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Strategic Nutritional Enhancement in Rabi Pulses: A Pragmatic Breeding Approach

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ABSTRACT

Rabi pulses like chickpeas (Cicer arietinum L.), lentils (Lens culinaris Medik.), pigeonpeas (Cajanus cajan L.), and cowpeas (Vigna unguiculata L.) serve as fundamental sources of protein, iron, zinc, and dietary fiber. Nonetheless, antinutritional factors (ANFs) including phytic acid, tannins, protease inhibitors, and raffinose family oligosaccharides impede nutrient absorption. This study encapsulates the molecular mechanisms underlying protein biosynthesis, encompassing seed storage protein accumulation, nitrogen assimilation via GS/GOGAT, and essential transcriptional regulators involved in seed development. Emphasis is placed on protein bioavailability rather than mere accumulation, with a focus on strategies to mitigate ANF synthesis through genomics-assisted breeding. Advanced techniques such as CRISPR/Cas9 gene editing, quantitative trait loci (QTL) mapping, and genome-wide association studies (GWAS) have been employed to identify promising targets for reducing ANFs and bolstering nutritional content. The integration of contemporary breeding approaches with processing techniques presents a viable pathway to cultivate nutritionally enhanced and climate-resilient pulse varieties, thereby supporting global food security and nutritional health.

INTRODUCTION

Rabi pulses, primarily including chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pigeonpea, and cowpea, are important winter-season legume crops contributing significantly to food and nutritional security, particularly in India, where they account for over 70% of pulse production (11.2 million tonnes in 2023). Although these crops are important sources of protein (19–32%) and important micronutrients like iron (Fe) and zinc (Zn), anti-nutritional elements like phytic acid, tannins, and polyphenolic compounds that lower mineral bioavailability frequently limit their nutritional benefits (FAO, 2023; Gupta et al., 2022; Upadhyaya, Bajaj, Das, et al., 2016). In developing economies, where micronutrient malnutrition affects over two billion people worldwide, pulses play a significant role in dietary protein intake. Therefore, crop biofortification is an efficient, long-term approach to addressing hidden hunger. (Andersson, 2017; A. B. Jha & Warkentin, 2020a; Y. Kumar et al., 2022)

Beyond mineral limitations, additional antinutritional compounds, including trypsin inhibitors, lectins, and raffinose-family oligosaccharides, negatively affect protein digestibility and consumer acceptance of pulse-based diets. (Abera et al., 2023; Acquah et al., 2021). The identification of genes and QTLs governing seed mineral accumulation and anti-nutritional factor biosynthesis has been expedited by recent developments in pulse genomics, including reference genome assemblies, dense SNP arrays, and molecular marker development. This has led to genomics-assisted nutritional improvement strategies. (Lonardi et al.,

2019; Upadhyaya, Bajaj, Narnoliya, et al., 2016; R. K. Varshney et al., 2013). Simultaneously, modern genome-editing technologies such as CRISPR-Cas9 offer new approaches to alter nutritional-quality pathways in pulse crops precisely. (Chandana et al., 2022; U. C. Jha et al., 2024; C. Singh et al., 2023a).

Although previous studies have examined individual aspects of pulse nutrition and crop-specific biofortification, this review provides a cross-species synthesis of nutritional enhancement strategies for four major Rabi pulses, integrating conventional breeding, genomics-assisted breeding, GWAS, QTL mapping, haplotype-based breeding, and gene-editing approaches. Particular emphasis is placed on major nutritional QTL hotspots identified in chickpea and lentil, as well as emerging breeding strategies and current technological bottlenecks, thereby providing a comprehensive framework for future biofortification research and breeding programmes. (A. B. Jha & Warkentin, 2020a; U. C. Jha et al., 2024; J. Kumar et al., 2024; Roorkiwal et al., 2021; Sab et al., 2020a)

NUTRITIONAL AND MINERAL COMPOSITION IN PULSES

Rabi pulses have high protein content, complex carbohydrates, dietary fibre, and micronutrient density, with variation across species, varieties, and growing conditions. (U. C. Jha et al., 2024; Roorkiwal et al., 2021). The nutritional and anti-nutritional composition of rabi pulse crops is listed in Table 1

Table 1. Nutritional and anti-nutritional composition of four rabi pulse crops.

Crop	Protein (%DW)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Ca (mg kg^{-1})	Phytate (g kg^{-1})	Tannin (g kg^{-1})	TI (TIU mg^{-1})	RFOs (gkg^{-1})	Reference
Chickpea (*C. arietinum*)	20–24	40–87	11–55	800–1,400	3.5–9.0	<1–80	3–8	30–60	(Jukanti et al., 2012; Roorkiwal et al., 2022a; Samineni et al., 2022)
Lentil (*L. culinaris*)	19–32	17–184	9–55	560–950	3.7–14.0	2–15	3–8	20–45	(Aboutayeb et al., 2023; J. Kumar et al., 2024; Thavarajah et al., 2009a)
Red Gram (*C. cajan*)	19–28	40–75	25–50	1,000–1,500	6.0–11.0	<1–20	2–10	15–35	(Mukherjee et al., 2023; Purwanti et al., 2026)
Cowpea (*V. unguiculata*)	18–32	50–90	26–55	850–1,200	4.0–10.0	<1–25	5–15	30–60	(Coelho et al., 2023; Dhanasekar et al., 2021; Gonçalves et al., 2016)

TI = Trypsin Inhibitor; TIU = Trypsin Inhibitor Units. Tannin values reflect the whole seed, including the seed coat. Phytate values represent myo-inositol hexakisphosphate salts quantified by HPLC or colorimetric methods. RFOs include raffinose, stachyose, and verbascose combined.

Chickpea

Cicer arietinum (chickpea) is a major winter pulse crop cultivated globally, with annual production exceeding 15 million metric tonnes. (FAOSTAT, 2022) It is an important dietary protein source because of its high biological value and balanced amino acid composition. However, it is deficient in sulphur-containing amino acids such as methionine and cystine. Desi types generally possess higher protein content (22–24%) than kabuli types (20–22%) (Jukanti et al., 2012). Total seed protein ranges from 19–27% and is mainly composed of globulins, along with albumins, glutelins, and prolamins, with major storage proteins including vicilin, legumin, and convicilin. These proteins are rich in arginine, glutamic acid, and aspartic acid, giving chickpea a superior nutritional quality among pulses. (Ferreira et al., 2019; Yegrem, 2021). Carbohydrates account for 50–60%, primarily starch with high amylose content, while lipids range from 3.8–10%, mainly unsaturated fatty acids. (Begum et al., 2023; Grasso et al., 2022). Chickpea also provides B-complex vitamins, vitamin E, and minerals including potassium, phosphorus, calcium, iron, and zinc. Bioactive compounds include isoflavones, saponins, phytosterols, phytic acid, and trypsin inhibitors, which affect digestibility and nutritional quality. (Martoccia et al., 2025; Mondor et al., 2009; Roorkiwal et al., 2022b).

Lentil

Lens culinaris (lentil) is an important pulse crop with a global production of about 7 million tonnes in 2023 (FAO, 2023). Seeds contain 23–32% protein, primarily globulins including legumin and vicilin, providing lysine-rich protein but limited sulphur amino acids. (Dhull et al., 2023). Carbohydrates constitute 55–65%, mainly starch and dietary fibre, while fat content remains low (~1–3%) with predominance of unsaturated fatty acids. (Joehnke et al., 2021). Lentils are rich in folate, thiamine, vitamin B6, vitamin E, and minerals such as iron, zinc, magnesium, potassium, and phosphorus. Important phytochemicals include polyphenols, saponins, phytosterols, phytic acid, and protease inhibitors that influence mineral bioavailability and protein digestibility. (Popova & Mihaylova, 2019; Salim et al., 2023; J. Singh & Basu, 2012).

Cowpea

Vigna unguiculata (cowpea) is widely cultivated in tropical regions, with annual production exceeding 8–9 million tonnes (FAO, 2023; Gondwe et al., 2019). Seeds contain 20–25.5% protein, mainly globulins such as vicilin and legumin, with high lysine but low methionine and cysteine content (Jayathilake et al., 2018). Carbohydrates contribute 55–63% of seed composition, whereas lipid content is low (1.5–2.6%) and rich in linoleic and oleic acid (Duraipandian et al., 2022). Cowpea supplies B-complex vitamins, vitamin E, and essential minerals including potassium, phosphorus, magnesium, calcium, iron, and zinc. Major bioactive compounds include phytosterols, tannins, saponins, phytate, and trypsin inhibitors, many of which are reduced by cooking (Jayathilake et al., 2018).

