

ENHANCING AMINO ACID QUALITY IN CEREALS: GENETIC AND BREEDING PERSPECTIVES

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ABSTRACT

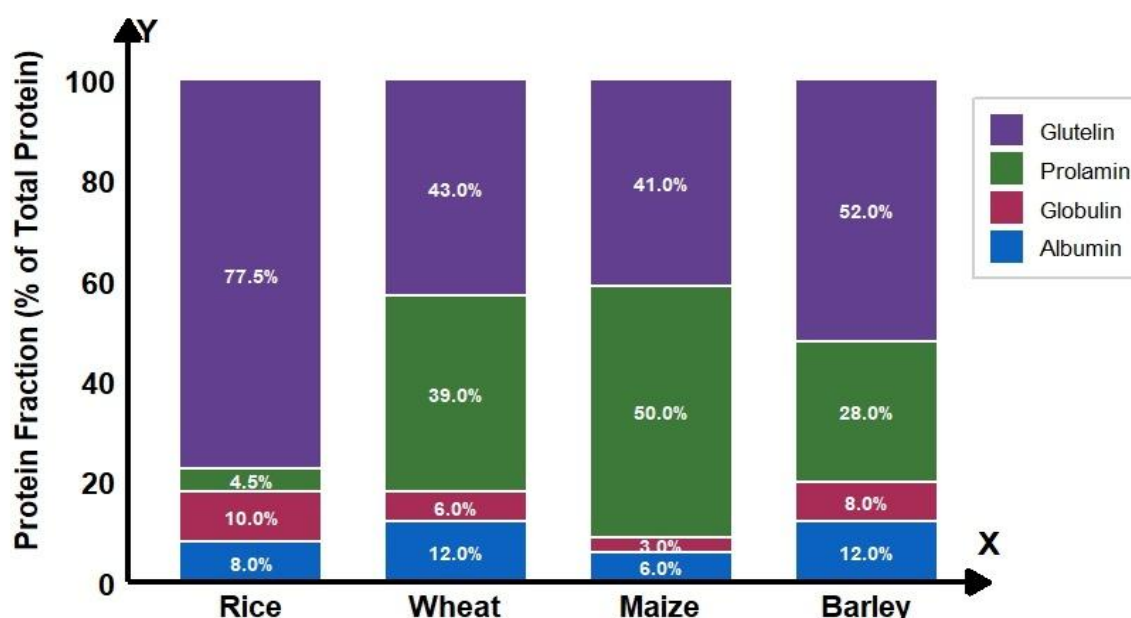
Cereal grains are central to global food security, yet their nutritional quality is frequently constrained by inadequate levels of essential amino acids, particularly lysine, methionine and tryptophan. Improving amino acid composition is an important component of developing nutritionally enhanced and climate-resilient cereal crops. This review critically examines the genetic, biochemical and breeding factors governing amino acid profiles in major cereals, with emphasis on rice, wheat, maize and barley. The major metabolic pathways underlying essential amino acid biosynthesis, together with genes involved in synthesis, transport and storage are discussed in relation to grain nutritional quality. Advances in chromatographic, spectroscopic and bioanalytical approaches have improved the accuracy and throughput of amino acid profiling, while genomics, transcriptomics, proteomics and metabolomics have accelerated the discovery of regulatory genes, genomic regions and metabolic networks. Conventional breeding, induced mutagenesis, marker-assisted selection, quantitative trait locus mapping, genomic approaches and precise genome editing have expanded opportunities for improving limiting amino acids, with quality protein maize providing an established example of successful nutritional enhancement. However, trade-offs among amino acid composition, grain yield, protein content, environmental stability and genotype-by-environment interactions remain major challenges. Future breeding strategies should integrate diverse germplasm, multi-omics information, high-throughput phenotyping and predictive approaches to develop stable, high-yielding cereals with superior protein quality and nutritional value.

KEYWORDS: Amino acid profiling, Essential amino acids, Cereal crops, Grain protein quality, Genomics-assisted breeding

1. INTRODUCTION

Cereals represent a fundamental component of global food security, serving as the primary source of dietary energy and making a significant contribution to protein intake for a large proportion of the world's population (Avelar *et al.*, 2024, Scharff *et al.*, 2022). Besides supplying calories, cereal proteins contribute to growth, tissue repair, enzyme synthesis, immune function and metabolic regulation. Their importance is particularly high in low-income populations where access to animal proteins is limited (ElShamey *et al.*, 2025). Cereal proteins are classified into albumins (water-soluble; enzymatic and metabolic proteins), globulins (salt-soluble; rich in lysine and nutritionally superior), prolamins (alcohol-soluble storage proteins such as gliadin in wheat, zein in maize, hordein in barley) and glutelins (alkali-soluble proteins such as glutenin in wheat and oryzenin in rice) (Rasheed *et al.*, 2020). Cereal proteins are nutritionally limited because the dominant prolamins are rich in glutamine and proline but deficient in essential amino acids such as lysine, tryptophan, methionine and threonine resulting in amino acid imbalance and reduced biological value (Fig 1). The imbalance and limited availability of essential amino acids in many cereal grains represents a major nutritional constraint, posing significant challenges for human and animal nutrition, especially in populations heavily dependent on cereal-based diets (Pott *et al.*, 2021). The widespread prevalence of protein malnutrition, often known as "hidden hunger" due to insufficient intake of essential amino acids, emphasizes the pressing need for genetic and metabolic strategies to improve the nutritional quality of staple crop species (Pott *et al.*, 2021). Global demand for dietary protein is projected to rise markedly by 2050 due to population growth and changing consumption patterns, placing increasing pressure on agricultural systems to enhance the production of nutritious foods (Safdar *et al.*, 2023). Improving the essential amino acid composition of cereal grains is an important strategy to enhance protein quality and address protein malnutrition, particularly in regions where cereals constitute the major component of the human diet (Devi *et al.*, 2023). Addressing this challenge requires a multifaceted approach that integrates conventional breeding strategies with advanced molecular genetics and biotechnological interventions to develop cereal varieties with improved protein quality. Biofortification, a cost-

effective strategy that genetically enhances nutrient content in crops, offers a sustainable solution to combat micronutrient deficiencies and protein malnutrition in vulnerable populations (Swamy *et al.*, 2021). Moreover, recent advances in omics technologies, particularly genomics and metabolomics, have facilitated the dissection of complex genetic architectures underlying metabolic traits, enabling the identification of novel targets for crop nutritional improvement (Chen *et al.*, 2021, Kudapa *et al.*, 2023). This approach directly aligns with Sustainable Development Goal 2 by improving the nutritional density of staple foods, thereby contributing to food security and public health outcomes (Sheera *et al.*, 2025, Zulfiqar *et al.*, 2024). Ultimately, this review aims to present a comprehensive and critical synthesis of recent scientific advances and their translational potential for developing nutritionally improved cereal crops.



Source: Haard 1999

Fig. 1 Protein fractions in major cereals

2. Importance of amino acids in cereals

Amino acids, the fundamental building blocks of proteins, play indispensable roles in metabolic processes, growth, and physiological development in humans and animals, necessitating adequate dietary intake to maintain health (Wang *et al.*, 2023). Cereals, although serving as primary sources of dietary energy, are often deficient in certain essential amino acids, particularly lysine, methionine, and tryptophan, which cannot be synthesized by the human body and must be supplied through diet. Such imbalances in amino acid composition influence protein quality, affecting its bioavailability and utilization, and may contribute to malnutrition in populations heavily dependent on cereal-based diets (Salaria *et al.*, 2022). The deficiency of these essential amino acids reduce protein synthesis and hinders optimal physiological functions resulting in stunted growth, impaired cognitive development, and compromised immune responses, particularly in vulnerable populations (Jha *et al.*, 2024, Trovato *et al.*, 2021). Beyond their role in protein synthesis, amino acids also serve as precursors for various secondary metabolites, hormones, and signalling molecules essential for plant growth, development, and stress resilience, thus influencing overall crop productivity and nutritional value (Liu *et al.*, 2023). Moreover, certain amino acids and their derivatives play crucial roles in plant defence mechanisms, acting as osmo-protectants and antioxidants, which indirectly affect nutrient accumulation under adverse environmental conditions (Raza *et al.*, 2025). Consequently, improving amino acid profiles in cereals not only addresses human nutritional needs but also potentially enhances crop resilience and overall nitrogen use efficiency (The *et al.*, 2021, Trovato *et al.*, 2021). Nitrogen is a vital component of amino acids, and enhancing nitrogen use efficiency in cereals is critical for sustainable agriculture, reducing the environmental impact of fertilizer application, and ensuring efficient amino acid synthesis (Sandhu *et al.*, 2021, Trovato *et al.*, 2021). Understanding the complex interplay between nitrogen metabolism, amino acid biosynthesis, and storage protein accumulation is important for developing cereal varieties with improved nutritional quality (Ou *et al.*, 2025).

3. Amino acid composition and nutritional value of major cereals

3.1. Amino acid profiles of wheat, rice, maize, and barley

Cereal-based diets often lack essential nutrients and micronutrients, contributing to malnutrition in many developing regions (Sandhu *et al.*, 2023). The amino acid composition varies considerably among different cereal species. Table 1 presents the amino acid profile of major cereal grains highlighting the significant differences in the amounts of essential and non-essential amino acids. Wheat and maize proteins are notably deficient in lysine and tryptophan. At the same time, rice has a slightly better amino acid balance, still presents

limiting levels of lysine and threonine. In contrast, legumes are often deficient in sulphur-containing amino acids, but frequently offer compensatory levels of lysine, emphasizing the complementary nutritional benefits of combining cereals with legume-based diets (Langyan *et al.*, 2022). Plant proteins from cereals, legumes, and pseudo-cereals differ considerably in composition and nutritional quality, highlighting the importance of understanding their amino acid profiles for improving dietary protein utilization (Zhang, Boateng, *et al.*, 2024). For instance, Quality Protein Maize (QPM) is a successful breeding achievement, exhibiting elevated lysine (265 mg to 408 mg/100 g maize) and tryptophan (67 mg to 75 mg/100 g maize) concentrations through conventional breeding, demonstrating the potential to enhance nutritional value in staple crops (Langyan *et al.*, 2022). Similarly, barley grain proteins contain high levels of lysine-deficient storage proteins (hordeins), which reduce the nutritional quality of the grain; therefore, genetic approaches targeting these proteins have been explored to improve amino acid composition (Bose *et al.*, 2021).

