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Nutritional improvement of cereal crops to combat hidden hunger during COVID-19 pandemic: Progress and prospects

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Contents

1. Introduction

With the increasing global population, it has become more challenging to ensure food and nutrition security for the ever-growing population. Climate change, global warming, reduction in agricultural land, and an ongoing pandemic are the major constraints in the adequate production of agricultural food supply. According to the World Health Organization (WHO), around 47 million children below the age of 5 are wasted globally,

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from which 14.3 million children are severely affected. Apart from human health and economic crises, pandemics impart severe impact on agricultural productivity and food availability (Mumtaz et al., 2021). The current coronavirus outbreak has further intensified the global hunger index and the already existing nutritional crisis (Muthamilarasan and Prasad, 2021). Therefore, the term nutritional security has come into the context, which needs to be focused on and prioritized on the agenda of plant breeders. Since the major components of nutrients, including carbohydrates, proteins, fats, vitamins, fibers, micro-, and macronutrients, are not produced in the human body, they must absorb from external dietary sources rich in them (Reider et al., 2020). The per-day recommended values of the essential micronutrients like zinc, iron, and vitamin A are 15 mg, 15 mg, and 600 μg, respectively. These nutrients are yet to be fortified in commonly utilized staple crops, including rice and wheat, which contains suboptimal fraction only (Gaikwad et al., 2020; Ram et al., 2020). However, several underutilized cereals, including millets, contain ample micronutrients, vitamins, dietary fibers, micro- and macronutrients that can be supplemented in a diet plan (Dhaka et al., 2021; Shyamli et al., 2021).

Advancements in genetics and genomics studies have led to detecting genes, alleles, and QTLs underlying complex nutritional traits in cereals. These genetic determinants can further be utilized in the breeding program to enhance grain nutritional content. Besides molecular breeding, several biotechnological approaches, including recombinant DNA technologies and targeted genome editing, are also reported to improve cereals' nutritional potential (Singh et al., 2020). During the last decade, the application of linkage disequilibrium (LD) based association mapping (AM), often termed genome-wide association studies (GWAS), has been increased significantly in cereals focusing mainly on nutrient traits (Gupta et al., 2014; Jaiswal et al., 2019). A GWAS in a crop can be performed with two primary purposes: firstly, to dissect marker-trait associations (MTAs) for a given phenotype, and secondly, to identify all the available QTLs, alleles, and interactions among them. The results obtained are an overview of the genetic basis of the trait, and the marker-trait associations which are most auspicious can be recognized and shortlisted for further analysis. MTAs for several nutrition-related traits have now been carried out in several cereal species. The recent advancements in whole-genome sequencing platforms have also prompted the application of GWAS study with fine resolution (Xiao et al., 2017). Improved computational biology tools have now been generated to extract more meaningful information from genome sequencing data and the

high-throughput phenotypic evaluation of a large number of accessions performed using advanced phenomics platforms. Following MTAs through GWAS, the reverse genetics approach is used to validate the molecular function of identified candidate gene(s). This includes gene silencing through RNA interference (RNAi), retrotransposon-mediated gene disruption, targeted genome editing using CRISPR/Cas9, and variants of CRISPR/Cas technology (Singh et al., 2021). The present chapter discusses the prerequisite genomic resources and various approaches to association mapping. Also, the chapter elaborates on integrating association mapping with multiple omics technologies, applying improvement of nutritional traits, and providing a roadmap for crop improvement.

2. Population structure and genetic base-broadening

Genetic resources in terms of germplasm collection form the base for crop improvement (Fig. 1). The commencement of an association mapping study should begin with gathering all the necessary information about the genotypes that are to be included in it. The selection of germplasms is a critical determinant of a prolific association study (Yu et al., 2006). Prior knowledge regarding genetic diversity, degree of genome-wide LD, and

Fig. 1 Next-generation genomics approaches available for improving the nutritional profile of crops by exploiting genetic diversity.