Pigeon Pea

Cajanus cajan (pigeon pea) is an important pulse crop, with global production of about 4.5–5 million tonnes, of which India contributes nearly 70–80% (FAO, 2023; Talari & Shakappa, 2018). Seeds contain 20–25% protein, mainly storage globulins including vicilin and legumin, and provide lysine-rich protein, though methionine and tryptophan remain limiting. (Talari & Shakappa, 2018). Carbohydrates account for 62–67%, while lipid content is low (0.4–1.5%) and mainly consists of polyunsaturated fatty acids. (Rizvi et al., 2022). Pigeon pea is also a source of B vitamins, vitamin C, carotenoids, and minerals such as potassium, phosphorus, magnesium, calcium, iron, and zinc. (Anaemene, 2020; Bamidele & Akanbi, 2015). Bioactive compounds include isoflavones, saponins, phytosterols, phytic acid, tannins, and protease inhibitors, with processing reducing antinutritional effects. (Godbole et al., 1994).

PROTEIN FORMATION AND ACCUMULATION PATHWAYS IN RABI PULSES

Nitrogen Assimilation

Nitrogen assimilation forms the foundation of protein biosynthesis in Rabi pulses. Nitrate, the major nitrogen source, is absorbed through low-affinity **NRT1** transporters under high nitrogen conditions and high-affinity **NRT2** transporters under nitrogen deficiency. (Xu et al., 2024). In pea (*Pisum sativum* L.), **PsNRT2.3** interacts with **PsNAR2** to enhance nitrate uptake under low nitrogen conditions. (Chen et al., 2024). Nitrate is reduced to nitrite by **nitrate reductase (NR)** and further to ammonium by **nitrite reductase (NiR)**. Ammonium is then assimilated via the **GS/GOGAT cycle**, producing glutamine and glutamate that serve as precursors for amino acid and seed protein synthesis. (Liu et al., 2022). Improving **the activities of GS, GOGAT, NR, NiR, and AMT transporters** is a major target for increasing seed protein content.

Amino Acid Biosynthesis

Assimilated nitrogen is converted into amino acids required for protein formation. **Methionine** is synthesised via the aspartate pathway, involving CGS, CBL, and methionine synthase (MS), in which substrate competition with threonine creates a metabolic bottleneck. (Hanafy et al., 2013). **Cysteine** biosynthesis depends on **serine acetyltransferase (SAT)**, whereas lysine synthesis depends on **DHDPS**. Branched-chain amino acids are synthesised through the **AHAS** and **BCAT** pathways. Rabi pulses are often deficient in sulfur-containing amino acids, making these biosynthetic genes important targets for breeding. (Cordoba et al., 2025).

Seed Storage Protein Biosynthesis

Final seed protein accumulation depends on storage protein biosynthesis. In pulses, **globulins** constitute nearly 65% of total protein, mainly as **7S vicilins, 11S legumins, and convicilins** (Cordoba et al., 2025). During seed maturation, storage protein genes are transcribed, translated on the rough ER, processed in the Golgi apparatus, and deposited into **protein storage vacuoles (PSVs)** (Müntz et al., 2001). In pea, vicilin and legumin are encoded by multigene families influencing protein stability and functionality (Brhane & Hammenhag, 2024). Chickpea studies show that vicilin and legumin isoforms undergo post-translational processing during seed development (Di Francesco et al., 2024).

Protein Folding and Packaging

After synthesis, storage proteins undergo folding and trafficking before accumulation. In the **endoplasmic reticulum (ER)**, molecular chaperones such as **BiP, PDI, and HSP70** assist correct folding and disulfide bond formation. Proteins are then transported through the Golgi to storage vacuoles. Seed maturation regulators **LEC1,**

LEC2, ABI3, and FUS3 coordinate protein accumulation, desiccation tolerance, and dormancy development (Verdier & Thompson, 2008). Improper folding reduces protein stability and seed protein accumulation.

Carbon–Nitrogen Balance

Protein accumulation depends on balanced carbon and nitrogen metabolism. Amino acids such as glutamate and aspartate generated through the **GS/GOGAT pathway** contribute significantly to seed protein composition. Increased nitrogen supply promotes aspartate accumulation by suppressing degradation pathways. (Liu et al., 2022). Carbon from photosynthesis is partitioned between starch synthesis via **AGPase** and **SUSY**, or protein biosynthesis. Breeding strategies reducing starch pathway activity while enhancing nitrogen assimilation may improve protein accumulation. (A. K. Singh et al., 2025).

Protein Degradation

Seed protein content can decline due to degradation during seed maturation. The **ubiquitin–26S proteasome system** regulates protein turnover, where **E1, E2, and E3 ligases** tag proteins for degradation (V. Varshney & Majee, 2022). During embryo development, proteasome activity decreases while autophagy increases, regulating proteome balance. (Yu & Hua, 2022). **Vacuolar cysteine proteases** also degrade storage proteins. Reducing degradation pathway activity may help retain higher net seed protein content.

Regulatory Transcription Factors Controlling Protein Network

Regulatory transcription factors control protein accumulation. The major seed maturation regulators **ABI3, FUS3, LEC1, and LEC2** interact with seed-specific promoter elements and regulate storage protein synthesis. (Kroj et al., 2003) In chickpea, transcription factor families, including bZIP, NAC, AP2/ERBP, and DOF, coordinate the expression of seed storage protein genes, while **ABI5** regulates late embryogenesis and seed longevity. (Verma & Bhatia, 2019). Manipulating these regulatory networks through breeding and gene editing offers strong potential for improving seed protein accumulation. (A. K. Singh et al., 2025)

ANTI-NUTRITIONAL FACTOR REGULATING PROTEIN BIOAVAILABILITY IN PULSES

Protein nutritional quality is reduced by anti-nutritional factors (ANFs). These are secondary metabolites or proteins that interfere with nutrient digestion, absorption, or utilisation. (Y. Kumar et al., 2022) Germination studies in chickpea, pea, and lentil reported reductions in **phytic acid, trypsin inhibitors, and tannins**, improving protein digestibility (Yılmaz Tuncel et al., 2025). Genes **MIPS, IPK1, and ITPK** regulate phytate synthesis. **Kunitz trypsin inhibitor (KTI)** and **Bowman–Birk inhibitor (BBI)** inhibit digestive enzymes, while tannin biosynthesis involves **CHS, DFR, and ANR** genes. Gene editing of these pathways can improve protein bioavailability (Kim et al., 2025). Some important ANF chemical structures are shown in Fig 1.

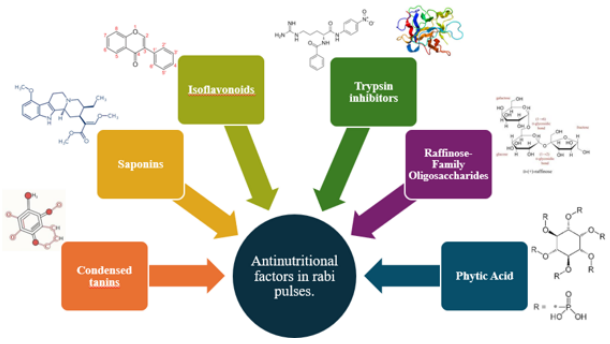

Figure 1. Diagrammatic representation of anti- nutritional factors in rabi pulses

Phytic Acid

Phytic acid (**myo-inositol hexakisphosphate; IP6**) is the principal phosphorus storage compound in seeds and a major anti-nutritional factor limiting nutrient bioavailability in Rabi pulses. Its biosynthesis occurs mainly through the **lipid-independent pathway**, where **glucose-6-phosphate** is converted to **myo-inositol-3-phosphate** by **myo-inositol-3-phosphate synthase (MIPS)**, followed by sequential phosphorylation by **ITPK** and **IPK2**, with **IPK1** catalysing the final rate-limiting step of IP6 formation. (Frittelli et al., 2023) The synthesised phytic acid is stored in protein bodies via MRP ABC transporters, where it accumulates alongside storage proteins.

