Nutrigenomics in Cereals

12

Shashank Kumar Yadav, Pragya Yadav, and Viswanathan Chinnusamy

Abstract

The sequencing of human genome has opened floodgates of immense knowledge and opportunities in the realm of personalized nutrition and health. Nutrigenomics is a rapidly emerging field that employs tools of bioinformatics, genomics, metabolomics, proteomics, epigenomics, and transcriptomics to bridge the existing gap and build up a holistic understanding of the interaction of dietary components and genes at the molecular level. Recent scientific evidence has fortified that genetic polymorphism plays a key role in daily nutritional requirements, metabolic response to food, and potency of dietary factors in response to diseases. Every individual has a categorical response to nutrients which results in nutrient impairment leading to alteration of gene expression. Several reports have highlighted that nutrients like carbohydrates, amino acids, fatty acids, vitamins, and minerals play a pivotal role in the regulation of gene expression. Cereal crops predominantly constitute 50% of daily dietary energy and protein source for majority of the worlds population. With the advent of molecular biology tools like genetic engineering, genome editing, and marker-assisted breeding, cereal crops are being enriched in order to maximize their nutritional potential while minimizing the anti-nutrient contents. The chapter highlights the potential role of cereals in nutrigenomics with emphasis on the current advances and challenges in the field.

Keywords

Gene-diet interactions · Nutritive value of cereals · Functional foods · Omics · Non-communicable diseases · Genome engineering

S. K. Yadav (✉) · P. Yadav · V. Chinnusamy
Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

R. Deshmukh et al. (eds.), *Biofortification in Cereals*,
https://doi.org/10.1007/978-981-19-4308-9_12

12.1 Introduction

Nutrigenomics is "the study of bidirectional interactions between genes and diet". Deciphering of the human genome in 2001 marked the beginning of a revolutionary era in the realm of nutrigenomics, particularly its impact on human health. The central dogmatic flow of genetic information from gene expression to protein synthesis is largely influenced by lifestyle, and diet particularly nutrients and non-nutrient components present therein. The novel example for the crosstalk between the human genome and its environment includes genome-food interaction which eventually translates into a healthy or diseased state on the basis of a given genome. Phenotype, which represents the physical characteristics or observable traits of an organism, is governed by the spatio-temporal expression of a gene or multiple genes. Apart from genetic expression, phenotypic characteristics are also influenced by nutrition, e.g. cardiovascular diseases are mainly caused by cholesterol present in the food. Cereals have remained an indispensable part of the human diet since the dawn of agriculture, but the true nutritive potential of cereals has largely remained unexplored. In the present chapter, emerging paradigms of nutritional genomics with respect to cereals are discussed as they comprise the functional food market. Emphasis has been laid on leveraging the role of genomics tools to produce better food for improving human nutrition and health and thereby delivering societal and economic benefits.

12.2 Nutrition Value of Cereals

Cereals and cereal products have always been an indispensable part of most human diets since the dawn of agriculture till the present irrespective of socio-economic and geographical divergence across the world. The importance of cereals and cereal products can be accessed from the fact that cereal crops are cultivated across 50% of harvested area worldwide [FAO Food Outlook], contributing to the greatest degree to the global food security, which extends to approximately 2800 million tons annually [FAO 2020]. Cereals and cereal products alone contribute to 75% of the daily dietary energy and nutritional requirement of the global population as they are a mammoth source of macronutrients, primarily carbohydrates (75%), proteins (5–15%), and fat (1–5%) inclusive of other micronutrients such as vitamins and minerals (World Health Organization 2003). Nutritive significance of major cereal crops has been summarized in Table 12.1. Cereals solely impart 10,000–15,000 kJ/kg of energy, which is approximately 15–20 times more than fruits and vegetables. Moreover, cereals are the paramount source of dietary fibres and bioactive compounds, particularly as whole grains with augmented health benefits (Hall et al. 2017).

Globally, standard nutritional guidelines are being devised emphasizing upon the embodiment of a larger proportion of whole grains in diet for boosting health (EU Science Hub n.d.; U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015; Dietitians Association of Australia (DAA) n.d.).