population structure in the population are requisite in determining the marker density, mapping resolution, selection of statistical methods for association analysis, and the strength of identified marker-trait associations (Zhu et al., 2008). The association mapping population can be divided into five groups based on population structure and relatedness: (i) ideal sample set with fine population structure and familial relatedness, (ii) multifamily sample set, (iii) sample set with population structure and but without familial relatedness, (iv) sample set consisting both population structure and familial relatedness, and (v) sample set with a strong population structure and familial relatedness. Based on the source of the population, it can be divided into germplasm bank collection, elite germplasm, and synthetic populations (Breseghello and Sorrells, 2006). From the germplasm bank collection, genotypes from the core collection can provide a source of genetic diversity due to the encompassing of a vast allele diversity and a population size that is easy to handle, making it a good source for association studies. The ploidy level should be evaluated of accessions from a plant species whose genetic architecture is unknown, specifically if the composed population comprises wild accessions collected from a germplasm bank. This will reduce the effort to distinguish the effects of functional polymorphisms from allele dosage.

Biparental populations, multiparental populations [nested association mapping (NAM) population, multiparent advanced generation intercross (MAGIC) population, and random-open-parent association mapping (ROAM) population], and breeding populations are already being used for GWAS and linkage studies. Biparental mapping population generated by crossing two contrasting parents. It includes F_2, $F_{2:3}$, backcross populations, advanced backcross populations, Double haploids population (DH), Near Isogenic Lines (NILs), and Recombinant Inbred Lines (RILs). The biparental population possesses a lower level of allelic variations and segregates only for a limited number of traits (Tripodi, 2021). The selection of any of these populations depends on the nature of the breeding system (inbred vs outbred) of particular crop species; for example, a multiparental population such as NAM is applicable for the cross-pollinated species like maize but may not be as suitable for an autogamous species. Various variants of the NAM population, including backcross NAM (BC-NAM), doubled haploid NAM (DH-NAM), and advanced backcross NAM (AB-NAM), have also been generated for crop plants (Li et al., 2016; Nice et al., 2016). It was suggested that a multiple-hybrid population (MHP) could also be applied for cross-pollinated species than the biparental or multiparental population (Wang et al., 2017). A comparative analysis of different mapping populations used for the GWAS analysis is illustrated in Table 1.

Table 1 Comparative analysis of mapping populations used for GWAS study in plants.

Features	Biparental mapping	NAM	MAGIC
Number of parents involved	2	>2	8
Mapping resolution	Low	High	High
Power of mapping	High	High	Moderate
Recombination derived LD	Yes	Yes	Yes
Historical LD	No	Yes	Yes
Chance to detect rare QTLs	High	High	Moderate
Multiple alleles detection	Yes	Yes	Up to 2 alleles
Genetic base and parental diversity	Narrow	Board	Moderate

3. Genotyping approaches for association mapping

DNA markers are utilized to analyze linkage maps, association studies, quantitative trait loci (QTL) findings, and marker-assisted selection (MAS) in crops. Before the next-generation sequencing (NGS) era, the digestion-based markers such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), and Amplified Fragment Length Polymorphisms (AFLPs). Advancement in NGS technology has enhanced computational capabilities. The introduction of new rapid genotyping technologies in the past decade has prompted the identification of high-throughput heritable genomic markers for most essential crops. This has illuminated the path of discovering causal genetic polymorphic factors for any phenotype. The application of large numbers of high-throughput genotypic markers within the entire set of the population has shifted the population genomics to the level of pangenome genetics. This allows the generation of several types of structural marker systems, including copy number variations (CNVs), presence/absence variations (PAVs), insertion-site-based polymorphisms (ISBPs), InDels, transposons, and epigenetic variations.

NGS technology is primarily based on large-scale parallel sequencing and imaging methods to produce as large as 100 billion bases in a single run (van Dijk et al., 2018). Several advanced platforms are available for NGS technology, including the Roche 454 FLX Titanium, Ion Torrent PGM, Illumina MiSeq, and HiSeq2500. Most of these platforms efficiently generate dependable DNA sequences and show high coverage on average, GC-rich, and a modest level of AT ratio in the genome. However, these

factors affect the quality of the results generated and the application in which it has to be utilized. The amount of genotypic data generated through these platforms often creates analytical and statistical challenges, requiring a suitable computational program for accurate analysis. There is an affluence of single nucleotide polymorphisms (SNPs) due to the advancements of NGS techniques, and hence these have become the most extensively exploited markers for association studies. The identification and genotyping of SNPs at whole-genome levels in a given set of the population is known as genotyping-by-sequencing (GBS). GBS has rapidly gained popularity because of its comparatively low cost, even in the species lacking the reference genome sequence (Scheben et al., 2017). This technique detects nucleotide sequences and scores SNPs simultaneously, which surpasses the DNA marker development procedure.