Phytic acid strongly chelates essential minerals, including **Fe, Zn, Ca, and Mg**, forming insoluble complexes that resist gastrointestinal digestion and reduce both **mineral bioavailability and protein digestibility** (Abera et al., 2023; Sarkhel & Roy, 2022). Across Rabi pulses, its nutritional impact is substantial: in **chickpea** phytate levels of **3.5–9.0 g kg^{-1} DW** restrict zinc absorption; in **lentil**, phytate: iron molar ratios of **4–20** reduce iron uptake; in **pigeon pea**, phytate-bound phosphorus constitutes **70–80% of total seed phosphorus**; while in **cowpea**, phytate: zinc ratios of **10–30** significantly impair zinc bioavailability (Aboutayeb et al., 2023; Mukherjee et al., 2023; Raizada et al., 2023; Sab et al., 2020a). Processing methods such as **soaking, cooking, fermentation, and germination** can partially reduce phytate levels, with cooking reported to reduce phytate by **37–38%** and soaking by **12–16%** in legumes. (Abera et al., 2023; Y. Kumar et al., 2022)

Breeding low-phytic acid (LPA) genotypes offers a sustainable solution. Field pea **lpa mutant lines** showed **27–55% lower phytate content** with improved iron bioavailability. (Lindsay et al., 2021) Similarly, **IPK1 suppression** via RNAi reduced phytic acid while increasing iron and zinc accumulation, making MIPS and IPK1 important targets for CRISPR/Cas9, RNAi, and marker-assisted breeding to improve protein digestibility and micronutrient availability in Rabi pulses. (Aggarwal et al., 2018; A. K. Singh et al., 2025)

Condensed Tannins and Protease Inhibitors

Among the major antinutritional factors limiting protein utilisation in Rabi pulses, condensed tannins and protease inhibitors are the most biochemically significant. Condensed tannins (proanthocyanidins) are polymeric flavonoids synthesised through the phenylpropanoid/flavonoid pathway, beginning with phenylalanine ammonia-lyase (PAL) and proceeding through enzymes such as chalcone synthase (CHS), flavanone-3-hydroxylase (F3H), dihydroflavonol reductase (DFR), leucoanthocyanidin reductase (LAR), and anthocyanidin reductase (ANR), producing catechin and epicatechin monomers that polymerise and accumulate mainly in seed coats. (Jonker & Yu, 2017) In lentil, genes including CHS, F3H, F3'H, DFR, and ANR show stage-specific expression during seed development and are regulated by bHLH transcription factors that control tannin biosynthesis. (Koura et al., 2023). Nutritionally, condensed tannins reduce protein bioavailability by forming insoluble tannin–protein complexes with storage proteins such as globulins, legumins, and vicilins, decreasing enzymatic digestion. Germination studies in pulses showed significant reductions in tannins and improved protein digestibility, confirming their direct antinutritional role(Yılmaz Tuncel et al., 2025). Breeding approaches targeting CHS, DFR, and ANR through CRISPR/Cas9 or selection of naturally low-tannin genotypes offer promising strategies to improve protein quality. (Cordoba et al., 2025).

Protease inhibitors represent another major antinutritional barrier to protein utilisation in pulses. They mainly occur as Kunitz trypsin inhibitors (KTIs) and Bowman–Birk inhibitors (BBIs), both of which are widely distributed in chickpea, lentil, and pea seeds. KTIs are ~21 kDa proteins inhibiting trypsin, whereas BBIs are smaller (~7–8 kDa) proteins capable of inhibiting both trypsin and chymotrypsin. These inhibitors remain partially active even after cooking, limiting complete protein digestion. (Vorster et al., 2023). Mechanistically, they bind digestive proteases in stable 1:1 complexes, blocking protein hydrolysis and reducing nutrient absorption. Gene-editing studies showed that CRISPR/Cas9-mediated silencing of BBI genes reduced trypsin inhibitor activity by 62–75% and significantly improved protein digestibility without affecting seed composition. (Kim et al., 2025). Similarly, KTI1 and KTI3 knockout lines showed reduced inhibitor activity without affecting growth or seed quality, demonstrating the breeding potential of low-inhibitor genotypes. (Wang et al., 2023). In addition, RNAi and multiplex CRISPR approaches targeting both KTI and BBI gene families provide promising strategies for developing pulse varieties with enhanced protein digestibility. (Kafer et al., 2026)

Raffinose-Family Oligosaccharides

Raffinose family oligosaccharides (RFOs), including raffinose, stachyose, verbascose, and chickpea-specific ciceritol, are major soluble carbohydrates in pulse seeds but act as important anti-nutritional factors limiting pulse consumption. RFO biosynthesis occurs through a galactosyl transfer pathway initiated by *Galactinol Synthase (GolS)*, which produces galactinol as the key precursor, followed by sequential conversion to raffinose and stachyose by *Raffinose Synthase (RS)* and *Stachyose Synthase (STS)*, respectively. Genes such as *GolS*, *RS2*, *RS3*, and *STS* regulate seed RFO accumulation, with *RS2* identified as a major determinant of low-RFO phenotypes in legumes. (Elango, Rajendran, et al., 2022) In chickpea (*Cicer arietinum*), substantial variation in seed RFO concentration has been reported, indicating strong potential for breeding low-RFO genotypes while retaining desirable seed quality traits.(Elango, Wang, et al., 2022)

The antinutritional effect of RFOs arises because humans and monogastric animals lack α-galactosidase, which prevents the digestion of α-1,6-glycosidic bonds, allowing undigested oligosaccharides to undergo microbial fermentation in the large intestine, producing gases such as CO_2, H_2, and CH_4 that lead to bloating and flatulence. (Samtiya et al., 2020) Processing methods, including soaking, germination, and malting, substantially reduce RFO concentration. At the same time, recent CRISPR/Cas9 editing of *the GolS and RS2/RS3 genes in soybean has demonstrated significant reductions in raffinose and stachyose without affecting agronomic performance, highlighting molecular breeding as a promising strategy to improve the nutritional acceptability of pulses*. (Cao et al., 2022; Le et al., 2020)

GENETIC AND MOLECULAR APPROACHES TO BIOFORTIFICATION AND REDUCTION OF ANTI-NUTRITIONAL FACTORS

The discovery of genes, QTL, and molecular markers linked with mineral accumulation, phytate biosynthesis, tannin production, and amino acid profiles has been made easier by the availability of reference genome sequences for

chickpea (~738 Mb), pigeon pea (~833 Mb), lentil (~4.1 Gb), and cowpea (~620 Mb).(Lonardi et al., 2019; R. K. Varshney et al., 2012, 2013). A thorough framework for the uptake and storage of micronutrients in legumes to promote biofortification was provided by (Roorkiwal et al., 2021), who determined that the main targets for breeding with the identification of key transporter genes, transcription factors, and regulatory networks, such as ferritin, NAS (nicotianamine synthase), YSL (yellow stripe 1-like), VIT (vacuolar iron transporter), and ZIP (zinc-regulated/iron-regulated transporter-like protein) family genes. Large-scale screening of breeding populations for mineral traits is now possible due to genomic selection, speed breeding, and high-throughput, non-destructive phenotyping techniques like X-ray fluorescence spectrometry and hyperspectral photography. (U. C. Jha et al., 2024)

Breeding Approaches

Conventional Breeding Approaches

Conventional breeding approaches remain the foundation of pulse crop improvement because they are cost-effective, do not require sophisticated laboratory infrastructure, and produce varieties acceptable across regulatory environments worldwide. (Farida Traoré et al., 2022; R. K. Singh et al., 2022). In rabi pulses, conventional breeding has delivered numerous improved varieties with higher protein and mineral content and lower ANF profiles through systematic germplasm evaluation, hybridisation, pedigree selection, backcrossing, mutation breeding, and ideotype-based selection. (U. C. Jha et al., 2024; Saxena et al., 2023) A review on pulse biofortification confirmed that in India, biofortified lentil varieties L4704, Pusa Vaibhav (102 mg kg^{-1} Fe), and IPL 220 (113 mg kg^{-1} Fe), as well as cowpea varieties Pant Lobia-1 to Pant Lobia-4, were developed and released for cultivation through conventional pedigree-based selection programmes. (A. B. Jha & Warkentin, 2020b)

Hybridization

Hybridisation is the most fundamental tool in pulse crop improvement for nutrition, combining favourable alleles from diverse parents into elite genetic backgrounds. (Saxena et al., 2023; R. K. Singh et al., 2022). In chickpea, hybridisation programmes have successfully combined high Fe and Zn from desi germplasm donors, including ICCC 32, MNK-1, and ICC 4958, with agronomically adapted Kabuli or desi backgrounds, using significant heritable variation in seed micronutrients documented across the reference set.(Farida Traoré et al., 2022; Samineni et al., 2022). The continuous variation in protein content across crosses between high- and low-protein chickpea lines indicates that additive gene action predominantly governs protein accumulation, making pedigree selection in the F_3–F_6 generations the primary strategy.(U. C. Jha et al., 2024)

In pigeonpea, high Fe (>100 mg kg-1) and Zn (>60 mg kg-1) content traits from secondary and tertiary gene pools have been transferred into commercial lines through hybridisation with wild relatives, particularly *Cajanus scarabaeoides*, *C. platycarpus*, and *C. reticulatus*. (Saxena et al., 2023)successfully increased protein content from 20–22% to 28–30% by crossing wild donors with cultivated varieties, thereby demonstrating the feasibility of this approach. In lentil, the interspecific cross Eston × IG 72815 (*Lens ervoides*) created RIL populations segregating high Fe, with wild-species alleles at QTL qFe2.1 on chromosome LcLG2 absent in the cultivated pool. (A. Singh et al., 2017a) Cowpea hybridisation programmes at IITA crossed high-Fe accessions (IT97K-499-35, IT00K-901) with drought-tolerant and disease-resistant parents, thereby developing multi-trait lines through pedigree selection in F_5–F_8 generations that meet mineral targets. (Andersson, 2017; Dhanasekar et al., 2021).

