true Fe bioavailability. Some studies report phytic acid concentration but other food matrix factors are not considered. A recent study reports lentil genotypes with superior Fe concentrations with phenolic and phytic acid profiles for enhanced Fe bioavailability (Table 15.3; Johnson et al. 2013a). Ten lentil commercial cultivars grown in two locations and years were tested for total Fe, phytic acid, ascorbic acid, gallic acid, and chlorogenic acid. Total lentil seed Fe concentration across genotypes was 56–70 mg/kg with low concentrations of phytic acid (6.3–8.7 mg/kg).

Table 15.3 Seed Fe (mg/kg), phytic acid (PA; mg/g), ascorbic acid (AA; mg/kg), gallic acid (GA; mg/kg), and chlorogenic acid (CLA; mg/kg) concentrations as well as Fe bioavailability (using Caco-2 cell culture model; ng ferritin/mg protein) of lentils grown in the USA and Canada

Genotype	Fe	PA	AA	GA	CLA	Fe bioavailability	Reference
CDC Greenland	56.7	8.7	69.4	24.4	20.9	–	Johnson et al. (2013a)
	61.0	6.1	–	–	–	12.2	Della Valle et al. (2013a)
CDC Lemay	67.9	8.5	67.2	24.4	12.1	–	Johnson et al. (2013a)
	75.3	–	–	–	–	3.4	Della Valle et al. (2013a)
CDC Red Rider	56.3	8.4	62.4	22.8	16.8		Johnson et al. (2013a)
	53.4	–	–	–	–	2.5	Della Valle et al. (2013b)
CDC Redberry	56.8	7.3	71.5	22.2	14.8	–	Johnson et al. (2013a)
	68.8	6.6	–	–	–	7.8	Della Valle et al. (2013a)
CDC Richlea	56.3	6.8	69.7	27.1	18.8	–	Johnson et al. (2013a)
CDC Rosetown	62.3	6.8	62.3	24.8	19.0	–	Johnson et al. (2013a)
	71.8	6.5	–	–	–	8.4	Della Valle et al. (2013a)
CDC Rouleau	61.3	6.7	75.7	22.2	12.7	–	Johnson et al. (2013a)
	86.2	–	–	–	–	3.0	Della Valle et al. (2013b)
CDC Viceroy	61.3	7.7	76.6	24.4	18.6		Johnson et al. (2013a)
	74.4	–	–	–	–	3.5	Della Valle et al. (2013b)
Pennell	69.5	7.5	81.8	27.7	18	–	Johnson et al. (2013a)
Riveland	62.3	6.3	87.4	29.5	22.2	–	Johnson et al. (2013a)

Data from Johnson et al. (2013a), Della Valle et al. (2013a, b)

Overall, lentil cultivars Pennell and Rivel may have higher Fe bioavailability based on food matrix factors; however, the true Fe bioavailability has yet to be tested.

Iron is divided into two categories from a nutritional point of view: heme and nonheme. The heme form absorbs as a stable porphyrin complex that is unaffected by antinutrients including phytic acid, tannins, and phenolics. However, nonheme Fe from plant-based diets is easily transformed by antinutrient compounds. For example, antinutrients are capable of binding nonheme Fe and producing insoluble Fe complexes in the intestinal lumen, resulting in inhibition of Fe absorption. Two Fe-binding proteins occur naturally in foods—lactoferrin (animal foods) and phytoferritin (plant foods)—in which Fe is separated from chelates by a protein coat and made less sensitive to antinutrient factors present in the food matrix. Legume phytoferritin contains 1,800–2,200 atoms of Fe per molecule depending on the growing location and environment (Zhao 2010). Therefore, delivering considerable amounts of bioavailable Fe is possible by enhancing the phytoferritin levels in food legumes.

15.4.2 Lentil Prebiotics

Prebiotics are an important nutritional component of foods. The concept of prebiotics is based on dynamic processes of the microbiota in the intestinal tract, which comprises over 1,000 known species. These commensal microbes can be manipulated to benefit (or worsen) human health via the substrates that are made available for fermentation (undigested food components). A prebiotic is selectively fermented when consumed, altering the composition and/or activity of the microbiota to yield health benefits to the human host. A variety of health benefits may be provided from prebiotics, including immunostimulation (Guigoz et al. 2002), prevention of intestinal infections (Bosscher et al. 2006), increased bioavailability of minerals (Mg, Ca, Fe, and others) (Franck 2006), and reduced risk of osteoporosis (Abrams et al. 2005) and metabolic diseases.

Many carbohydrates that are now considered prebiotics were documented in lentil decades ago; however, discoveries of putative health benefits have cast their presence in a new light. Some prebiotic carbohydrates, such as raffinose family oligosaccharides (RFO) and resistant starch (RS), had been viewed as antinutrients. Breeders and food processors sought to remove RFO due to its implication in flatulence (Frias et al. 1999) and RS to enhance the available energy from food (Vidal-Valverde and Frias 1992). However, researchers have now begun to see prebiotic carbohydrates as a breeding trait not only for potential plant health and protection (Peters et al. 2007) but also for human health (Huynh et al. 2008).