Table 1 Amino acid profiles of major cereals

Amino acid (g/100g of sample)	Rice	Wheat	Maize	Barley	Source
Aspartic acid	0.72	0.70	0.56	0.62	(Tomičić <i>et al.</i> , 2022)
Threonine*	0.27	0.37	0.35	0.33	
Serine	0.38	0.63	0.49	0.40	
Glutamic acid	1.39	2.94	1.68	2.87	
Proline	0.37	1.28	0.76	1.17	
Glycine	0.47	0.52	0.33	0.46	
Alanine	0.45	0.47	0.55	0.37	
Cystine	0.12	0.22	0.18	0.24	
Valine*	0.39	0.57	0.43	0.44	
Methionine*	0.21	0.19	0.24	0.16	
Isoleucine*	0.34	0.51	0.30	0.31	
Leucine*	0.66	0.82	0.92	0.72	
Tyrosine	0.23	0.27	0.26	0.21	
Phenylalanine*	0.42	0.66	0.46	0.52	
Histidine*	0.16	0.25	0.22	0.23	
Lysine*	0.23	0.36	0.29	0.33	
Arginine	0.59	0.65	0.48	0.58	
Tryptophan*	1.1	1.0	0.6	1.7	(Haard 1999)

(* indicates essential amino acids)

3.2. Essential Amino Acid Deficiencies in Cereals

Certain important amino acids, including tryptophan and lysine, are naturally in lower concentration in cereal grains, which poses a serious nutritional problem because humans cannot produce these amino acids on their own and must obtain them through diet (Korth *et al.*, 2022). The lack of these limiting amino acids significantly leads to protein malnutrition and related health problems since they are essential for tissue repair, enzyme activity, protein synthesis, and general physiological function (Garrido-Galand *et al.*, 2021). The variation in essential amino acid composition among major cereal grains and their comparison with FAO ideal amino acid requirements are illustrated in Fig. 2. Rice, wheat and maize contain comparatively lower levels of lysine and tryptophan. This imbalance reduces the overall protein quality of cereal-based diets and emphasizes the need for complementary protein sources to achieve balanced nutrition.

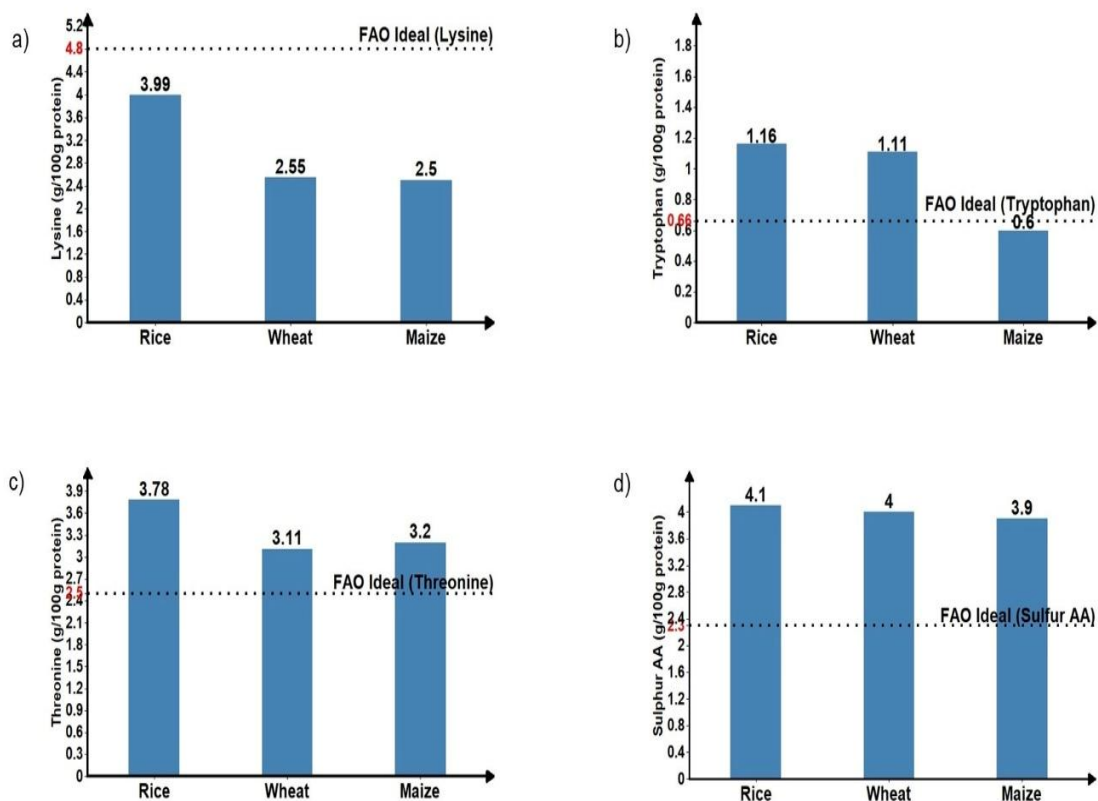


Fig. 2 Comparison between essential amino acid composition among major cereal grains and FAO ideal amino acid requirements in adults

3.3. Factors influencing amino acid content

The complex interplay of genetic, environmental, and developmental factors profoundly influences the amino acid content and protein quality in cereal grains. Genetic factors, encompassing gene expression regulation and allelic variations of genes involved in amino acid biosynthesis and storage protein synthesis, play a predominant role in determining the intrinsic amino acid profile of a particular cereal variety (Feng 2024).

Environmental factors, such as soil nitrogen availability, temperature, and water stress, can significantly modulate amino acid accumulation, often impacting the balance of essential amino acids within the grain. Furthermore, post-harvest handling and storage conditions can also influence protein integrity and amino acid profiles, underscoring the need for a holistic approach to nutritional improvement (Nsafon *et al.*, 2020). For example, Sulphur fertilization can improve the composition and quality of wheat storage proteins, while Selenium supplementation may influence functional properties of plant proteins, highlighting the potential of mineral-based interventions to enhance the nutritional and functional quality of cereal and legume proteins (Abdalla *et al.*, 2025). Therefore, a multi-pronged strategy integrating genetic manipulation with optimized agronomic practices is essential to achieve robust and stable improvements in the amino acid composition of cereal crops.

4. Methods for amino acid profiling

4.1. Chromatographic techniques

Accurate and high-throughput quantification of amino acids is fundamental for genetic improvement programs, with chromatographic techniques forming the backbone of such analyses. These methods, particularly high-performance liquid chromatography and gas chromatography, offer precise separation and detection of individual amino acids, enabling detailed profiling essential for identifying genetic variations and breeding targets. Specifically, HPLC, often coupled with UV or fluorescence detection after pre- or post-column derivatization, is widely employed due to its versatility and sensitivity in quantifying both free and protein-bound amino acids (Buha *et al.*, 2011). Conversely, gas chromatography-mass spectrometry provides superior resolution and definitive structural identification, proving invaluable for complex matrices and the analysis of a broader range of amino acid derivatives. Despite their analytical power, these methods involve extensive sample preparation, such as hydrolysis and derivatization, which can be time-consuming and limit their throughput in large-scale breeding programs.

4.2. Spectroscopic methods

Beyond chromatographic methods, spectroscopic techniques offer rapid, non-destructive, and often high-throughput alternatives for estimating amino acid content and protein quality, albeit typically with less precision for individual amino acids. Near-infrared reflectance (NIR) spectroscopy, for instance, has emerged as a

promising tool, enabling rapid prediction of overall protein content and, in some cases, specific amino acid concentrations in intact seeds or ground samples (Fontaine *et al.*, 2001). Genetic variation in amino acid concentration and protein digestibility has been explored through quantitative trait loci (QTL) analysis in pea, providing insights for improving protein quality through breeding (Zhou *et al.*, 2023). This technology exploits the absorption of infrared light by specific chemical bonds within amino acids to generate spectral fingerprints, which can then be correlated with actual amino acid values using chemometric models (Qi *et al.*, 2025). While NIR spectroscopy is amenable to high-throughput screening, its limitations in precisely quantifying specific amino acids, particularly sulphur-containing ones, necessitate the exploration of complementary or more advanced techniques (Kim *et al.*, 2024). Nuclear magnetic resonance spectroscopy and Fourier-transform infrared spectroscopy represent additional spectroscopic avenues, providing detailed structural information and quantitative data on amino acid profiles with minimal sample preparation, thus offering potential for enhanced throughput in breeding contexts. However, these spectroscopic methods, while rapid, often lack the precision to quantify individual amino acids directly and reliably, particularly essential amino acids that are present in lower concentrations (Cavdaroglu *et al.*, 2025).

4.3. Bioanalytical approaches

Bioanalytical approaches supplement physicochemical methods by assessing the functional significances of amino acid profiles, often involving *in vitro* or *in vivo* digestibility studies and protein quality evaluations such as Protein Digestibility Corrected Amino Acid Score or Digestible Indispensable Amino Acid Score (Mondal *et al.*, 2023). These techniques directly influence the nutritional impact of cereal proteins by offering vital insights into the bioavailability and usage of amino acids. Furthermore, advanced omics technologies, such as proteomics and metabolomics, offer comprehensive insights into protein expression patterns and metabolic pathways influencing amino acid biosynthesis and accumulation. Proteomics, in particular, can identify variations in storage protein composition and the enzymes involved in amino acid metabolism, while metabolomics can quantify the full spectrum of amino acids and related precursors, providing a deeper understanding of the genetic and biochemical underpinning of amino acid profiles (Kaur *et al.*, 2021). Despite their comprehensive nature, the high cost, technical complexity, and extensive data analysis required for omics technologies currently limit their routine application in large-scale breeding programs.

5. Genetic basis of amino acid biosynthesis

Understanding the genetic architecture underlying amino acid biosynthesis is important for targeted breeding strategies aimed at enhancing the nutritional quality of cereal crops. The intricate pathways involved in the synthesis of essential amino acids, often conserved across plant species, represent key targets for genetic manipulation to improve the nutritional value of staple cereals.

5.1. Metabolic pathways

Metabolic pathways governing essential amino acid biosynthesis play a crucial role in determining the nutritional quality of cereal grains (Fig. 3). In particular, the aspartate family pathway, which regulates the synthesis of lysine, threonine, and methionine, and the shikimate pathway, responsible for the formation of aromatic amino acids such as tryptophan and phenylalanine, are of considerable importance because these amino acids are frequently deficient in major cereal crops. The limited availability of essential amino acids in cereals such as maize and wheat has been widely recognized as a major nutritional constraint, necessitating the identification of genes and metabolic networks controlling their biosynthesis to facilitate targeted crop improvement strategies (Pott *et al.*, 2021).

A classic example of genetic regulation of amino acid composition is the *opaque-2* (*o2*) gene in maize, which has been extensively exploited in quality protein maize breeding programs. The recessive mutation at the *opaque-2* locus alters the expression of endosperm storage proteins and significantly increases the accumulation of lysine and tryptophan in maize kernels, thereby enhancing their nutritional value compared with conventional maize varieties (Terera and Mcebisi 2023).

Beyond this well-characterized locus, advances in metabolomics-assisted genome-wide association studies have revealed additional genes and metabolic regulators controlling amino acid composition in cereal grains. For example, genetic variation in enzymes involved in branched-chain amino acid metabolism, such as acetolactate synthase, has been associated with changes in leucine concentration, highlighting the complex genetic architecture underlying amino acid accumulation (Pott *et al.*, 2021).