In major legumes, the genetic control of seed protein mainly involves dominance, partial dominance, additive, and non-additive gene actions. (Saxena et al., 2023) Fe, Zn, and protein broad-sense heritability values in rabi pulses typically range from 0.55 to 0.80 across multiple environments, suggesting that selection within segregating populations derived from well-chosen hybridisation crosses can yield significant genetic gains. (Samineni et al., 2022; Thavarajah et al., 2009b). A study on genetic variability for grain protein, Fe, and Zn content in the chickpea reference set confirmed that protein content ranges from 17 to 22%, Fe from 48 to 57 mg kg^{-1}, and Zn from 35 to 43 mg kg^{-1} in available germplasm, with narrow ranges that highlight the importance of using diverse parents, including wild relatives, in hybridisation programs. (Roorkiwal et al., 2022a)

Mutation Breeding

Mutation breeding through induced mutagenesis is an effective tool for generating new variation in pulse crops with limited genetic diversity, especially for improving nutritional quality traits that are underrepresented in current germplasm collections. (Amri-Tiliouine et al., 2018; Jegadeesan & Punniyamoorthy, 2023). Ethyl methanesulphonate (EMS), gamma irradiation (Cobalt-60 or Caesium-137), sodium azide, and fast neutrons are the main mutagenic agents applied to pulse crops; EMS mainly causes GC-to-AT base pair transitions (90–99% of mutations), whereas gamma rays produce a wider range of effects, including deletions, inversions, and chromosomal rearrangements. (Amri-Tiliouine et al., 2018)

In chickpea, a study by Datta *et al*. (2018) (Amri-Tiliouine et al., 2018)demonstrated that treating the Kabuli genotype Ghab4 with 280 grey of gamma irradiation induced significant phenotypic diversity. TILLING (Targeting Induced Local Lesions IN Genomes) analysis of 768 M_2 progenies (obtained with 0.2% EMS) revealed a mutation frequency of one per 165 kb, which is 1.6 times higher than in Arabidopsis, confirming the population's suitability for nutritional mutant screening. (Amri-Tiliouine et al., 2018) Specifically, regarding nutritional quality, the high-protein mutant Hyprosala of chickpea (*Cicer arietinum* L.) derived through gamma irradiation showed a 3–4% higher protein content than its parent, representing one of the earliest documented successes in mutation breeding for protein quality in the species. (Jegadeesan & Punniyamoorthy, 2023) In lentil, mutation breeding using EMS, caffeine, and combined treatments in M_3 generations generated 18 mutant lines with significantly increased protein and mineral contents (Fe, Zn, Cu) compared to the control, demonstrating the utility of chemical mutagenesis for micronutrient trait modification in this species. (Laskar et al., 2018) In cowpea, induced mutagenesis studies confirmed that EMS and gamma rays caused significant variation in tannin, flavonoid, and phytate content across M_2 mutant genotypes, with broad-sense heritability values of 0.86–0.99 and narrow-sense heritability of 0.06–0.50 for tannin and phytate. This indicates that some mutant lines with reduced ANF content could be directly selected in advanced generations. (Elharadallou et al., 2015)

A targeted approach called Targeting Induced Local Lesions IN Genomes (TILLING), combining EMS mutagenesis with reverse-genetic mutation discovery, represents the most precise form of mutation breeding. It has been proposed as a non-transgenic complement to CRISPR for identifying natural or induced alleles at phytate pathway genes (CaIPK1, CaMIPS) and tannin biosynthesis regulators.(U. C. Jha et al., 2024)

Backcross Selection

Backcross-based introgression programmes, including conventional marker-free backcrossing (CB) and marker-assisted backcross breeding (MABC), transfer nutritional quality QTL from donors to elite backgrounds while minimising linkage drag(A. B. Jha & Warkentin, 2020a; R. K. Singh et al., 2022) Conventional backcross involves crossing F_1 progeny to the recurrent parent, then selfing, achieving >98.4% genome recovery after BC_3 with background selection. (R. K. Singh et al., 2022). In chickpeas, this method transfers high-Fe alleles from ICC 4958 into elite cultivars, using XRF-based seed Fe phenotyping and genome–wide SSR markers. (U. C. Jha et al., 2024; Samineni et al., 2022) In pigeonpea, wild *Cajanus platycarpus* is used as an Fe and Zn donor to produce BC_3F_3 progeny with mineral levels 15–25% higher than those of the checks, while maintaining good agronomic performance. (Mukherjee et al., 2023). In lentil, MABC with SNP markers at QTLs SdFe2.1 and SdFe6.1 is used at ICARDA to transfer high-Fe alleles from donors into Syrian and South Asian backgrounds, with BC_2F_3 lines evaluated in Bangladesh and India. (Salaria et al., 2022)

Ideotype Breeding

Ideotype breeding involves designing an ideal plant architecture to optimise resource use for specific quality and yield goals. (R. K. Singh et al., 2022) In Rabi pulses, a nutritional ideotype includes high seed protein (>25%), elevated iron (>90 mg kg^{-1} in lentils; >66 mg kg^{-1} in cowpeas), zinc (>55 mg kg^{-1} in lentils; >44 mg kg^{-1} in cowpeas), low phytate (<6 g kg^{-1}), reduced tannin (<2 g kg^{-1}), short cooking time (<25 min), and high dehulling recovery (>75%). These traits must combine with good agronomic performance and stability across environments. (Andersson, 2017; U. C. Jha et al., 2024) The chickpea ideotype, formalised at ICRISAT, uses selection indices that combine mineral content, protein, performance, and stress tolerance to segregate populations. (U. C. Jha et al., 2024) An induced brachytic mutant with shorter internodes could be a compact, high-density ideotype to increase mineral partitioning. In cowpea, ideotypes include white-seeded, early-maturing, high-Fe and Zn varieties like IT 97 K-499-35 and IT 00 K-901, with low tannin as a proxy for mineral and protein bioavailability.(Dhanasekar et al., 2021). For lentil, the ideotype features a red cotyledon (low tannin), high Fe and Zn, and low phytate, guiding the development of varieties such as IPL 220, CDC Vantage, and BARI Masur-8. (J. Kumar et al., 2024; Podder et al., 2021)

Genomics-Assisted Breeding Approaches

Genomics-assisted breeding (GAB) employs molecular markers, dense genetic maps, whole-genome sequences, and statistical models to improve the efficiency, precision, and speed of pulse crop enhancement for nutritional quality traits. The provision of high-quality reference genomes for all four crops discussed: chickpea, pigeonpea, lentil, and cowpea alongside genotyping-by-sequencing (GBS), SNP (single nucleotide polymorphism) arrays, and comprehensive bioinformatics pipelines for large-scale marker-trait association analysis has significantly accelerated and improved the accuracy of nutritional biofortification breeding. Fig 2. illustrates the GWAS process in pulses. The primary GAB methodologies used to assess the nutritional quality of rabi pulses include QTL mapping, GWAS, haplotype breeding, genomic selection, and genome editing, each with unique advantages and limitations in mapping resolution, population

requirements, and practical feasibility. genomic selection and genome editing, each offering distinct advantages and limitations in mapping resolution, population requirements, and practical implementation. (R. K. Singh et al., 2022)

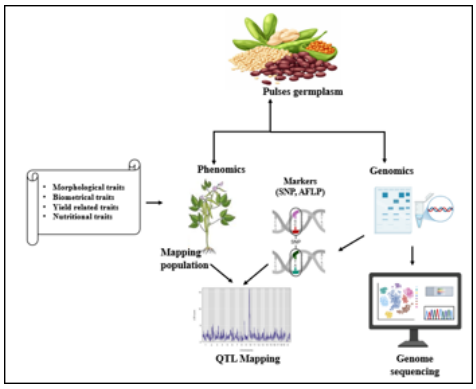

Figure 2. Schematic representation of genome-wide association studies (GWAS) in pulses.

