Chapter 5

Contribution of Conventional Breeding Approaches in Legumes Biofortification

Hafiz Muhammad Ahmad, Sadaf Zahra, Sadaf Oranab, Shazia Arif, Shehnaz Zakia, Gul-E-Zahra, Aamir Raina, Muhammad Zubair Khan, Flavien Shimira, Ghassan Zahid, Saira Bano, and Mahmood-Ur-Rahman

Abstract Deficiency of important micronutrients in human diet is usually known as hidden hunger. Globally, malnutrition affects the life of about 2 billion people. Especially, the life of pregnant women and children of developing countries is affected very badly. In the past, plant breeders majorly focused to increase the crop productivity by improving resistance against biotic and abiotic stresses. A very less attention was given to improve the nutritional accumulations of crops. Recently, biofortification of crop plants has been considered an objective of major breeding

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H. M. Ahmad (✉) · S. Zahra · Mahmood-Ur-Rahman
Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Faisalabad, Pakistan

S. Oranab
Department of Biochemistry, Government College University Faisalabad, Faisalabad, Pakistan

S. Arif · M. Z. Khan
Department of Plant Breeding and Molecular Genetics, University of Poonch, Rawalakot, Pakistan

S. Zakia
Botanical Science Division, Pakistan Museum of Natural History, Islamabad, Pakistan

Gul-E-Zahra
Institute of Botany, University of the Punjab, Lahore, Pakistan

A. Raina
Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, India

F. Shimira
Department of Horticulture, Institute of Natural and Applied Sciences, Çukurova University, Adana, Turkey

G. Zahid
Department of Biotechnology, Institute of Natural and Applied sciences, Çukurova University, Adana, Turkey

S. Bano
Department of Plant Breeding and Genetics, University of Sargodha, Sargodha, Pakistan

M. A. Nadeem et al. (eds.), *Legumes Biofortification*,
https://doi.org/10.1007/978-3-031-33957-8_5

programmes by combining conventional breeding and genetic engineering tools. This chapter focuses on various approaches adopted by conventional breeding programmes to fortify the food legumes for bioavailability, translocation and uptake of micronutrients. We also highlighted the strategies of legume breeders to improve the vitamins and diminish the anti-nutrients. In the end, we shed light on the challenges and limitations of conventional breeding approaches to fortify legumes.

1 Introduction

Legumes are considered the powerhouse of energy, standing among the important staple food crops after cereals grown by human beings in various civilizations worldwide (Mousavi-Derazmahalleh et al. 2019). Legume seeds are believed to be the most economical meat substitute supplying valuable proteins with essential amino acids profile, carbohydrates, vitamins and important minerals required for the proper functioning of the human body (Didinger and Thompson 2021; Roorkiwal et al. 2021). Food legumes such as beans, mung bean, broad bean, chickpea, lentils, lupins, peanuts and other podded plants are widely used in human diet (Ahmad et al. 2012; Didinger and Thompson 2021). Legumes seeds are rich source of good quality proteins and dietary fibres that generally contain essential amino acid lysine (Didinger and Thompson 2021). Protein contents of peas and beans are about 17–20%, whereas lupin and soybeans contain 38–45% proteins that is important for normal body growth and development (Mahto et al. 2022). The daily dietary reference value of nutrient components for adults is 8 to 18 mg for Fe, 8 to 11 mg for Zn and 750 mg of Ca depending on gender, which is usually not possible to fulfil and hence results in micronutrient deficiency (O'Neill et al. 2020). Besides their potential roles in the sustainability of healthy food systems, legumes also contribute to human nutrition, food security and are also associated with reduced risk factors for chronic disease (Kurek et al. 2022). A study on human health proved that use of lentil in daily diet may increase the blood selenium concentration (Thavarajah et al. 2010). According to FAO stat 2018, about 92 million tons pulses are produced globally out of which 42 million tons are produced in Asia (Kumar and Pandey 2020).

Malnutrition of micronutrients usually caused due to supply of insufficient or poor quality of nutrients in daily diet (Mahto et al. 2022). The scarcity of micronutrients in the diet is also termed hidden hunger that adversely affects the body's normal development and physical functions like immunodeficiency, retarded physical and mental growth (Shahzad et al. 2021). Malnutrition also results in different infectious diseases like malaria, diarrhoea, measles in developing countries (Shahzad et al. 2021). Deficiencies of β-carotene, folic acid, Fe and Zn are global issues and affect more than two billion people in Asia, Africa and Latin America (Shahzad et al. 2021). Globally, there are approximately 32.8% of pregnant ladies, 32.5% of non-pregnant ladies and 41.7% of youngsters under the age of 5 are suffering from iron deficiency and potentially leading to restriction in intrauterine development, low birth weight, protein malnutrition and persistent energy deficiency (Kumar and Pandey 2020). The industrialized solution for this micronutrient deficiency is food fortification by enhancing nutrient content and biofortification.

