REVIEW

Meeting the challenge of developing food crops with improved nutritional quality and food safety: leveraging proteomics and related omics techniques

Shalu Jain · Anjana Rustagi · Deepak Kumar · Mohd Aslam Yusuf ·
Shashi Shekhar · Neera Bhalla Sarin

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Abstract Eliminating malnutrition remains an imminent priority in our efforts to achieve food security and providing adequate calories, proteins, and micronutrients to the growing world population. Malnutrition may be attributed to socio-economic factors (poverty and limited accessibility to nutritional food), dietary preferences, inherent nutrient profiles of traditional food crops, and to a combination of all such factors. Modern advancements in “omics” technology have made it possible to reliably predict, diagnose, and suggest ways to remedy the low protein content and bioavailability of key micronutrients in food crops. In this review, we briefly describe how proteomics techniques can potentially be used for improving the

nutrient profile of major crops, through high throughput multiplexed assays. Food safety is another important issue where proteomics and related platforms can offer solution for absolute quantitation of food allergens and mycotoxins present in the plant-based food. The purpose of the present review is to discuss the proteomic-based strategies in food crops to meet the challenges of overcoming malnutrition throughout the world.

Keywords Food quality · Malnutrition · Micronutrients · Proteomics · Toxins

S. Jain
Department of Plant Pathology, North Dakota State University, Fargo, ND, USA

A. Rustagi
Department of Botany, Gargi College, New Delhi, India

D. Kumar
Department of Botany, Central University of Jammu, Jammu & Kashmir, India

M. A. Yusuf
Department of Bioengineering, Integral University, Lucknow, India

S. Shekhar · N. B. Sarin (✉)
School of Life Sciences, Jawaharlal Nehru University, New Delhi, India
e-mail: neerasarin@rediffmail.com

Introduction

Providing safe, nutritious, and affordable food continues to be a major challenge for agriculture. With the world population expected to surpass 9 billion by 2050, a steep increase in global food demand would be unavoidable (<http://www.un.org/en/development/desa/news/population/2015-report.html>). The efforts directed towards tackling the demand for food have, however, increased the reliance on a few major crops, thereby, decreasing the diversity in diet (Massawe et al. 2016). While the Green Revolution starting in the 1940s in Mexico and spreading to Asia in the 1970s helped tremendously in increasing the world food grain production, similar efforts have not been made on a global scale for delivering adequate levels of

micronutrients (minerals and vitamins). As such, malnutrition still pervades human societies and warrants immediate preventive measures. Although the most prominent images that the term malnutrition conjures are of famished and starving individuals, malnutrition is not just a problem of the poor and underdeveloped nations. It is rather a complex and multifaceted condition caused by inadequate or inappropriate nutrition and affects both developed and developing countries. The prevalence of micronutrient deficiencies has increased, with more than two billion people currently suffering from chronic clinical conditions (Nair et al. 2016). Dietary supplementation and food diversification have traditionally been used to amend micronutrient profiles. However, such efforts have had limited success in developing countries because of the lack of social and economic infrastructure, which reduces accessibility. Biofortification is a food-based approach to increase the micronutrient content of staple food crops, using conventional breeding or transgenic methods (Bouis and Saltzman 2017). It takes a considerable amount of time, sometimes up to a decade, before a biofortified crop is released and become widely available pending consumer acceptance. Recent reviews highlighted the progress and perspectives in nutritionally enhanced food crops using biofortification methods (Garg et al. 2018).

During the last few decades, various “omics” technologies have revolutionized biological research. Proteomics, which is the study of the total protein complement present within a defined biological setting as a function of prevalent environmental conditions, referred to as the “proteome” (Wilkins et al. 1996), has emerged as a powerful component of biotechnological workflow in research on crop improvement (Eldakak et al. 2013). Proteomic studies provide an array of tools for deciphering critical properties associated with food quality, composition, and physiological and biosynthetic pathways of nutrient accrual and assimilation. During the many stages of biofortification, proteome techniques can be applied for systematic search of genomic resources and new marker proteins/peptides and pathways associated with food quality networks, thus, accelerating the development of improved varieties. Alternatively, accurate and reliable proteomic tools can be used to validate crops developed through conventional breeding and genetic modification, augmented for protein

