Siddharth Tiwari
Baljeet Singh *Editors*

Harnessing Crop Biofortification for Sustainable Agriculture

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This book is dedicated to the diligent efforts of scientists, researchers, and farmers worldwide, who work tirelessly for the betterment of their nations. Their dedication, passion, and perseverance in advancing agricultural knowledge and practices are indispensable in shaping a sustainable, nutritious food-secure future for all.

Preface

In a world grappling with malnutrition and food insecurity, the biofortification of crops has emerged as a promising solution to address widespread nutrient deficiencies. Hidden hunger (micronutrient deficiencies), a prevalent global issue, refers to the chronic deficiency of essential micronutrients despite sufficient caloric intake. With over 2 billion affected individuals, particularly women and children in low-income countries, it impairs physical and cognitive development, increases disease susceptibility, and diminishes productivity.

Crop biofortification seems a sustainable solution to reduce the global burden of hidden hunger. The background and purpose of this book, *Harnessing Crop Biofortification for Sustainable Agriculture*, aim to provide a comprehensive and authoritative resource on the advancements, challenges, and potential of crop biofortification. This book is rooted in the urgent need to tackle global malnutrition.

Despite significant progress in agriculture and food production, millions of people still suffer from deficiencies in vital micronutrients such as iron, zinc, and vitamin A. These deficiencies have severe consequences for human health, leading to impaired growth, compromised immune systems, and increased susceptibility to diseases. Conventional approaches to combat malnutrition, such as dietary diversification or nutrient supplementation, often face practical and economic limitations.

Biofortification offers a sustainable and cost-effective approach to address these challenges. By enhancing the nutrient content of crops through conventional breeding techniques or modern biotechnological tools, biofortification provides an opportunity to improve the nutritional quality of staple food crops. This approach ensures a continuous supply of nutrient-rich crops to human populations, especially in regions with limited resources.

The primary goal of this book is to consolidate the most recent research, perspectives, and hands-on knowledge concerning crop biofortification. It seeks to act as an inclusive manual for professionals across various domains, including scientists, researchers, policymakers, scholars, and practitioners engaged in agriculture, nutrition, and public health. Covering a diverse array of subjects, ranging from the fundamental science and methodologies underpinning biofortification to the intricate dynamics of nutrient bioavailability and its repercussions on human health, the book provides an extensive exploration of the field. Additionally, it delves into crucial aspects such as strategies for expanding biofortification initiatives, policy frameworks, and future trajectories for the development and deployment of biofortified crops.

Ultimately, the overarching aim of this book is to play a pivotal role in the global endeavor to enhance nutrition and alleviate health challenges associated with malnutrition. It seeks to achieve this objective by advocating for sustainable and readily accessible solutions through the promotion of crop biofortification. By spotlighting the potential of biofortified crops to address nutritional deficiencies effectively, the book endeavors to foster widespread adoption of this approach, thereby making significant strides towards improving public health on a global scale.

This book is meticulously crafted to fulfill several pivotal objectives such as:

- Providing an exhaustive exploration of crop biofortification, encompassing all relevant aspects and developments.
- Integrating the latest breakthroughs and research insights into the discourse, ensuring a comprehensive understanding of the subject matter.
- Offering practical guidance and actionable strategies for the successful implementation of biofortification initiatives, thereby facilitating real-world impact.
- Adopting a multidisciplinary approach that synthesizes perspectives from diverse fields, fostering a holistic understanding of the complexities involved.

In essence, this book emerges as an indispensable resource for a wide spectrum of stakeholders, including researchers, academics, extension workers, policymakers, students, and professionals engaged in agriculture, nutrition, and health sectors. By advocating for diets abundant in essential nutrients and promoting biofortification, it endeavors to benefit individuals at every stage of the value chain, thereby contributing significantly to the enhancement of global nutrition and well-being.

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Contents

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Siddharth Tiwari is a Scientist-F at National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India. He received his doctoral degree in 2008 from CSIR-National Botanical Research Institute (CSIR-NBRI), Lucknow, U.P., India. His area of specialization includes Plant Tissue Culture, Molecular Biology, and Genetic Engineering. During his doctoral research, Dr. Tiwari made significant contributions, including the development of a groundnut-seed-expressed edible vaccine against cholera and rabies, for which he received the BioAsia Innovation Young Scientist Award in 2010. Additionally, he developed insect-resistant transgenic groundnut against the polyphagous foliage insect *Spodoptera litura*, earning him the 97th Indian Science Congress Association (ISCA) Young Scientist Award in 2010. He joined DBT-NABI in July 2010 and presently serving as a Scientist-F. Dr. Tiwari's research focuses on the metabolic engineering of staple crops like banana and wheat, utilizing transgenic and genome editing. He is the pioneer in banana genome editing and developed nutritionally enriched banana. He has published over 50 research articles, delivered over 100 invited lectures, and supervised 7 Ph.D. students and over 60 graduate/postgraduate trainees in various disciplines of plant biotechnology. He has edited two books and was elected as a fellow of the prestigious Plant Tissue Culture Association of India.

