DRAFT THEMATIC STUDY

for

THE THIRD REPORT ON THE STATE OF THE WORLD'S PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

(Not for Quotation or Citation)

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Plant Genetic Resources for Food and Agriculture for Enhanced Nutrition

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List of Abbreviations

CBD	:	UN Convention on Biodiversity
CGIAR	:	Consultative Group on International Agricultural Research
CIP	:	International Potato Centre
CWR	:	Crop wild relatives
EGS	:	Early Generation Seeds
FAO	:	Food and Agriculture Organization of the United Nations
GM	:	Genetic Modification
GWAS	:	Genome-wide Association Studies
ICRISAT	:	International Crops Research Institute for the Semi-Arid Tropics
LMIC	:	Low- and Middle-Income Countries
MABB	:	Marker-assisted backcross breeding
MAGIC	:	Multi-parent Advanced Generation Inter-cross
MAS	:	Marker Assisted Selection
MVD	:	Mutant Varieties Database
NDCS	:	Non-Communicable Diseases
NUS	:	Neglected and Underutilized Species
PABRA	:	Pan African Bean Research Alliance
PGRFA	:	Plant Genetic Resources for Food and Agriculture
QDS	:	Quality Declared Seeds
QPM	:	Quality Protein Maize
QTLs	:	Quantitative Trait Loci
SDGs	:	Sustainable development Goals
SNP	:	Single Nucleotide Polymorphisms
WHO	:	World Health Organization

Abstract

Many people are unable to afford a healthy diet, leading to food insecurity and malnutrition, and characterized by undernourishment, micronutrient deficiencies and/or obesity. It is estimated that about 80 percent of the food consumed by humans are derived from plants. Cereals, legumes, fruits, vegetables, roots and tubers and nuts, when adequately combined, present valuable nutrition profiles, supplying energy, proteins, micronutrients, macro nutrients, vitamins, and essential amino acids. Despite the nutritional diversity of cultivated crops and wild food plants, a few starchy staples provide the majority of calories but tend to be low in essential vitamins and minerals. Plant genetic resources for food and agriculture (PGRFA) are essential for increasing dietary diversity, hence enhancing nutritional status. These resources have been harnessed through plant breeding for enhancing the nutritional contents of many food crops contributing to improved diets and health of millions of people globally. Substantial progress has been made in unlocking the genetic potential of PGRFA to develop nutrient-rich crops. Breeding for improved nutrition often combines conventional breeding with modern biotechnologies, including genome sequencing, induced mutagenesis, genetic engineering and genome editing. Successes in biofortification include enhancing the content of micronutrients, mineral elements, proteins and oils. Plant breeders have successfully developed varieties of staple crops containing higher concentrations of iron, zinc, provitamin A and protein. This study explores the importance of conservation and sustainable use of PGRFA towards enhanced nutrition.

1. Introduction

Far too many people, especially in Asia, Africa and Latin America and the Caribbean, suffer from food insecurity and malnutrition, a situation that has been worsening progressively since 2015 (FAO et al., 2021). In 2021, about 2.3 billion people (approximately 30 percent) of the global population, suffered from hunger while almost 3.1 billion people were unable to afford a healthy diet in 2020 (FAO et al., 2022). Globally, malnutrition affects over two billion people with premature mortality and morbidity mainly attributed to sub-optimal diets (Afshin et al., 2019; GBD, 2019). In 2021, 149.2 million (22 percent) children under 5 years of age were stunted, 45.4 million (6.7 percent) wasted and 38.9 (5.7 percent) million overweight globally (Development Initiatives, 2021; FAO et al., 2021).

In line with these statistics, global trends indicate also a shift from traditional diets to an increased consumption of highly refined foods, red and processed meat, sugar and sweetened beverages with limited consumption of pulses, nuts, fruits and vegetables (Afshin et al., 2019). There is increasing reliance on just a few starchy staples, which provide a large share of energy but relatively low amounts of essential vitamins and minerals, frequently resulting in hidden hunger among populations in low- and middle-income countries (LMIC). Consequently, many populations are faced with the triple burden of malnutrition characterized by undernourishment, micronutrient deficiencies and obesity (Christian and Dake, 2022). Iodine, vitamin A, iron and zinc are the major micronutrients lacking in many diets. At the same time, excessive consumption of trans- and saturated fats, sugar, sodium and cholesterol has been associated with the increasing disease burden and mortality (Fern et al., 2015).

The 2021 Global Nutrition Report highlighted the urgent need for large-scale changes toward healthy and sustainable diets to feed the growing population (Development Initiatives, 2021). About 80 percent of the food consumed by humans is plant-based, implying that crops and other food plants are critically important for providing healthy diets for all as means to attaining universal food security and nutrition as committed to through the Sustainable Development Goals (United Nations, 2023; Bhatia et al., 2021).

The conservation and sustainable use of plant genetic resources for food and agriculture (PGRFA) are essential in addressing the above-mentioned nutritional constraints. PGRFA include improved crop varieties, farmers' varieties/landraces, crop wild relatives and wild food plants. These resources may be found on farmers' fields; conserved through their propagules in genebanks, i.e. *ex situ* collections; safeguarded in their natural habitats, i.e. *in situ*, or as breeding materials in experimental fields.