Biofortification is an approach to enhancing the dietary value of crops with the assistance of transgenic techniques like breeding and agronomic practices. The approaches used for biofortification aimed to target and regulate the metabolic pathways such as transportation, root uptake mechanism, remobilization, storage and enhanced minerals concentration (Roorkiwal et al. 2021). Biofortification acts as an advanced technology involving the transfer of the genes directly in selected genotype to reduce mineral deficiency. Efficient biofortification could be achieved by increasing the bioavailability of micronutrients and their concentration, improving the retention of minerals and decreasing the concentration of nutrients that minimize their absorption (Roorkiwal et al. 2021). Vitamins C, D, and E, choline, niacin and provitamin A act as promoters and increase the absorption of Se, P, Zn, Ca, Fe, methionine and tryptophan, while certain polyphenols and phytate decrease the micronutrient bioavailability to crops (Rehman et al. 2019; Shahzad et al. 2021). Transgenic approaches to protein, vitamins and mineral contents have been significantly observed in pigeon pea, common beans, field pea and chicken pea (Rehman et al. 2019). Biofortified crops proved better to withstand adverse environmental conditions with a higher concentration of micronutrients. Biofortification by using techniques of plant breeding is one of the most effective and economic approaches among different strategies to reduce the micronutrient deficiencies, and it could easily be available to people living in rural and remote areas where the access to fortified food is limited (Jha and Warkentin 2020). Biofortification has improved legumes' nutritional quality and contents in the last decade, although numerous demanding situations should be addressed to maximize the successful use of biofortified foods. Mass selection technique was successfully adopted to identify the dry pea genotypes with improved yield and nutritional contents and the results indicated that there was huge diversity in minerals and phytic acid contents among genotypes (Thavarajah et al. 2022). Biofortification refers to crops with increased nutrient density developed using different modern and conventional breeding approaches. Nowadays access to nutritionally balanced food is important for the overall growth and development of organisms including human beings. However, most food crops lack adequate amounts of critical micronutrients; increasing nutrient density is important to mitigate the adverse effects of malnutrition. The number of undernourished people increased from 636.8 million to 811 million over the last 10 years (FAO 2022) (Fig. 5.1). An estimated 22% of children (149.2 million) under the age of five suffered from stunting due to severe acute malnutrition and is expected to worsen under the shadow of the COVID-19 pandemic. Malnutrition is an underlying cause of death of more than 2.6 million children each year, which accounts for one-third of total child deaths worldwide. It is also a leading cause of physical and mental developmental disorders, diseases and premature deaths (Development Initiatives 2018). With such a gloomy scenario it is important to have major changes in agriculture and its allied sectors. The first and most important change would be a shift from producing more food quantities to producing nutritionally rich food in

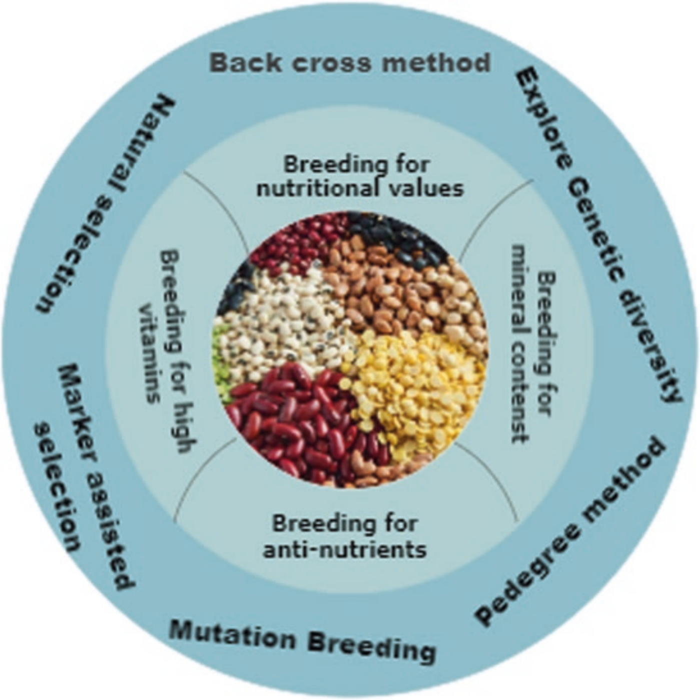

Fig. 5.1 Conventional breeding techniques to improve the food legumes

adequate amounts. Here, the biofortification of crops would play a pivotal role in providing adequate quantities of nutrients to the poor population. The biofortified cereals, legumes, vegetables and fruits are providing adequate levels of micronutrients to targeted populations. Crops are the major source of nutrients in the developing world and their biofortification of crops would play a vital role in making the nutritionally available food for the masses (Table 5.1).