Genome-Wide Association Study (GWAS)

Genome-wide association studies utilise historical recombination events in genetically diverse natural populations to map trait-associated loci with higher resolution than bi-parental QTL mapping. This approach enables the identification of SNP markers and candidate genes applicable across the germplasm pool. (Roorkiwal et al., 2022a; B. Singh et al., 2023). In chickpea, a GWAS of 258 accessions for 12 nutritional traits- protein, Zn, Ca, Mg, P, Mn, and B-vitamins- using a multi-locus mixed model (MLMM) and BLINK algorithm identified significant marker-trait associations (MTAs) on all eight chromosomes. A thiamine biosynthesis gene (AT1G22940 homologue) was co-localised with protein and Zn MTAs on chromosome CaLG01.(Roorkiwal et al., 2022a) A second GWAS of chickpea, employing the Axiom 50K CicerSNP array in 147 core-set accessions, identified 7,277 high-quality SNPs and significant MTAs for Zn, Cu, Fe, and Mn. Validated marker-trait associations for Zn were found on chromosomes LG1, LG2, LG3, LG4, and LG7, and for Fe on LG1, LG3, LG4, LG5, and LG7. (Fayaz et al., 2022) A multi-environment GWAS in 258 chickpea lines under heat and drought stress detected significant MTAs for Fe and Zn on chromosomes CaLG01, CaLG04, CaLG06, and CaLG07. This demonstrates that environmental stress significantly influences mineral accumulation and highlights the need for multi-environment QTL to achieve stable biofortification. (Samineni et al., 2022)

A GWAS on lentil, using GBS-derived SNPs across 95 diverse genotypes, identified 23 SNPs linked to Fe content across six chromosomes (LcLG1, LcLG2, LcLG4, LcLG5, LcLG6, LcLG7). Additionally, 17 SNPs were associated with Zn, with candidate genes including NAS and ferritin orthologues based on their physical proximity in the lentil genome assembly. (B. Singh et al., 2023) In a separate marker-trait association study involving 96 lentil accessions, including the biofortified variety IPL 220—73, SSR markers revealed PBALC 13 (on chromosome LcLG3; PVE 9–11%) for Fe and PBALC 206 (on chromosome LcLG5; PVE 14–21%) for Zn. A significant SNP was also linked to the galactosyltransferase gene Lcu. 2RBY.1g019390 (on chromosome LcLG2) for RFO content, marking the first published molecular marker for oligosaccharide reduction in lentil. (J. Kumar et al., 2024) In cowpea, GWAS using the 60K Cowpea iSelect Consortium Array on 374 diverse accessions identified significant associations for seed Fe on chromosomes Vu01, Vu04, and Vu08, and for Zn on Vu03 and Vu06. Candidate genes included VuNAS1, VuNAS3, VuFER1, VuZIP3, and VuZIP12.(Lonardi et al., 2019) In pigeonpea, GWAS on the ICRISAT mini-core diversity panel found MTAs for protein on chromosomes CcLG01, CcLG05, and CcLG08, and for Fe and Zn on CcLG02, CcLG04, CcLG07, and CcLG09, with NAS gene orthologues and ferritin-encoding genes as key functional candidates. (Mukherjee et al., 2023) These GWAS results are detailed in Table 2 of this review.

Table 2.

Summary of GWAS for nutritional traits in rabi pulses.

Trait	Association Panel Name2	SNPs Used	Population Size	Candidate Gene	Role of SNP / Candidate Gene	Chromosome No.	Reference
CHICKPEA (*Cicer arietinum* L.)							
Seed Fe, Zn	92 desi and kabuli accessions (GBS and gene-based SNPs)	16,591 SNPs (GBS + candidate gene)	92	YSL1 (yellow stripe-like 1 protein)	Fe phloem loading: strong association with Fe and Zn	CaLG03	(Upadhyaya, Bajaj, Das, et al., 2016) https://doi.org/10.3389/fpls.2016.00302
Seed Fe, Zn	92 desi and kabuli accessions	16,591 SNPs	92	LEA (late embryogenesis abundant protein)	Seed storage protein; associated with Fe and Zn accumulation	CaLG04	(Upadhyaya, Bajaj, Das, et al., 2016) https://doi.org/10.3389/fpls.2016.00302
Seed Fe, Zn	92 desi and kabuli accessions	16,591 SNPs	92	VPS (vacuolar protein sorting protein)	Seed vesicle trafficking; metal compartmentalisation	CaLG05	(Upadhyaya, Bajaj, Das, et al., 2016) https://doi.org/10.3389/fpls.2016.00302
Seed Zn, Cu, Fe, Mn	Chickpea core set (Axiom 50K CicerSNP array)	7,277 high-quality SNPs	147	NA	SNPs on all 8 chromosomes; validated MTAs for Zn (LG1,2,3,4,7) and Fe (LG1,3,4,5,7)	LG1–LG8	(Fayaz et al., 2022) https://doi.org/10.1038/s41598-022-14487-1

12 nutritional traits (protein, Zn, B-vitamins, phytic acid, etc.)	Chickpea reference set (MLM, MLMM, BLINK)	NA	258	AT1G22940 (TH1 homologue)	Thiamine biosynthesis; co-localised with protein and Zn MTA on CaLG01	CaLG01, CaLG04, CaLG06	(Roorkiwal et al., 2022a) https://doi.org/10.3389/fpls.2022.843911
Seed Ca, Mg, P, Mn, Zn	256 diverse chickpea accessions	NA	256	Ionic homeostasis genes (calcium ATPase, calreticulin)	Ca/Mg transport and compartmentalisation in the seed	CaLG01, CaLG06	(Srungarapu et al., 2022) https://doi.org/10.3390/cells11152457
Seed Fe, Zn (heat/drought)	Multi-env GWAS (258 lines × 2 env)	NA	258	NA	Heat and drought modulate mineral MTA stability on CaLG04, CaLG07	CaLG01,CaLG04,CaLG06,CaLG07	(Samineni et al., 2022) https://doi.org/10.1016/j.envexpbot.2021.104688
LENTIL (*Lens culinaris* Medik.)							
Seed Fe, Zn	96 diverse Indian and Mediterranean accessions (73 SSRs)	73 SSRs	96	PBALC 13 (SSR); GLLC 563	Fe transport (YSL family); Zn chelation (ZIP family inferred)	LcLG3, LcLG5	(A. Singh et al., 2017b)https://doi.org/10.1371/journal.pone.0188296
Seed Fe, Zn (GWAS)	95 diverse genotypes (GBS)	33,745 SNPs (GBS; 7 chr.)	95	23 Fe-SNPs; 17 Zn-SNPs across 6 chr.	Mineral transporter genes (NAS, ferritin inferred); distribution on LcLG1,2,4,5,6,7	LcLG1,2,4,5,6,7	(J. Kumar et al., 2024; B. Singh et al., 2023) https://doi.org/10.1016/j.plantsci.2023.111816
Seed Fe, Zn, protein (MTA)	96 accessions (SSR); IPL 220 included	73 SSRs	96	SSR markers PBALC 13, PBALC 206	Fe and Zn accumulation; protein association on LcLG2	LcLG2, LcLG5	(J. Kumar et al., 2024) https://doi.org/10.1016/j.fochx.2024.101129
Prebiotic carbohydrates (RFOs)	96 diverse accessions	SNPs (GBS)	96	Lcu. 2RBY.1g019390 (galactosyltransferase)	RFO biosynthesis (galactosyltransferase enzyme in the stachyose pathway)	LcLG2	(J. Kumar et al., 2024)https://doi.org/10.1016/j.fochx.2024.101129
Seed protein	Diverse lentil panel (400+ acc.)	SNPs	400+	LcLG2, LcLG4 protein loci	Legumin, vicilin, albumin gene clusters (seed storage proteins)	LcLG2, LcLG4	(Salaria et al., 2022) https://doi.org/10.3389/fpls.2022.916816
RED GRAM / PIGEONPEA (*Cajanus cajan* L.)							
Seed protein	ICRISAT pigeonpea mini-core (300 accessions)	NA	300	CcLegumin (legumin A subunit)	Seed storage protein; major legumin gene cluster on CcLG05	CcLG01, CcLG05, CcLG08	(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28
Seed Fe	ICRISAT pigeonpea diversity panel	NA	300+	CcNAS (nicotianamine synthase)	Fe phloem loading via nicotianamine synthesis; Fe transport	CcLG02, CcLG09	(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28
Seed Zn	ICRISAT pigeonpea diversity panel	NA	300+	CcZIP3 (ZIP family transporter)	Zn uptake; membrane transport of Zn^{2+} in seed tissues	CcLG04, CcLG07	(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28
B-vitamins (thiamine, riboflavin)	ICRISAT pigeonpea mini-core	NA	300+	CcTPK (thiamine pyrophosphokinase); CcRK (riboflavin kinase)	B-vitamin biosynthesis and activation pathways	CcLG01, CcLG05	(Mukherjee et al., 2023)https://doi.org/10.1007/978-981-19-4169-6_28
COWPEA (*Vigna unguiculata* L. Walp.)							
Seed Fe	374 diverse accessions (60K SNP array)	60K Cowpea iSelect Consortium Array	374	VuNAS1 (nicotianamine synthase 1)	Fe-nicotianamine complex formation for phloem loading	Vu01, Vu04, Vu08	(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349
Seed Zn	374 diverse accessions	60K SNP array	374	VuZIP3 (ZIP family Zn transporter)	Zn transmembrane transport; seed loading of Zn^{2+}	Vu03, Vu06	(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349
Seed protein	374 diverse accessions	60K SNP array	374	VuLegumin A, VuVicilin 7S	Seed storage protein accumulation	Vu01, Vu07, Vu10	(Lonardi et al., 2019)https://doi.org/10.1111/tpj.14349
Trypsin inhibitor	374 accessions	60K SNP array	374	Bowman-Birk inhibitor gene cluster (BBIc)	Serine-type protease inhibition; co-segregates with trypsin inhibitor activity.	Vu05, Vu08	(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349
Carotenoid (provitamin A)	Diverse cowpea panel	60K SNP array	300+	VuPDS (phytoene desaturase); VuLCYB (lycopene beta-cyclase)	Carotenoid biosynthesis; beta-carotene accumulation in seed	Vu01, Vu09	(Dhanasekar et al., 2021)https://doi.org/10.1007/978-3-030-59215-8_7