Quality seeds and planting materials are the result of a number of interrelated activities. These begin with the conservation of PGRFA, the breeding of progressively superior crop varieties and finally the availability of the quality seeds and planting materials to farmers. The purpose of this study is to explore the contributions of PGRFA to nutrition. It provides a review of the documented and potential contributions of PGRFA to enhanced nutrition, encompassing enhancements of nutritional qualities of improved crop varieties through plant breeding, and providing evidence for more diversified diets resulting from a greater availability and consumption of local fruits, vegetables and pulses.

2. Plants for healthy diets

Plant-based diets offer an opportunity for adaptation and mitigation of climate change while enjoying the health benefits (IPCC, 2019; Jarmul, 2020; Springmann et al., 2018). A food regimen rich in plant-based meals and with fewer animal source ingredients confers both improved health and environmental benefits (Willett et al., 2019). However, fruit and vegetable production and consumption are significantly below the threshold recommended by FAO and World Health Organization (WHO) in 88 percent of the world's countries (Kalmpourtzidou, Eilander and Talsma, 2020; Mason-D'Croz *et al.*, 2019). Additionally, both legume and nut intake is more than two-thirds below the recommended two servings per day (Development Initiatives, 2021), with the presence of anti-nutrients in most legumes further inhibiting the bioavailability of the little plant protein consumed (Samtiya, Aluko and Dhewa, 2020). Low-income countries have the lowest intakes of nutritious foods, such as fruits and vegetables (Development Initiatives, 2021).

Wheat, rice and maize provide an estimated 42 percent of the world's food calories and 37 percent of protein intake (FAO, 2021). In Africa, for example, the consumption of traditional staples such as millets and sorghum has declined in favour of wheat despite their superior nutritional composition (see Table 1).

Table 1: Nutrient composition of major cereals (rice, wheat and maize) compared to traditional food crops in Africa and Asia (Content/100g)

Nutrient	Pearl millet	Sorghum	Finger millet	Foxtail millet	Proso millet	Barnyard millet	Kodo millet	Rice (milled)	Maize	Wheat flour
Energy (kcal)	361	349	328	331	341	397	309	345	342	346
Protein (g)	11.6	10.4	7.3	12.3	7.7	6.2	8.3	6.8	11.1	12.1
Fat(g)	5.0	1.9	1.3	4.3	4.7	2.2	1.4	0.4	3.6	1.7
Calcium(mg)	42.0	25.0	344	31.0	17.0	20.0	27.0	10.0	10.0	48.0
Iron(mg)	8.0	4.1	3.9	2.8	9.3	5.0	0.5	3.2	2.3	4.9
Zinc(mg)	**3.1**	**1.6**	**2.3**	**2.4**	3.7	3.0	0.7	1.4	2.8	2.2
Thiamine(mg)	0.33	0.37	0.42	0.59	0.21	0.33	0.33	0.06	0.42	0.49
Riboflavin(mg)	0.25	0.13	0.19	0.11	0.01	0.10	0.09	0.06	0.10	0.17
Folic acid(mg)	45.5	20	18.3	15.0	9.0	-	23.1	8.0	20	36.6
Fiber(g)	1.2	1.6	3.6	8.0	7.6	9.8	9.0	0.2	2.7	1.2

Source: Adhikari et al., (2017)

Globally, legumes provide an inexpensive protein source and are considered the second most important food source after cereals (Kouris-Blazos and Belski, 2016). Legumes are rich in proteins with essential amino acids, complex carbohydrates, dietary fibre, unsaturated fats, vitamins and essential minerals (Rebello et al., 2014). Apart from their nutritional value, diets rich in legumes also have added health benefits such as prevention of cardiovascular diseases, hypertension, dyslipidaemia, cancer, and microbial infections (Cicero et al., 2017 ; Ndidi et al., 2014). The demand for legumes has increased due to growing consumer awareness of their nutrition and health benefits as well as demand for alternatives to animal proteins (meat alternatives). Table 2 shows the amino acid profiles of common legumes. While cereals have low levels of lysine, legumes generally have low levels of essential sulphur-containing amino acids. Consequently, the protein quality of plant-based foods can be achieved by consuming meals containing both legumes and cereals (Kouris-Blazos and Belski, 2016).

Table 2: Amino acid profiles of commonly consumed legumes globally- expressed as g/100 g protein