and micronutrient portfolios. Diverse biotechnology platforms that combine proteomics and genomics approaches along with databases of various plant species have been widely used to study the changes in gene/protein abundance related to different macro and micronutrients (Eldakak et al. 2013). Proteomics and many other related advances in plant biotechnology such as genomics for gene discovery and gene editing have contributed significantly to development and validation in nutritional quality of food crops and food safety (De Steur et al. 2017; Francis et al. 2017). These techniques have emerged as an inevitable tool for improving the nutritional quality of food for millions of people around the globe (Garg et al. 2018). An urgent need is to experimentally integrate current proteomic and other high throughput methods to enhance the production of nutrient-rich foods, with a focus on eliminating malnutrition and micronutrient deficiency in staple crops like wheat, maize, rice, potato, and soybean, thus, ensuring sustained nutritional security. The aim of this review is to collate the knowledge regarding the current applications of proteomics and related techniques for genetic improvement of crops with regard to traits related to food quality, nutritional value and food safety.

Proteomic tools shed light on food quality, safety, and nutritional values

Proteomic studies aim to explain the quantity, functionality, and spatial and temporal location of proteins within an organism. The tools of proteomics involve two complementary, gel-based or gel-free, approaches. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), a gel based technique, is widely used in conjunction with Matrix Assisted Laser Desorption/Ionization mass spectrometry (MALDI-MS) or tandem mass spectrometry. Although this approach is prized for its robustness, parallelism and high resolution, there are also certain limitations, such as insensitivity to low-abundance proteins, inability to characterize the entire proteome in one gel, and lower efficiency in the analysis of hydrophobic proteins (Rabilloud et al. 2010). Moreover, gel based methods are time-consuming and labor intensive. Most of these limitations could be addressed by gel-free proteomic techniques, which are promising for samples in which proteins of interest are less

abundant. Liquid chromatography (LC) is very efficient in separating proteins and peptides in complex mixtures. The advanced MS systems provide more sensitive and more accurate protein quantification. However, gel-free MS based proteomics requires considerable investment in expensive MS instruments and associated infrastructure and highly-skilled personnel to run the facility. Although, both gel- and gel-free approaches are of great importance, no single approach is sufficient to dissect complex samples for obtaining qualitative and quantitative data of all the protein components (Abdallah et al. 2012).

Recently, protein microarrays have been utilized for obtaining information about the analytes, ligands, receptors, antibody affinity-based interactions, and binding partners, thus, permitting high-throughput analysis (Romanov et al. 2014). Protein microarrays are being routinely used for protein–protein interaction analyses, biochemical pathway mapping, biomarker identification, host–microbe interaction studies, detection of infectious diseases, drug screening, vaccine development, enzyme–substrate profiling, and immuno-profiling (Romanov et al. 2014). Such protein microarray datasets encompass both the classical and functional proteome analysis (Lodha et al. 2013). Another high throughput method called quantitative dot blot (QDB) analysis opens new avenues for proteomic research through its ability to measure both the absolute and the relative quantity of individual proteins in a sample (Tian et al. 2017). The QDB has been used in clinical studies so far, but this technique can be helpful in studying protein expression in a high-throughput manner for food quality, safety, and nutritional analysis. Data obtained from proteomics experiments need to be analyzed using prior information available in databases. There are a large number of protein databases, such as Global Proteome Machine Database (<http://gpmdb.thegpm.org/>), PeptideAtlas (<http://www.peptideatlas.org/>), PRIDE database (<https://www.ebi.ac.uk/pride/archive/>), MaxQB (<http://www.uniprot.org/database/DB-0186>), and Model Organism Protein Expression Database (<http://moped.proteinspire.org>), which can be exploited to get relevant information (Perez-Riverol et al. 2015). All the information on peptide/protein identification and quantification values needs to be deposited and adequately annotated in public databases and repositories. Earlier reviews by Lodha et al. (2013), and Schubert et al. (2017) have elaborately

explained the development of various gel and non-gel based techniques and their challenges, limitations, and future possibilities in molecular systems biology, clinical research, and personalized medicine. Many of these techniques have been harnessed in food industries to characterize and standardize raw materials, to analyze pre- and post-processing changes in protein composition, to check variations in different batches of production, and for quality control and safety assessment of the final food products (Gasosokac et al. 2010). A schematic summary of the current proteomics-based tools, which are predominantly used in the analyses of major food crops for quality and food safety traits, is provided in Fig. 1. This shows the potential of proteomics techniques to select breeding lines for protein quality (Pompa et al. 2013), gluten (Hajas et al. 2018), fiber (Yahata et al. 2005), fructan (Verspreet et al. 2015), essential amino acids such as lysine (Dahl-Lassen et al. 2018), lipid transfer proteins (Stevenson et al. 2015) as well as range of phytochemicals such as anthocyanins and flavonoids (Liu et al. 2010). Further efforts need to be directed towards the discovery of peptide markers to help us understand the underlying mechanisms for more nutritional traits and to have high-throughput and low cost methods for accurate identification of breeding lines in early generations.