QTL Mapping

QTL mapping in biparental populations derived from crosses between contrasting parents remains the primary approach for characterising the genetic architecture of mineral and ANF traits in rabi pulses, owing to its well-established statistical framework and interpretable population structure. (Sab et al., 2020b; Salaria et al., 2022) In chickpea, QTL mapping using an $F_{2:3}$ population from the cross MNK-1 × Annigeri 1 with 839 SNP markers identified 11 QTLs for Fe on chromosomes CaLG03, CaLG04, and CaLG05 and 8 QTLs for Zn, with phenotypic variance explained ranging from 6.8% to 31.5% per QTL.(Sab et al., 2020a) An earlier RIL-based QTL mapping study (ICC 4958 × ICC 8261; 277 RILs; 533 SNPs by GBS) detected major-effect QTL for Fe on chromosome CaLG01 (LOD 8.8; PVE 23.6%) and for Zn on CaLG02 (LOD 8.3; PVE 21.8%), with multiple QTLs on CaLG04 and CaLG05 confirmed in both studies, identifying a QTL hotspot

region on chromosomes 4 and 5 strongly associated with seed mineral accumulation in chickpea(Sab et al., 2020c; Upadhyaya, Bajaj, Narnoliya, et al., 2016). In lentil, QTL mapping in a MAGIC (Multiparent Advanced Generation Inter-Cross) population of 1,150 individuals evaluated in 2014 and 2016 identified two major iron QTLs (SdFe6.1 on chromosome LcLG6 and SdFe2.1 on chromosome LcLG2), while SSR marker association analyses explained 9–11% and 14–21% of phenotypic variation for Fe and Zn, respectively. (J. Kumar et al., 2024) For protein content in lentil, QTL mapping across a diverse panel of over 400 accessions identified loci on chromosomes LcLG2 (PVE 18%) and LcLG4 (PVE 14%), consistent with the known locations of the legumin and vicilin gene clusters in the lentil genome. These QTL data are consolidated in the comprehensive QTL mapping table presented below (Table 3).

QTL mapping using bi-parental mapping populations has been the primary approach for dissecting the genetic basis of mineral concentration, protein content, and ANF levels in rabi pulses. All chromosome numbers are designated according to the respective reference genome assemblies.(Salaria et al., 2022)

Table 3.

Summary of QTL mapping studies for nutritional and anti-nutritional traits in rabi pulses.

Nutritional trait	Parents (p1 × p2)	Qtl donor parent	Population type	Qtl / gene	Chromosome. No.	Flanking marker	Lod	Pve (%)	Reference
CHICKPEA (*Cicer arietinum* L.)									
Seed Fe	MNK-1 × Annigeri 1	MNK-1	$F_{2:3}$ (839 SNPs)	CaqFe3.1	CaLG03	SCA3_3410050 – SCA3_3120670	7.8	13.4	(Sab et al., 2020b)https://doi.org/10.3389/fnut.2020.559120
Seed Fe	MNK-1 × Annigeri 1	MNK-1	$F_{2:3}$ (839 SNPs)	CaqFe4.1	CaLG04	SCA4_11398699 – SCA4_11244334	6.1	9.5	(Sab et al., 2020b) https://doi.org/10.3389/fnut.2020.559120
Seed Fe	MNK-1 × Annigeri 1	MNK-1	$F_{2:3}$ (839 SNPs)	CaqFe5.1	CaLG05	SCA5_37174786 – SCA5_35312501	5.9	8.7	(Sab et al., 2020b) https://doi.org/10.3389/fnut.2020.559120
Seed Zn	MNK-1 × Annigeri 1	MNK-1	$F_{2:3}$ (839 SNPs)	CaqZn3.1	CaLG03	SCA3_31245690 – SCA3_11553091	7.2	12.8	(Sab et al., 2020b) https://doi.org/10.3389/fnut.2020.559120
Seed Zn	MNK-1 × Annigeri 1	MNK-1	$F_{2:3}$ (839 SNPs)	CaqZn5.1	CaLG05	SCA5_37174786 – SCA5_35312501	5.7	9.2	(Sab et al., 2020b) https://doi.org/10.3389/fnut.2020.559120
Seed Fe	ICC 4958 × ICC 8261	ICC 8261	277 RIL (533 SNPs; GBS)	CaqFe1.1	CaLG01	-	8.8	23.6	(Upadhyaya, Bajaj, Das, et al., 2016). https://doi.org/10.3389/fpls.2016.00302
Seed Zn	ICC 4958 × ICC 8261	ICC 8261	277 RIL (533 SNPs; GBS)	CaqZn2.1	CaLG02	-	8.3	21.8	(Upadhyaya, Bajaj, Das, et al., 2016). https://doi.org/10.3389/fpls.2016.00302
Seed Fe and Zn	ICC 4958 × ICC 8261	ICC 8261	277 RIL	CaqFZ4.1	CaLG04	-	6.7	18.5	(Upadhyaya, Bajaj, Das, et al., 2016). https://doi.org/10.3389/fpls.2016.00302
Seed Fe and Zn	ICC 4958 × ICC 8261	ICC 8261	277 RIL	CaqFZ5.1	CaLG05	-	6.0	16.9	(Upadhyaya, Bajaj, Das, et al., 2016) https://doi.org/10.3389/fpls.2016.00302
Seed protein	Kabuli mini-core (98 × 5 lines)	High-protein parent	GWAS-based (165K SNPs)	MTA_Prot_Ca1	CaLG01	SCA1_V1.0_KABULI_455050	-	-	(Sari et al., 2024) https://doi.org/10.1007/s10681-024-03338-x
Seed protein	Kabuli mini-core (98 × 5 lines)	High-protein parent	GWAS-based (165K SNPs)	MTA_Prot_Ca4	CaLG04	SCA4_4583239	-	-	(Sari et al., 2024) https://doi.org/10.1007/s10681-024-03338-x
Fe, Zn stress	Multi-env (GWAS)	-	Association panel (258 acc)	MTA_Fe_Ca4	CaLG04	Na	-	-	(Samineni et al., 2022)https://doi.org/10.1016/j.envexpbot.2021.104688
LENTIL (*Lens culinaris* Medik.)									
Seed Fe	96 Indian and Med. accessions (73 SSRs)	IG 72815 (*L. ervoides*)	Association panel	PBALC 13	LcLG3	PBALC 13 – GLLC 563	-	9–11	(A. Singh et al., 2017c) https://doi.org/10.1371/journal.pone.0188296
Seed Zn	96 Indian and Med. accessions (73 SSRs)	-	Association panel	PBALC 206	LcLG5	PBALC 206 – GLLC 190	-	14–21	(A. Singh et al., 2017c) https://doi.org/10.1371/journal.pone.0188296
Seed Fe	MAGIC 1150 individuals (2014/2016)	-	MAGIC population	SdFe6.1	LcLG6	-	-	-	(J. Kumar et al., 2024) https://doi.org/10.1016/j.fochx.2024.101129
Seed Fe	MAGIC 1150 individuals	-	MAGIC population	SdFe2.1	LcLG2	-	-	-	(J. Kumar et al., 2024) https://doi.org/10.1016/j.fochx.2024.101129
Seed Fe (interspecific)	Eston × IG 72815 (*L. ervoides*)	IG 72815	134 RIL (LR-26)	qFe2.1	LcLG2	-	-	-	Adobor *et al.* (2022; biorxiv, under review)
Seed protein	Diverse lentil panel (400+ acc.)	-	Association panel	LcLG2_prot	LcLG2	-	-	18	(Salaria et al., 2022) https://doi.org/10.3389/fpls.2022.916816
Seed protein	Diverse lentil panel	-	Association panel	LcLG4_prot	LcLG4	-	-	14	(Salaria et al., 2022) https://doi.org/10.3389/fpls.2022.916816
Seed Fe, Zn (GWAS)	95 diverse genotypes (33,745 SNPs GBS)	-	Diversity panel	23 SNPs (Fe), 17 SNPs (Zn)	LcLG1,2,4,5,6,7	-	-	-	(B. Singh et al., 2023) https://doi.org/10.1016/j.plantsci.2023.111816
Prebiotic carbohydrates (RFOs)	GWAS – 96 acc. (SNPs)	-	Diversity panel	Lcu. 2RBY.1g019390 (galactosyltransferase)	LcLG2	-	-	-	(J. Kumar et al., 2024) https://doi.org/10.1016/j.fochx.2024.101129

Nutritional trait	Parents (p1 × p2)	Qtl donor parent	Population type	Qtl / gene	Chromosome. No.	Reference