Amino acid	Bambara nuts	Cowpea	Soybean	Adzuki bean	Lupins	Lima beans	Lentils	Chickpea	Broad beans	Kidney beans
Arginine	4.0	1.6	7.2	1.3	3.9	2.2	2.2	1.8	0.7	1.5
Aspartic acid	5.0	2.8	11.7	2.4	3.9	2.9	3.1	2.3	0.8	2.9
Histidine	2.2	0.7	2.5	0.5	1.0	0.6	0.8	0.5	0.2	0.7
Serine	3.2	1.2	5.1	1.0	1.9	1.1	1.3	1.0	0.3	1.3
Glutamic Acid	16.5	4.5	18.7	3.1	8.7	4.2	4.4	3.4	1.3	3.6
Proline	3.2	1.1	5.5	0.9	1.5	1.0	1.2	0.8	0.3	1.0
Glycine	3.3	1.0	4.2	0.8	1.5	1.1	1.1	0.8	0.3	0.9
Alanine	3.5	1.1	4.3	1.2	1.3	1.1	1.2	0.8	0.3	1.0
Lysine*	3.0	1.6	6.4	1.5	1.9	1.8	2.0	1.3	0.5	1.6
Threonine*	2.5	0.9	3.9	0.7	1.3	0.9	1.0	0.7	0.3	1.0
Valine *	3.8	1.1	4.8	1.0	1.5	1.2	1.4	0.8	0.3	1.2
Isoleucine*	3.8	1.0	4.5	0.8	1.6	1.0	1.2	0.8	0.3	1.0
Leucine*	6.8	1.8	7.8	1.7	2.7	1.8	2.0	1.4	0.6	1.9
Tyrosine*	3.2	0.8	3.1	0.6	1.4	0.7	0.8	0.5	0.2	0.7
Phenylalanine*	4.3	1.4	4.9	1.1	1.4	1.1	1.4	1.0	0.3	1.3

Tryptophan*	0.7	0.3	1.3	0.9	0.3	0.3	0.3	0.2	0.1	0.3
Cystine**	0.5	0.3	1.3	0.2	0.4	0.4	0.4	0.3	0.1	0.3
Methionine**	2.0	0.3	1.3	0.2	0.3	0.3	0.2	0.3	0.1	0.4

*Essential amino acid; **Essential sulphur containing amino acid (Source: Maphosa and Jideani, 2017).

Globally, there are approximately 1,100 vegetable species that are edible (Meldrum et al., 2018) and over 1,250 fruit species (van Zonnevel et al., 2023). Tables 3 and 4 illustrate the nutrient profiles of selected indigenous fruits and vegetables which demonstrates that the human nutrient requirements can be met by incorporating them in the usual diets. For instance, *Grewia tenax* fruits, commonly found in semi-arid and sub-humid tropical climates in Africa, the Arabian Peninsula and Asia, could meet the daily iron requirements of children while providing substantial quantities of calcium. Baobab (*Adansonia digitata* L.) *and Sclerocarya birrea* Hochst also contain substantial quantities of Vitamin C (Table 3). Similarly, slender leaves and spider plant are vegetables containing high levels of iron and calcium (Table 4).

Table 3: Nutrient profile comparisons of selected African indigenous and exotic fruits (per 100g edible portion)

Species	Energy (Kcal)	Protein (g)	Vitamin C (mg)	Vitamin A (RE*) (µg)	Iron (mg)	Calcium (mg)
Indigenous fruits						
Adansonia digitata L.	327	2.5	**126-509**	0.03-0.06	6.2	275
Dacryodes edulis	263	4.6	19	n.a.	0.8	43
Grewia tenax (Forrsk.) Fiori	n.a.	3.6	n.a.	n.a.	**7.4-20.8**	**610**
Irvingia gabonensis(Kernels)	697	8.5	n.a.	n.a.	3.4	120
Sclerocarya birrea Hochst.	225	0.7	**85-319**	0.035	3.4	35
Tamarindus indica L.	275	3.6	11-20	0.01-0.06	3.1	192
Ziziphus mauritania Lam.	184	0.4	3-14	0.07	0.8	23
Exotic Fruits						
Guava *(Psidium guajava L.)*	68	2.6	**228.3**	0.031	0.3	18
Mango *(Mangifera indica L.)*	65	0.5	27.7	0.038	0.1	10
Orange *(Citrus sinensis L.)*	47	0.9	53.0	0.008	0.1	40
Pawpaw *(Carica papaya L.)*	39	0.6	62.0	**0.135**	0.1	24

* RE = retinol equivalents. Source: Kehlenbeck et al., (2013); Stadlmayr et al., (2013)

Table 4: Micronutrient content of selected Indigenous Leafy Vegetables (ILVs) commonly consumed in Asia, Africa and the Middle East (mg/100 g)

Indigenous Leafy Vegetable	*Micronutrient content (mg/100 g)*						
	Ca	**P**	**Fe**	**Mg**	**Na**	**K**	**Vit C**
Amaranth	323.70	89.00	7.50	122.00	230.00	341.00	50.00
Cowpea Leaves	428.01	17.23	9.62	46.73	31.25	81.25	8.00
Nightshade	100.47	62.50	8.63	**461.00**	74.22	100.00	54.00
Slender leaves	**1234.40**	11.25	**28.13**	155.00	22.66	162.50	-
Spider plant	**1484.40**	48.95	**29.67**	47.50	18.75	75.00	-
Lamb's quarters	309	72	1.2	34	43	452	80
Purslane	65	44	1.99	68	-	494	21
Blackjack	-	-	15	-	-	-	63
Jew's mallow	208	83	4.76	64	-	559	37
Pumpkin leaves	15	41	0.87	15	4	170	43
Chinese cabbage	77	29	0.31	13	8	238	27

Source: Mungofa et al., (2022).

3. Conservation of wild PGRFA for improved nutrition

Wild PGRFA, which include wild food plants and crop wild relatives, continue to evolve features in their natural environments (FAO, 2017). Preserving wild PGRFA in their natural habitats (i.e., *in situ*) enhances the resilience of food systems to global challenges by providing a diverse pool of genetic resources that can be utilized to address nutrition security (Pathirana and Carimi, 2022).