Analysis of protein composition

Nutritional quality of food is reliant on a complex mixture of biochemical compounds, such as lipids, carbohydrates, proteins, minerals, vitamins, phenolics, and organic acids (Hu 2002). A combination of different techniques is required for the specific analyses of these compounds (Fig. 1). Globally, approximately half of the dietary protein supply comes from cereal seeds (faostat.fao.org). A critical understanding of proteome dynamics is important for ensuring adequate protein content and quality of seeds, grain legumes, and pseudocereals consumed as food and feed (De Ron et al. 2017). The analysis of differential protein expression using large-scale quantitative proteomic approaches can help in identifying inherent patterns that make some genotypes more allegenic or anti-nutritional and can provide valuable insights towards the development of more suitable, non-allergenic, and nutritious crop cultivars.

Fig. 1 Tools and techniques used in food quality analysis. Two dimensional gel electrophoresis (2D-PAGE), differential gel electrophoresis (2D-DIGE), isotope coded affinity Tags (ICAT), isobaric tagged for relative and absolute quantification (iTRAQ) are coupled to mass spectrometry (MS), which include matrix-assisted laser desorption/ionization (MALDI), Quadrupole

Time-of-flight (qTOF), parallel reaction monitoring (PRM), and selected reaction monitoring (SRM). Other methods such as high performance liquid chromatography (HPLC), gas chromatography (GC), capillary zone electrophoresis (CZE), and micellar electro kinetic chromatography (MEKC) can be combined with MS to analyze food components

Food borne allergies are major clinical concern affecting food quality and safety. Proteins are considered to be the dominant biomolecules responsible for such allergic reactions. In this context, use of proteomic techniques, with focus on qualitative and quantitative assessment of allergens in foods of plant origin, has gained importance. Some cereals such as wheat, rye, and barley have gluten proteins, which can cause hypersensitivity reactions in a large number of people. In a study by Hajas et al. (2018), protein composition analysis was conducted in 23 common wheat cultivars and five of these cultivars were found suitable for developing gluten reference material. Within the legumes, peanut seeds are widely used as a source of high quality protein and oil throughout the world. However, clinical studies on peanut allergy have frequently reported antigenic cross-reactivity attributed to proteins closely related to the Bet v 1-like proteins, profilins, and nonspecific lipid transfer proteins (Table 1). Enzyme-linked immunosorbent assay (ELISA) and liquid chromatography (LC)

coupled with MS are two routinely used methods for allergen analysis. Recently, flow cytometry has been used for the identification of allergens because of its potential for detecting multiple allergens in a remarkably short time. It is also known as Multi Analyte Profiling (xMAP) technology, which is an automated fluorescent microsphere based multiplex immunoassay (Cho et al. 2015; Pedersen et al. 2017). The xMAP technology enhances the ease of use and automation through binding of multiple allergens onto different beads. Proteomics tools and techniques have been useful to determine the primary structure of approximately 850 allergens (Nony et al. 2016). A targeted proteomics approach combining multiple reaction monitoring (MRM) and labeled standard peptides is commonly used to determine accurate allergen levels in important crops such as soybean, wheat, and maize (Houston et al. 2011; Rogniaux et al. 2015; Stevenson et al. 2015).