Baljeet Singh is a distinguished scientist and researcher in the field of plant molecular biology and genome editing. With a strong academic background and extensive research experience, he has been at the forefront of innovative projects aimed at enhancing crop biofortification. Currently serving as a Project Scientist at the National Agri-Food Biotechnology Institute (NABI), he is actively involved in a significant project titled "Development and Transfer of Technology from the Queensland University of Technology, Australia to India for Biofortification in Banana." In this capacity, he is focused on the molecular characterization of transgenic events and the development of biofortified banana through genome editing. Previously, Dr. Singh worked on a project centered around "Targeted editing of potato genome to develop variety-specific true potato seed (TPS)." He has completed a Ph.D. on the genome-wide association mapping for micronutrients in tetraploid potato. With a diverse research portfolio, Dr. Singh has contributed to various crop nutrition-related projects at the prestigious ICAR—Central Potato Research Institute, Shimla, India. Dr. Singh also excels in scientific writing, as demonstrated by a publication record that includes articles, reviews, and book chapters.

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Importance of Nutrient Requirements of Humans

1

Raghu Pullakhandam, Ravindranadh Palika, C. N. Neeraja, and G. Bhanuprakash Reddy

1.1 Introduction

The age-old notion that food profoundly impacts general health has gained robust support from an emerging body of epidemiological, clinical, and basic research, highlighting the importance of optimal nutrition for human health. Thus, understanding the nutrient requirements of humans is very essential for researchers, healthcare professionals, and policy makers to address dietary deficiencies, prevent chronic diseases, and support best health outcomes with optimal nutrition (Meyers et al. 2006; Nair and Augustine 2018). Based on the requirement in terms of functionality and quantity, nutrients are categorized as essential or non-essential, and macronutrients and micronutrients. Essential nutrients are substances that our bodies cannot synthesize on their own and must be obtained from our diet (Barasi 2003). Macronutrients such as carbohydrates, proteins, and fats provide the body with energy and building blocks for tissues and cells. Micronutrients, including vitamins and minerals, are required in smaller amounts but play essential roles in metabolic processes (Barasi 2003). The dietary fibre (soluble and insoluble), abundant in plant foods, although is not absorbed into blood, helps in digestion, bowel movements, and maintaining healthy gut microbiome (Gill et al. 2021). Dietary fibre also helps in regulating the blood cholesterol and glucose levels, and thus reduces the risk of chronic age-related diseases (Koç et al. 2020). In addition, a plethora of bioactive food components such as polyphenols/flavonoids, phytosterols, and carotenoids have gained significant attention in recent years due to their potential to prevent a wide range of diseases and promote overall well-being

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possibly mediated by their anti-inflammatory and antioxidant properties (Dillard and German 2000; González et al. 2011).

The critical importance of diet in determining the health and disease spurred considerable basic and epidemiological research towards unravelling the components of the diet that yield positive or negative health outcomes. The basic research contributed to the understanding of the functional role of nutrients such as substrates for generating energy and regulatory roles in influencing the genome, transcriptome, and proteome either directly or indirectly (Reddy et al. 2018). On the other hand, epidemiological and clinical studies provided evidence for the health benefits of regular intake of diets rich in fruits and vegetables (Liu 2003, 2013; Slavin 2012; Fardet and Rock 2014). Together, this knowledge immensely helped in understanding the physiological function of nutrients and establishment of nutrient or disease-specific biomarkers. However, translating this knowledge to the entire human subject, especially in a general population, is necessary but remained a complex question to address (Reddy et al. 2018). The first question in this direction is to understand how much nutrient one needs to consume to be healthy; in other words what is the requirement of specific nutrient (s) according to age, gender, physiological state, and physical activity level. Several countries including India set the requirements of key nutrients for different age, gender, and physiological groups called dietary reference intakes (DRIs), and presumably these DRIs form the basis for food fortification programmes (WHO 1967, 1988; Trumbo et al. 2001; Meyers et al. 2006; King and Garza 2007; EFSA 2015, 2017; FSSAI 2018). Most likely these DRIs are also relevant and useful to biofortification approaches. However, understanding DRIs-related terminology along with methodology in arriving at these metrics is essential for their contextual interpretation and decision making by the different stakeholders. For instance, in the biofortification context, what should be the level of nutrient one should aim to achieve to substantially improve the intakes that results in quantifiable impact in target population? In the following sections we attempted to provide the basic concepts of nutrient requirements such as nutrient homeostasis, approaches involved in deriving nutrient requirements, and applications/implications of these nutrient requirements to crop biofortification for sustainable agriculture.