4. High-throughput phenotyping platforms and statistical tools

Along with genotyping, phenotyping is another crucial factor that decides the fate of a successful association study. Accurate phenotyping is an extensively distributed and arduous step that requires large-scale management and quantification of plants. It involves maintaining hundreds or thousands of plants grown at different geographical locations in diverse environments over multiple years. The data obtained from these experiments is utilized in studies conducted to delineate factors governing stress, yield, quality, and nutritional aspects in crops. There have been rapid advancements in genotyping facilities; however, phenotyping technologies have not been developed to that extent and consume much more time and employees. The experimental field design is a critical parameter for an efficient association study. The data generated from an adequately designed field experiment will be more reliable, and statistical deductions will be more. The foundation of a reliable experimental design depends on the size of the experimental unit, replication, blocking, and randomization (Casler, 2015). Other factors are to be considered while carrying out phenotypic analysis, including photoperiod sensitivity, flowering time, pathogen susceptibility, water, and fertilizer applications.

High-throughput phenotyping (HTP) methods can provide precise and rapid results for a large set of accessions. It is generally known as phenomics, and it utilizes automated technologies to measure phenotypic traits (Rafalski,

2010). However, not all phenotypic traits can be visually evaluated and thus create a genotype-phenotype bottleneck. Nevertheless, image-based phenomics provides a promising platform for the visually scored traits to record the dynamic changes occurring throughout the experiment. Examples for some of the existing phenomics platforms are International Plant Phenomics Network (IPPN), European Plant Phenotyping Network (EPPN), Australian Plant Phenomics Facility, French Plant Phenomics Network (FPPN), German Plant Phenotyping Network (DPPN), High-Throughput Rice Phenotyping Facility (HRPF), Green Crop Network (GCN), Biotron Experimental Climate Change Research Facility, Laboratory of Plant Ecophysiological responses to Environmental Stresses (LEPSE), The Australian Plant Phenomics Facility, and LeasyScan at ICRISAT (Knecht et al., 2016). These noninvasive HTP platforms utilize advanced technologies including infrared thermography and imagery, fluorescent spectroscopy, 3D reconstruction, light detection and ranging (LIDAR), magnetic resonance imaging, positron emission tomography, canopy spectral reflectance, nuclear magnetic resonance, hyperspectral imaging, and digital RGB imaging for the measurement and assessment of various traits like plant growth, structure, water relations and other dynamic attributes (Mir et al., 2019). This whole process generates large-scale data and thus requires its systematic storage, management, and retrieval. New methods of data mining and machine learning can provide valuable assistance in managing large datasets. HTP has been used in many crop studies. Campbell et al. (2015) used temporal imaging in 378 diverse rice lines to understand the factors behind salinity-induced growth in plants. Further, a greenhouse-based HTP platform helped assess water usage and projected shoot area in 357 rice genotypes (Baba et al., 2020). The genetic information combined with HTP data can provide more depth in genotype-trait association analysis.

Several software are available to carry out association mapping, and new methodologies and programs keep developing for the same. The frequently used software package for plants is Trait Analysis by aSSociation, Evolution, and Linkage (TASSEL). It provides a user-friendly interface and gets updated with new methods and features (Bradbury et al., 2007). Other software, including R, STRUCTURE, Genome Association and Prediction Integrated Tool (GAPIT), Spatial Pattern Analysis of Genetic Diversity (SPAGeDi), and others along with their applications related to association studies are listed in Table 2.

Table 2 List of software used in association mapping analysis.