2 Exploitation of Genetic Variations for Micronutrients Improvement

Genetic variation can be used to improve the targeted nutritional traits by selecting favourable alleles (Kumar et al. 2016). The exploitation of genetic variation is a tool to start new research strategies for the biofortification of legumes using the wild relatives to fight hidden hunger and provide nutritious food to a major portion of the global population. Genetic variation uses conventional breeding methods along with modern genomic approaches to exploit the genetic diversity to boost up the micronutrient contents in legume seeds (Roorkiwal et al. 2021; Mahto et al. 2022). Existing genetic diversity, trait inheritance, gene activity, trait linkage, accessible

Table 5.1 Nutritional contents of various food legumes

Name of legumes	Protein contents	Lipid contents (%)	Vitamins	CHOs (%)	Dietary fibres	References
Glycine max	37–42	17–19	A, D, E and K	35	20	Kumar and Pandey (2020)
Cicer arietinum	17–22	4–8	Folic acid, tocopherol, vitamin B complex	40–60	18–22	Madurapperumage et al. (2021)
Vigna mongo	20–25	2.1–2.7	A, B6 and E	55–65	7	Kumar and Pandey (2020)
Vicia faba	26.1–38	0.7–3.2	Folates, vitamin C	55.1–71.4	6.4–34.9	Martineau-Côté et al. (2022)
Cajanus cajan	19.5–22.9	1.49	Folates, vitamin B complex, thiamin	62.78	10	Talari and Shakappa (2018)
Pisum sativum	22.24–31.59	1.66–2.22	Thiamin, riboflavin, niacin, vitamin B6	27.80 to 34.78	16.81–40.63	Brigide et al. (2014), Kumar and Pandey (2020)
Lens culinaris	23.3–25.88	1.93–2.15	β-Carotene	54.08–55.81	6.99–8.14	Kumar et al. (2016)
Arachis hypogaea	25.80	49.24	Folates, niacin, pyridoxine	16.13	8.5	Arya et al. (2016)

screening procedures and diagnostic tools are all utilized to assess prospective genetic gains (Baker et al. 2019). Lot of genetic diversity is available between current legumes and their wild relatives (Rehman et al. 2019). However, wild species contain a high number of beneficial foreign genes that are no longer found in the farmed gene pool. Efforts to gather and protect wild relatives of diverse food legume crops in national and international gene banks have been underway. Many species have previously shown cross-compatibility with cultivated varieties in a number of studies (Kumar et al. 2016). Because of this compatibility, foreign genes from wild species have been effectively introgressed which are regulated by significant genes. Different breeding techniques may be used to exploit this genetic diversity to boost the micronutrients content of food legumes. A good genetic biofortification technique requires both high micronutrients concentration and high yield under different environmental circumstances. Plant breeding techniques are used to screen the large genetic variation available for selective traits. However, advanced genetic approaches such as quantitative trait loci (QTL) mapping and marker-assisted selection are used to identify the genes controlling micronutrients concentration in a particular crop. The exploitation of genetic variation can be a sustainable solution for malnutrition by linking agriculture to nutrition and health. Micronutrients comprise very small portion of the total weight; therefore, successful breeding programme with precise estimation is prerequisite (Brigide et al. 2014; Shahzad et al.

2021). So malnutrition could be mitigated by a precise breeding method, including identifying and utilizing the factors affecting the nutritional traits (Roorkiwal et al. 2021). An alternative is the selection of plant varieties with superior ability to accumulate certain nutrients in the grain. Variations in seed coat colour also affect the nutrients contents in food legumes. Research reported that the white-coloured seed coat of the Andean bean possessed lower contents of phenolic compounds and antioxidant activities than genotypes with mottled or red seed coat genotypes (Rehman et al. 2019). In food legumes several fortified varieties have been released throughout the world through conventional breeding (Rehman et al. 2019).

3 Breeding for Micronutrients Improvement

Breeding of high nutrients cultivars, rich in Zn, Fe, Ca, K and the substances make the bioavailability of these nutrients is an effective way to minimize the nutrient deficiency (Marques et al. 2021; Shahzad et al. 2021). Different strategies are being used to develop the biofortified legume crops. The biofortification strategy strives to enrich legumes with minerals such as Fe, Zn, Sr, I and others so that individuals who eat such grains get more of them (Madurapperumage et al. 2021; Mahto et al. 2022). Even though legumes are rich in mineral contents, however their bioavailability is very low, consequently dwindling the use of legumes as a mineral source (Praharaj et al. 2021). Because micronutrient deficiency or hidden hunger is more common in low-income nations, where customers have limited purchasing power, they can't afford vitamin supplements or a micronutrient-rich diet (Rehman et al. 2019). Improved nutritional status of regularly consumed food grains is the best sustainable method for treating micronutrient deficiency in such circumstances (Rehman et al. 2019; Kumar and Pandey 2020). A successful biofortification method should guarantee that grain yield is enhanced or maintained, as well as the grain micronutrients contents are increased for considerable beneficial health effects, and grain performance is consistent across settings (Praharaj et al. 2021). By creating genotypes with high levels of Zn in edible plant portions, plant breeding and/or transgenic techniques give a promising and long-term strategy to alleviate micronutrient deficiencies (Xia et al. 2020). Though establishing a genotype is expensive and complex, it provides a long-term advantage as there are no recurrent costs. Because considerable genetic variety exists in the germplasms of key cereal crops, breeding for high micronutrients concentration is conceivable (Jha and Warkentin 2020). Identifying appropriate genetic variation and selecting parents, long-term crossing and backcrossing, stabilization of target characteristics across multiple climates conditions and acclimatization of biofortified genotypes to regional agricultural management practices are the minimum stages required in breeding (Maqbool et al. 2020). Improved nutritional quantity was reported in mung bean when this crop was crossed with mash bean through interspecific hybridization (Abbas et al. 2019).