1.2 Nutrient Homeostasis

Nutrient homeostasis refers to the maintenance of a balance or equilibrium of essential nutrients required for optimal health and functioning. It involves several complex regulatory mechanisms that ensure adequate levels of nutrients available for cellular processes while preventing excess or deficiency. These mechanisms operate at multiple levels, including absorption, distribution, utilization, and excretion of nutrients. Nutrient homeostasis is crucial for sustaining various physiological processes, including metabolism, growth, repair, and overall well-being. A simplified nutrient homeostasis model is depicted in Fig. 1.1. Cooking and gastrointestinal digestion facilitate the mechanical breakdown of complex food molecules (increases

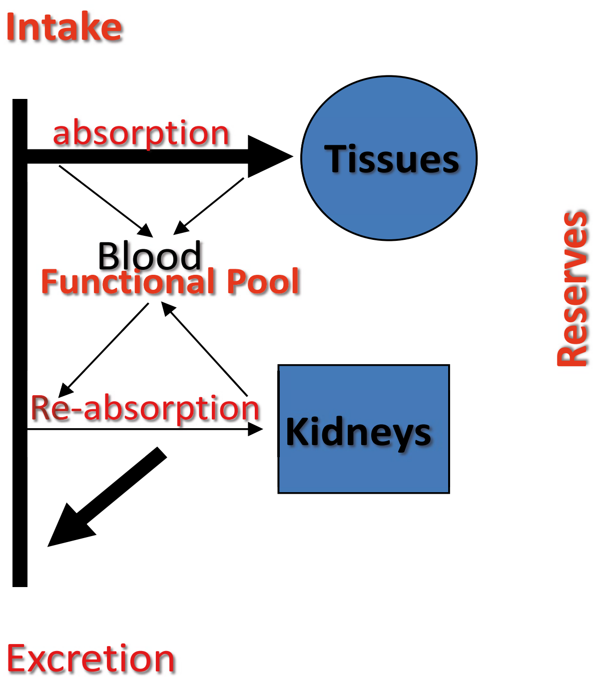

Fig. 1.1 Simplified framework of nutrient homeostasis: The dietary nutrients are absorbed into the blood, either mobilized to functional pools (i.e. blood) or tissue storage (i.e. liver) pools or excreted via urine/faeces as determined by the nutrient status. During deficiency the absorption of nutrient increases, and excretion is reduced. During adequate nutrient status or excessive intakes, the absorption is inhibited and excretion is increased. The concerted regulation of absorption, tissue pool mobilization and excretion ensures nutrient balance and thus compensates the fluctuation in dietary intakes

the surface area that helps in further enzymatic digestion) and facilitate the release of nutrients by breaking down complex carbohydrates, fats, and proteins into their monomeric units, such as sugars, fatty acids, and peptides/amino acids by the action of amylases, lipases, and gastric pepsin, respectively (Treuting et al. 2018; Sensoy 2021). At the end of gastric digestion, the "Chyme" enters the small intestine where fatty acids are micellarized with the help of pancreatic lipases and bile salts, and proteins/peptides are further digested by the action of pancreatic proteases (trypsin, chymotrypsin). The nutrients in the chyme are then absorbed through the small intestine either by facilitated (through a membrane receptor, i.e. iron and zinc) or passive diffusion (micellar fatty acids and other fat-soluble vitamins A, D, E, and K) process into the blood stream (Kiela 2016; ICMR 2020). Once in the blood stream, the nutrients are channelled to the tissue pools for storage and subsequent utilization as per the physiological needs; otherwise excess nutrients or their catabolic products are excreted through renal filtration or pancreatic secretions into the faeces (Bourne 2012; Kondaiah et al. 2019; ICMR 2020). In addition, there could be other excretory pathways such as menstrual losses in women and excretion through milk in lactating mothers (ICMR 2020). The term "Nutrient Balance" denotes a condition where the amount of absorbed nutrient is exactly equal to that of excreted (ICMR 2020), meaning that the nutrient concentration in functional compartments (i.e. blood) and storage tissue pools is saturated (optimum level), and any additional absorbed nutrient is excreted. This is the condition all the regulatory homeostatic networks of specific nutrient in the body strives hard to establish, even in the face of dynamic fluctuations in dietary intakes (Kondaiah et al. 2019). In other words, nutrient homeostasis refers to the body's inherent capacity to regulate and maintain a stable concentration of nutrients within a narrow range (concentration required for their metabolic functions) despite changes in the external environment or internal conditions. This happens at multiple levels, where lower dietary intakes are compensated by increasing the absorption, reducing the excretion, and mobilization of tissue reserves to functional pools (Trumbo et al. 2001; Meyers et al. 2006; EFSA 2015, 2017; ICMR 2020). Conversely, when the intakes are high, there is reduction in absorption and elevation in excretion and/or higher mobilization of the nutrient to the stores. It is to be noted that the homeostatic mechanisms of each nutrient are unique. For instance, there is no known obligatory excretory pathway for iron and thus its homeostasis is solely regulated by controlling absorption (Anderson and Frazer 2017). On the other hand, for zinc, though absorption increases during lower intakes, there is no mechanism of inhibiting the absorption during excess (Pullakhandam et al. 2021, 2023). This is compensated by increased zinc excretion through pancreatic juices into the intestine, subsequently in faeces. Similarly, the regulation of B-vitamin(s) homeostasis is achieved by excretion through urine (Christensen and Willnow 1999; Birn 2006). The reabsorption of some nutrients such as glucose, amino acids, and electrolytes in proximal renal tubules also contributes to their homeostasis (Rosner 2011).