Similarly, genomic analyses have identified novel candidate genes linked to essential amino acid metabolism, including arogenate dehydratase, which regulates phenylalanine biosynthesis, and the transcription factor *Opaque2*, which influences the lysine-to-total amino acid ratio in maize kernels (Pott *et al.*, 2021). The identification of such candidate genes and regulatory loci provides valuable opportunities for modern crop improvement programs.

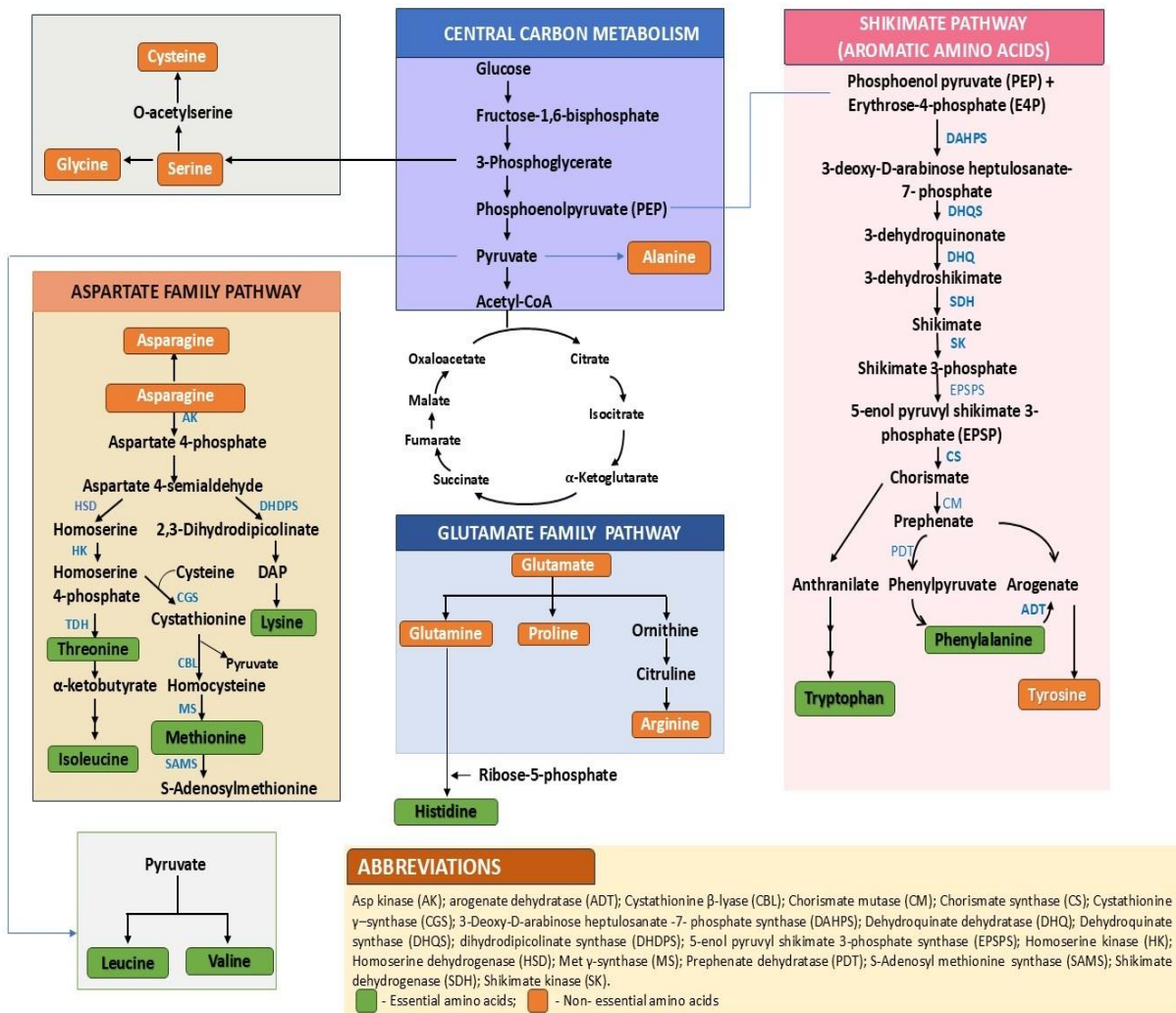


Fig. 3 Metabolic pathways of amino acid biosynthesis

5.2. Genes Regulating Synthesis and Transport

Beyond amino acid biosynthesis, their transport, allocation, and storage within plant tissues- particularly during grain development- play a critical role in determining the final amino acid composition and nutritional quality of cereal grains. Amino acid content (AAC) is an important nutritional trait in cereals, and understanding its genetic architecture is essential for improving grain nutritional value through modern breeding strategies. Genome-wide association and haplotype-based analyses in rice have identified several candidate genes regulating AAC, highlighting the complex genetic control underlying amino acid accumulation in developing grains (Wang *et al.*, 2023). Among these, *OsAUX5*, a transmembrane amino acid transporter, regulates the accumulation of essential amino acids such as valine, leucine, and phenylalanine, demonstrating the importance of transporter-mediated processes in shaping grain amino acid composition (Wang *et al.*, 2023). These findings emphasize the significance of source-sink transport mechanisms during grain filling and suggest that manipulation of amino acid transport pathways could enhance the deposition of nutritionally important amino acids in cereal endosperm.

In addition to transport processes, grain protein accumulation is regulated by coordinated metabolic and transcriptional networks during seed development. Integrated transcriptomic and metabolomic analyses have shown that genes involved in central carbon metabolism, glycolysis, the tricarboxylic acid cycle, and amino acid biosynthesis exhibit dynamic expression during the grain-filling stage, a key period determining protein accumulation and grain nutritional quality (Ji *et al.*, 2025). Advances in functional genomics and metabolic pathway engineering further highlight the potential of manipulating key metabolic pathways and regulatory genes to improve nutritional quality and stress resilience in cereal crops (Liu *et al.*, 2023).

Genetic variation controlling grain protein composition also plays a fundamental role in determining cereal nutritional quality. In rice, grain protein content (GPC) is a complex quantitative trait governed by multiple genomic loci and candidate genes associated with protein synthesis and accumulation in the endosperm. QTL mapping studies have identified several genomic regions contributing to GPC variation, illustrating the polygenic nature of protein accumulation and its influence on grain quality traits (Bharali *et al.*, 2025). Rice grain protein is predominantly composed of storage proteins such as glutelins and prolamins, which largely determine the nutritional and physicochemical characteristics of milled rice (Bharali *et al.*, 2025). Moreover,

amino acid transporters belonging to the amino acid permease (AAP) family play crucial roles in nitrogen allocation and grain development. Functional studies have demonstrated that *OsAAP14* regulates rice tillering, while *OsAAP15* enhances panicle branching and grain yield by modulating tyrosine and valine concentrations in the panicle (Jin *et al.*, 2024). These transporters, along with those involved in the remobilization of amino acids from vegetative tissues, constitute complex regulatory networks that can be strategically manipulated to improve the protein quality of cereal crops through genetic improvement.

5.3. Omics-based Approaches in Amino Acid Regulation

Transcriptome analyses have enabled the identification of key genes and metabolic pathways associated with amino acid biosynthesis and nitrogen distribution within plant tissues, thereby providing insights into the regulation of essential metabolic processes influencing plant growth and productivity (Li *et al.*, 2025). In cereal crops, integrated transcriptomic and metabolomic investigations have further revealed dynamic changes in gene expression and metabolite accumulation associated with amino acid metabolism and grain protein formation during seed development (Liu *et al.*, 2022). Notably, recent multi-omics studies have demonstrated that nitrogen availability plays a critical role in regulating amino acid metabolism and seed storage protein synthesis through coordinated transcriptional and metabolic adjustments during grain development (Ou *et al.*, 2025).

Integrated transcriptomic and metabolomic analyses have demonstrated that sulphur availability significantly influences the expression of genes involved in amino acid metabolism and protein synthesis, thereby affecting grain nutritional quality in wheat. In particular, sulphur plays a crucial role in the synthesis of sulphur-containing amino acids such as cysteine and methionine, which are essential for both protein quality and functional properties of wheat flour (Liu *et al.*, 2022). Advances in omics-based technologies, including genomics, proteomics, and metabolomics, have further expanded our understanding of the complex regulatory networks governing nutrient metabolism and grain composition. These approaches enable the identification of key metabolic pathways and candidate genes associated with important nutritional traits, thereby facilitating crop biofortification strategies (Kudapa *et al.*, 2023). The integration of diverse germplasm resources with genomics, transcriptomics, proteomics, metabolomics and molecular marker-assisted selection has significantly accelerated the development of Nutritionally enhanced cereal cultivars. These integrated breeding approaches facilitate the identification of superior alleles and genomic regions associated with amino acid biosynthesis, stress tolerance and yield-related traits, thereby enabling the development of high-yielding, disease-resistant, climate-resilient and nutrition enriched cereal varieties (Fig. 4).

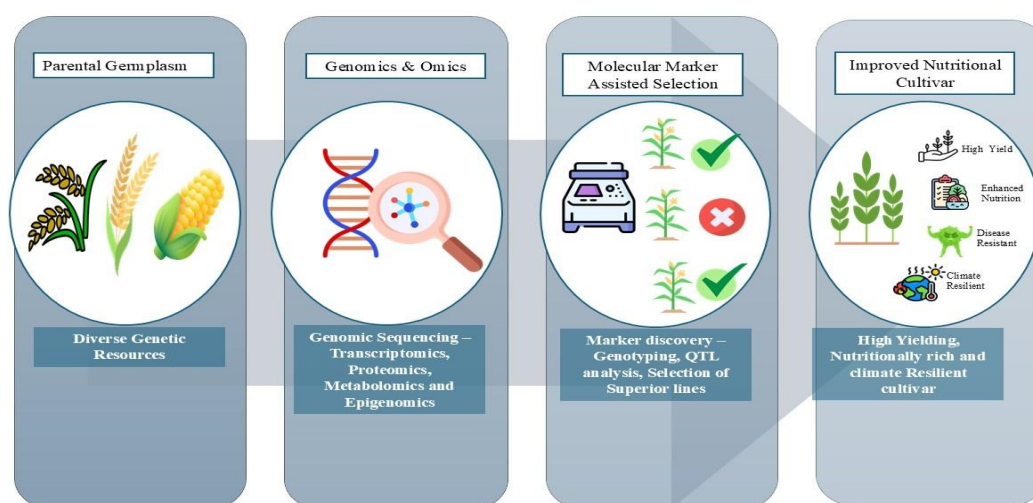


Fig. 4 Integrated breeding approaches for development of high-yielding, disease-resistant, climate-resilient and nutrition enriched cereal varieties

6. Genetic improvement strategies

6.1. Classical breeding

Conventional breeding strategies for improving amino acid composition in cereal crops have primarily relied on the exploitation of natural genetic variation within diverse germplasm resources. These approaches typically involve hybridization between genetically diverse accessions followed by phenotypic selection to identify genotypes with superior nutritional traits. Landraces and crop wild relatives often serve as valuable sources of favourable alleles, which can be introgressed into elite breeding lines through successive backcrossing and selection to enhance grain nutritional quality (Zenda *et al.*, 2021). Such strategies have contributed to the development of crop varieties with improved protein composition and essential amino acid profiles (Table 2). In maize, for instance, breeding programs targeting enhanced methionine content have utilized natural variation and mutations affecting seed storage proteins, particularly zeins, which regulate sulphur-containing amino acid accumulation in the endosperm (Devi *et al.*, 2023). However, the polygenic nature of amino acid inheritance and the significant impact of genotype-by-environment ($G \times E$) interactions necessitate advanced diagnostic tools to ensure stable and targeted biofortification. Therefore, advanced analytical techniques, including chromatographic and spectroscopic approaches, are increasingly employed for high-resolution profiling of

amino acids. The integration of conventional breeding with such advanced phenotyping tools enhances the efficiency of selection and accelerates the development of nutritionally improved cereal cultivars.