4 Breeding for Vitamin-Enriched Legumes

Vitamins are organic compounds vital for energy production, but inadequacy of vitamins is directly linked with various human health losses (Madurapperumage et al. 2021). Such losses can be effectively overcome by delivering vitamin-rich food to an impoverished population. Biofortification through conventional breeding is the most effective approach which enables various food plants to be enriched with such vitamins (O'Neill et al. 2020). Using new breeding techniques, including modification of metabolic pathways of vitamins, can enhance the biofortification progress in a variety of plant species like legumes. The diversity of wild relatives and cross compatible species-rich in essential vitamins can be used by selective breeding to increase such nutrients in legume seeds (Garg et al. 2018). The planned identifications of specific factors associated with nutritional traits and their proper utilization in a selective breeding program could help in mitigating malnutrition. The important vitamins found in legumes belong to the vitamins B-group like folate, thiamin, niacin and riboflavin. Folic acid is also present in abundant amount; however, the availability of vitamin C and ascorbic acid is very lower in legumes (Rehman et al. 2019). It is suggested that if legumes are used in combination with foods high in vitamin C, iron absorption can be increased. The biochemical composition of the legumes differs among various forms of seeds exhibiting high variability in case of their vitamin content. The contents of lipo-soluble vitamins are also very low in legumes, except α-tocopherol (vitamin E) in soybean and peanut. The γ-tocopherol form in a few legumes is most abundant, with the highest level being described in peas, pigeon peas and lentils (Amarovicz 2009). Various strategies comprising metabolic engineering, classical breeding and mutation breeding have been initiated to enhance the vitamin contents of legumes (Mène-Saffrané and Pellaud 2017). Furthermore, enrichment in α-tocopherol contents can be obtained by traditional breeding utilizing naturally high α-tocopherol level alleles detected in QTL studies of legume germplasms and then by introgression into commercial varieties to increase vitamin E (Mène-Saffrané and Pellaud 2017). Some legumes seeds are a good source of carotenoids, most commonly β-carotene, cryptoxanthin, lutein and zeaxanthin; for example, the concentration of β-carotene in the case of chickpea was more in comparison with some crops genetically modified for β-carotene. The traits for carotenoid contents have high heritability that is not much affected by the environment (Abbo et al. 2005). Therefore, identifying such barriers more likely connected with the carotenoids biosynthesis pathway will improve the modifying strategies for producing legumes enriched with carotenoids (Schmidt et al. 2015). This trait is highly heritable, and variations found in legume germplasm for this trait can be used in breeding programs. In chickpea cross of wild relatives with Israeli cultivar showed more β-carotene and lupine contents as compared to their parent lines (Abbo et al. 2005). Seed coat colour is important trait that influence the of β-carotene and lupin contents in soybean so this trait may be keep in mind while breeding the legumes for higher carotene contents (Gebregziabher et al. 2022).