Software package	Focus	Remarks	Website
TASSEL (Trait Analysis by aSSociation, Evolution and Linkage)	Association analysis	Freely available. It calculates marker-traits associations, evolutionary relationships, and linkage disequilibrium	https://www.maizegenetics.net/tassel
R	General	Freely available for statistical computing and graphics	http://www.r-project.org/
STRUCTURE	Population structure	Widely used and freely available for evaluating population structure analysis	https://web.stanford.edu/group/pritchardlab/structure.html
GAPIT (Genome Association and Prediction Integrated Tool)	Association analysis	Widely used and freely available for GWAS and Genomic Selection (GS). Methods used are Q+K, CMLM, FarmCPU and BLINK	http://www.zzlab.net/GAPIT/
SAS (Statistical Analysis System)	General	Used for data mining, statistical analysis and simulation	http://www.sas.com
SPAGeDi (Spatial Pattern Analysis of Genetic Diversity)	Population relatedness	Freely available for characterizing spatial genetic structure of mapped population	http://ebe.ulb.ac.be/ebe/SPAGeDi.html
GenAlEx	Population relatedness	Runs within Microsoft Excel and provides both frequency-based and distance-based analyses	https://biology-assets.anu.edu.au/GenAlEx/Welcome.html
Matapax (MArker-Trait Association Platform And eXplorer)	Association analysis	Web-based freely available program that utilizes R library EMMA and GAPIT	http://matapax.mpimp-golm.mpg.de/
GenAMap	Association analysis	Freely available. Visual analytics system for structured association mapping	http://sailing.cs.cmu.edu/genamap

Table 2 List of software used in association mapping analysis.—cont'd

Software package	Focus	Remarks	Website
Pascal (Pathway scoring algorithm)	Enrichment analysis	Computes gene and pathway scores from marker-trait association statistics	https://www2.unil.ch/cbg/index.php?title=Pascal
NAM (Nested Association Mapping)	Association analysis	Designed to carry out association studies in NAM populations through likelihood and Bayesian methods	https://cran.r-project.org/web/packages/NAM/index.html
SNPEVG	Visualization tool	Graphical tool for visualization of SNP effects in GWAS	https://animalgene.umn.edu/snpevg
EMMAX (Efficient Mixed-Model Association eXpedited)	Association analysis	Utilize variance component model for considering sample structure in association mapping	http://genetics.cs.ucla.edu/emmax/
PLINK	Association analysis	Open source tool that can handle large datasets	http://zzz.bwh.harvard.edu/plink/
HaploView	Haplotype analysis	User friendly tool for analysis and visualization of LD haplotype maps	https://www.broadinstitute.org/haploview/haploview

5. Integrating omics with association mapping

Other than genomics, different omics approaches, including transcriptomics, proteomics, and metabolomics, are also essential to understanding gene function. An integrated omics approach can be employed together with association mapping studies to decipher the underlying mechanism of phenotype variation. Global transcriptome analysis is used to determine the transcripts abundance, comparative variation in gene expression, and epigenetic silencing (Singh et al., 2021). With the advancements in NGS technology, RNA-Seq based expression analysis has been available for many crop plants. Unlike genome sequencing, RNA-Seq is not largely affected by the

organism's ploidy or large genome complexity. By analyzing the transcript sequence of a large number of accessions, SNP associated with genic regions can be analyzed. Thus, it can be utilized to conduct a GWAS study to delineate the effect of the difference in gene and regulatory sequence on desirable traits. The significant advantage of a transcriptome-wide association study (TWAS) is its applicability to crops with limited genomic resources. Several TWAS has been carried out in various crops, including rapeseed (Tang et al., 2021), maize (Jia et al., 2020; Kremling et al., 2019), rice (Anacleto et al., 2019), barley (Sharma Poudel et al., 2019), and others. The integrated use of GWAS and transcriptome data is much more reliable evidence of the association of genic regions to the desirable trait. Similarly, metabolite-based GWAS, often called mGWAS, is a recently emerging area and gaining popularity as metabolites directly affect plant phenotype. Considering the relative accumulation of a specific metabolite in the accessions of a population as trait, mGWAS can be performed. The major platforms used for the separation, detection, characterization, and quantification of metabolites are based on chromatography coupled to mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy. Metabolome profiling integrated with advanced genomics to elucidate the genomic loci controlling natural diversity in the abundance of specific metabolites. Several such studies have been carried out in various crop species, including wheat (Chen et al., 2020; Shi et al., 2020), maize (Zhou et al., 2019), rice (Matsuda et al., 2015), and others. These studies suggest that the metabolites can bridge genotype and phenotype for a specific trait. The integrative approach consisting of GBS, metabolic profiling, and transcriptome analysis can provide an in-depth mechanism behind the cause of natural variation in a given population. Fig. 2 briefly illustrates the strategies of integrating omics with association mapping and further crop improvement through marker-assisted breeding (MAB).