Table 2 Commercial release of genotypes with improved amino acid composition and enhanced grain protein quality in major cereal crops

CROP	VARIETIES & HYBRIDS	QUALITY TRAITS	SOURCE
RICE	BPT Rice 2841 (Swarna x IRGC18195 x MTU1081)	High protein, lysine	Veni <i>et al.</i> , (2025)
	HAHP_101	High lysine	Badoni <i>et al.</i> , (2024)
	Milyang 320	High lysine	Lee and Masclaux-Daubresse (2021)
	Dharial mutant-derived lines (Induced mutation by EMS)	High protein, lysine	
	CR-Dhan 310 and CR-Dhan 311	High protein	Chattopadhyay <i>et al.</i> , (2019)
WHEAT	SNZM 1	High valine, threonine, phenylalanine	Zhu <i>et al.</i> , (2025)
	QYZ-1	High protein, lysine, methionine	
	Blue wheat 20072	High threonine, lysine	
	Atlas 50 and Atlas 66	High protein	Shewry and Hey (2015)
	ZY4, ZY5, ZY11, ZY17-1, ZY17-2, ZY21, ZY22 (Interspecific hybridization)	High in hydrolysed amino acids	Guo <i>et al.</i> , (2013)
MAIZE	Vivek QPM 9	High lysine, tryptophan	Gupta <i>et al.</i> , (2013)
	Pusa Vivek QPM 9 Improved	High lysine, tryptophan	Yadava <i>et al.</i> , (2020)
	Pusa HM 4 Improved	High lysine, tryptophan	
	Pusa HM 8 Improved	High lysine, tryptophan	
	Pusa HM 9 Improved	High lysine, tryptophan	
	Pusa HQPM 1 Improved	High provitamin-A, lysine, tryptophan	
	Pusa HQPM 5 Improved		
	Pusa HQPM 7 Improved		
	Pusa Biofortified Maize Hybrid		
	LQMH 1, LQMH 2, LQMH 3	High lysine, tryptophan	
	Malviya Swarn Makka 1	High lysine, tryptophan	
	Opaque-2 (Spontaneous mutation)	High lysine, tryptophan	Emerson <i>et al.</i> , (1935)
	Opaque-7 (Spontaneous mutation)	High lysine, tryptophan	Misra <i>et al.</i> , (1972)
	Floury-2 (Spontaneous mutation)	High lysine and S-amino acids	Bjarnason <i>et al.</i> , (1992)
	Opaque7749, Opaque7455 [o11] (Spontaneous mutation)	High lysine, methionine	Nelson (1981)
BARLEY	Hiproly (Spontaneous mutation)	High protein, lysine	Rasmussen <i>et al.</i> , (1988), Jood and Singh (2001)
	Notch-1 (Induced mutation by EMS)	High protein, lysine	Jood and Singh (2001)
	Notch-2 (Induced mutation by EMS)	High protein, lysine	Jood and Singh (2001)
	Riso 1508 (Induced mutation by EI)	High lysine	Jood and Singh (2001)
	Lysimax	High lysine	Gabert <i>et al.</i> , (1995)
	Ca 700202	High lysine	Newman <i>et al.</i> , (1990)
SORGHUM	IS 11167 (Spontaneous mutation)	High lysine	Singh and Axtell (1973)
	IS 11758 (Spontaneous mutation)	High lysine	Singh and Axtell (1973)
	P-721 opaque	High lysine	Fano (2017)

	(Induced mutation by DES)			
	P-721 vitreous (Induced mutation by DES)		High lysine	Fano (2017)
PEARL MILLET	PMiGAP016, PMiGAP081, PMiGAP167, PMiGAP271, PMiGAP306, PMiGAP331, PMiGAP332	PMiGAP071, PMiGAP155, PMiGAP263, PMiGAP281, PMiGAP311,	High protein, high EAA especially in leucine, isoleucine, methionine, tryptophan	Singh <i>et al.</i> , (2024)

6.2. Mutagenesis and TILLING

Beyond conventional breeding approaches, induced mutagenesis has been widely employed to generate novel genetic variability for improving amino acid composition in cereal crops. Chemical mutagens such as ethyl methane sulphonate (EMS) and physical mutagens, including gamma irradiation, are capable of introducing genome-wide mutations, thereby producing diverse phenotypic variants that can be exploited for nutritional improvement (Xin 2023). Such mutagenesis-based approaches have historically contributed to the identification of nutritionally enhanced mutants, including high-lysine maize genotypes such as *opaque-2*, which significantly altered endosperm protein composition by reducing zein fractions and increasing non-zein storage proteins, thereby improving essential amino acid balance (Mertz *et al.*, 1964, Prasanna *et al.*, 2001). The integration of mutagenesis with advanced reverse-genetics platforms, particularly Targeting Induced Local Lesions in Genomes (TILLING), enables the efficient detection of point mutations affecting genes involved in amino acid metabolism and protein synthesis (Till *et al.*, 2004). In parallel, modern genome-editing technologies such as CRISPR/Cas9 have revolutionized crop improvement by enabling precise modification of genes controlling metabolic pathways, including those associated with asparagine synthesis and nitrogen metabolism in cereal grains (Oddy *et al.*, 2021). These technologies allow the generation of targeted allelic diversity that may not be readily available within natural germplasm resources. Furthermore, the integration of mutagenesis strategies with next-generation sequencing (NGS) platforms facilitates rapid identification and characterisation of causal mutations underlying desirable phenotypes (Hudson 2022, Xin 2023). High-throughput sequencing of mutant populations has greatly accelerated functional gene discovery and the development of improved crop varieties. Collectively, the synergistic application of mutagenesis, reverse genetics, genome editing, and genomic sequencing provides powerful tools for enhancing amino acid composition and overall nutritional quality in cereal crops.

6.3. Marker-assisted selection

The integration of molecular markers tightly linked to desirable amino acid traits has emerged as a powerful strategy to accelerate breeding cycles and enhance selection efficiency by enabling early identification of superior genotypes. Marker systems such as single-nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) have been widely employed for the detection of quantitative trait loci (QTLs) associated with amino acid composition and grain quality traits in crop species. These markers facilitate the identification of candidate genes controlling complex nutritional traits and enable more precise selection in breeding populations (Ación *et al.*, 2023, Li *et al.*, 2024). For instance, high-density genetic mapping and QTL analysis have successfully identified genomic regions regulating essential amino acid content in soybean, thereby providing valuable resources for marker-assisted selection in crop improvement programs (Li *et al.*, 2024). In maize, genomics-assisted breeding has been effectively applied to introgress nutritionally important genes such as *waxy1* (*wx1*) and *opaque2* (*o2*), resulting in hybrids with enhanced lysine and tryptophan levels while maintaining desirable agronomic performance (Talukder *et al.*, 2022). The use of gene-based markers in marker-assisted backcross breeding significantly reduces the reliance on labour-intensive phenotypic screening and accelerates the recovery of elite genetic backgrounds. Furthermore, the integration of genomic selection and other advanced molecular breeding approaches has improved the efficiency of crop improvement programs by enabling the simultaneous selection of multiple complex traits, including grain quality and stress tolerance in maize (Kim *et al.*, 2026). Collectively, genomics-assisted breeding strategies provide an efficient framework for the rapid development of nutritionally enhanced cereal cultivars.

6.4. QTL identification

The identification and characterisation of quantitative trait loci (QTLs) and candidate genes controlling amino acid composition are important objectives in genomics, linking phenotypic variation to underlying molecular mechanisms. QTL mapping and genome-wide association studies (GWAS) have revealed that amino acid accumulation in cereal grains is governed by complex polygenic architectures, with numerous loci contributing to metabolic variation across the genome. In maize, extensive genomic analyses have identified hundreds of loci associated with kernel amino acid composition. For instance, Deng *et al.*, (2017) reported 528 loci linked with 308 candidate genes controlling amino acid variation, while Wen *et al.*, (2018) identified 153 significant loci influencing 61 primary metabolites, including 23 amino acids. These studies highlight the intricate genetic control of essential amino acids such as lysine, methionine, and tryptophan, which are critical determinants of grain nutritional quality.

Similarly, in rice, genomic studies have identified multiple chromosomal regions associated with amino acid content, demonstrating the quantitative inheritance of this trait. Numerous QTLs associated with essential and

non-essential amino acid composition have been identified across major cereal crops including rice, wheat, maize and barley using diverse mapping populations and molecular marker systems. These QTLs are distributed across multiple chromosomes and associated with key amino acids such as lysine, methionine, tryptophan, threonine and branched-chain amino acids highlighting the complex genetic architecture in grain nutritional quality (Table 3). For instance, several mapping studies have detected numerous QTLs controlling different amino acid components, with major clusters frequently reported on chromosomes 1, 7, and 9 (Wang *et al.*, 2023).

Limitations: Despite the increasing number of identified QTLs, the cloning of genes underlying amino acid variation remains limited because classical map-based cloning of quantitative traits is often laborious and time-consuming (Wang *et al.*, 2023). To overcome these limitations, haplotype-based association studies and high-density genomic analyses are increasingly used to directly identify candidate genes and key natural variations responsible for amino acid traits. These approaches enable researchers to transition from broad QTL intervals to specific genes involved in amino acid metabolism, such as those associated with the aspartate-derived pathway controlling lysine and methionine biosynthesis (Pott *et al.*, 2021). Furthermore, epistatic interactions among loci often contribute significantly to metabolic variation, underscoring the complexity of genetic regulation governing amino acid accumulation in grains (Pott *et al.*, 2021). Major QTLs identified in rice, wheat, maize and barley for amino acid related traits.