In the context of pathophysiology, the nutrients are categorized as type 1 (iron, vitamin-A, and B-vitamins) and type 2 (protein, zinc, magnesium, phosphorus, and potassium). This categorization is mainly based on manifestations of nutrient deficiencies on child growth (Golden 1995). During type 1 nutrient deficiencies the growth continues by utilizing the tissue reserves resulting in measurable reduction in functional pools such as blood or plasma followed by clinical manifestation such as anaemia (iron), night blindness (vitamin A), scurvy (vitamin C), rickets (vitamin D), Beri Beri (thiamine), etc. (Golden 1995). In contrast, when there is deficiency of type 2 nutrients such as protein and zinc, the body's growth is hampered and appetite is reduced, resulting in stunting, wasting, and energy malnutrition, without marked changes in whole-body nutrient levels. Notably, repletion of type 2 nutrients results in rapid linear growth. Therefore, the diagnosis of subclinical forms of type 1 nutrients is possible through measurement of their levels in blood or tissues; the same is not always the case with type 2 nutrients.

1.3 Absorption and Bioavailability of Nutrients

Absorption and bioavailability are often referred to or interpreted as one and the same, but they are distinctly different processes (Benito and Miller 1998). The absorption of a nutrient refers to the proportion of dietary nutrient passed through the small intestine into the blood stream, which is influenced by the amount of nutrient in the diet, solubility of nutrient after gastrointestinal digestion, levels of nutrient transporter expressed at both apical and basolateral sides of polarized intestinal epithelium and relative levels of other competing nutrients. For example, a high zinc and calcium levels or high phytic acid levels in the chyme interfere with iron absorption at the enterocyte (Kondaiah et al. 2019) and the amount of dietary fat or xanthophylls influences the absorption of carotenoids (Dube et al. 2018). The bioavailability extends beyond absorption, and it represents comprehensive knowledge of nutrient utilization including its impact on the biochemical and physiological function. For example, the proportion of iron appearing in the blood after ingesting food represents its absorption, while the proportion of iron that is incorporated into blood haemoglobin refers to its bioavailability (Benito and Miller 1998). Therefore, apart from factors that influence absorption, other physiological factors will also influence the bioavailability. Anaemic subjects have higher bioavailability of iron compared to non-anaemic subjects, if the underlying cause of anaemia is iron deficiency. Although absorption and bioavailability are not the same, it can be safely assumed they are inter-related (Benito and Miller 1998), at least when equilibrium or balance is achieved. For example, under ideal conditions, a food with higher iron absorption will always result in its higher bioavailability. For instance, inclusion of ascorbic acid (vitamin C) increases the absorption of iron in intestinal enterocytes and bioavailability in human subjects (Thankachan et al. 2008; Nair et al. 2013).

In the context of biofortification, considering the large number of breeding at different stages of development, it is technically difficult to assess the absorption and/or bioavailability in human subjects. To overcome this limitation several in vitro models are suggested, and are being used for assessing the relative absorption/bioavailability of nutrient using simulated digestion models, referred to as “bioaccessibility”, and these are validated against observations in clinical studies (Au and Reddy 2000; Peijnenburg and Jager 2003; Sreenivasulu et al. 2008). However, one pitfall in assessing bioaccessibility is, it is always relative to some reference nutrient/variety, and can only help in ranking the foods/varieties to select best for subsequent testing in animal/clinical studies.