In addition to RFO and RS, prebiotic carbohydrates in lentil include sugar alcohols and small quantities of fructooligosaccharides (FOS) (Bhatty 1988; Wang et al. 2009; Biesiekierski et al. 2011; Johnson et al. 2013b). Variation in seed RFO concentrations ranges from 1.8 to 7.5 % (Martinez-Villaluenga et al. 2008). Results of a 2-year replicated field study reveal genetic variability across ten commercial lentil genotypes (Johnson et al. 2013b). This variation is also observed in concentrations of two sugar alcohols, sorbitol (1,036–1,349 mg/100 g) and mannitol (158–294 mg/100 g), across genotypes (Johnson et al. 2013b). The varietal range in RS concentration in cooked lentil ranges varies from 3.7 to 5.1 g/100 g dry matter (Wang et al. 2009). FOS compounds nystose and kestose have been reported in lentil but at low concentrations and without significant genetic variation (Biesiekierski et al. 2011; Johnson et al. 2013b). A common finding accompanying genetic variation of expression of prebiotic carbohydrates is the influence of environmental factors on their concentrations in lentil seeds (Tahir et al. 2011; Johnson et al. 2013b). While the literature clearly suggests that the accumulation of these compounds in lentil can be manipulated through breeding efforts and by adapting plants to diverse locations, the potential effects of doing so have not been studied.

Prior to the development of effective breeding strategies to optimize prebiotic concentrations in lentil, a thorough understanding of changes that these compounds undergo during post-harvest storage, processing, and cooking procedures is required. RS and RFO (and FOS and sugar alcohols; unpublished data) all accumulate primarily in the cotyledon (Wang et al. 2009). The traditional consumption of lentil, either as a whole seed or dehulled and split, may have nutritional importance. Removal of the seed coat will concentrate prebiotic carbohydrates in the prepared food. Moreover, the fraction of starch that is resistant to human enzymes is highly sensitive to preparation procedures (Hoover and Zhou 2003). Cooling of boiled lentils nearly doubles the RS concentration, a phenomenon that is not reversed with reheating (Fig. 15.2).

15.4.3 Lentil Folates

Folate is another important micronutrient with respect to the prevention of preterm delivery, low birth weight, fetal growth retardation, and developmental neural tube defects (NTDs). Folate fortification and supplementation approaches have been attempted, but conflicting results regarding the ability to prevent NTDs and to increase the folate status at population levels in different countries have raised doubts about the continuous use of folic acid. The inability of folic acid to prevent NTDs and safety concerns with respect to too much folate in vulnerable population groups (e.g., children) demands alternative approaches to supply daily folates. Biofortification of staple crops with highly bioavailable folates might be a solution to folate deficiency. Lentils are naturally rich in folates, with levels in selected Canadian- and US-grown commercial lentil genotypes, quantified as folic acid equivalents, ranging from 275 to 622 μg/100 g (data not shown).

A recent study indicates that there is potential for genetic biofortification of lentils with bioavailable folates, quantified as tetrahydrofolate (Sen Gupta et al. 2013). Folate concentration in ten commercial lentil cultivars grown in North Dakota, USA, ranged from 216 to 290 μg/100 g,

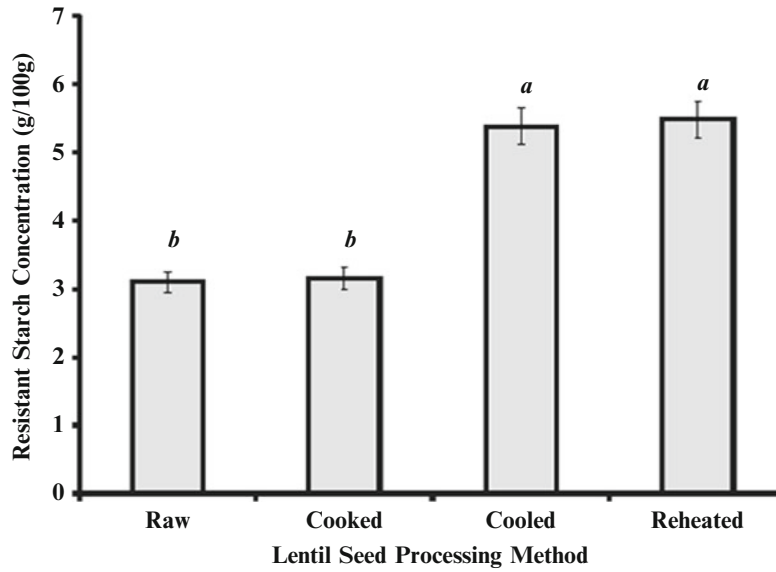

Fig. 15.2 Mean resistant starch concentration in raw, cooked, cooled, and reheated lentil products by whole and split market classes. Error bars are based on $p < 0.05$ ($n = 96$)

which would provide 54–73 % of the recommended daily allowance of dietary folate for adults (Sen Gupta et al. 2013). In addition, lentils have a higher folate concentration than chickpeas (42–125 μg/100 g), yellow field peas (41–55 μg/100 g), and green field peas (50–202 μg/100 g) (Sen Gupta et al. 2013). Further increases in folate levels and, more specifically, increases in bioavailable folate forms are possible through accurate identification and selection of genetic material and location sourcing. Overall, this limited set of data shows that a significant genetic effect could be further enhanced by careful selection of growing location (Sen Gupta et al. 2013).