Table 3 QTLs identified for cereals

RICE							
POPULATION/ CROSS	POPULATION SIZE	QTL	TRAIT	CHROMOSOME	MARKERS	SOURCE	
Zhenshan97 x Nanyangzhan	RIL population (190 lines)	<i>qAA.1</i>	EAA/Asp/Thr/Glu/Gly/Ala/Cys/Tyr/Pro	1	RM472-RM104	Wang <i>et al.</i> , (2008)	
Zhenshan97B x Delong208	RIL population (188 lines)	<i>qAa1</i>	Asp/Thr/Ser/Glu/Gly/Ala/Val/Ile/Leu/Phe/His/ Arg/Pro/TAA		RM493-RM562	Zhong (2011)	
Milyang23 x Tong88-7	F ₁₅ RILs	<i>qAAC1.1</i> ; <i>qAAC1.2</i> ; <i>qAAC1.6</i>	His		2886294-3539052; 4301442-4494576; 39162572-39234399	Jang <i>et al.</i> , (2020)	
		<i>qAAC1.3</i> ; <i>qAAC1.4</i> ; <i>qAAC1.5</i>	Ser		4632918-4708475; 5154287-5197935; 37112359-37711021		
ZS97 x MY 46	RIL Population (247 lines)	<i>qAsp1</i>	Asp		RG146-RM24	Xie <i>et al.</i> , (2020)	
Zhenshan97 x Nanyangzhan	RIL population (190 lines)	<i>qAA.2</i>	Val/Ile/Leu/His/Phe	2	RM324-RM301, RM322-RM521	Wang <i>et al.</i> , (2008)	
Milyang23 x Tong88-7	F ₁₅ RILs	<i>qAAC2.1</i> ; <i>qAAC2.2</i>	Ser		4065908-4220624; 18092703-18131695	Jang <i>et al.</i> , (2020)	
ZS97 x MY 46	RIL Population (247 lines)	<i>qThr2</i>	Thr		RM263-RM6	Xie <i>et al.</i> , (2020)	
		<i>qSer2</i>	Ser		RM6-RM240		
		<i>qVal2</i>	Val		RM6-RM240		
		<i>qIle2</i>	Ile		RM240-RZ123		
		<i>qTyr2</i>	Tyr		RZ318-RM263		
Milyang23 x Tong88-7	F ₁₅ RILs	<i>qAAC3.1</i>	Ala	3	4542873-4614153	Jang <i>et al.</i> , (2020)	
		<i>qAAC3.2</i>	Gly		5140419-5225129		
Dasanbyeon TR22183	172 F ₁₄ RILs	<i>qAla3</i>	Ala		id3015453-id3016090	Yoo (2017)	
		<i>qVal3</i>	Val		id3015453-id3016090		
		<i>qLeu3</i>	Leu		id3015453-id3016090		
		<i>qPhe3</i>	Phe		id3015453-id3016090		
		<i>qLys3</i>	Lys		id3015453-id3016090		
Zhenshan97 x Nanyangzhan	RIL population (190 lines)	<i>qAA.3</i>	Tyr		RM520-RM468	Wang <i>et al.</i> , (2008)	

ZS97 x MY 46	RIL Population (247 lines)	<i>qGlu3</i>	Glu		RZ328-RZ575	Xie <i>et al.</i> , (2020)
		<i>qArg3</i>	Arg		RZ328-RZ575	
ZS97 x MY 46	RIL Population (247 lines)	<i>qThr4</i>	Thr	4	RG776A-RG620	Xie <i>et al.</i> , (2020)
		<i>qGly4</i>	Gly		RM3317-RM401	
		<i>qVal4</i>	Val		RM303-RG214	
		<i>qIle4</i>	Ile		RM3317-RM401	
		<i>qPhe4</i>	Phe		RM303-RG214	
		<i>qHis4</i>	His		RG454-RM273	
Zhenshan97 x Nanyangzhan	RIL population (190 lines)	<i>qAA.4</i>	Thr/Gly/His/Arg		RM348-RM131	Wang <i>et al.</i> , (2008)
Milyang23 x Tong88-7	F ₁₅ RILs	<i>qAAC6.1</i>	Ala/Arg/Asx/Glx/Gly/Ile/Leu /Met/Phe/Tyr/Val	6	3515361-3658340	Jang <i>et al.</i> , (2020)
		<i>qAAC6.2</i>	Met		4192641-4230522	
ZS97 x MY 46	RIL Population (247 lines)	<i>qAsp6</i>	Asp		RM225-RM6917	Xie <i>et al.</i> , (2020)
		<i>qThr6</i>	Thr		RM225-RM6917	
		<i>qSer6</i>	Ser		RM225-RM6917	
		<i>qGlu6</i>	Glu		RM225-RM6917	
		<i>qGly6</i>	Gly		RM225-RM6917	
		<i>qAla6</i>	Ala		RM253-RM276	
		<i>qVal6</i>	Val		RM225-RM6917	
		<i>qMet6</i>	Met		RM190- RZ516	
		<i>qLeu6</i>	Leu		RM6917-RZ450	
		<i>qTyr6</i>	Tyr		RZ398- RM204	
		<i>qPhe6</i>	Phe		RM225-RM6917	
		<i>qLys6</i>	Lys		RM225-RM6917	
		<i>qHis6</i>	His		RZ398- RM225	
		<i>qArg6</i>	Arg		RM225-RM6917	
		<i>qPro6.1</i>	Pro		RM225-RM6917	
		<i>qPro6.2</i>	Pro		RZ667-RM19784	
Milyang23 x Tong88-7	RIL population (190 lines)	<i>qAA.7</i>	Pro/Gly/Met/Arg	7	RM125-RM214	Wang <i>et al.</i> , (2008)
Zhenshan97B x Delong208	RIL population (188 lines)	<i>qAa7</i>	Asp/Thr/Ser/Glu/Gly/Ala/Cys/Val /Met/Ile/Phe/Arg/Pro/Taa		MRG186–MRG4499	Zhong (2011)
Milyang23 x Tong88-7	F ₁₅ RILs	<i>qAAC7.1</i>	Arg/Ile/Leu/Phe/Ala/Asx/Glx /Ser/Tyr/Val		4856196-5206110	Jang <i>et al.</i> , (2020)
		<i>qAAC7.2</i>	Thr		5627191-5803738	
		<i>qAAC7.3</i>	Gly		11635687-14689865	
ZS97 x MY 46	RIL Population (247 lines)	<i>qAsp7</i>	Asp		RM3325-RM1243	Xie <i>et al.</i> , (2020)
		<i>qThr7</i>	Thr		RM3325-RM1243	
		<i>qSer7</i>	Ser		RM1243-RM3859	
		<i>qGly7.2</i>	Gly		RM3325-RM1243	
		<i>qCys7</i>	Cys		RZ721-RZ395	
		<i>qVal7</i>	Val		RM3325-RM1243	
		<i>qTyr7.1;</i> <i>qTyr7.2</i>	Tyr		RM1243-RM3859; RZ626-RG650	
		<i>qPhe7</i>	Phe		RM3325-RM1243	
		<i>qArg7</i>	Arg		RM3325-RM1243	

		<i>qPro7.1</i> ; <i>qPro7.2</i>	Pro		RZ471-RM320; RM336-RZ264	
Zhenshan97 x Nanyangzhan	RIL population (190 lines)	<i>qAA.8</i>	Cys	8	RM447-RM458	Wang <i>et al.</i> , (2008)
Milyang23 x Tong88-7	F ₁₅ RILs	<i>qAAC8.1</i>	Gly		19156876-26250116	Jang <i>et al.</i> , (2020)
		<i>qAAC8.2</i>	Lys		26398897-26510920	
ZS97 x MY 46	RIL Population (247 lines)	<i>qSer8</i>	Ser		RG108-RIX4	Xie <i>et al.</i> , (2020)
Zhenshan97B x Delong208	RIL population (188 lines)	<i>qAa9</i>	Asp/Thr/Ser/Gly/Val/Ile/Phe/Lys/ Total	9	RM328-RM107	Zhong (2011)
ZS97 x MY 46	RIL Population (247 lines)	<i>qCys9</i>	Cys		RM105-RM3700	Xie <i>et al.</i> , (2020)
		<i>qMet9</i>	Met		RZ698-RM296	
		<i>qPhe9</i>	Phe		RM257-RM242	
		<i>qLys9</i>	Lys		RM242-RM108	
		<i>qHis9</i>	His		RM242-RM108	
Zhenshan97 x Nanyangzhan	RIL population (190 lines)	<i>qAA.10</i>	Cys/Leu/Ile/Phe	10	RM467-RM271	Wang <i>et al.</i> , (2008)
ZS97 x MY 46	RIL Population (247 lines)	<i>qPro10</i>	Pro		RM5348-RM1859	Xie <i>et al.</i> , (2020)
ZS97 x MY 46	RIL Population (247 lines)	<i>qThr11</i>	Thr	11	RM287-RM209	Xie <i>et al.</i> , (2020)
		<i>qGlu11</i>	Glu		RZ816-RM332	
		<i>qGly11</i>	Gly		RM287-RM209	
		<i>qMet11</i>	Met		RZ816-RM332	
		<i>qIle11</i>	Ile		RG167-RM287	
ZS97 x MY 46	RIL Population (247 lines)	<i>qCys12</i>	Cys	12	RG81-S13126	Xie <i>et al.</i> , (2020)
		<i>qTyr12</i>	Tyr		RM20-RG81	
		<i>qLys12</i>	Lys		RM20-RG81	
		<i>qHis12</i>	His		RM20-RG81	

WHEAT						
POPULATION/ CROSS	POPULATION SIZE	QTL	TRAIT	CHROMOSOME	MARKERS	SOURCE
Huapei 3 x Yumai 57	DH population (168 lines)	<i>QLys1A-1</i>	Lys	1A	Xwmc550-Xbarc269	Jiang <i>et al.</i> , (2013)
		<i>QLys1A-2</i>	Lys		Xgwm498-Xcwem6.2	
		<i>QThr1A</i>	Thr		Xgwm498-Xcwem6.2	
		<i>QHis1A</i>	His		Xgwm498-Xcwem6.2	
		<i>QHis1B</i>	His	1B	Xgwm582-Xcfe026.2	
		<i>QGlu1D</i>	Glu	1D	Xwmc429-Xcfd19	
Huapei 3 x Yumai 57	DH population (168 lines)	<i>QLys2A</i>	Lys	2A	Xwmc455-Xgwm515	Jiang <i>et al.</i> , (2013)
		<i>QVal2A</i>	Val		Xgwm448-Xwmc455	
		<i>QArg2B-1</i>	Arg	2B	Xwmc661-Xbarc200	
		<i>QArg2B-2</i>	Arg		Xgwm388-Xbarc101	
		<i>QMet2B</i>	Met		Xcwem55-Xbarc129.1	
		<i>QPro2D-1</i>	Pro	2D	Xcfd53-Xwmc18	
		<i>QPro2D-2</i>	Pro		Xcfd161-Xgwm311.2	
Huapei 3 x Yumai 57	DH population (168 lines)	<i>QLys3A</i>	Lys	3A	Xbarc356-Xwmc489.2	Jiang <i>et al.</i> , (2013)
		<i>QThr3A</i>	Thr		Xbarc356-Xwmc489.2	
		<i>QIle3A</i>	Ile		Xgwm155-Xcfa2170	
		<i>QVal3A</i>	Val		Xbarc356-Xwmc489.2, Xgwm155-Xcfa2170	
		<i>QLeu3A</i>	Leu		Xbarc86-Xwmc21	
		<i>QPro3A</i>	Pro		Xbarc356-Xwmc489.2	
		<i>QGlu3A</i>	Glu		Xswes107-Xbarc86, Xbarc356-Xwmc489.2	
		<i>QAsp3A</i>	Asp		Xbarc356-Xwmc489.2	