5 Breeding for Anti-nutrients

Anti-nutrients generate indigestible complexes with nutrients and proteins and impair the bioavailability of micronutrients to human body. Abundant intake of anti-nutrients through diet may become toxic to the body (Samtiya et al. 2020; Martineau-Côté et al. 2022). Anti-nutrients can have a large negative impact on food nutrition; hence, lowering these contents in meal is an important objective to boost human nutrition. Most legumes contains anti-nutritional factors, such as tannins, phytic acid, digestive enzyme inhibitors, oxalate and lectins that can decrease the bioavailability and uptake of proteins and minerals during digestion and induce toxic effects (Martineau-Côté et al. 2022). Anti-nutrient compounds restrict the bioavailability of essential micronutrients, ultimately resulting in malnutrition and various diseases like anaemia, beriberi, night blindness, rickets and scurvy more prevalent among the population. Plant molecular biology and genetic modification techniques currently allow for the reduction or elimination of anti-nutrients in staple plant foods and a large boost of promoter substance levels in these foods. When trying to develop food crops as sources of micronutrients for humans, plant breeders and molecular scientists should attentively examine the approach of boosting promoter chemicals in food crops. Biofortification is a balanced method to combat mineral shortages. It entails improving the nutritional content of food crops using either classic plant breeding or current biotechnology (Jha and Warkentin 2020). In the last decade, biofortification via plant breeding has increased the nutrient quality of pulse crops and has gained traction. Several studies on pulse crops have found genetic diversity for critical micronutrients in accessible gene pools, with successful breeding lines employed in breeding and related genotypic markers for marker-assisted breeding selection (A.M. Pérez-de-Castro et al. 2012). For accurate marker–trait association, gene discovery, functional marker creation and their deployment in routine breeding programmes, next-generation sequencing (NGS) and genotyping technologies must be applied (Scheben et al. 2018). Anti-nutritional agents such as aponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, raffinose and goitrogens are present in edible crops (Samtiya et al. 2020). Anti-nutritional substances mix with nutrients and create lower nutrient bioavailability, a big problem. Other variables, such as trypsin inhibitors and phytates, found mostly in legumes and grains, limit the digestibility of nutrients and mineral assimilation. Wild lima beans (*Phaseolus lunatus*) contains a toxic compound cyanogenic glycoside its high quantity consumption may cause respiratory distress in human body (Shlichta et al. 2014). Another legume grass pea (*Lathyrus sativus*) possess a non-protein amino acid β-N-oxalyl-l-α,β-diaminopropionic acid which causes lower limb paralysis (Yigzaw et al. 2001). Consumption of alkaloid glycoside present in faba bean (*Vicia faba*) causes hemolysis (Crépon et al. 2010). An anti-nutrient compound trypsin present in mung bean (*Vigna radiata*) is the cause of indigestibility and reduce the bioavailability of minerals (Ullah et al. 2014). A comparative study on lentil crop showed that phytic acid concentration is influenced by temperature. Higher the temperature lower will be the phytic acid concentration whereas in cool temperature phytic concentration reduced in same genotypes (Thavarajah et al. 2010).

6 Identification of QTL/Genes for Nutrients/Vitamins Accumulation

Traditionally, screening for beneficial alleles influencing improved concentration for specific nutritional properties was applied in legumes to determine existing natural variation. This assisted in identifying genetic variability that may be used as a donor for transferring helpful genes into the background of cultivated genotypes, as well as for usage as a biofortified variety if the detected variant is already a high producing cultivar. Legume cultivars were tested over many years and in several places and resulted in the generation of maps that illustrated variability for certain micronutrients using geostatistics (AbdelRahman et al. 2016). Advances in tissue culture techniques, on the other hand, may now be ready to aid in embryo rescue, which encouraged alien gene expression from the secondary gene pool. Furthermore, evolutionary pressures cause modifications in wild species germplasm in the direction of probable cross-compatibility with farmed species. As a result, formerly incompatible wild and domesticated species can now cross (Singh et al. 2021). It's time to revisit the crossability links between modern legume cultivars and wild species. As a result, pre-breeding activities are critical, especially among wild species that carry important foreign genes for biofortification features. Moreover, wild relatives are major sources of novel variety generated by recombination breeding techniques (Lyzenga et al. 2021). Furthermore, the utilization of multi-parent populations has aided in the discovery of quantitative trait loci (QTL). As a result, a number of QTL with high mapping resolution have been found for breeding programs. The markers for this QTL are a valuable tool for enhancing selection efficiency in breeding projects employing marker-assisted selection (Phan and Sim 2017). For QTL detection in wild populations or germplasm collections, association mapping is a powerful technique that utilizes past recombination events. In comparison to linkage analysis, this mapping technique offers various advantages, including improved mapping accuracy, reduced time and a larger number of alleles to mine (Hu et al. 2016). For nutritional qualities, there is genetic heterogeneity among cultivated germplasm that may be used in breeding operations. Several nutritional properties in lentil were strongly influenced by environmental factors and the genetic composition of a given genotype (Kahraman et al. 2004). As a result, efforts should be focused on location-specific nutritional characteristic breeding. Several crops have identified wild relatives as possible providers of nutritional characteristics. However, wild relatives have been discovered as important genetic resources for other agronomical qualities, but such variants have not yet been investigated in lentils (Kumar et al. 2016). Hence, wild relatives will be prioritized in the future for providing appropriate pre-breeding materials for biofortification-related features. Genomic analysis is becoming an important aspect of breeding efforts. In the case of legumes, significant progress has already been done in terms of developing genetic resources. However, in the genetic biofortification of legume crops, these genomic resources have not been completely used. Through clustering, molecular markers may be utilized to identify and map genomic areas that affect the expression of nutritional characteristics. These advancements may make it easier to dissect the complicated genetics that regulate dietary characteristics. The markers linked with beneficial genes/QTL impacting biofortification features can be employed in marker-assisted breeding to generate biofortified legume cultivars promptly and cost-effectively in the near future (Kahraman et al. 2004). QTL mapping to explain the genetic determinants for seed mineral deposition in soybean seeds, explicitly for phosphorus (P), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) minerals. The study made extensive use of recombinant inbred lines and cultivated soybean accessions (Wang et al. 2022). Considering several genetic loci are engaged in the metabolic pathways of mineral deposition in seeds, the molecular mechanisms regulating mineral element deposition are genetically sophisticated. These discoveries will lay the groundwork for mineral biofortification, mostly through MAS breeding (Wang et al. 2022).