6. Application of association mapping in genetic improvement of nutritional traits

Association mapping has primarily been used for exploring polymorphisms governing variations in the desired trait in cereals. There are many studies reported where association mapping has been applied in determining genetic regulators of agronomic and physiological traits in various crops (Dang et al., 2014; Kikuchi et al., 2017; Maurer et al., 2016; Melandri et al., 2020; Pauli et al., 2014; Sahoo et al., 2020; Setter et al., 2011;

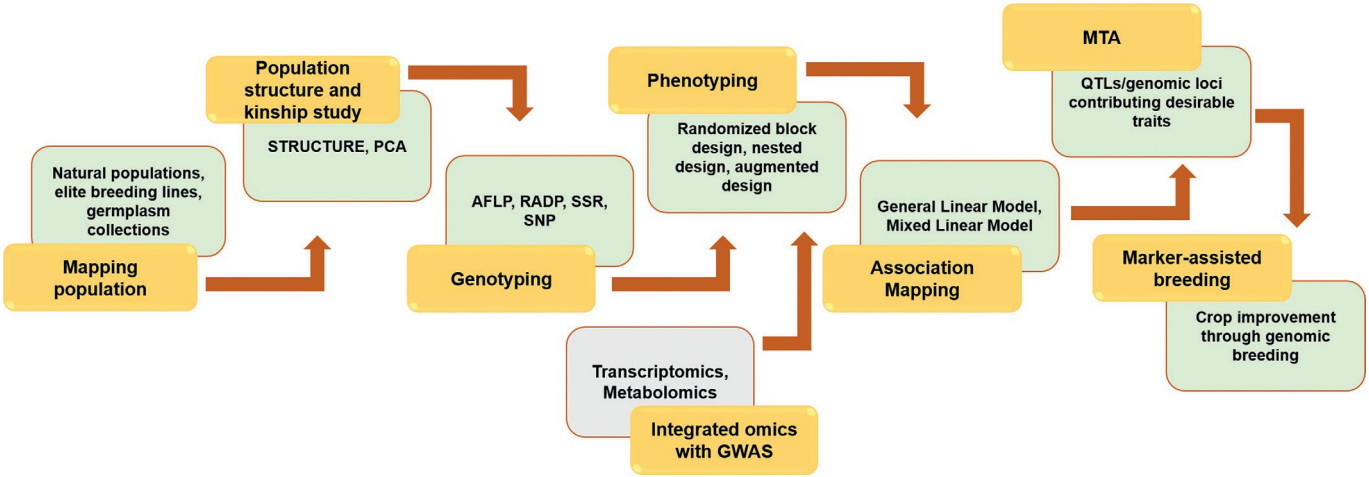

Fig. 2 Schematic representation of the steps involved in association analysis and integration of various omics approaches.

Tian et al., 2011; Zhang et al., 2014). In this section, we have focused on applying association mapping in delineating nutritional traits in cereal crops.