		<i>QSer3A</i>	Ser		Xswes107-Xbarc86, Xbarc86-Xwmc21				
		QGly3A	Gly		Xbarc356-Xwmc489.2, Xbarc86-Xwmc21				
		QAla3A	Ala		Xbarc356-Xwmc489.2, Xgwm155-Xcfa2170				
		QTyr3A	Tyr		Xbarc86-Xwmc21, Xcfa2170-Xbarc51				
		QArg3A	Arg		Xbarc356-Xwmc489.2				
		QCys3D	Cys		3D		Xgdm72-Xbarc1119		
		QMet3D	Met		Xgdm72-Xbarc1119				
Huapei 3 x Yumai 57	DH population (168 lines)	QGlu4A	Glu	4A	Xwmc313-Xwmc497	Jiang <i>et al.</i> , (2013)			
		QAsp4A-1	Asp	Xbarc343-Xwmc313					
		QAsp4A-2	Asp	Xbarc362-Xbarc078					
		QIle4B	Ile	4B	Xwmc413-Xcfd39.2				
		QPhe4B	Phe	Xwmc47-Xwmc413					
		QVal4B	Val	Xwmc125-Xwmc47					
		QPhe4D	Phe	4D	Xcfe254-BE293342				
Huapei 3 x Yumai 57	DH population (168 lines)	QPhe5A-1	Phe	5A	Xswes45-Xbarc180	Jiang <i>et al.</i> , (2013)			
		QPhe5A-2	Phe	Xcwem40-Xbarc358.2					
		QThr5B	Thr	5B	Xbarc36-Xbarc140				
		QGly5B	Gly	Xbarc36-Xbarc140					
		QHis5B	His	Xbarc36-Xbarc140					
		QArg5B	Arg	Xbarc36-Xbarc140					
		Huapei 3 x Yumai 57	DH population (168 lines)	QLys6A	Lys		6A	Xgwm82-Xwmc553	Jiang <i>et al.</i> , (2013)
QPro6A	Pro			Xcfe179.1-Xswes170.2					
QPhe6A	Phe			Xwmc553-Xgwm732					
QTyr6A	Tyr			Xwmc553-Xgwm732					
QHis6A	His			Xwmc553-Xgwm732					
QLys6B	Lys			6B	Xwmc74-Xgwm58				
QAsp6B	Asp			Xcfa2187-Xgwm219					
QGly6B	Gly			Xwmc74-Xgwm58					
QHis6B	His			Xwmc415-Xswes170.1					
QThr6B	Thr			Xwmc74-Xgwm58					
QGlu6B-1	Glu			Xbarc1129-Xcfa2257					
QGlu6B-2	Glu			Xwmc74-Xgwm58					
QAsp6B	Asp			Xwmc74-Xgwm58					
QGly6B	Gly			Xwmc74-Xgwm58					
QArg6B	Arg			Xwmc74-Xgwm58					
QIle6D	Ile			6D	Xswes861.1-Xgwm681				
QPhe6D	Phe			Xubc808-Xswes679.1					
QPhe6D-1	Phe			Xcfd42-Xcfd13					
QPhe6D-2	Phe			Xswes861.1-Xgwm681					
QLeu6D	Leu			Xswes861.1-Xgwm681					
QAsp6D	Asp			Xswes861.1-Xgwm681					
QPro6D	Pro			Xgwm681-Xubc808					
QSer6D	Ser			Xgwm681-Xubc808					
Huapei 3 x Yumai 57	DH population (168 lines)			QAsp7B	Asp	7B	Xwmc273.1-Xcfd22.1	Jiang <i>et al.</i> , (2013)	
				QHis7B	His	Xgwm333-Xwmc10			
				QGlu7D	Glu	7D	Xgwm428-Xcfd175		
				QSer7D	Ser	Xgwm428-Xcfd175			
				QGly7D	Gly	Xgdm67-Xwmc634			
				QArg7D	Arg	Xgdm67-Xwmc634			
				QLeu7D	Leu	Xgdm67-Xwmc634			
				QGly7D	Glv	Xgdm67-Xwmc634			

MAIZE						
POPULATION/ CROSS	POPULATION SIZE	QTL	TRAIT	CHROMOSOME	MARKER/INTERVAL (Mbp)	SOURCE
DQL 2104-1 x DQL 2034	109 F _{2:3}	<i>qTRP9.1</i>	Trp	9	umc1258-bnlg1209	Jasrotia <i>et al.</i> , (2025)
AF109	248 DH lines	<i>qLYS-1-1</i>	Lys	3	59.07-97.50	Zhang, Wen, <i>et</i>

(KB319003 x AJ317001)		<i>qLYS-1-2</i>		4	37.64–65.21	<i>al.</i> , (2024)
		<i>qLYS-1-3</i>		9	129.70-137.80	
AF116 (KB519007 x KB717001)	190 DH lines	<i>qLYS-2-1</i>	Lys	3	161.43–169.00	
		<i>qLYS-2-2</i>		9	130.80-149.39	
AF129 (KB320005 x KB120001)	316 DH lines	<i>qLYS-3-1</i>	Lys	1	267.37-271.96	
		<i>qLYS-3-2</i>		2	210.90-219.60	
		<i>qLYS-3-3</i>		6	117.49-141.72	
AF170 (JinQingWL2 x KB319004)	265 DH lines	<i>qLYS-4-1</i>	Lys	1	143.21-159.07	
		<i>qLYS-4-2</i>		5	12.70-13.55	
		<i>qLYS-4-3</i>		9	93.07-125.24	
		<i>qLYS-4-4</i>		10	13.61–17.92	
B73 x By804, Kui3 x B77, Zong3 x Yu87-1	3 RIL populations	<i>qMet2</i>	Met	2	PZE-102080745	Devi et al.,2023 Deng et al.,2017
		<i>qMet2.1</i>			SYN1715	
		<i>qMet3</i>		3	PZE-103099938	
		<i>qMet4</i>		4	SYN2317	
		<i>qMet4.1</i>			PZE-104138961	
		<i>qMet8</i>		8	PZE-108087230	
		<i>qMet/Total1</i>	Met/Total amino acids	1	ZM013506-0433	
		<i>qMet/Total2</i>		2	PZE-102080745	
		<i>qMet/Total3</i>		3	SYN37729	
		<i>qMet/Total3.1</i>			PZE-103075963	
		<i>qMet/Total3.2</i>			PUT-163a-101384606-6	
		<i>qMet/Total6</i>		6	PZE-106006541	
		<i>qMet/Total7</i>		7	SYNGENTA3646	
B73o2 x CML161	146 RILs	<i>qMet5</i>	Met	5	bnlg1046	Rojas et al.,2010
		<i>qMet7</i>		7	umc2142	
		<i>qMet8</i>		8	umc1304	
Oh545o2 x Oh51Ao2	F2:3 progenies	<i>qFAA</i>	FAA	2L	bmc1633, bmc1329	Wang and Larkins (2001)
		<i>qFAA</i>	FAA	2S	bmc1537, bmc2248	
		<i>qFAA</i>	FAA	3S	bmc1904, bmc2136, bmc1452	
		<i>qFAA</i>	FAA	7L	bmc2328b, phi045	

TABLE 1

POPULATION/ CROSS	POPULATION SIZE	QTL	TRAIT	CHROMOSOME	MARKERS	SOURCE
IMLEL9 x mooner No.3	F ₉ RILs (193 families)	QHIS. KAAS-2H	His	2H	Scssr03381-Scssr07759	Yan <i>et al.</i> , (2021)
		QHIS. KAAS-4H		4H	HVBAMMGB84- BMAG0808	
		QHIS. KAAS-7H		7H	GBM1303-GMS056	

(TAA-Total amino acids, FAA-Free amino acids, EAA-Essential amino acids, Ala-Alanine, Arg-Arginine, Asx-Asparagine and Aspartic acid, Cys-Cysteine, Glx-Glutamic acid and Glutamine, Gly-Glycine, His-Histidine, Ile-Isoleucine, Leu-Leucine, Lys-Lysine, Met- Methionine, Phe-Phenylalanine, Pro-Proline, Ser-Serine, Thr-Threonine, Trp-Tryptophan, Tyr-Tyrosine, Val-Valine)

6.5. Fine mapping

The refinement of broad quantitative trait loci (QTL) intervals into precise genetic determinants is a crucial step in identifying polymorphisms underlying variation in amino acid composition in cereal grains. Because cereal genomes often exhibit substantial linkage disequilibrium, distinguishing functional variants from closely linked markers requires high-resolution genetic mapping approaches. Strategies such as the development of near-isogenic lines, heterogeneous inbred families, and fine-mapping populations have been widely employed to reduce QTL intervals and facilitate the identification of candidate genes controlling metabolic traits. In maize, the identification of QTLs associated with methionine accumulation has provided important insights into the genomic regions regulating sulphur-rich storage proteins such as δ -zeins, which are key determinants of kernel protein quality (Devi *et al.*, 2023). These discoveries offer promising opportunities for developing maize lines with enhanced methionine content through targeted breeding and molecular interventions.

In parallel, genome-wide association studies (GWAS) have emerged as a powerful complementary approach to traditional linkage mapping by exploiting natural genetic diversity and high-density single nucleotide polymorphism markers to identify genomic loci associated with metabolic traits (Pandey *et al.*, 2023). GWAS enables the detection of loci influencing amino acid composition with improved mapping resolution by leveraging historical recombination events present in diverse germplasm collections. However, the effectiveness of this approach can be influenced by factors such as population structure and the extent of linkage disequilibrium within the study population.

6.6. GWAS studies

Genome-wide association studies (GWAS) have substantially advanced the dissection of complex metabolic traits by utilizing high-density molecular markers and diverse germplasm panels to identify genomic regions associated with metabolite variation. The rapid development of next-generation sequencing technologies has enabled large-scale SNP genotyping, thereby facilitating the identification of loci governing metabolic pathways and nutritional traits in crop plants (Pott *et al.*, 2021). Unlike traditional biparental mapping populations that capture only limited allelic diversity, association mapping panels exploit natural genetic variation across diverse accessions, significantly improving mapping resolution and enabling the detection of multiple loci influencing metabolite accumulation. In maize, integrative genomic and metabolomic analyses have revealed numerous loci associated with amino acid variation, including genes regulating lysine and phenylalanine accumulation as well as the lysine-to-total amino acid ratio during kernel development (Pott *et al.*, 2021). These findings highlight the complex genetic architecture underlying essential amino acid biosynthesis in cereal grains.