1.4 Nutrient Requirements

One intuitive question is what is the amount of a specific nutrient that one needs to consume to be healthy or at balance? The answer is rather complex as the requirements are varied among individual subjects due to several poorly understood physiological reasons, called inter-individual variation. Even among the same individual, growth spurts during childhood, adolescence, and maintaining health throughout life impact the need for specific nutrients, and therefore nutrient requirements also vary across different life stages (Trumbo et al. 2001; Meyers et al. 2006, EFSA 2015, 2017). Moreover, the nutrient absorption and metabolism may also vary in populations due to multiple reasons, including differences in the dietary pattern, diet quality, and bioavailability (Reddy et al. 2018). Therefore, it is logical that requirements vary within an individual throughout different stages of life (child, adolescent, adult and elderly, pregnancy, lactation, menstruation etc.), and there is an associated variation in the requirements between individuals of the population (inter-individual variation). Therefore, in a population context, nutrient requirements are always a series of numbers which are expected to follow a statistically normal distribution.

The Institute of Medicine (IOM, now National Academy of Medicine, USA) and European Food Safety Authority (EFSA) frame and revise the nutrient requirements periodically for their respective populations (Trumbo et al. 2001; Meyers et al. 2006; EFSA 2015, 2017). The Food and Agricultural Organization (FAO)/the World Health Organization (WHO) set the nutrient requirements contextualized to different countries (Allen et al. 2020). The ICMR-National Institute of Nutrition (ICMR-NIN), India, first published the nutrient requirements in the year 1968, revised in 1978, 1985, 2010, and more recently in 2020 (ICMR 2020). These periodic revisions are made specifically to account for contemporary scientific knowledge from across the world. The ICMR-2020 nutrient requirements differ from the previous recommendations in the sense that these recommendations proposed the use of Estimated Average Requirements (EAR), Recommended Dietary Allowances (RDA), and Tolerable Upper limits (TUL). The methodologies adopted for deriving the nutrient requirement are unique for each nutrient and are elaborated elsewhere (ICMR 2020). In the context of biofortification and for the benefit of agricultural research fraternity, a brief account of general methodology that helps the reader in understanding different metrics of nutrient requirements and their possible applications is presented below.

As explained in the nutrient homeostasis section (Fig. 1.1), the human body has tremendous capacity to adjust to natural fluctuations in dietary intakes by regulating the absorption and excretion of nutrients. Let us assume that under an ideal condition (an apparent healthy condition with respect to target nutrient) it is expected that the amount of absorbed nutrient is always equal to that of excreted nutrient. Therefore, if one precisely measures the amount of excreted nutrient in a healthy subject, it is possible to estimate what is actually required for that individual (Rao 2010; Swaminathan et al. 2016; ICMR 2020). Since the excretion or loss of nutrient takes place through several routes, one has to measure these losses through all possible routes and pool them, referred to as factorial computation (Swaminathan et al. 2016; Ghosh et al. 2019a, b; ICMR 2020). Due to a variety of reasons including physiology and genetics, the excretion of a nutrient varies even among healthy subjects. Therefore, it is also required to measure the excretion/losses of nutrient in a set of subjects that ideally represents the target population. The resultant data provides the distribution of excretion/losses from all possible routes, which are then pooled to estimate the average losses and its associated deviation (Ghosh et al. 2019a, b; ICMR 2020). Since excretion reflects the requirement at balance (ICMR 2020), we consider this as the requirement distribution of population, and generating this data is the essential first step in deriving the nutrient requirements. This basal requirement can then be adjusted for body weight (per kg) and can be applied to other age groups (by adjusting for body weight), if there is no specific data available. Additional factors such as tissue stores, tissue mass, and catabolic rate of nutrients wherever necessary for specific nutrients are also taken into account in this factorial computation, where necessary (ICMR 2020).

Now that we have a basal physiological requirement (per kg body weight), next step is to compute the requirements related to growth in children or foetal growth in pregnant women. This is computed based on new tissue accretion per day (g/day), which is then converted to additional requirement by imputing that tissue concentration of the nutrient and added to the requirement distribution generating new and specific requirement for other population subgroups (Trumbo et al. 2001; Meyers et al. 2006; ICMR 2020). To account for lactation-related losses, the average amount of breast milk excreted and its concentration of nutrient are added to the basal requirements (ICMR 2020). After the physiological requirement distribution for specific population subgroups is derived, next step is to adjust for the bioavailability for deriving the dietary requirement. As described in the previous section, only a fraction of nutrient present in the diet is absorbed due to a variety of factors such as physico-chemical properties of nutrient, the food matrix in which it is present, and culinary practice (ICMR 2020). To account for this, the physiological requirements are adjusted for absorption/bioavailability (experimentally measured) to derive the dietary requirements (Table 1.1). Please note that dietary requirements are much higher compared to physiological requirements, as the fractional absorption/bioavailability of nutrient is lower; for instance the bioavailability of iron and zinc is only 8% and 23%, respectively, from habitual diets of Indians. The physiological and dietary requirements of iron and zinc are listed in Table 1.1, to appreciate the critical role of bioavailability in influencing the nutrient requirements.