6.1 Protein content

A minicore collection was selected from the rice global population collection of the US Department of Agriculture (USDA). Through association mapping, variation in protein content (5.4–11.9%) helped identifying eight nucleotide markers that were linked with genes of protein precursors (Bryant et al., 2013). Laidò et al. (2014) selected 970 polymorphic DArT markers for marker-phenotypes association analysis in 230 inbred wheat lines. The generalized linear model (GLM) and mixed linear model (MLM) approach of association mapping identified 44 MTAs for protein content that identified 39 putative QTLs. From these QTLs, one was determined to be significantly linked to the Gpc-B1 gene. This gene plays an essential role in determining protein content in both tetraploid and hexaploid wheat. This gene is translated into a NAC-domain transcription factor that increases the rate of senescence, which culminates in the increased mobilization of nitrogen into the grain. In another study, a diverse panel of 390 sorghum accessions was evaluated for variation in grain nutritional traits, including starch, protein, and fat content. Using near-infrared spectroscopy (NIRS), protein content was measured that fell in the range of 8.1–18.8% and identified a QTL through GWAS analysis (Rhodes et al., 2017). Besides the grain content, protein quality is also an essential factor determining essential amino acid content and protein digestibility. Out of the 20 amino acids, 9 are regarded as essential amino acids as the human body cannot synthesize them and hence are to supplemented through diet. These include lysine, tryptophan, threonine, methionine, phenylalanine, isoleucine, leucine, valine, and histidine. In cereals, the amino acids lysine, threonine, and tryptophan are present in a limiting amount, and therefore, studies have been conducted to biofortify their amount. *Opaque2 modifiers* (*Opm*) gene belongs to bZIP transcription factor family and has a role in regulating tryptophan content in grains. A total of 74 genic and 46 genomic SSR markers were used in association mapping to identify QTLs tightly linked to *Opm* variation in tryptophan content. The study has led to the identification of two QTLs linked to tryptophan content and a QTL for total protein content (Babu et al., 2014). Protein content in barley grain is crucial for determining its quality when used for malt and food applications. Genetic determinants governing protein grain contents in 99 wild and 59 cultivated

barley have been identified, and markers generated through diversity arrays technology (DArT) were used for association mapping. From the associated 5, 7, 6, 5, 6, and 8 markers that were localized on the 1, 2, 3, 5, 6, and 7 chromosomes, respectively, *HvNAM1* and *HvNAM2* were selected as candidate genes for further protein content improvement (Cai et al., 2013).

6.2 Carbohydrate and related traits

The chief carbohydrate component in cereal grains is starch, which is also the primary energy source for humankind. Apart from starch, cereal grains also contain nonstarch polysaccharides like arabinoxylans and β-glucans. β-Glucans are sometimes considered antinutritional factors due to hindrance in absorption (Rudi et al., 2006). However, their functions as an immunomodulator, serum cholesterol reducer, and prebiotic effect have attracted attention. MTAs for β-glucan content in 230 tetraploid wheat genotypes utilizing SNPs retrieved from the 90k-iSelect array were generated. This led to the identification of potential candidate genes that might have a role in regulating β-glucan content in grains through carbon partitioning (Marcotuli et al., 2016). The identified putative genes were glycosyl hydrolases endo-β-(1,4)-glucanase, (1,4)-β-xylan endohydrolase, and xylanase inhibitor protein I. To unfold genomic regions governing protein content and cooking quality parameters in rice, Wang et al. (2017) have selected an association panel of 22,488 SNPs from 258 diverse rice accessions. Association studies had led to the determination of 19 QTLs regulating the 4 characters, including gel consistency, gelatinization temperature, apparent amylose content, and protein content. These 19 QTLs were linked to genes of the starch synthesis pathway. In another study, a diverse panel of 591 landraces constituting 2.9 billion SNP and 0.39 billion InDels were investigated to discover regions governing grain width of raw and cooked rice grain. Novel GWi7.1, GL3.1, GWi5.1, and GWi11.1 genomic regions were identified, controlling the desired trait (Misra et al., 2017). Further, to identify genetic determinants for amylose content in maize, Li et al. (2018) used a collection of 464 maize varieties incorporating 9 million SNPs markers. The GWAS study led to identifying 39 putative genes having a role as transcription factors, glycosidases, glycosyltransferases, and hydrolases that might regulate the amylose content in grains. Glycemic index (GI) is an essential nutritional trait, and to discern its genetic regulators, an association panel of 305 diverse resequenced Indica accessions was explored. Combined with a transcriptome-wide association study (TWAS), GWAS indicated a

novel factor ascertained at locus LOC_Os05g03600 responsible for the variation in the desired trait (Anacleto et al., 2019).