3.1 Wild food plants – sources of essential nutrients

Wild food plants, often found in and around forest habitats, are frequently consumed during periods of food shortage (Kehlenbeck et al., 2013). Different parts of these plants are utilized as food, including stems, roots, shoots, leaves, fruits, seeds, and buds, (Shaheen et al., 2017). These edible wild plants are often high in nutritional value (see some examples in Table 5).

Table 5: Selected wild food plants with their nutrient profile

Wild plant for food	Macro and micronutrients	Region where commonly consumed	Reference
Purslane (*Portulaca oleracea* L.)	High in omega-3 fatty acids, vitamins A, C, E, and B-complex vitamins (including folate), and minerals like calcium, iron, magnesium, and potassium. phenolic acids, flavonoids, tannins	Mediterranean, Middle East, and Asia	Xiang et al., 2005
Wild leek (*Allium ampeloprasum* L.)		Mediterranean, Middle East, and Asia	Kim et al., 2018
Nettles (*Urtica dioica* L.)	Rich in vitamins A, C, and K, as well as minerals like calcium, iron, and potassium. Nettles are also a good source of protein and dietary fiber.	Europe, North America, Asia, and North Africa	Duma et al., 2014
Wild Asparagus (*Asparagus racemosus* Willd.)	A good source of vitamins A, C, E, and K, as well as minerals like calcium, magnesium, and potassium.	Found in various parts of the world, including Asia and Africa.	Guarrera and Savo, 2013
Amaranth (*Amaranthus* spp.)	Rich in protein, dietary fiber, vitamins A and C, calcium, iron, magnesium, and phosphorus.	Cultivated and consumed in many parts of the world, including South America, Africa, and Asia.	Duguma, 2020
Bamboo Shoots (*Bambusa* spp.)	Rich in dietary fiber, vitamins A, B6, and E, as well as minerals like potassium, calcium, and magnesium.	Consumed in Asia, particularly in countries like China, Japan, and India.	Satter et al., 2016
False daisy (*Eclipta alba L)*	Rich in iron, zinc, copper, magnesium, calcium, sodium and potassium		Rana et al., 2019
Chinese chaste tree (*Vitex negundo* L**)**			Afolayan and Jimoh, 2009
Chaff flower (*Achyranthes aspera* L)			Gupta et al., 2005 et al 2005
Moringa (*Moringa stenopetala* (Baker f.) Cufod.)	Rich in zinc, iron, copper and calcium	Consumed in many parts of Africa	Abuye et al., 2003
Star flower *(Borago officinalis* L)	Rich in vitamin C, vitamin B1-B2-B3	Consumed in many parts of Africa and Mediterranean	Dresler et al., 2017
common mallow (*Malva sylvestris* L.)	Rich in anthocyanins (malvidin), vitamin C, alkaloids, and phenolic compounds	Consumed in many parts of Mediterranean	Mohajer et al., 2016
Nabag fruit *(Ziziphus spina-christi* (L.) Desf.)	A good source of zinc, iron, copper, magnesium and calcium		Osman and Ahmed, 2009

3.2 Crop wild relatives (CWR) – sources of traits used in plant breeding

Crop wild relatives (CWR) are wild plant species that are closely related to domesticated crops and have not been modified by human selection (FAO, 2017; Ahmad et al., 2021). These species play a crucial role in providing a diverse pool of traits with the potential to improve nutrition in domesticated crops. CWR have evolved under various environmental conditions, making them genetically diverse and adapted to different climates, soils, and biotic stresses. Some CWR possess essential nutrients and bioactive compounds that are lacking in certain cultivated crops (Murthy and Paek, 2021). High levels of genetic variability exist for nutrition traits in crop progenitors and wild relatives which can be incorporated into improved nutritionally rich varieties through biofortification strategies.

Identifying those PGRFA high in vitamin and mineral content from available germplasm is necessary for breeding biofortified crops, which can be found in CWR (Gaikwad et al., 2020). For example, studies have shown that some wild wheat relatives have significantly higher Fe and Zn than cultivated wheat (Chhuneja et al., 2006). In wild rice, the red pericarp has been shown to have high levels of proanthocyanidins and anthocyanins which are health-promoting nutrients (Zhu et al., 2019). In another instance, the use of wild relatives of carrots has been used to enhance beta-carotene content (a precursor of vitamin A) in cultivated carrot varieties. These examples highlight the importance of CWR in breeding as they offer a repertoire of nutrient encoding genes for biofortification of staples.

4. On-farm management of cultivated PGRFA for improved nutrition

Diversification of cropping systems allows for the cultivation of a broader range of crop varieties, including traditional and locally adapted ones, thus conserving genetic diversity on-farm. This diversity acts as a reservoir of valuable traits, including those related to nutrition, which is a vital resource for researchers, plant breeders and farmers (Dempewolf et al., 2023). Different crop types have varying nutritional profiles and their inclusion in these cropping systems has the potential of ensuring a steady supply of essential nutrients even under changing environmental conditions (Drewnowski and Popkin, 1997). Initiatives such as community seed banks promote the conservation and exchange of diverse crop varieties, including those with high nutritional value and adapted to local conditions. These seed banks can also safeguard traditional knowledge related to crop diversity, contributing to the continuity of diversified cropping systems (Ceccarelli, 2015; Demeulenaere and Bonneuil, 2014; Padulosi et al., 2011). This includes information on selecting, growing, and processing various crops, which can optimize their nutritional value and utilization (Powell et al., 2015).