7 Marker-Assisted Selection for Dietary Improvement of Legumes

Marker-assisted selection (MAS) enables rapid identification of elite breeding material based on DNA, RNA and/or protein markers, making this method genotype-based selection (Wang et al. 2022). These markers can be found throughout the gene of interest or linked to a gene that determines a trait of interest (Boopathi 2013). Further people more rely on conventionally biofortified crops as compared to genetically modified crops (Marques et al. 2021). MAS assists breeders in increasing selection efficiency, precision and intensity, as well as the selection of satisfactory gene combinations in early generations, leading to increased genetic gain. Nevertheless, before employing a marker for the selection of promising crops in a segregating generation, the magnitude of the target QTL's effects and precise chromosomal position are critical for exploring the benefits of MAS. Markers linked to a trait must be validated across multiple genetic backgrounds. To increase the accessibility of genomic resources and data in important species, the use of extremely polymorphic and reproducible markers like SNP and SSR in marker–trait association assessment is a key problem for MAS utilization in breeding programs. Furthermore, the cooperation between biotechnologists and plant breeders should be improved to allow for a more systematic and efficient use of MAS. Countering these obstacles may enable the adoption of challenging genomic data sets and the production of enhanced cultivars involving MAS by breeders. In cereal crops, marker-assisted pyramiding has been widely used to combine multiple genes and QTLs (Ahmad et al. 2015). This combination is seen in legume crops, such as soybean, whereas successful gene and QTL pyramiding may be dependent on a close marker–trait association. A well-established and close relationship between markers and target traits has already been described in pulses. As a result, breeders are working actively to employ them for marker-assisted pyramiding in pulse crops and legumes (Ahmad et al. 2018).

MAS is often used in cultivar development and genetic enhancement. For instance, in faba bean (*Vicia faba L.*), the primer focus was on the development of molecular markers for selecting resistance to diseases. MAS is currently being utilized to improve seed quality in faba beans by targeting zero tannins. Thus, these polyphenolic substances had an inhibition effect on the activity of digestive enzymes in humans. To develop the tannin-free cultivars, breeders are looking for the presence of two recessive genes, *zt-1* and *zt-2*, responsible for tannin absence. A reliable SCAR marker such as SCC5551, releasing a high accuracy prediction of the *zt-1* gene, was approved in a sizable population of faba beans, demonstrating its potential as a cost-effective tool for MAS. Similarly, a practical SCAR marker associated to the *zt-2* gene was identified, which led to the elaboration of tannin-free faba bean cultivars. The abovementioned discoveries will enable gene pyramiding and intensify the development of new faba bean cultivars with optimized nutritional value for immediate consumption (Sallam et al. 2016). Furthermore, recent advancements in whole-genome sequencing and comparative genomic approaches, particularly for lentil (*Lens esculenta*), have greatly assisted in the mapping of genes and QTLs for important agronomic traits in the lentil, as well as the elaboration of functional markers for MAS. Using QTL analysis, genes controlling Fe uptake in lentil populations have been identified. Several molecular markers, comprising SSRs, AFLPs and SNPs, have been used in population genotyping to develop biofortified lentil cultivars. Polymorphic SSR markers were employed in the same fashion to characterize a dozen lentil genotypes with stable Fe and Zn concentrations in their grain (Kumar et al. 2016). Accuracy rate of MAS and genomic selection (GS) for the seed weight (SW) trait using a genome-wide association study in a large population of soybean (Glycine max) based on a high number of single nucleotide polymorphisms (SNPs). In the mixed model that included these loci, they discovered two minor-effect loci associated with SW that explained 83.4% of the phenotypic variation. They also reported high prediction precisions for GS and MAS using cross-validation, with 0.75–0.87 and 0.62–0.75, respectively. Their findings could contribute to the discovery of genes controlling the SW trait in soybean. Likewise, moreover, Yang et al. (2015) used next-generation sequencing (NGS) to develop a significant number of reliable SSR markers for MAS in pea breeding, retaining approximately 841 stable amplifications of perceptible polymorphisms within 24 genotypes of cultivated pea (*Pisum sativum* L.) and wild relatives (*P. fulvum* Sm.). Alongside that, 33 polymorphic SSR markers were highlighted as being compelling in F2 generation. This discovery may be useful for future research into pea quality enhancement and the development of biofortified pea cultivars. Several reports have highlighted advancements in chickpea (*Cicer arietinum*) research, and a few SNP markers controlling iron and zinc concentrations have also been discovered (Kumar et al. 2016). The common bean is one of the legume crops being used in the large strategy to combat zinc deficiency in developing countries. This strategy is built on a combination of conventional breeding and MAS. Thus, zinc content in the seeds of legume crops is regulated by several genes engaged in mineral uptake, transport and deposition from soils to seeds. Numerous DNA markers and quantitative trait loci (QTLs) linked with seed zinc accumulation have already been identified and validated in common beans. There are approximately 28 different QTLs associated with grain zinc content and their associated SSR/SNP markers that have already been observed in common bean. Studies focusing on the zinc content of common bean seeds are now incorporating other molecular techniques, such as the Genome Wide Association Study (GWAS). As a result, this technique employs diverse crop germplasm to scan the entire genome to provide a clear perception of the candidate genes responsible for the expression of the trait of interest (Abbas et al. 2019). Other QTL-based studies in common bean revealed moderate phenotypic variation for Fe and Zn concentrations in seeds (Kumar et al. 2016).