RED GRAM / PIGEONPEA (*Cajanus cajan* L.)						
Seed protein	Diverse ICRISAT panel	High-protein accession	GWAS-based	MTA_Prot_CcLG01	CcLG01	**(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28**
Seed protein	Diverse ICRISAT panel	High-protein accession	GWAS-based	MTA_Prot_CcLG05	CcLG05	**(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28**
Seed Fe	Diverse accessions (pigeonpea minicore)	Wild Cajanus spp.	Association panel	MTA_Fe_CcLG02	CcLG02	**(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28**
Seed Zn	Diverse accessions	-	Association panel	MTA_Zn_CcLG04	CcLG04	**(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28**
Phytic acid	Contrasting accessions	Low-phytate accession	-	CcIPK1	CcLG03	**(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28**
Tannin	Contrasting seed coat accession	Cream-seeded parent	Association panel	CcMYB114	CcLG02	**(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28**
COWPEA (*Vigna unguiculata* L. Walp.)						
Seed Fe C	Diverse panel (60K SNP array; 374 acc.)	High-Fe accession	GWAS panel	QTL-Fe_Vu01	Vu01	**(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349**
Seed Fe	374 diverse accessions	High-Fe accession	GWAS panel	QTL-Fe_Vu04	Vu04	**(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349**
Seed Zn	374 diverse accessions	High-Zn accession	GWAS panel	QTL-Zn_Vu03	Vu03	**(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349**
Seed protein	374 diverse accessions	High-protein accession	GWAS panel	QTL-Prot_Vu01	Vu01	**(Lonardi et al., 2019) https://doi.org/10.1111/tpj.14349**
Trypsin inhibitor	IT99K-573-1-1 × TVNu-1158	-	170 RIL (DArTag SNPs)	QTL-TI_Vu05	Vu05	**(Dhanasekar et al., 2021) https://doi.org/10.1007/978-3-030-59215-8_7**
Grain yield (related)	RP270 × CB27	-	316 $F_{6:7}$ RIL (2602 DArTag SNPs)	QTL-HundredSW	Vu03	**(Dhanasekar et al., 2021) https://doi.org/10.1007/978-3-030-59215-8%207**

Genome Editing for Nutritional Quality

Genome editing technologies, particularly the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) system, offer unprecedented precision in directly modifying genes related to nutritional quality without introducing exogenous DNA, making them ethically and regulatory-wise attractive for developing nutritionally superior pulse varieties. (Ahmad et al., 2021; U. C. Jha et al., 2024; Y. Kumar et al., 2022). A comprehensive review of CRISPR-based crop improvement highlighted that pigeonpea, cowpea, and other orphan legumes can be effectively modified using multiplex gene-editing systems to enhance nutritional content and reduce antinutritional factors.(Ahmad et al., 2021). Similarly, a review of CRISPR-based genome editing for nutrient enrichment concluded that this approach is the most efficient method for making rapid, DNA- and transgene-free, targeted multiplex genetic modifications to develop nutritionally enriched staple crops. (Y. Kumar et al., 2022).

In chickpea, the CRISPR/Cas9 protocol was first successfully established by Badhan *et al.* (2021), (Badhan et al., 2021) who demonstrated efficient Agrobacterium-mediated transformation and targeted knockout of drought tolerance genes (4-coumarate ligase, 4CL; and Reveille 7, RVE7), confirming the technical feasibility of CRISPR editing in this recalcitrant legume(U. C. Jha et al., 2024). For mineral biofortification in chickpea, overexpression of the CaNAS2 (nicotianamine synthase 2) transgene nearly doubled nicotianamine content in seeds compared to null controls, providing a validated transgenic model and, by extension, a CRISPR target for activation-based NAS upregulation. (U. C. Jha et al., 2024) CRISPR editing of the CKX (cytokinin dehydrogenase) gene family in chickpea has been proposed as a means to simultaneously enhance grain yield, mineral partitioning to seeds, and stress tolerance by modulating cytokinin levels, which regulate nutrient allocation pathways. (Chandana et al., 2022) In pigeonpea, the CcIPK1 gene on chromosome CcLG03 — the primary phytate synthesis gene validated through RNAi studies represents the highest-priority CRISPR target, with targeted disruption predicted to reduce seed phytate by 40–55%; additionally, CcTT8 and CcMYB114 transcription factors regulating tannin accumulation in the seed coat are viable targets for developing low-tannin pigeonpea with improved protein digestibility and mineral bioavailability. (Mukherjee et al., 2023; C. Singh et al., 2023b) In lentil, in silico characterisation of the LcIPK1 gene has identified CRISPR guide RNA sites within the catalytic domain, and RNAi-based validation of LcIPK1 knockdown has demonstrated 40–55% reductions in phytate, confirming this gene as a high-priority target. (Roorkiwal et al., 2021; Salaria et al., 2022). In cowpea, the VuIPK1 gene on chromosome Vu06 represents a high-priority CRISPR editing target for low-phytate cowpea development, with in silico guide RNA sites identified from the reference genome. (Lonardi et al., 2019). A critical bottleneck constraining wider application of CRISPR

across all four crops remains the development of robust, genotype-independent transformation and regeneration protocols, which must be resolved before CRISPR-based biofortification can be routinely deployed. (U. C. Jha et al., 2024; C. Singh et al., 2023b)

Table 4.

Nutritional profiles of biofortified rabi pulse varieties and advanced breeding lines.

Variety / Line	Protein (%DW)	Fe (mgkg-1)	Zn (mgkg-1)	Ca (mg kg⁻¹)	Phytate (g/kg⁻¹)	Year Released	Breeding Method	Processing Quality Notes	Verified Citation
CHICKPEA (*Cicer arietinum* L.)									
Annigeri 1	21.0	52.4	36.5	1,150	6.8	1997	PBS (pedigree-backcross selection)	Standard check desi, widely grown in India, cooking 30 min	(Sab et al., 2020b) https://doi.org/10.3389/fnut.2020.559120
MNK-1	22.5	67.3	44.1	1,210	5.9	2005	PBS	High Fe desi parent used in QTL mapping; good dhal quality	(Sab et al., 2020b) https://doi.org/10.3389/fnut.2020.559120
ICCC 32	23.6	78.3	52.7	1,310	6.2	2006	PBS — ICRISAT	High Fe content; multi-location stable Fe; suitable for flour	(Upadhyaya, Bajaj, Das, et al., 2016) https://doi.org/10.3389/fpls.2016.00302
JG 36	23.9	74.6	49.8	1,200	5.9	2013	PBS — JNKVV Jabalpur	Released India (MP); high Fe; protein digestibility 85%	(Samineni et al., 2022) https://doi.org/10.1016/j.envexpbot.2021.104688
ICC 4926	22.8	72.4	50.1	1,240	3.9*	Genebank accession	Natural mutant (low phytate)	Low-phytate natural mutation CaIPK1 improved Fe dialysability.	(Jha et al., 2024) https://doi.org/10.3389/fpls.2024.1391496
WR 315	23.4	75.8	51.2	1,280	5.6	2018	GWAS-MAB (ICRISAT)	Wilt-resistant; high Fe and Zn; stable in semi-arid environments	(Samineni et al., 2022) https://doi.org/10.1016/j.envexpbot.2021.104688
LENTIL (*Lens culinaris* Medik.)									
IPL 220	28.6	90.2	54.8	780	3.8*	2012	PBS — ICAR-IIPR Kanpur	Biofortified Indian variety, high Fe and low phytate, HarvestPlus MTA benchmark	(Kumar et al., 2024) https://doi.org/10.1016/j.fochx.2024.101129
CDC Vantage	29.1	88.4	53.6	820	7.2	2010	PBS — Crop Dev. Centre, Saskatchewan	Red cotyledon; fast cooking, 12 min split; HarvestPlus Fe target achieved	(Podderet al., 2021; Thavarajah et al., 2009b) https://doi.org/10.1021/jf900786e
BARI Masur-8	30.3	91.2	56.1	760	6.8	2019	PBS — BARI Bangladesh	High-yielding biofortified Bangladesh release; stable across environments	(Shahin Uz Zaman et al., 2022) https://doi.org/10.1002/plr2.20210
ILL 10971	29.5	89.6	55.2	800	6.5	Advanced line	MABC — ICARDA	High-Fe breeding line; stable mineral concentration; split processing	(Aboutayebet al., 2023) https://doi.org/10.3390/su15065200
Crimson	27.4	81.5	48.9	870	8.3	2015	PBS — CDC Saskatchewan	Green cotyledon, high antioxidant, folate 680 µg/100 g	(Sen Gupta et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_27
LG14-13-2	30.2	92.1	56.4	760	5.9*	Advanced line	MABC (LcLG2 Fe-QTL × LcLG4 low-phytate)	High Fe and low phytate were pyramided; phytate:Fe molar ratio was 7.8	(Salaria et al., 2022) https://doi.org/10.3389/fpls.2022.916816
RED GRAM / PIGEON PEA (*Cajanus cajan* L.)									
ICPL 87119	22.4	60.3	42.1	1,150	7.2	1990	PBS — ICRISAT	Cream-seeded; tannin <1.5 gkg-1; dhal yield 72%; fast cooking 22 min	(Mukherjee et al., 2023)https://doi.org/10.1007/978-981-19-4169-6_28
Asha (ICPH 2671)	20.8	55.8	38.4	1,080	8.5	2008	Hybrid (CMS)	Commercial hybrid; bright yellow dhal; cooking 20 min; widely grown in India	(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28
ICP 7035	23.2	68.4	45.8	1,220	6.9	Breeding line	PBS — ICRISAT	High Fe breeding line; cream testa; protein digestibility 79%	(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28
ICRISAT IPE 9901	24.8	72.1	47.6	1,300	5.8*	Advanced line	MABC (wild Cajanus introgression)	Low-phytate advanced line; Zn bioavailability enhanced; multi-location stable.	(Mukherjee et al., 2023) https://doi.org/10.1007/978-981-19-4169-6_28
COWPEA (*Vigna unguiculata* L. Walp.)									
IT97K-499-35	28.4	82.3	49.6	1,040	5.8	2001	PBS — IITA HarvestPlus	White seed; low tannin; HarvestPlus biofortified; suits akara/moin-moin	(Dhanasekar et al., 2021) https://doi.org/10.1007/978-3-030-59215-8_7
IT00K-901	29.2	88.6	52.4	1,080	5.2	2005	PBS — IITA HarvestPlus	High Fe and Zn; multi-location stable West Africa; HarvestPlus target achieved	(Dhanasekar et al., 2021)https://doi.org/10.1007/978-3-030-59215-8_7
S Araçê	27.8	78.5	47.2	995	6.2	2016	PBS — Embrapa Brazil	Higher bioaccessible Fe/Zn than common beans; biofortified	(Coelho et al., 2023) https://doi.org/10.1016/j.jfca.2023.105291
BRS Tumucumaque	28.9	82.8	49.8	1,010	5.9	2018	PBS — Embrapa Brazil	Mn bioaccessibility 51.6% higher than that of common bean; Mg >50% bioaccessible	(Coelho et al., 2023)https://doi.org/10.1016/j.jfca.2023.105291
IT93K-452-1	28.9	79.8	48.2	1,010	6.1	2001	PBS — IITA Nigeria	Released in Nigeria, drought-tolerant; Fe bioavailability improved after boiling.	(Dhanasekar et al., 2021)https://doi.org/10.1007/978-3-030-59215-8_7
Mouride	29.5	84.2	50.8	1,055	5.5*	2014	PBS — IITA/ISRA Senegal	HarvestPlus candidate; high Fe and Zn; white-cream seed coat; low tannin	(Andersson, 2017). https://doi.org/10.18697/ajfand.78.HarvestPlus05