4.1 Farmers' varieties and landraces

Smallholder farmers traditionally cultivate a range of well-adapted local varieties that are often genetically and phenotypically heterogeneous, are adapted to the environmental conditions of the areas of their cultivation. These farmers' varieties/landraces tend to be preferred for their agronomic and culinary qualities or for their cultural values (FAO, 2019; Furman, Noorani and Mba, 2021; Mba et al., 2021). On-farm crop diversity can also contribute to increased dietary diversity for local communities, especially in regions where people rely heavily on a few staple crops (Sthapit et al., 2012). A more diverse diet can improve overall health and nutrition, as it provides a broader range of essential nutrients (Ickowitz et al., 2019).

4.2 Neglected and under-utilized species

Neglected and under-utilized species (NUS) are those plant species plant species which have been under-researched and under-represented in agricultural and nutritional practices (Padulosi et al., 2013; Li and Siddique, 2018; Li, Yadav and Siddique, 2020). The exact number of NUS that can be utilized for agriculture, food, and nutrition is still unknown (Hunter et al., 2019). NUS such as teff (Cheng et al., 2017); buckwheat (Christa and Soral-Śmietana, 2008); enset (Bosha et al., 2016); underutilized roots and tubers (Olango et al., 2013) have potential to contribute to dietary health by providing valuable macronutrients (carbohydrates, proteins and fats) and micronutrients (vitamins and minerals), (Bioversity International., 2017; Dulloo et al., 2014; WHO/CBD, 2015).

5. Plant Breeding for Improved Nutrition Outcomes

Plant breeding is carried out to improve crops for essential nutrients to develop biofortified crops, with the aim of increasing their nutritional value (Garg et al., 2018a). The success of breeding is largely driven by the degree of genetic variation that exists in cultivated species and their wild relatives, which enables breeders to create novel plant gene combination critical to generate desirable trait expressions (Glaszmann et al., 2010), including improved nutritional food values desired to sustain human health. Conventional breeding is currently the most frequently employed technique for developing genotypes and varieties that are nutrient-enriched. However, it is typically labour- and resource-intensive, and can take years to complete (Prado et al., 2014).

Breeding for enhanced nutrient content (biofortification) in crops has been successful in increasing the content of micronutrients (vitamin A, copper, zinc, iron, iodine, molybdenum, selenium, nickel, cobalt), mineral elements (sulphur, chlorine, phosphorus, calcium, magnesium, and sodium) and protein and oil (Banerjee et al., 2023). In the last few decades, breeding has combined classical breeding with modern biotechnology strategies (induced mutation, marker assisted selection, genome wide assisted selection, genomic selection, new plant breeding techniques like genetic engineering and genome editing). This section presents advances in conventional breeding and biotechnology that have contributed to nutritional enhancement in food crops.

5.1 Pre-breeding

A considerable proportion of valuable alleles, including those for nutrition, that have not been harnessed into elite gene pools needs to be introduced for the development of nutrition dense improved varieties (Chatzav et al., 2010). It implies that cultivars must be crossed with distant relatives for the introduction of the desired trait (micronutrient and mineral content) and therefore it often requires to overcome several challenges associated with interspecific wide crosses (e.g. cross incompatibility, hybrid sterility, etc.). Pre-breeding is the transfer of desirable traits from non-adapted materials to generate intermediate materials, which are then used in breeding improved crop varieties (Abebe and Tafa, 2021). Through the use of biotechnology, useful genes from CWR are being efficiently used in biofortification through the identification of optimum combinations of favourable alleles for targeted trait based on sequence information and gene editing tools (Bohra et al., 2022).

5.2 Conventional plant breeding

Conventional breeding can improve nutrient levels in staple crops to target levels required for enhancing human nutrition without sacrificing yield or farmer-preferred agronomic features. The Harvest Plus Program of the CGIAR (HarvestPlus), for example, has undertaken conventional breeding approaches to deliver biofortified crops for Fe, Zn, provitamin A and protein. For example, it has been possible to identify high Zn parental lines for use in breeding (Andersson et al., 2017; Sharma et al., 2020). PGRFA variability has been exploited to enhance micronutrient density for other staples such as sweet potato, beans, rice, and cassava (Beebe et al., 2000; Chavez et al., 2000; Hagenimana and Low, 2000; Kimani and Warsame, 2019; Maziya-Dixon et al., 2000). Sorghum germplasm also showed high variability for iron and zinc (Guild and Stangoulis, 2021; Satish et al., 2016; Sen et al., 2019; Upadhyaya et al., 2016).