8 Mutation Breeding for Nutrient-Enhanced Legumes

Different approaches have been designed and implemented from time to time to achieve the goals of biofortification. Among various approaches mutation breeding has played a vital role in developing biofortified crops. More than 1150 mutant varieties with improved nutritional quality have been developed and officially released (MVD-2022). Mutation breeding has proven a suitable technique for improving various attributes such as yielding potential, adaptability, stress tolerance and nutrient quality of economically important crops such as cowpea (Rasik et al. 2022; Raina et al. 2022a, b), faba bean (Khursheed et al. 2018a, 2019), urdbean (Goyal et al. 2021a, b), mung bean (Wani et al. 2017), chickpea (Laskar et al. 2015; Raina et al. 2017), lentil (Laskar et al. 2018; Raina et al. 2022a) and black cumin (Tantray et al. 2017; Amin et al. 2019). However, one of the most important attributes is the biofortification of crops. The success of mutation breeding aimed at biofortification is determined by several factors such as selection of appropriate mutagen, mutagen dose and duration of the treatment. The dose employed must be optimum that could induce maximum frequency of mutation with least biological damage (Goyal et al. 2019, 2020a, b). In addition to mutagen attributes, selection of plant material is also important. Depending on the degree of utilization, the crop can be selected, and it is always preferred to improve the grain quality and nutritional status of staple crops (Raina et al. 2016, 2018). Developing crops with increased nutrient density may prove a sustainable approach to mitigate the devastating impact of malnutrition. Several workers have employed mutation breeding technique to improve the nutritional status of several crops. For instance, Raina et al. (2020) treated two varieties of cowpea with different doses of gamma rays and sodium azide employed individually and in combination. They were successful in developing 11 mutant lines with increased micronutrient concentration. Similarly, Laskar et al. (2018) developed lentil mutant lines that showed increased micronutrient density. They treated traditional lentil cultivars with different single and combined doses of gamma rays and hydrazine hydrates. Khursheed et al. (2018b) while working with mutagenized faba bean reported few biofortified lines and emphasized the role of mutation breeding in developing biofortified crops. Wang et al., 2022 attempted to incorporate zinc biofortification in rice through mutation

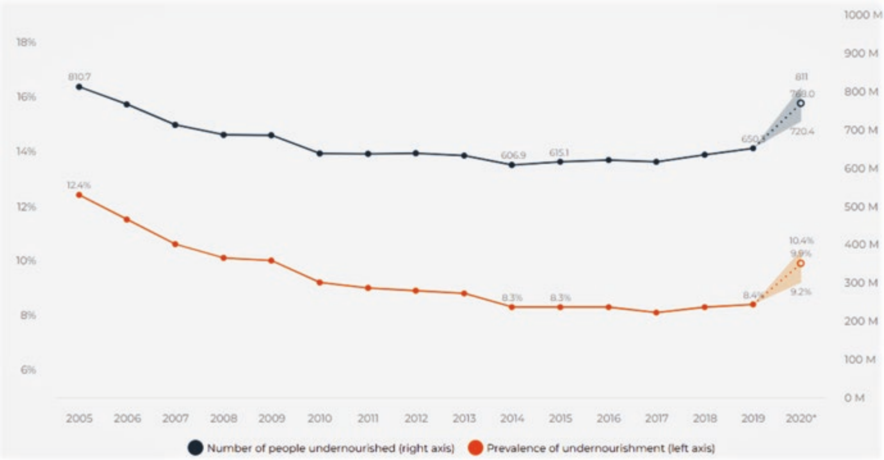

Fig. 5.2 The number of undernourished people in the world continued to rise in 2020. Between 720 and 811 million people in the world faced hunger in 2020. NOTES: * Projected values for 2020 in the figure are illustrated by dotted lines. Shaded areas show lower and upper bounds of the estimated range that considers statistical uncertainty. (Source: FAO)

breeding by treating nine popular rice varieties with ethyl methanesulphonate. They were successful in isolating few mutant lines with increased zinc content. On the basis of the literature, it is evident that mutation breeding has played a vital role in accomplishing the goals of biofortification of crops; however, the technique has been employed in limited crops. Therefore, more crops should be mutagenized to observe the mutagen-induced alterations in the contents of micro and macronutrients. This way the devastating effects of malnutrition can be mitigated to a greater extent (Fig. 5.2).