As shown in Fig. 1.2, when we plot the individual requirements of subjects of a specific population subgroup, we end up with a distribution of requirements which is expected to follow normal distribution or it can be normalized using appropriate statistical methods (Ghosh et al. 2019a, b). The mean/average of this dietary requirement distribution is referred to as estimated average requirement (EAR), where the actual requirement of 50% of the population falls either above and below this level, while the upper 97.5th centile (+2SD to the mean) of this distribution is referred to as recommended dietary allowance (RDA). It is very important to note that RDA refers to the maximum requirement of population, and requirements of 97.5% of the population usually fall below this level (Swaminathan et al. 2016; ICMR 2020; Tattari et al. 2022). Another metric in ICMR-2020 nutrient requirements is TUL (tolerable upper limit), derived from toxicological framework and the intakes beyond this level increase the risk of toxicity (ICMR 2020; Tattari et al. 2022; Ghosh et al. 2023). The TUL serves as a guide to keep a ceiling on the upper levels of specific nutrient being consumed from all possible sources, and is extremely relevant in unsupervised settings such as food (bio) fortification.

1.5 Application of Nutrient Requirements

The science-based knowledge of nutrient requirements and their interpretations have multi-dimensional applications. It would be useful for various stakeholders including policymakers, regulators, academicians, dieticians, and food industry. As mentioned in the previous sections, the knowledge of nutrient requirements helps to

Table 1.1 Dietary requirements of zinc and iron for different physiological groups (ICMR 2020)

Category/Age	Bodyweight (kg)	Physiological requirement (mg/day)		Bioavailability (%)		Zinc (mg/day)		Iron (mg/day)		TUL (mg/day)	
		Zinc	Iron	Zinc	Iron	EAR	RDA	EAR	RDA	Zn	Iron
Men	65	3.25	0.91	23	8	14.1	17	11	19	40	45
Women (WRA)	55	2.53	1.20	23	8	11.0	13.2	15	29	40	45
Pregnant women	55	3.0	2.55	25	12	12.0	14.5	21	27	40	45
Lactating women (0-6 m)	55	3.53	1.28	30	8	11.8	14.1	16	23	40	45
Infants											
(0–6 m)	5.8	–	–	–	–	–	–	–	–	4	40
6–12 m	8.5	0.486	0.33	23	15	2.1	2.5	2	3	5	40
Children											
1–3 y	12.9	0.64	0.38	23	6	2.8	3.3	6	8	7	40
4–6 y	18.3	0.85	0.49	23	6	3.7	4.5	8	11	12	40
7–9 y	25.3	1.13	0.61	23	6	4.9	5.9	10	15	12	40
Adolescents											
Boys 10–12 y	34.9	1.62	0.93	23	8	7.0	8.5	12	16	23	40
Girls 10–12 y	36.4	1.64	1.27	23	8	7.1	8.5	16	28	23	40
Boys 13–15 y	50.5	2.75	1.23	23	8	11.9	14.3	15	22	34	45
Girls 13–15 y	49.6	2.46	1.39	23	8	10.7	12.8	17	30	34	45
Boys 16–18 y	64.4	3.38	1.45	23	8	14.7	17.6	18	26	34	45
Girls 16–18 y	55.7	2.72	1.46	23	8	11.8	14.2	18	32	34	45

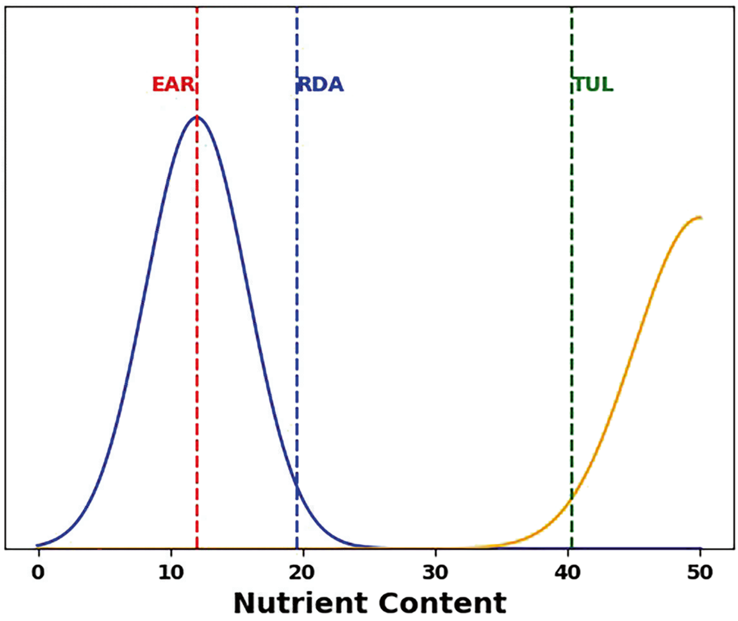

Fig. 1.2 Distribution of nutrient requirements: The nutrient requirements vary in individual subjects of a population, and are expected to follow normal distribution. The mean of this distribution is referred to as estimated average requirement (EAR), and the 97.5% centile of this distribution is referred to as recommended dietary allowance (RDA). The tolerable upper limit (TUL) is derived from toxicological studies; beyond this level of intake increases the risk of toxicity