Plant breeders have successfully developed varieties of staples with iron concentrations that are two to five times higher than those of traditional commercial types (Boy, 2017). Several nutrient rich varieties have been released in many countries all over the world. High zinc varieties of wheat were released in India and Pakistan (Singh et al., 2017). Through public–private partnerships, HarvestPlus has reached more than 50 000 wheat farmers in the Eastern Gangetic Plain of India (Velu et al., 2015). In recent years, India has released a number of quality protein maize (QPM) hybrids with high levels of the essential amino acids lysine and tryptophan. ICRISAT has released biofortified millet and sorghum. CIAT and HarvestPlus has released iron biofortified common bean in many countries in

Africa. Similarly, CIP released orange-fleshed sweet potato varieties in many countries in Africa, South America and China which attracted global acknowledgement which earned its scientists the 2016 World Food prize. An example of the impact of these varieties is elucidated in Box 1.

BOX 1: Addressing vitamin A deficiency in lactating mothers and infants through biofortification

One of the major health issues in underdeveloped nations is vitamin A deficiency, which can result in permanent blindness. Studies on the effectiveness of provitamin A supplementation found that eating foods containing vitamin A biofortification increased the amount of circulating beta-carotene and had a moderate impact on serum retinol, a marker of vitamin A status. Consuming Orange Fleshed Sweet potatoes (OFSP) can significantly raise vitamin A body reserves in people of all ages (Low et al., 2007). When used as a staple crop, biofortified provitamin A maize is an effective source of vitamin A. The total body stores of vitamin A in the children in the orange maize group considerably increased over the course of three months compared to those in the control group, according to an efficacy research carried out in Zambia with 5-7-year-old children (Gannon et al., 2014). Orange maize consumption has been shown to dramatically enhance visual function in children who are just mildly vitamin A deficient and to increase total body vitamin A reserves as effectively as supplementation (Palmer et al., 2016a). In Kenya, a modest provitamin A cassava has been developed and an effectiveness study with children aged 5 to 13 has been completed. In this study, the yellow cassava group outperformed the control group in terms of small but significant gains in vitamin A status as determined by serum retinol and beta-carotene. In Nigeria, a larger-scale efficacy experiment is being conducted (Talsma et al., 2016a)

Children's vitamin A status across age groups in Zambia is considerably improved by consuming vitamin A-rich orange sweet potatoes (OSP) (Sakala et al., 2018). A large-scale effectiveness study conducted in Uganda found that after four growing seasons, the introduction of OSP to farming households significantly improved the vitamin A status of children who were deficient at the beginning of the study (9.5 percent reduction in low serum retinol prevalence). This improvement was attributed to children's vitamin A intake among women and children (Hotz et al., 2012b). Regular OSP use also decreased child morbidity (Hotz et al., 2012a). A study conducted in Zambia among school-aged children (ages 5 to 6) discovered that switching from ordinary maize to vitamin A maize dramatically increased the kids' vitamin A status (Palmer et al., 2016b). The vitamin A content of the breast milk produced by Zambian mothers who consumed vitamin A-rich maize twice daily for three months improved, and the prevalence of low vitamin A concentration in breast milk decreased by more than 50 percent (Palmer et al., 2021). In eastern Kenya, children aged five to thirteen who ate boiling and mashed vitamin A cassava saw a slight but nutritionally significant improvement in their vitamin A status over the course of 4.5 months (Talsma et al., 2016b). After 3.5 months, pre-school children (3-5 years old) in Nigeria who had been consuming vitamin A-rich cassava twice a day had increased vitamin A and iron status (serum retinol) levels (Afolami et al., 2021). Children's vitamin A status across age groups in Zambia is considerably improved by consuming vitamin A-rich orange sweet potatoes (OSP) (Sakala et al., 2018).

5.3 Marker-aided selection

Molecular markers are important for crop improvement programs and pave the way to efficiently dissect genetics of target traits in breeding. Several molecular markers have been used to study nutrition and other economic traits (Kumar et al., 2021; Mir and Varshney, 2013; Sihag et al., 2021; Tyagi et al., 2019). Molecular markers identify a particular gene locus and aid their application in marker-aided breeding which has been applied to nutrition traits (Sarkar et al., 2021). Particularly, the discovery of genes/quantitative trait loci (QTLs) related to critical nutrients and their successful deployment in elite breeding lines through marker-assisted breeding in order to address the issue of nutritional inadequacy is important. QTLs have been identified for protein content, vitamins, macronutrients, micronutrients, minerals, oil content, and important amino acids in main food crops (Gaikwad et al., 2020). The identification and tagging of QTLs, especially those associated with micronutrients, has allowed marker assisted selection (MAS) for these traits to rapidly accelerate development of nutrient dense crop varieties (Ortiz-Monasterio et al., 2007). Single nucleotide polymorphisms (SNP), advances in whole genome sequencing and their applications, have rapidly evolved to enhance the accuracy of plant breeding, while decreasing the time involved. Genome-wide association mapping (GWAS), genomic selection and whole genome sequence data facilitate the identification of markers tightly linked to nutritional trait of interest.

QTLs for mineral elements have been reported in many studies using diverse genetic mapping populations. Examples include those for vitamin A, Fe, Zn and minor trace elements, which have facilitated biofortification leading to the release of crop varieties with high nutritional value. For example, golden rice, maize, and cassava have high levels of vitamin A (Palmer et al., 2016c). Pro-vitamin A QTLs were mapped to chromosome 10 in maize (Babu, 2013; Suwarno et al., 2015). Additionally, SSR markers in maize have been used to identify QTL for carotene content in recombinant inbred line (RIL) populations (Yan et al., 2010). Further, four major genetic markers encoding lycopene (Zeng et al., 2015) were localized and used in MAS resulting in the doubling the b-carotene concentrations (Tavares and Rodriguez-Amaya, 1994).