6.3 Micronutrients

A total of 17,937 SNP markers have been used for 246 wheat genotypes to identify markers associated with micronutrients like zinc, iron, and beta-carotene and grain protein content (Kumar et al., 2018). Several marker-trait associations were identified using SLST, MLMM, MTMM, and mvLMM methods, from which nine highly associated and significant MTAs were selected for further investigation. For calcium content in wheat, MTAs were carried out using 7761 SNP generated from 90k iSELECT ILLUMINA chip and 7762 mapped SNPs from a 35k Affymetrix chip (Alomari et al., 2017). In total, 276 SNPs with positive allele effect led to identifying candidate genes located in the proximity of associated SNPs, which participate in transport and uptake of calcium. In barley, a set of 336 genotypes utilizing 6519 SNP markers, GWAS resulted in the identification of 2 QTLs to be associated with calcium, 2 with barium, 3 with sulfur, 4 with sodium, 4 with copper, 11 with iron, 2 with potassium, 3 with magnesium, and 3 with zinc (Gyawali et al., 2017). In the case of millets, 74 marker-trait associations were identified in foxtail millet for 10 micronutrients (Ni, K, Ca, B, Mg, P, S, Zn, Mn, and Fe) using an association panel of 96 diverse accessions and 10k SNPs generated through resequencing (Jaiswal et al., 2019). Further, Puranik et al. (2020) utilized an association panel of 190 finger millet genotypes to identify MTAs for micronutrients like iron, zinc, calcium, magnesium, potassium, and sodium, along with protein content. Through GLM and MLM approach, 34 high confidence MTAs were identified, from which 18 markers were found to be linked with genes having a role in the binding and movement of metal ions.

6.4 Secondary metabolites

Polyphenols are compounds imparting antioxidant benefits to the human body. In sorghum, phenols, proanthocyanidins, and 3-deoxyanthocyanidins contents were studied in a panel of 381 accessions. Utilizing 404,628 SNPs for GWAS analysis, novel QTLs were detected that contained homologs of Pr1 and TT16 (Rhodes et al., 2014). An interesting study conducted by Colasuonno et al. (2017) identified 24 candidate genes for the carotenoid synthesis pathway, a precursor in the synthesis of precursors of vitamin A. The identified QTLs and genes might provide an insight into the development

of advanced high nutritional wheat varieties. Noteworthy, maize grains are limited in tocopherols, with vitamin E activity. Through the GWAS study, an SNP marker was mapped 85 kb upstream to a *Zea mays* VTE4, which codes for a γ-tocopherol methyltransferase. Additionally, two InDels markers were also found to be associated, out of which one was located to the promoter area and possibly regulating transcriptionally (Li et al., 2012). The candidate-gene approach of GWAS lycopene epsilon cyclase (LCYE) and β-carotene hydroxylase 1 (CRTRB1) had already been identified in the maize breeding scheme of CIMMYT. Further, high density 476,000 SNPs assisted in identifying genomic regions possessing a salient role in accumulating precursors of isoprenoids, namely, GGPS1, GGPS2, and DXS1. Also, genes like *HYD5*, *CCD1*, and *ZEP1*, responsible for hydroxylation and carotenoid degradation, were identified (Suwarno et al., 2015).

7. Biofortification using conventional and genomic approaches

Biofortification aims to improve the nutritional qualities of a crop plant. Both the conventional breeding and recent genomics approaches have been successfully applied in the biofortification of cereal crops. The conventional approach mainly includes mineral fertilization through soil or foliar application and crossing contrasting parents to obtain desired progenies with improved nutrient bioavailability. The genomics approach initiates with genotyping of the population to identify genomic determinants responsible for improved nutritional traits and completes with introgression to the elite species either through molecular breeding or genetic engineering/genome editing to get desired lines (Singh et al., 2020). To eradicate micronutrient deficiency, HarvestPlus biofortification program has floated since the early 2000s employing conventional breeding and genomics approaches. More than 240 crops with improved iron, zinc, and vitamin A have been released under the program, and several are in the delivery phase (https://www.harvestplus.org/knowledge-market/publications). In India, research was mainly focused on developing zinc and iron-enriched crops and, to date, has been able to release zinc fortified wheat varieties, BHU-6 (Chitra) and BHU-3; and iron-enriched pearl millet varieties including ICMH 1201 (Shakti-1201) and ICTP 8203-Fe-10-2 (Dhanashakti). The program had set a target of reaching more than 1 billion people with a supply of biofortified crops to combat hidden hunger by 2030.