Table 1 Recent large-scale proteomics-based studies in most common food crops for allergen analysis

Crop	Technique	Nutrient trait studied	References
Maize	MALDI-TOF/TOF MS	Allergen Bet v 1.01C	Huang et al. (2012)
Maize	Isoform-specific tandem mass spectrometry	Allergen Analysis	Stevenson et al. (2015)
Peanut	Linear trap Mass spectroscopy	Allergen content	Stevenson et al. (2009)
Peanut	Data-independent acquisition coupled with ion mobility mass spectrometry–mass spectrometry (DIA–IM–MS)	Allergen composition of raw peanuts and roasted peanut flour	Johnson et al. (2016)
Rice	2DE, tandem MS and multidimensional protein identification technology (MudPIT)	Allergen identification	Koller et al. (2002)
Soybean	LC–MS/MS	Allergen analysis	Houston et al. (2011)
Soybean	2DE, LC–MS/MS	Allergen and antinutritional profiling	Seo and Cho (2016)
Wheat	2-DE/MS	Gluten protein quality in grain	Pompa et al. (2013)
Wheat	Targeted MS/MS	Allergen analysis	Rogniaux et al. (2015)
Wheat	Linear ion trap quadrupole (LTQ)	Gluten profiling	Bromilow et al. (2017)
Wheat, rye, barley, oats	RP-HPLC, SDS-PAGE, LC-ESI-QTOF-MS and untargeted LC-MS/MS	Gluten protein characterization	Schalk et al. (2017)

Analysis of essential and nonessential trace elements

Another important aspect of food quality is dependent on accurate estimation and profiling of micronutrient content. The micronutrient concentration in food depends on the environmental persistence of trace elements, whereas the accrual of these trace elements directly depends on their respective source, edaphic and climatic conditions, plant genotype, and agro-technical conditions. Many analytical tools, including enzyme-based techniques, have been developed for nutrient analyses in food samples. Enzyme-based assays enable selective, sensitive, and accurate determination of both macro and micronutrients in foods. Spectroscopic and chromatographic methods have become very popular for separation and identification of food components because of their high

reproducibility and sensitivity (Koeberl et al. 2014). Microwave plasma atomic emission spectrometry methods can be used for determination of boron, phosphorus, and molybdenum content (Jia et al. 2011). Similarly, biological and immunological methods are also used for detection of specific targets in food samples. In recent years, the application of enzyme-based technology has received a boost with the development of biosensors. Bioavailability of essential minerals and toxic contaminants can be tested in different environments using the whole cell-based biosensors (Gui et al. 2017). Because of low mobility and non-degradability, some metallic trace elements, such as lead and cadmium, tend to accumulate in soils and the consumption of large amount of food grown on contaminated land can be toxic. The bioavailability of such metallic trace elements (zinc, lead, and cadmium) in soil was efficiently analyzed using biosensors

(Hurdebise et al. 2015). Disposable electrode printed (DEP)-On-Go is a handheld device that offers simple and affordable detection of multiple trace heavy metals in field, water, or food samples (Biyani et al. 2017).

Analysis of microbial contaminants

The microflora associated with both pre- and post-harvest food products includes molds, yeasts, and bacteria. Some fungi produce toxic metabolic products, known as mycotoxins, and have been the focus of research during the past several years. Some mycotoxins, such as fumonisin, aflatoxin, and ochratoxin in particular, have been associated with human pathologies. Several techniques, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), MS, enzyme-linked immunosorbent assay (ELISA), and electrochemical immunosensors, have been used for detecting and quantifying mycotoxins in food (Imam et al. 2016). Recent developments in “omic” strategies and techniques have enabled the buildup of comprehensive data sets of microbial secreted proteins and other metabolites for study of their interaction with host crops (Imam et al. 2016). Single protein identification using SDS-PAGE, protein fractionation using 2D gel electrophoresis, and total protein digest identification using shotgun proteomics and tandem LC–MS can be utilized to generate microbial constituent protein data sets (Carberry et al. 2006). Quantitative isobaric tag for relative and absolute quantification (iTRAQ)-based LC–MS/MS proteomics analysis of *Aspergillus niger* helped in the identification of novel hydrolytic enzymes (Adav et al. 2010). Therefore, this technique has potential relevance in the microbial secretome analysis to unlock the pathways related to microbial toxins and is also advantageous over 2D gel electrophoresis because of its ability to detect proteins, which are low in abundance or are highly acidic, highly basic, or highly hydrophobic in nature. The effect of a mycotoxin, ochratoxin A, on *Arabidopsis thaliana* plants was studied using this technique (Wang et al. 2017). Another technique called enzyme immunoassay screen, is also useful to detect mycotoxin in food and was used to detect a mycotoxin called tenuazonic acid in sorghum grains and sorghum-based infant food (Gross et al. 2017).