assess nutritional status of individuals and populations in terms of adequacy or inadequacy. In turn it also aids in planning the diet/meal to meet the essential nutrients requirements, either at an individual level or a population level. Information on nutrient requirements is essential for many food/nutrients supplementation or intervention programs/schemes and to regulate the food policy programs. Further, changes over time in economic status vis-à-vis purchasing capacity clubbed with epidemiological transition can lead to changes in dietary habits and consumption patterns. These paradigm shifts necessitate revisiting nutrient requirements periodically in addressing the nutrition and health issues in population.

As an example, let us consider the nutritional status of an individual adult man, whose actual dietary intake of iron is about 9 mg/day as against the EAR of 11 and RDA of 19. Since we do not know the exact nutrient requirement for an individual (often the case) and 50% of the population's requirements fall below the EAR, it is not possible to directly judge if this person meets or does not meet his requirement. However, it is possible to predict the probability of him being adequate or inadequate by measuring the area under the curve (AUC) left side (adequate) or right side (inadequate) (Swaminathan et al. 2016, 2019; Ghosh et al. 2023). Nevertheless, to make sure that he meets this requirement, it is ideal to shift his intakes closer to the RDA to reduce the probability of being inadequate whereas for population, this could be achieved through estimating the population intakes below and above the EAR (and not RDA) or by statistically computing the probability of inadequacy by comparing the intake and requirement distribution of population (ICMR 2020; Ghosh et al. 2023). To reduce the inadequacy of population by means of food (bio) fortification, it is ideal to iteratively model the population usual intakes with additional allowances such that inadequacy is reduced, while reaching the TUL is minimized (Ghosh et al. 2023).

1.6 Food Fortification and Biofortification

Food fortification entails addition of exogenous nutrient(s) of interest to foods, while biofortification attempts to increase the nutrient content of food through crop breeding practices, either by genetic engineering or marker-assisted selection breeding methods. The goal of either of these approaches is to enrich the nutrient content of grain/food that will help to increase the dietary intakes and results in measurable impact in reducing the clinical or sub-clinical forms of nutritional deficiencies in populations. WHO suggested that food fortification is one of the sustainable and cost-effective strategies to combat micronutrient malnutrition (Dary and Hurrell 2006; Pullakhandam et al. 2023), and the same should be applicable to biofortification. However, the additional advantage of biofortification is that it is self-sustainable, because once desirable traits/genes are established, transferred, and integrated into agriculture practices, additional interventions are not required. In contrast, traditional food fortification requires continued availability (continuous investment) of purified or synthetic food grade nutrients, centralized processing facilities, vigilance on standards being adhered to, and massive distribution efforts to reach the target population. Nevertheless, in traditional fortification it is possible to enrich multiple micronutrients in a single chosen food source at the specified levels, which may be difficult (if not impossible) with biofortification approaches.

The WHO stated that the development of successful food fortification programs is conditional on three important criteria: 1. There is evidence on the deficiency of target nutrient in population to an extent that it signifies as public health problem (i.e. >40% prevalence for anaemia, >20% for vitamin A and zinc); 2. Evidence of dietary inadequacy in target population 3. Evidence from randomized controlled trials that the fortified food consumption produces a measurable impact in target population (Dary and Hurrell 2006; Pullakhandam et al. 2023). As discussed in the nutrient homeostasis section, the body has an inherent capacity to adjust/adapt its mechanisms to use nutrients, even when the dietary intake is low (by increasing absorption and reducing excretion), and thus manifestation of clinical symptoms of its deficiency in high proportions in a population is a clear indication of deficiency. But sub-clinical micronutrient deficiencies measured solely based on specific blood biomarkers are likely to be influenced by several factors. Many biomarkers of nutrition in the body are influenced (either increase or decrease) by infection or sub-clinical inflammation portraying a false picture of deficiency (Tomkins 2003; Thurnham and Northrop-Clewes 2016). For example, inflammation induces the serum ferritin, a diagnostic marker of iron stores in the body, and thereby incorrectly postulates iron deficiency (ID) conditions (under estimates iron deficiency), unless it is corrected for inflammation by additional markers such as C-reactive protein (CRP) or α(1)-acid glycoprotein (AGP); the opposite is true for zinc and vitamin A (Namaste et al. 2017; Kulkarni et al. 2021; Pullakhandam et al. 2021). The next important factor to consider is the dietary intakes of target nutrients, although homeostasis adjustments can cope up with short-term fluctuations in intake, chronic low intakes manifest in clinical or sub-clinical deficiencies of nutrients. Therefore, estimating the dietary inadequacy across population groups (against age and gender-specific EARs) and their possible relationships with the extent of deficiencies can help to understand the role of low dietary intakes in the aetiology of deficiencies, although causal association of low intakes is still not established (Swaminathan et al. 2016; ICMR 2020; Kulkarni et al. 2021; Reddy et al. 2021; Ghosh et al. 2023). For example, children from rural areas showed lower iron deficiency but higher anaemia prevalence, while children from urban areas showed lower prevalence of anaemia, and yet higher prevalence of iron deficiency (Kulkarni et al. 2021). This could be partly due to inefficient utilization of iron in the body in rural children, for the reasons that are incompletely understood. Therefore, it is important to ensure the impact of supplementing the fortified food in reducing the deficiency, based on randomized controlled trials or evidence synthesized through systematic reviews of these trials. This will help in establishing the causal role of low dietary intakes on nutrient deficiencies and provides quantitative information on expected benefits, required for policy.