Similarly, in rice, GWAS approaches have been effectively applied to unravel the genetic basis of free amino acid content using large panels of diverse accessions and millions of genome-wide SNP markers. Studies involving hundreds of rice accessions have identified numerous quantitative trait nucleotides associated with variations in branched-chain and aromatic amino acids such as valine, leucine, isoleucine, arginine, and tryptophan (He *et al.*, 2022). These results demonstrate that amino acid accumulation is typically governed by numerous small-effect loci distributed across the genome, reflecting the polygenic nature of metabolic traits. The prevalence of such minor loci complicates breeding for amino acid enhancement, as their cumulative effects often obscure the detection of major QTLs unless high statistical power and large populations are employed.

To overcome these limitations, researchers increasingly integrate metabolomics with genomic analyses through metabolite-based genome-wide association studies (mGWAS), which directly correlate metabolic phenotypes with genetic variation. In rice, mGWAS approaches have enabled the quantitative profiling of hundreds of metabolites across diverse natural populations, leading to the identification of numerous loci associated with specific amino acid levels that remain undetected through conventional phenotypic selection (Feng *et al.*, 2025, He *et al.*, 2022). Such studies have identified stable quantitative trait nucleotides and candidate genes involved in primary metabolic processes, particularly those associated with amino acid biosynthesis and tryptophan metabolism (He *et al.*, 2022).

6.7. Genomic selection

Genomic selection (GS) represents a significant advancement over conventional marker-assisted breeding by utilizing genome-wide molecular markers to predict genomic estimated breeding values (GEBVs) for complex quantitative traits. Unlike traditional approaches that target only a few major quantitative trait loci, GS captures the cumulative effects of numerous small-effect loci distributed across the genome, thereby enabling a more comprehensive assessment of genetic potential. Genome-wide association studies combined with genomic prediction models have been successfully used to identify genomic regions associated with amino acid composition and other nutritional quality traits in crop species (Pandey *et al.*, 2023). By integrating high-density genotypic information with phenotypic data, genomic selection models allow breeders to predict the performance of untested genotypes and accelerate genetic gain in breeding populations (Sinha *et al.*, 2021).

Furthermore, genomic prediction approaches effectively capture the collective influence of minor genes and environmental interactions that govern complex agronomic and quality traits, thereby improving prediction accuracy compared with traditional marker-assisted selection (Kumar *et al.*, 2024). These models have also demonstrated strong potential for predicting traits such as root system architecture and other agronomic characteristics, highlighting their value for enhancing selection efficiency in plant breeding programs (Sharma *et al.*, 2021). The increasing availability of high-throughput genotyping platforms, next-generation sequencing technologies, and advanced statistical models has further expanded the application of genomic selection across diverse crop improvement programs (Naqvi *et al.*, 2022). Collectively, genomic selection provides a robust

genomics-assisted breeding framework for accelerating the development of crop varieties with improved nutritional quality and enhanced agronomic performance.

7. Biotechnological interventions

Genetic engineering provides powerful avenues for introducing or enhancing genes encoding enzymes involved in amino acid biosynthesis, storage protein modification, or catabolism, thereby directly altering the amino acid composition in cereal grains. These interventions often involve the overexpression of key enzymes in specific biosynthetic pathways, leading to increased accumulation of target essential amino acids, or the silencing of genes that degrade these valuable compounds.

7.1. Metabolic engineering

This strategy involves the systematic alteration of enzyme activities or gene expression within biosynthetic pathways to channel metabolic flux towards increased synthesis of specific amino acids (Yuan and Alper 2019). This can include overexpression of genes encoding rate-limiting enzymes, introduction of novel enzymes from other organisms, or downregulation of competing pathways, exemplified by enhancing lysine synthesis through the modification of aspartate kinase activity (Jakobsen *et al.*, 2009). For instance, modifications to the aspartate pathway have been successfully implemented in maize to elevate lysine and threonine levels, while overexpression of dihydrodipicolinate synthase has led to significant increases in lysine content in rice. Conversely, reducing the expression of genes involved in the catabolism of essential amino acids, such as *lysine ketoglutarate reductase/saccharopine dehydrogenase* (LKR/SDH), can also lead to their increased accumulation (Arruda and Neshich 2012). Furthermore, metabolic engineering has proven effective in increasing the accumulation of sulphur-containing amino acids, like methionine, by modifying the cystathionine gamma-synthase pathway and enhancing the uptake and assimilation of sulphate. These targeted manipulations aim to bypass the inherent feedback inhibition mechanisms regulating amino acid biosynthesis, thereby enabling a sustained increase in the accumulation of target essential amino acids (Hesse *et al.*, 2004, Avraham *et al.*, 2005). Conversely, approaches that involve silencing genes responsible for antinutritional factors or those that reduce amino acid bioavailability, such as phytate, also contribute to an improved nutritional profile (Shahzad *et al.*, 2021). In addition, removal of feedback inhibition through engineering of feedback-insensitive enzymes, amplification of metabolic pathways by overexpressing rate-limiting enzymes, enhancement of precursor supply from central carbon metabolism, elimination of competing catabolic pathways to improve metabolic flux toward amino acid biosynthesis, cofactor balancing, engineering of amino acid transport systems, regulatory network rewiring, seed-specific expression control, and modification of storage proteins have further contributed to increasing the accumulation of protein-bound essential amino acids and improving the nutritional quality of cereal grains (Fig.5).

7.2. CRISPR/Cas applications

The CRISPR/Cas genome-editing system has emerged as a transformative tool for precise manipulation of plant genomes, enabling targeted improvement of nutritional traits in major cereal crops. Owing to its high efficiency, simplicity, and precision, CRISPR technology allows the introduction of specific genetic modifications such as gene knockouts, insertions, and base substitutions, thereby facilitating the targeted enhancement of metabolic pathways associated with amino acid biosynthesis and protein quality (Kumar *et al.*, 2022). In recent years, CRISPR-mediated genome editing has been widely employed to modify genes involved in crop nutritional quality, including the regulation of storage proteins and metabolic enzymes that influence the amino acid composition of grains (Tuncel *et al.*, 2023). By enabling the precise modification of key regulatory genes, this technology provides opportunities to improve essential amino acid content, enhance nutrient bioavailability, and reduce undesirable compounds in edible plant tissues.

Furthermore, genome-editing approaches have demonstrated considerable potential to improve crop performance across diverse environmental conditions while enhancing nutritional attributes. CRISPR-based engineering of metabolic pathways can facilitate the targeted accumulation of beneficial metabolites and improve overall grain nutritional profiles (Das *et al.*, 2024). These approaches also enable the modification of specific genes associated with stress tolerance, which is critical for maintaining nutritional stability under changing climatic conditions (Mohamed *et al.*, 2024). In addition, the integration of CRISPR technology with genomics-assisted breeding strategies provides a powerful platform for accelerating crop biofortification programs. Collectively, CRISPR-based genome editing represents a promising frontier in crop improvement, offering unprecedented opportunities to develop climate-resilient cereal cultivars with enhanced amino acid composition and improved nutritional quality to address global food security challenges.

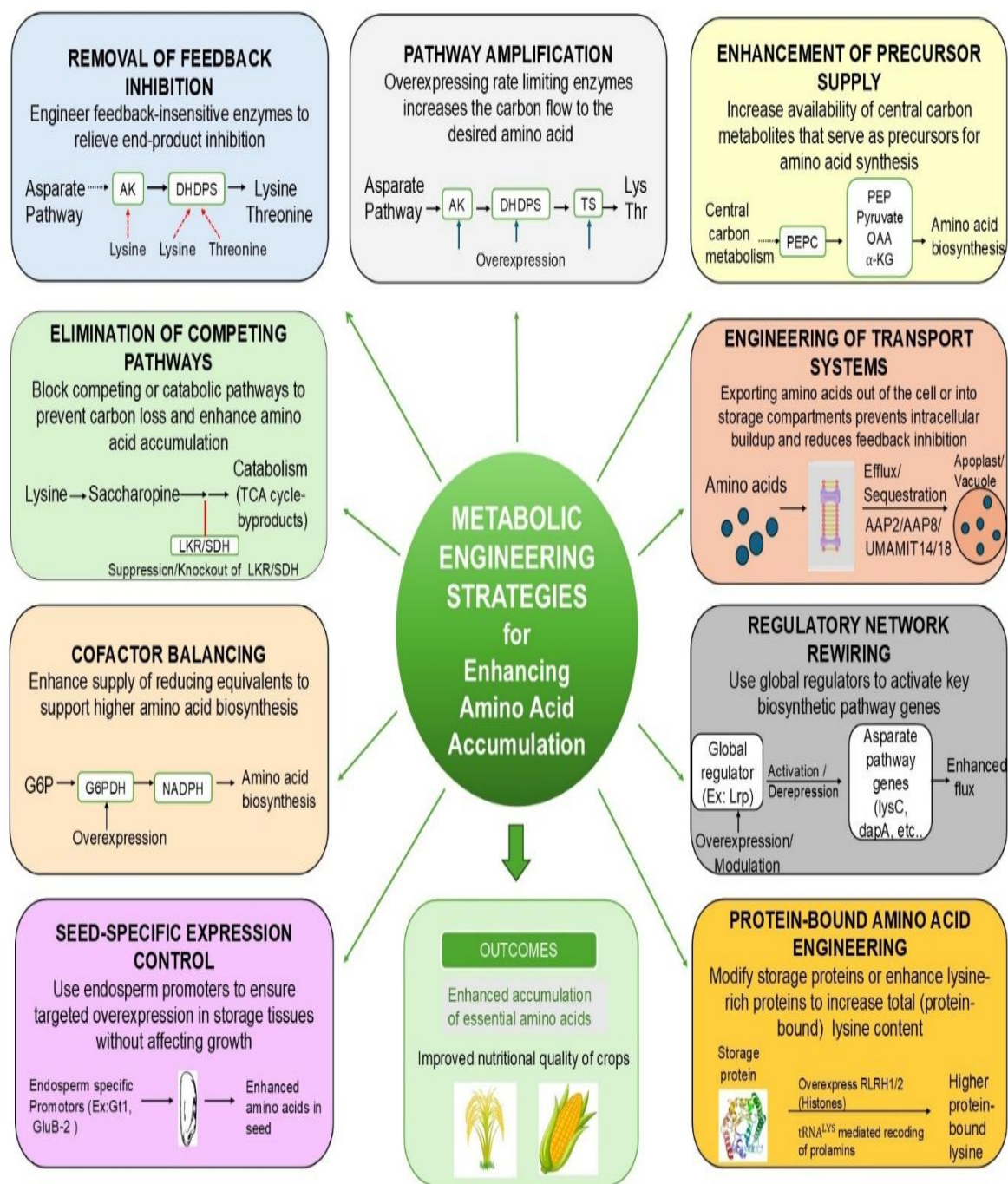


Fig.5 Metabolic engineering strategies for enhancing amino acid accumulation

8. CHALLENGES AND OPPORTUNITIES

8.1. Yield vs Protein Trade-offs

The persistent negative correlation between grain yield and grain protein content remains a major physiological constraint in cereal breeding. This trade-off is largely attributed to the imbalance between carbon accumulation and nitrogen assimilation during the grain-filling stage, where rapid starch deposition dilutes the concentration of grain proteins as yield potential increases (Oury and Godin 2007, Bogard *et al.*, 2013). Consequently, breeding strategies that prioritize yield improvement alone may inadvertently reduce grain nutritional quality, particularly the levels of essential amino acids such as lysine and tryptophan that are already limited in most cereal grains (Swamy *et al.*, 2021). This phenomenon, commonly referred to as the protein dilution effect, poses a significant challenge for developing cultivars that simultaneously achieve high productivity and superior nutritional value. Improving nitrogen uptake efficiency and enhancing the remobilization of nitrogen from vegetative tissues to developing grains therefore represent critical targets for crop improvement. In this context, the identification of key genetic regulators controlling nitrogen remobilization offers promising opportunities for overcoming the yield–protein trade-off. Notably, NAC transcription factor genes such as NAM-A1 and NAM-B1 have been shown to accelerate leaf senescence and promote efficient translocation of nitrogen to developing

grains, thereby increasing grain protein concentration without substantially compromising yield (Uauy *et al.*, 2006, Borrill *et al.*, 2019, Taub *et al.*, 2008). The strategic incorporation of such functional alleles through modern breeding approaches could therefore facilitate the simultaneous enhancement of yield and nutritional quality in cereal crops.