15.5 Phenotyping Method for Micronutrient Analysis

15.5.1 Minerals

Mineral profiles of plant seeds can be analyzed by previously described modified HNO_3-H_2O_2 method (Thavarajah et al. 2009a). Finely ground seed sample (500 mg) digestion with nitric acid (70 % HNO_3) and hydrogen peroxide (30 %) leads to release of mineral micronutrients to the digested solution. The minerals in the digested solution can be carried out by atomic absorption spectroscopy or inductively coupled plasma emission spectroscopy.

15.5.2 Phytic Acid (PA)

PA involves prior PA extraction from sample prior to analysis (Thavarajah et al. 2009b). Simply, 0.5 M hydrochloric acid (HCl) addition to finely powdered sample and then heating in a boiling water bath enable PA extraction. The extracted PA could be decomplexed by addition of stronger acid (12.0 M HCl). For accurate PA identification and quantification, high-performance anion exchange (HPAE) with a conductivity detector was found to be a better method. In this procedure, an OmniPac Pax-100 anion exchange column with an OmniPac Pax-100 (8 μm) guard column (Dionex, Sunnyvale, CA, USA) with gradient mobile phase consisting of sodium hydroxide, deionized water-isopropanol, and water provide very good resolution of PA from other phytates. However, this method needs a strong anion suppressor because of higher conductivities of alkaline mobile phase.

15.5.3 Phenolics

Extraction and quantification of phenolic compounds could be carried out by the previously described method (Duenas et al. 2002). Phenolic compounds were extracted using methanol/water/acetic acid. The extracted phenolic can be analyzed by high-performance liquid chromatography (HPLC) system with photodiode array detection.

15.5.4 Folates

A finely ground sample needs to be dispersed in an extraction buffer consisting of potassium phosphate with sodium ascorbate and 2-mercaptoethanol. For foliate analysis, tri-enzyme treatment method is used. In this method, the homogenized seed samples need to be treated with enzymes -amylase, protease, and conjugase to release the folates from sample matrixes. The extracted folates can be analyzed on RP-HPLC (reversed-phase high-performance liquid chromatography) with fluorescent or mass detectors. This method has been described earlier (Sen Gupta et al. 2013).

15.6 Future Direction on Nutritional and Antinutritional Phenotyping

Both caloric and micronutrient malnutrition are important health concerns in developing countries. A lack of micronutrients in daily diets is a cause of many of the devastating health impacts felt by billions of people worldwide. Food systems based on a calorie-focused approach need urgent changes to control both malnutrition and associated diseases.

Past approaches to prevent micronutrient deficiency, including fortification, supplementation, and dietary diversification, have been met with limited success. However, biofortification of crops with micronutrients appears to be a sustainable solution to reduce micronutrient deficiencies. As such crops are developed and disseminated and become available to common consumers, biofortified foods could become a source of daily micronutrient requirements. To this end, pulse crops provide great promise. In particular, lentil is a protein-rich, medium-energy crop with a range of micronutrients. It has a high iron concentration with low levels of phytic acid and other iron bioavailability inhibitors and, hence, greater iron bioavailability. In addition, it is a rich source of prebiotics including fructose family oligosaccharides and resistant starches. While lentil may be an ideal crop for increasing bioavailable iron content, it is also a medium-energy food source that would serve to also reduce caloric malnutrition. Recent research clearly shows that certain lentil varieties have superior nutrient profiles, providing further opportunities for increased biofortification and bioavailability efforts. To seize these opportunities, extensive characterization of nutritional and anti-nutritional traits in different cultivars, including wild genotypes, may be required. This would be a fundamental step toward a systematic and cost-effective way to advance biofortification/bioavailability efforts.

Lentil is a pulse crop with several advantages: superior iron and prebiotic carbohydrate profiles, short cooking time (~10–20 min), and nitrogen-fixing abilities. Therefore, incorporation of lentil into future food systems may provide benefits beyond a food-based solution to malnutrition. Other crops, including other pulse crops, also need to be studied to provide greater diversity to food systems and consumer choices. Thus, intensive phenotypic characterization is essential. Resource allocation and shifting of "calorie"-focused thinking are urgently needed toward the development of food systems that will have sustainable human and environmental health benefits.

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