Proteomics and other omics techniques as tools to identify genes and molecular markers for biofortification

Proteomics and functional genomics based techniques can play a major role in nutritional enhancement of crops in the current scenario by facilitating the identification of candidate genes and pathways affecting micronutrient, quality, and protein accumulation. Different approaches, such as targeted proteomic technologies, nuclear magnetic resonance, next generation sequencing, and microarray technologies generate massive genome, gene expression, protein, and metabolome profiling, depicting a global and in-depth analysis of gene and gene networks. However, proteomics studies have an edge over omics techniques because it has capacity to reveal proteins, the functional molecules in a cell that are closely associated with the phenotype. However, major challenges for proteomics are throughput capacity and cost. The proteomics in conjunction with other related techniques fathoms massive information including the identification of novel genes, which could be potentially tapped for bio-refinement of current cultivars and nutraceutical breeding. Plant traits are result of the interactions between different metabolic and regulatory pathways, requiring a number of posttranscriptional events such as alternative splicing, proper folding, transport and localization, assembly into complexes, translational or posttranslational regulation. Therefore, identification of protein QTLs (pQTL) is desirable to predict the outcome of genetic crosses (Rödiger and Baginsky 2018). Recently, these efforts have been extended into some plant studies such as genome-wide mapping of protein abundance was conducted in 98 maize inbred lines (Jiang et al. 2018). Study demonstrated that there were some unique pQTLs distinct from eQTLs suggesting that diverse regulatory genetic mechanisms influence gene expression phenotypes at many different levels in a natural maize population. In another study, Acharjee et al. (2018) identified pQTL across the potato genome and detected pQTLs that co-localized with phenotypic QTLs for several quality related traits (including starch content and cold sweetening) in a mapping population consisting of 98 progeny originated from a dihybrid cross in potato. However, cataloging all the proteins present in a cell is a Herculean task compared to the sequencing of genome. For this reason, “multi-

omics” platforms are most suitable for the discovery and characterization of candidate genes for nutritional enhancement and bioavailability of nutrients in food and feed crops in the near future. Depending on the objective of study, a holistic “multi-omic” approach can be applied involving genomics, transcriptomics, proteomics and/or metabolomics to decipher pathways involved in quality traits of crops (Jiang et al. 2017). Metabolites are closer to phenotype of the species and can be measured easily in a high throughput manner from a small amount of sample. For this reason, proteomic techniques have been used in combination with metabolomic techniques. For instance, nutritional properties of different mung bean cultivars were evaluated using both the techniques (Hashiguchi et al. 2017). Similarly, an integrated multi-omics approach was used to study tissue-specificity related to rice seed quality (Galland et al. 2017). However, data analysis from thousands of samples and accurate quantification of unknown/known metabolites are technically challenging. Therefore, caution should be exercised to compare and combine the data obtained from different approaches (Ramalingam et al. 2015).

A proteomic study was conducted for exploiting the diversity of lupin seed storage proteins to find new sources of nutraceuticals for health and welfare (Cabello-Hurtado et al. 2016). The accumulation of storage proteins encoded by gliadin genes, which play an essential role in nutritional quality of flour was verified and quantified using proteomic methods (Zhang et al. 2015). Transcriptional and proteomic analyses of *opaque2* mutant in maize were conducted to study the effect of this mutation on the expression of lysine-rich proteins and genes involved in the biosynthesis of starch (Jia et al. 2013). Use of these genes might lead to the development of high lysine maize lines with improved nutritional quality. Genomics and protein based methods helped in the detection of higher amount of calmodulin protein in developing embryos of high grain calcium finger millet genotype, which correlated with higher accumulation of calcium (Kumar et al. 2014). Peptide mass fingerprinting and transcript analysis were used to identify another calcium binding protein, calreticulin, and to study its role in calcium accumulation during spike development in finger millet (Singh et al. 2016). Proteomic analysis helped in the identification of 14 differentially expressed proteins, including NAD-dependent isocitrate dehydrogenase, triticin precursor, LMW-s

glutenin subunit, and replication factor C-like protein during grain development between two Chinese bread wheat cultivars, which differed in gluten quality (Guo et al. 2012). Distelfeld et al. (2007) used molecular mapping along with plasma-optical emission spectroscopy to study grain protein content locus, *Gpc-B1*. The *Gpc-B1* locus encodes a NAC transcription factor and was found to mobilize protein, Zn, and Fe concentrations efficiently in the grain (Distelfeld et al. 2007). A combination of omics approaches facilitated the dissection of the pathway associated with ascorbic metabolic network in tomato, which can augment the vitamin C biofortification efforts (Rugieri et al. 2016). Thus, integration of proteomics, genomics, transcriptomics, and metabolomics strategies can be applied for identification and validation of other micronutrient traits and for advancement of biofortification in food crops.