9 Challenges and Limitations of Legumes Biofortification Through Conventional Breeding

Although there are several benefits of breeding for legumes biofortification through conventional breeding methods, numerous limitations exist, the most prominent of which is the dependence on availability of the diverse gene pool of the crop under focus (Jha and Warkentin 2020; Marques et al. 2021). Using conventional breeding methods, no crop can be biofortified in the absence of enough genetic diversity and only transgenic approaches remains helpful (Ahmad et al. 2012). Low levels of heritability and linkage drag also make conventional methods almost ineffective. It has also been observed that some of modern-day crops show lower nutritive quality in comparison with their wild relatives (Didinger and Thompson 2021). But, there are limitations of using wild relatives of several crops due to their immense underrepresentation in the global gene bank (Pandey et al. 2008), reproductive hindrances in the development before and after the formation of zygotes (Munguía-Rosas and Jácome-Flores 2020) and the probability of desired traits existing linked to the undesired traits (Joshi-Saha et al. 2022). The limited efforts for in situ conservation of wild relatives have resulted in very poor collection of such quality germplasm. The other major limitation of the conventional breeding is that it takes quite a long to even several years to breed and release a new desired variety since rigorous selection up to sixth generation is required to incorporate a quality trait into an agronomically desired cultivar (Sobia et al. 2014). Moreover, genotype vs. environment interactions make the job more complex to achieve the objective (Marques et al. 2021). To tackle such problems there are some potential ways such as molecular marker-assisted selections (MAS), seed-chipping-technology and genomic selection strategies can speed up the release of a desired quality legume cultivar. Currently, genomes of several legume crops have been sequenced with variable qualities, from reference genome to draft and the unassembled (Ha and Lee 2020). It will be very helpful in developing the molecular markers which could be utilized in high-throughput arrangements to identify the linkages/marker–trait associations of such markers for high-yielding legumes. Moreover, the whole-genome sequence data offers its use in genome-wide association studies (GWAS) to identify and incorporate the micronutrient genes in legumes in addition to the use of these markers in marker-assisted recurrent selection (MARS) and the marker-assisted backcross breeding (MABC). At present, molecular markers which are associated or linked with QTLs for zinc (Zn) and/or iron (Fe) have been identified in very few legume species (Joshi-Saha et al. 2022).

However, to achieve the success at large, a potential collaboration among plant breeders, genetic engineers and nutrition experts is crucial, and the biofortification of legumes has huge potential to overcome the hidden hunger among the poor and the developing states (Garg et al. 2018). In either case, to breed for a legume rich in nutritional quality, it is also central to understand the biochemical, physiological as well as molecular mechanisms of entire processes which are essential for their biofortification. Additionally, in order to break the negative linkage between yield or yield enhancing traits and the nutrient contents, there is an endless need to supplement the conventional breeding with the targeted and random mutagenesis. It has been reported that polyphenols and phytates are among such anti-nutrient substances which diminish the biological availability of the mineral nutrients. For example, polyphenols normally bind themselves with non-heme iron and minimize its absorption. Hence, to enhance the biological availability of the mineral nutrients, the lower phytic acid contents are considered very important. It has also been reported that the phytic acid found in numerous legumes is involved in controlling and regulation of different abiotic and biotic stress resistance system.

The ever-increasing demand of nutritionally rich legume crops is a challenge to be met. This challenge can be solved by incorporating the legume breeding projects into the national level breeding networks and programs. Moreover, policy reforms regarding markets and investments opportunities in legume are bitterly required which should be incentive and subsidies based on the farmers growing the biofortified legumes (Joshi-Saha et al. 2022).

10 Conclusion

Conventional breeding techniques have been intensively used to develop the legume varieties with improved nutritional contents. Some common bean varieties such as MAC42, MAC44, CAB2 and PVA1438 having high zinc and iron contents were developed through conventional breeding approaches in Bangladesh. Similarly, plant breeders released high zinc contents lentil varieties such as Alemaya, Shital, Sisir and Simal. Through molecular breeding approaches, several QTL identified for both nutrient and vitamins contents. These QTLs contain several genes related to nutrients and vitamins and can be used as candidates for future studies aimed at developing biofortified legume crops.

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