The efficiency of nitrogen remobilization and grain protein accumulation in cereals is strongly influenced by environmental conditions, as abiotic stresses can disrupt the metabolic coordination required for optimal nitrogen partitioning during grain filling. Environmental factors such as temperature fluctuations, drought stress, and nutrient availability significantly affect nitrogen metabolism, enzymatic activity, and amino acid accumulation, thereby influencing final grain nutritional quality (Sethi *et al.*, 2023). Elevated temperatures and other climatic stresses may impair starch and protein deposition in developing grains, highlighting the importance of regulatory gene networks controlling seed development and grain composition (Rossi and Yao 2022). Nitrogen availability also plays a critical role in regulating storage protein synthesis and amino acid metabolism during seed development, as demonstrated by integrated transcriptomic and metabolomic analyses of nitrogen-responsive pathways (Ou *et al.*, 2025).

Furthermore, the timing and progression of leaf senescence are key determinants of nitrogen remobilization efficiency and grain protein concentration. Senescence is regulated by complex genetic networks involving transcription factors that coordinate nutrient translocation from vegetative tissues to developing grains (Sandhu *et al.*, 2021). Among these regulators, the NAC transcription factor NAM-B1 has been shown to accelerate senescence and enhance the remobilization of nitrogen and micronutrients from leaves to grains, resulting in increased grain protein, iron, and zinc content (Andleeb *et al.*, 2023, Shoormij *et al.*, 2023). However, many modern wheat cultivars possess non-functional or mutated forms of this allele, limiting the efficiency of nutrient remobilization and highlighting the genetic consequences of intensive yield-focused breeding. In addition to genetic regulation, environmental stresses such as drought can induce premature senescence and trigger complex metabolic responses, including protein degradation and nitrogen remobilization to developing seeds (Hajibarat and Saidi 2022). Consequently, understanding the interaction between environmental stress factors and genetic regulation of senescence is essential for improving nitrogen use efficiency and enhancing amino acid composition in cereal grains.

8.2. Environmental Effects

Beyond temperature and water stress, broader edaphic and atmospheric conditions also play a critical role in determining amino acid composition in cereal grains through their influence on nutrient availability and metabolic regulation. Environmental factors such as soil fertility, nutrient balance, and atmospheric composition can substantially alter protein synthesis and amino acid accumulation in plants (Szepe *et al.*, 2021). In particular, the relative availability of nitrogen and sulphur strongly regulates the biosynthesis of several amino acids, especially sulphur-containing amino acids such as methionine and cysteine, which are essential for determining grain protein quality. In addition, increasing atmospheric carbon dioxide concentrations have been widely reported to reduce plant protein concentration through the carbon fertilization effect, whereby enhanced carbohydrate accumulation occurs without a proportional increase in nitrogen uptake (Taub *et al.*, 2008, Myers *et al.*, 2014). This imbalance between carbon assimilation and nitrogen acquisition leads to a dilution of grain protein concentration and often results in decreased levels of essential amino acids in staple crops.

The magnitude of this dilution effect is further influenced by soil nitrogen availability, which directly governs amino acid biosynthesis and storage protein formation in developing grains. When nitrogen supply is limited, plants prioritize carbohydrate accumulation over nitrogen-rich compounds, thereby exacerbating deficiencies in essential amino acids such as lysine and methionine (Wang *et al.*, 2023).

The metabolic cross-talk between carbon and nitrogen assimilation pathways represents a fundamental constraint in improving grain protein and amino acid composition in cereal crops. The synthesis of amino acids requires substantial energy and reductants, which are also utilized in photosynthetic carbon assimilation, thereby creating a physiological trade-off between carbon fixation and nitrogen metabolism (Spoorthi *et al.*, 2024). This metabolic competition becomes particularly relevant under conditions where the allocation of reducing power and energy must be balanced between growth and protein synthesis.

Environmental variability also introduces substantial heterogeneity in grain composition across agricultural landscapes. Large-scale analyses of soybean production systems have demonstrated pronounced geographical patterns in protein concentration and amino acid profiles, reflecting the combined influence of soil fertility, precipitation, and genotype–environment interactions (Cerrudo *et al.*, 2023). In wheat, environmental factors have been shown to account for a large proportion of the variation in grain free asparagine content, with studies attributing up to 80% of the observed variation to location effects and approximately 44% to environmental contributions (Oddy *et al.*, 2021). Such pronounced environmental plasticity highlights the necessity of incorporating genotype-by-environment (G×E) interactions into breeding strategies aimed at improving grain nutritional quality. The integration of genomics, transcriptomics, proteomics, and metabolomics have emerged as powerful tools for unravelling the complex regulatory networks governing amino acid biosynthesis and storage protein deposition in cereal grains (Liu *et al.*, 2022, Pott *et al.*, 2021).

9. Future perspectives

9.1. Wild relatives

The exploration of wild relatives and traditional landraces represents a critical strategy for broadening the genetic diversity available for improving amino acid composition and nutritional quality in cereal crops. Modern

breeding programs have historically focused on yield and processing traits, often resulting in a narrowing of genetic diversity within elite cultivars and limiting the availability of alleles associated with nutritional traits. In contrast, wild relatives and ancient cereal germplasm harbour extensive genetic variation that can be exploited to enhance grain nutritional quality and other agronomic traits (Olalekan 2024). The utilization of these genetic resources is increasingly facilitated by advances in plant genomics and high-throughput sequencing technologies, which enable the identification and characterization of novel alleles governing key metabolic and developmental traits. Recent developments in pangenome analysis have further expanded our understanding of crop genetic diversity by revealing extensive structural variations, copy-number variations, and presence–absence variations across diverse germplasm collections (Shi *et al.*, 2025, Jayakodi *et al.*, 2024). These genomic resources provide valuable opportunities for haplotype-based mining of beneficial alleles that may be absent in conventional reference genomes of domesticated crops.

Furthermore, pangenomic analyses of crop species have demonstrated that structural variation plays a crucial role in trait evolution and domestication, offering new targets for genomics-assisted breeding strategies aimed at improving both productivity and nutritional quality (Jayakodi *et al.*, 2024). Given the increasing challenges posed by climate change and global food insecurity, the development of climate-resilient and nutritionally enhanced cereal cultivars has become a central objective of modern crop improvement programs. Integrating genomic resources with advanced breeding approaches therefore offers promising opportunities for simultaneously enhancing stress tolerance, grain quality, and nutritional value in future cereal varieties (Zenda *et al.*, 2021).

9.2. Climate-resilient nutritionally enhanced cereals

Emerging evidence indicates that global climate change poses significant threats to crop productivity and nutritional security, particularly through rising temperatures, erratic precipitation patterns, and other environmental stresses that adversely affect plant growth and grain quality. These climatic changes are expected to intensify abiotic stresses such as drought, heat, and salinity, thereby disrupting plant metabolic processes and ultimately reducing the nutritional value of staple crops (Zenda *et al.*, 2021). Climate-induced environmental variability also increases the risk of nutritional insecurity, especially in regions already vulnerable to food shortages and limited access to nutrient-rich diets. Consequently, developing crop varieties capable of maintaining both productivity and nutritional quality under changing climatic conditions has become a central objective of modern crop improvement programs. Advances in plant genomics and molecular breeding now provide powerful tools to address these challenges.

In particular, improving nitrogen uptake, assimilation, and partitioning toward developing seeds is essential for enhancing grain protein content and amino acid composition in crop plants (The *et al.*, 2021). These physiological processes regulate the synthesis and transport of amino acids that ultimately determine seed nutritional quality. Moreover, the increasing frequency of climatic disturbances—such as heat stress and irregular rainfall patterns—demands breeding strategies that extend beyond yield improvement alone and incorporate complex traits including stress tolerance and nutrient density (Mir *et al.*, 2022). Recent developments in genomics-assisted breeding, including high-throughput genotyping, phenotyping, and molecular marker technologies, have significantly accelerated the identification of genes and genomic regions associated with agronomic performance and nutritional traits (Mir *et al.*, 2022).

10. CONCLUSION

This review has synthesized the complex interplay between genetic architecture, metabolic regulation, and breeding methodologies that govern amino acid profiles in major cereal crops, highlighting that the persistent deficiency of lysine, methionine, and tryptophan remains a challenging barrier to global food security. By integrating classical and molecular approaches, ranging from the success of Quality Protein Maize to the precision of CRISPR/Cas-mediated gene editing, emphasize the potential to reconfigure storage protein composition and enhance the bioavailability of essential amino acids. However, converting such laboratory breakthroughs into agronomically viable, widely adopted cultivars remains restricted by the intricate trade-offs between grain yield, protein content, and environmental stability, necessitating a paradigm shift toward integrated breeding strategies that harness multi-omics data and predictive modelling. Future research must focus on unravelling genotype-by-environment interactions to identify stable genetic factors of amino acid accumulation that can withstand the changing climate conditions, ensuring that nutritional gains are not diminished by abiotic stresses. Ultimately, achieving a sustainable and nutritionally secure food supply requires a coordinated effort to bridge the gap between molecular discovery and farmer adoption, ensuring that improved amino acid profiles are accessible to the populations that need them most.

Statements and Declarations

Author contributions

Jeyaprakash P: Conceptualization, Writing- review & editing. Gokul A: Writing- original draft. T. Thirumurugan, K. Sakthivel, M. Vijayakumar, S. Geethanjali, K. Geetha and CN Neeraja examined the manuscript's writing format and references. P. Jeyaprakash supervised the entire writing process of the article. All authors reviewed the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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