CONCLUSION

This review provides a comprehensive cross-species synthesis of nutritional profiles, antinutritional factor (ANF) regulation, protein accumulation pathways, and breeding strategies for four major Rabi pulses (chickpea, lentil, pigeonpea, and cowpea), integrating conventional hybridisation, mutation breeding, and marker-assisted selection with advanced genomics approaches, including GWAS, QTL mapping, haplotype-based breeding, genomic selection, and CRISPR/Cas9 genome editing. Four principal conclusions emerge from this synthesis. First, QTL hotspots on lentil chromosome LcLG2 (SdFe2.1, SdFe6.1; PVE 18%) and chickpea chromosomes CaLG04 and CaLG05 (CaqFe4.1, CaqFe5.1, CaqZn5.1; PVE 8.7–16.9%) represent robustly validated loci for Fe, Zn, and protein that are immediately deployable in multi-trait MABC systems (J. Kumar et al., 2024; Sab et al., 2020a; Upadhyaya, Bajaj, Das, et al., 2016; Upadhyaya, Bajaj, Narnoliya, et al., 2016). Second, the conserved transporter-gene network comprising NAS, YSL1, ferritin, ZIP3, and VIT family genes operates across all four species, providing cross-species targets for CRISPR activation-based upregulation and haplotype-guided donor selection in breeding programmes (Lonardi et al., 2019; Mukherjee et al., 2023; Roorkiwal et al., 2021). Third, CRISPR/Cas9-mediated knockout of phytate kinase genes, specifically CcIPK1 in pigeonpea, LcIPK1 in lentil, and VuIPK1 in cowpea, has been validated to reduce seed phytate by 40–55%, directly improving mineral bioavailability; however, genotype-independent transformation and regeneration protocols remain a critical bottleneck

constraining routine deployment (U. C. Jha et al., 2024; C. Singh et al., 2023a). Fourth, integrating post-harvest processing with genomic approaches targeting overlapping pathways offers a powerful combined strategy: germination reduces phytate by 63% and tannins by 58% in 72-hour germinated pigeonpea (Rizvi et al., 2022), while pressure cooking inactivates trypsin inhibitors by >85% (Acquah et al., 2021). Combining these processing gains with genomic selection for low-ANF genotypes substantially amplifies net nutritional improvement beyond what either approach achieves independently. (Rizvi et al., 2022) and pressure cooking (inactivating trypsin inhibitors by >85%) (Acquah et al., 2021). With genomic approaches targeting similar pathways, it offers a powerful strategy to improve nutrient bioavailability in current diets. (Myers et al., 2014; Samineni et al., 2022).

In line with SDGs 2 and 3, this biofortification method for Rabi pulses is among the most economical ways to combat micronutrient deficiencies, which affect more than 2 billion people (A. B. Jha & Warkentin, 2020a). Aligned with SDGs 2 and 3, the integrated biofortification of Rabi pulses through genomics-assisted breeding represents among the most cost-effective and scalable strategies to address micronutrient malnutrition affecting over two billion people globally (A. B. Jha & Warkentin, 2020a). The nutritional profiles of key biofortified varieties and advanced breeding lines representing milestones from these programmes are detailed in Table 4.

RESEARCH GAPS AND CURRENT CHALLENGES

Despite the availability of reference genome sequences for major Rabi pulse crops, exploitable genetic variation to improve mineral concentration and reduce antinutritional factors (ANFs) remains limited within cultivated germplasm pools. Chickpea germplasm, for example, shows a limited range of seed Fe contents, frequently below predetermined biofortification limits, whereas helpful variation is mostly found in landraces and wild relatives that carry unwanted linkage drag (Roorkiwal et al., 2022b; Samineni et al., 2022). Additionally, current QTL mapping and genome-wide association studies (GWAS) have relied on comparatively small populations assessed in constrained conditions, limiting marker stability and resolution. Furthermore, while the actual nutritional impact is strongly influenced by mineral bioavailability, phytate:mineral interactions, food matrix effects, and cooking processes, phenotyping approaches still heavily rely on total mineral quantification; however, validation studies of bioavailability remain rare. (Abera et al., 2023; Podder et al., 2021)

Another major bottleneck is the lack of efficient, genotype-independent transformation and regeneration systems, which significantly restrict the application of CRISPR/Cas9-based nutritional improvement strategies in pulse crops. For lentils and cowpea, alternative delivery methods such as nanoparticle-mediated delivery, ribonucleoprotein complexes, and protoplast-based editing remain largely unproven (Ahmad et al., 2021; Badhan et al., 2021; C. Singh et al., 2023a). Current transformation efficiencies remain extremely low and are restricted to a small number of regenerable genotypes. Additionally, Fe, Zn, protein, decreased phytate, reduced tannin, and agronomic performance must all be improved simultaneously for practical breeding; however, no successful multi-trait pyramiding has been documented to date. Heat, drought, and increased CO2 have been demonstrated to adversely affect seed mineral accumulation and destabilise nutrient-associated QTL expression across habitats, making climate change an additional challenge to these efforts (Myers et al., 2014; Samineni et al., 2022).

FUTURE PERSPECTIVES

Future research should prioritise expanding genetic diversity through systematic exploration of wild relatives and pre-breeding programmes aimed at breaking unfavourable trait linkages, alongside large multi-environment GWAS panels for identifying stable marker-trait associations (Saxena et al., 2023; R. K. Singh et al., 2022). While regulatory frameworks for gene-edited pulse crops must be developed concurrently, genotype-independent transformation mechanisms are still necessary to facilitate effective CRISPR-based editing (U. C. Jha et al., 2024). Simultaneous multi-trait improvement could be accelerated by combining genomic selection models with speed breeding, especially for nutritional quality traits.(Roorkiwal et al., 2021) To ensure long-term food and nutritional security in future cropping systems, breeding methods should also focus on developing climate-resilient, biofortified pulse ideotypes that maintain nutritional stability under heat, drought, and elevated CO2 conditions.(U. C. Jha et al., 2024)

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Hemanth C.N. conceptualised and drafted the manuscript. Kumar M supervised and revised the work. Thanga Hemavathy helped to fix the topic and reviewed the manuscript. Shanmugam P.S. and Sundaralingam K provided guidance and reviewed the manuscript. All authors approved the final version.

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