Fe and Zn content have also been identified by aid of molecular markers in double haploid (DH) and RIL populations (Gaikwad, 2020; Paudel et al., 2020). For example, major QTLs for Zn content have been mapped in bread wheat (Soman et al., 2014) and in rice (Biradar et al., 2007). QTLs for Fe concentration were found in in maize (Lung'aho et al., 2011). QTLs for minor trace elements have been identified in rice (Swamy et al., 2018), lentil (Ates et al., 2016) and chickpea (Jadhav et al., 2015).

GWAS has been applied to many genomes to identify those statistically associated with a specific trait (Uffelmann et al., 2021). This approach has substantially reduced the time for varietal development from 8-10 years under conventional breeding to 5-6 years. GWAS has facilitated quick development of markers (Tong et al., 2020) and increased marker density and resolution (Wang et al., 2018) for nutrition traits (Wu and Hu, 2012), as well as identifying gene families responsible for nutrient uptake, transport and accumulation (Alomari et al., 2021).

5.4 Induced mutations

The process of induced mutation aims to generate desirable traits without compromising the genetic integrity of the material, while reducing the risk of unintended deleterious alleles. The most common physical mutagen used is ionization radiation (e.g., gamma and X-rays). Chemical mutagen such as alkylating agents and azides have been widely used (Kathiria and Eudes, 2014). The International Atomic Energy Agency (IAEA) through the application of nuclear energy, has significantly advanced crop improvement using induced mutation.

Induced mutation has been employed for the development of QPM cultivars that contain twice as much lysine and tryptophan, two crucial amino acids, (Chakraborty and Paul, 2013). In rice, five mutants associated with increases in the content of protein, vitamins , amino acids and mineral elements have been reported (Zhang et al., 2007). Additionally, the increased bioavailability of phosphorus and micronutrient minerals in cereals and legumes has been made possible by the release of new mutant varieties of barley, wheat, rice, and soybean with low phytic acid (Chakraborty and Paul, 2013).

5.5 Genetic engineering

Genetic engineering, also known as transgenic plant breeding, has been used to develop biofortified crops with enhanced nutrient and agronomic features where the target nutrient does not naturally occur at the required levels. It has the advantage of facilitating the movement of desirable genes among phylogenetically distant and incompatible species. Genetic engineering offers the added opportunity to characterize gene function, which may then be used to engineer plant metabolism (Arya et al., 2020).

Genetic engineering in cereals has resulted in enhanced bioavailability of iron and zinc in edible seed. Aung et al. (2013) developed transgenic rice for the overexpression of the gene to enhance iron transport in order to increase iron accumulation in the endosperm. A high yielding transgenic indica rice was modified to express the ferritin gene from soybean, leading to increases in Fe and Zn concentrations (Paul et al., 2014).

Genetic engineering in other crops has also led to higher levels of bioavailability of iron and zinc. For example, transgenic chickpea (*Cicer arietinum* L.) showed increased iron transport and storage through a combination of chickpea nicotinamide synthase 2 (CaNAS2) and soybean (*Glycine max*) ferritin (GmFER) genes, thereby increasing iron bioavailability (Tan et al., 2015). The iron content in wheat has been enhanced by expression of the ferritin gene sourced from soybean (Drakakaki et al., 2000). Overexpression of the nicotinamide synthase gene has been shown to enhance Fe and Zn bioavailability in both wheat and maize (Beasley et al., 2019; Barma et al., 2019).

Transgenic approaches have also been used to increase the level of β-carotene in rice. For example, golden rice is one of the most successful examples of a genetically modified crop variety developed for the accumulation of high pro-vitamin A (Paine et al., 2005; Diretto et al., 2007). The level of β-carotene was improved using the orange (Or) gene in rice (Bai et al., 2016) and white fleshed sweet potato (Sankari et al., 2018). In bananas, genetic engineering increased provitamin A content (Paul et al., 2017) by expressing phytoene synthase under the control of the banana ubiquitin promoter (Ubi). Wheat was improved for provitamin A by expressing bacterial PSY and carotene desaturase genes CrtB, CrtI (Wang et al., 2014) and by suppressing the degradation of provitamin A, resulting in an increase of β-carotene levels (Zeng et al., 2015). Maize endosperm has been enriched with provitamin A (carotenoids) by expressing bacterial crtB (85) and multiple (5) carotenogenic genes (Manjeru et al., 2019).

Levels of several other vitamins have been improved in crops through genetic engineering. Rice was genetically modified to enhance folic acid content (up to 150-fold), important in addressing anaemia, particularly during pregnancy (Demis et al., 2019; Gorelova et al., 2019). In maize, Vitamin C (l-ascorbic acid) levels has been enhanced nearly 100-fold using genetic modification (Wang et al., 2010). Naqvi et al., (2009) developed maize with a 169-fold increase of beta-carotene, two-fold increase of folate and 6-fold of ascorbate by modifying three distinct metabolic pathways. In tomato, strawberry, and potato, the GDP-l-galactose phosphorylase gene was exploited to increase ascorbate, resulting in transgenic tomato plants displaying a 3- to 6-fold increase, while strawberry transgenic lines displayed a 2-fold increase (Scholes et al., 2012).