1.7 What Foods Need to Be Targeted for Biofortification

Consumption of balanced diet with a variety of foods (cereals, pulses, legumes, milk, and animal protein) along with fruits and vegetables is the natural and holistic way of achieving optimal nutrition and health (Kennedy 2004). But when a population is predisposed to a specific nutrient (s) deficit due to a particular dietary pattern, addressing this through evidence-based public health approach such as fortification is justified, and is also proven successful. For example, mandatory salt fortification with iodine virtually reduced its clinical (Goitre) and sub-clinical deficiencies (based on urinary iodine) to negligible levels (Rah et al. 2015). The enormous success of salt iodization can be attributed primarily to its centralized production and its regular and universal consumption across population groups along with regular food/meal. Therefore, the target foods for biofortification should be chosen based on the consumption pattern of the target population and the downstream food processing including culinary practise has minimal or no bearing on the nutrient content of the foods. Cereals such as rice and wheat are consumed across all population groups, making them ideal targets for biofortification of minerals, vitamins, and protein. The Indian Council of Agricultural Research (ICAR) along with various agricultural universities and research institutes has developed more than 120 biofortified crop varieties (wheat, rice, and maize), which are already being cultivated on

~ten million hectares, with more than 1000 tonnes of breeder seed already in production in India (Yadava et al. 2017). However, one should ensure to maintain similar sensory attributes of biofortified foods compared to conventional foods. For example, enrichment of carotene in golden rice through genetic manipulation (Al-Babili and Beyer 2005) is a technological marvel. But its acceptability as a food in communities due to change in sensory attribute (golden vs white colour) often requires education to convince the population about its potential benefits (Nestle and Greger 2001). Another important aspect is to target the enrichment of nutrient in the edible portion of the crop such that it actually increases the intakes in population. For example, rice polishing or wheat flour extraction (whole wheat flour vs maida) leads to leaching of nutrients (Dandago 2009; Oghbaei and Prakash 2016).

1.8 Potential Target Enrichment Levels and Bioavailability Considerations

Once the target food is chosen and specific enrichment in the edible portion is possible, the next step is to decide on the amount of target nutrient enrichment to be achieved for a desired impact in population. As discussed, EAR is the metric in population context, and one should aim to reduce the proportion of population inadequacy via nutrient enrichment in biofortified foods. This requires collaboration among biofortification scientists, public health professionals, and nutritionists to compute the required enrichment levels based on the knowledge of usual intake patterns of the population from all possible sources, and additional nutrient required to substantially reduce dietary inadequacy (Ghosh et al. 2023). There are also alternate possibilities, remember that dietary nutrient requirements are much higher compared to physiological requirements due to lower absorption/bioavailability (Table 1.1). For example, physiological requirement of iron in adult men is 0.91 mg/day, but the bioavailability of iron habitual foods is only 8%; this means one needs to consume 11 mg of iron from daily diet to meet his physiological requirement of ~1 mg. Alternatively, if we can improve the bioavailability of iron in foods to, let's say, 16%, the physiological requirement could be met by consuming only 5.5 mg dietary iron (half of current EAR), which appears to be possible. In fact, the reduction of phytic acid in *finger millet* or increasing the nicotianamine in rice (Zheng et al. 2010; Beasley et al. 2020; Reddy et al. 2022) has been shown to increase the bioavailability of iron. Interestingly, manipulating the source and supply of phosphorus in rice cultivation has been shown to influence grain phytic acid, iron, zinc content and their bioavailability (Su et al. 2018). Therefore, biofortification researchers should be aware that their target is not always the enrichment, but explore alternate possibilities such as manipulating the cultivation practices to optimize nutrient content and/or bioavailability which may be possible to achieve.