Molecular breeding is being integrated into conventional breeding schemes to get biofortified cultivars at enhanced speed and lower cost, as exemplified in the case of increase in iron content in rice (Paul et al. 2012). The identification, functional characterization, and validation of candidate genes related to quality traits are indispensable steps in the development of functional molecular markers and proteomics and other omics techniques can be applied to obtain this information. Functional molecular markers are preferred in marker-assisted breeding programs as they are directly associated with the trait and can give high rate of accuracy during selection in plant breeding programs. Several protein quality-related functional molecular markers have been developed in wheat (Kumar et al. 2018). Similarly, recent studies conducted in maize helped in the identification of molecular markers related to pro-vitamin A content (Azmach et al. 2013) and α -carotene content (Zhou et al. 2012). Molecular markers were developed in maize to select DGAT1-2 gene governing the oil content and quantity of oleic acid (Chai et al. 2012). In rice, some molecular markers were identified for grain nutritional quality traits, such as proteins, lipids, and mineral contents (Anuradha et al. 2012). These molecular markers can hasten the breeding process aimed at increasing the nutritional value of different crops. Furthermore, correct and easy assessment of biodiversity using molecular markers will assist in enhancing the micronutrient content in food crops

through informed integration of the available germplasm within the ongoing breeding programs.

Applications in analysis of genetically modified (GM) crops

Modern genetic engineering technologies, such as transgenics and genome editing, can significantly advance the biofortification programs because these techniques are cost efficient and less time consuming than the conventional breeding techniques (Francis et al. 2017). Newly developed genome editing techniques, such as the CRISPR–Cas9 system, can create variation at specific sites in the plant genome (Arora and Narula 2017). Significant research efforts have been focused on enhancing the nutritional value of crops through genetic engineering but none of the GM biofortified crops are available for commercial cultivation, till date, because of regulatory and ethical issues (De Steur et al. 2017). Extensive analysis of genetically modified (GM) crops is required before releasing them as a new cultivar because transgenic plants may have high expression of new proteins with allergenic potential. Proteomic techniques, in conjunction with other omic techniques, can be utilized to study newly expressed proteins, proteins with allergenic properties, and any off-target effects in GM crops in comparison to their non-GM counterparts. For example, GM wheat lines were compared with non-transformed wheat lines using a proteomic approach to analyze the expression of wheat allergy proteins, viz., wheat kernel albumins/globulins (A/G) and gluten proteins (Lupi et al. 2013). In a recent study, Liu et al. (2018) used iTRAQ comparative proteomic approach to evaluate unintended effects on proteomic profiles in GM soybean lines in comparison to their non-GM soybean lines. Thus, proteomics and other omics based approaches will play a significant role in the production of biofortified varieties of staple crops in a time and cost efficient manner.

Conclusion

Because of impending changes in the food availability and food consumption, maintaining nutritional requirements in a diet represents a major challenge worldwide. Proteomics is an emerging field with

cutting-edge technologies that can assist in the detection of food allergens, quantification of micronutrient content, and determination of protein composition in early stages of crop biofortification efforts. The recent advances in 2D-PAGE, MS/MS analysis, and other high throughput methods coupled to advanced protein databases have enhanced our understanding of protein networks, providing crucial inputs towards developing nutritionally enriched crops. Increased reproducibility and ability to detect low abundance molecules in cost efficient manner is achieved through high-throughput techniques. However, proteome maps and appropriate bioinformatics platforms for all food crops need to be developed in order to utilize the data efficiently. The integration of proteomics with other “omics” techniques has not only been instrumental in discovering gene functions related to nutritional aspects but also in identifying robust molecular markers to accelerate the development of superior germplasm. However, success of any program for ensuring food security lies in the continued use of “omics” tools and techniques to select, develop, and integrate nutritionally superior genotypes within our food supply chain and to avoid food contamination with microbial toxins in order to fight against malnutrition in both developing and developed countries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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