Through overexpression, silencing, and gene editing, the functional genomics approach is a rapid and widely utilized strategy to generate biofortified crop plants. Provitamin A enriched wheat plants have been generated by introducing bacterial carotenoid biosynthetic genes *CtrB* and *ctrI* (Wang et al., 2014). Similarly, lysine rice grains have been achieved by induced expression of endogenous rice lysine-rich histone proteins, RLRH1 and RLRH2 (Wong et al., 2015). In contrast to biofortification, bioelimination is also essential to reduce antinutrient substances from food grains. Phytic acid is one of such antinutrient in cereals that has the ability to chelate micronutrients and restrict the bioavailability of essential nutrients. Targeted silencing of *inositol pentakisphosphate kinase* (*TaIPK1*), a gene involved in the phytic acid biosynthesis pathway, has resulted in up to 58% reduced accumulation of phytic acid with enhanced grain mineral contents in wheat (Aggarwal et al., 2018). Similarly, CRISPR/Cas9-mediated knockdown of *Inositol 1,3,4-trisphosphate 5/6-kinase* (*OsITPK1–6*) has significantly lowered the levels of phytic acid in rice (Jiang et al., 2019). These studies further need to be expanded to millets and other cereals crops where phytic acid is a major nutritional constraint.

8. The way forward

Food scarcity and disease outbreaks have coexisted with us since the beginning of human evolution. Similar to the previous pandemics, COVID-19 pandemics have posed an intense threat to the food supply around various parts of the world. The majority of the human population is following social distancing, which has reduced the efficiency of enterprises and risks the farmers wealth. The other most important aspect of this pandemic is the vulnerability of marginal people, which require a high nutritious diet during infection and recovery. Since cereals share more than half of total food consumption worldwide, fortification of nutritional traits in them would be the prime concern of crop breeders.

The significance of improving nutritional traits in cereals is well understood, and multidimensional approaches are being used to address this. Undoubtedly, the association mapping approach has been beneficial in finding QTLs and genomic regions associated with nutritional traits in plants. During the last decade, remarkable advancements have been achieved in GWAS. However, there is a need for further advancements for more efficient use of the approach. Along with several advantages of association mapping, some restrictions still exist. One such limitation is the reproducibility

of the identified regions when the same association study is repeated. Another limitation is the occurrence of false positives and false negatives data. The risk of false positive occurs due to a strong association of the studied trait with population structure. To minimize this, it has been suggested to use the STRUCTURE program that utilizes structured associations. Further, incorporating principal component analysis (PCA) is also recommended to resolve issues related to population structure. When large-scale genetic markers are used, the errors in false associations increase, which requires the validation of MTAs distinctively. GWAS analysis identifies only the genomic locus associated with the desirable trait and does not identify the gene(s) responsible for phenotypic polymorphism. Genes flanking to the identified regions need further investigation to establish the gene-trait association. Integration of data from various omics is also being suggested for exploring the molecular mechanisms behind genotype-phenotype relationships. Many studies have been conducted in recent years to explore the genetic determinants governing nutrition-related traits in various crops; however, there is a need to increase further the number of studies in the area of cereal nutritional traits under the present scenario of the global pandemic and food scarcity. NGS technologies can be utilized in the generation of more detailed whole-genome scans. Still, high-throughput phenotyping platforms are not in the reach of most of the breeders. An efficient association mapping largely depends on the size of the population, standard of the genotyping and phenotyping, statistical methods used, and experimental validation of MTA. Altogether, association mapping provides a high-throughput platform to uncover the cause of phenotypic variation, which is widely used in crop improvement through molecular breeding. These studies can be combined with functional genomic approaches to generate more and more biofortified cereal crops targeting to achieve zero hunger.

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