5.6 Genome editing

Genome editing is a method for making specific changes to the DNA that involves addition, removal or alteration of DNA within the genome. Genome editing technologies offer viable options for addressing the challenges of malnutrition due to their ability to modify genomes in a precise way (Voytas and Gao, 2014). Sequence specific nucleases are utilized in plant genome editing to modify targeted genes with reproducibility, minimal target effects and no external gene sequence integration (Rosenthal et al., 2021). Deletions, insertions, single-nucleotide substitutions, and extensive fragment substitutions are key advantages offered by genome editing. Genome editing techniques frequently result in gene knockout mutants, gene replacement mutants, and gene insertion mutants, making them an effective tool for enhancing nutritional quality in food crops (Voytas and Gao, 2014).

Several techniques have evolved for genome editing in the last few decades and include the mega nucleases (otherwise also known as homing endonucleases) such as Zn-finger nucleases (ZFNs) and the transcription activator like effector nucleases (TALENs). ZFN technology and TALENs include complex designs that require customized protein for each DNA sequence and tend to be low in specificity, show low engineering feasibility, are less able to knock out genes and are unsuitable for RNA editing (Ghani et al., 2023; Salsman and Dellaire, 2016; Sun et al., 2018). Further, the total DNA-binding domain specificity of ZFN is limited to about 12–18 nucleotide sequences (Cebrian-Serrano and Davies, 2017). Recently, the palindromic repeat clusters (CRISPR/Cas) system has been efficiently used to target specific region of any genome controlling a specific trait for crop improvement.

Due to their effectiveness, high specificity, and multiplex ability, genome editing technologies such as CRISPR, have gained popularity as genomic tools for improving the nutritional content of food crops

(Gaikwad et al., 2020). CRISPR involving Cas 9/13 is a two-component system comprising of a guide RNA, which recognizes the target sequence of the genome and the CRISPR-associated endonuclease (Cas) that cuts the targeted sequence. CRISPR-Cas technology is thus highly preferred as it overcomes most of the challenges associated with the other gene editing technologies (Asmamaw and Zawdie, 2021).

Applications of CRISPR technologies for the improvement of nutrition quality have been reported in several crops. β-carotene content increased in rice calluses with the modification of the Orange (or) gene, using CRISPR/ Cas9 (Endo et al., 2019). Similarly, high carotenoid was developed by adding a carotenoid biosynthesis cassette using the CRISPR-Cas9 (Dong et al., 2020). Genome editing was used to knock out the OsVIT2 gene to drive increased Fe availability in rice (Dong et al., 2020; Zheng et al., 2021). In maize, genome editing has also been explored for the biofortification of maize phytoene synthase gene involved in carotenoid biosynthesis pathway (Zhu et al., 2016). Genome editing was also successfully used to modify nitrogen transporter gene OsNRT1.1B associated with protein accumulation in rice (Lu and Zhu, 2017). Additionally, the knockout of OsITPK1-6 by CRISPR-Cas9 resulted in low phytic acid accumulation in rice grain, which thus increased micronutrient availability (Jiang et al., 2019). Another successful case was the use of genome editing in increasing Zn concentration in wheat through targeted mutagenesis of the TaVIT2 gene (Mourad et al., 2019). The function of the OsZIP9 gene, was confirmed to be involved in Zn uptake and accumulation through CRISPR/Cas 9 system. Zinc-regulated transporters and iron-regulated transporters (ZIP) are targets for genome editing that holds great potential to increased iron and zinc uptake in pulses crops (Tan et al., 2015).

6. Conclusions, future perspectives

Conventional crop improvement has mainly addressed yield and agronomic traits and have not focused on enhanced nutrition. Overall, this has resulted in crop genepools with low variation for nutrition traits. Recently, however, new technologies have let to substantial progress in unlocking the genetic potential of PGRFA for the development of nutrient rich crops.

Genome sequencing, the use of induced mutagenesis and new plant breeding techniques, especially genetic engineering and genome editing, has contributed to enhance genetic variation for nutrition traits. The combination of conventional breeding with MAS, genetic engineering and genome editing strategies has been effective in increasing the number of varieties with improved nutrition traits that are available and accessible.

Molecular markers and advances in genome sequencing and their applications have rapidly evolved to enhance the accuracy of plant breeding, while decreasing the time involved to develop improved varieties. MAS has allowed for the identification of genes associated with traits of interest. The use of induced mutations and genome editing have increased the understanding of the genetic basis underlying the biochemical pathways associated with nutrition. Genetic engineering has enabled the incorporation of target genes from other genepools, such as CWR, into breeding lines.

In the future, sustainable agrifood systems will increasingly have to meet nutritional needs while adjusting to an ever-changing environment. Genetic gains for improving nutrition will also need to progress hand-in-hand with advances in other areas such as agronomy, soil health, water use efficiency and plant health to attain food security. In this context, PGRFA continue to be the basis for progressing towards these goals and reinforcing the need for their effective conservation and sustainable use.

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