Table 12.1 Nutritive content of major cereals

Cereal	Protein (g)	Fat (g)	Carbohydrate (g)	Minerals (g)	Calcium (g)	Fibre (g)	Energy (kcal)
Wheat (whole)	11.9	1.5	71.2	1.5	41	1.2	346
Rice (raw)	6.8	0.5	78.2	0.6	10	0.2	345
Finger millet	12.3	1.3	72	2.7	344	3.6	328
Foxtail millet	12.3	2.7	66	2.1	31	8	365
Proso millet	12.5	4.2	73	1.7	14	2.2	378
Pearl millet	11.6	5.0	67.5	2.3	42	1.2	361
Barley	13.6	1.2	74	1.5	26	2.4	352
Sorghum	10.4	1.9	72.6	1.6	25	1.6	349
Amaranth	14	7.0	65	1.5	37	2.7	371
Maize	9.2	4.7	72	1.9	1.2	7.3	365
Oat	16.9	6.9	66.3	1.8	4.0	10.6	389

Source: Gopalan C, Rama Sastri B.V., and Balasubramanian, S.C., 2004, Nutritive Value of Indian Foods, National Institute of Nutrition, ICMR, Hyderabad

One of the many pivotal functions of whole grains, recently discovered, highlights their prebiotic functionality for gut microflora, a principal component for the host's wellbeing (Dietitians Association of Australia (DAA) n.d.; Costabile et al. 2008). In native or unprocessed form, cereals are the key source of carbohydrates, proteins, fats, essential oils, fibre, minerals, and vitamins which upon post-harvest processing (dehusking and dehulling) for value enhancement and other commercial aspects like polishing results in loss of essential minerals and oils. For human consumption, cereals are routinely marketed in native form or as additives in processed food. As animal fodder, they are primarily consumed by poultry and livestock, which are ultimately consumed by humans as poultry products, dairy, and meat. Cereals also have multitudinal commercial objectives, such as in adhesives, oils, paper industry, textiles, laundering preparations, and cosmetics (Rosentrater and Evers 2018).

12.2.1 Composition and Nutritional Aspects of Cereals

12.2.1.1 Carbohydrates

Carbohydrates dominantly constitute approximately 80% of total dry matter in cereals, primarily as crude fibre and soluble carbohydrates. Cellulose, pentosans, and hemicelluloses are the key constituents of fibre, while starch forms the major portion of soluble carbohydrates across all cereals. Trace quantities of dextrin and other free sugars including glucose and disaccharides like sucrose and maltose are also present.

12.2.1.2 Protein

Protein accounts second after carbohydrate in terms of constituents, but its content varies across different species as well as lineage amongst the same species of cereals. Protein is omnipresent in different proportions across all tissues of cereal grain. Elevated concentration of protein occurs in embryo, scutellum, and aleurone layer compared to endosperm, pericarp, and testa. Categorically, protein in cereals belongs to albumin, globulin, prolamine (gliadins), and glutelin subclass which varies in different cereals.

12.2.1.3 Lipids

Lipids constitute 1–2% in major cereal crops like rice and wheat while 3% in maize with highest proportion present in germ and bran compared to other tissues of grain. Chemically lipids in cereals are mostly triglycerides of palmitic, oleic, and linoleic acid. Apart from these, cereals also are sources phospholipids and lecithin. Owing to the amount of cereals consumed, it is assessed that cereals solely can meet 50% of our essential fatty acid requirement while cereals in conjugation with pulses can suffice the essential fatty acid requirement.

12.2.1.4 Minerals

The majority of minerals (about 95%) naturally exist as sulphates and phosphates of potassium, magnesium, and calcium. Despite a substantial proportion of phosphorus and calcium present in phytin, these remain largely unabsorbed by the body. Phytates also block iron uptake by the body, but upon germination, phytate content declines sharply due to enzymatic breakdown leading to improved availability of iron to the body. Unprocessed cereals contain more phytates than refined or polished cereals. Moreover, cereals are also a rich source of essential trace elements like zinc, manganese, and copper.

12.2.1.5 Vitamins

Unprocessed and whole-grain cereals are a rich source of vitamins, particularly vitamin B. Since the majority of these vitamins are located on the bran, therefore polishing of grains considerably reduces the vitamin content of cereals. Apart from postharvest processing, parboiling of cereals leads to depletion of vitamin content present on the outer layer of grains. Except for maize, cereals lack either vitamin A or C. Cereal grains-derived oils are also a rich source of vitamin E.

12.2.1.6 Enzymes

Certain grains contain many enzymes such as proteases, amylases, oxio-reductase, and lipases which are of prime importance. Proteases are relatively more in the germ compared to other tissues, while amylase activity accentuates during germination. Lipase enzyme is primarily responsible for fatty acid metabolism during the storage of cereals.

12.3 Cereal and Cereal Product Contribution to the Diet

Being heterotrophs, humans depend upon different plants as a source of food and energy, where cereals occupy the paramount position amongst them [FAO 2002]. Cereals are the edible seeds or caryopsis, belonging to common grass members of the monocot family Poaceae, also known as Gramineae (Bender and Bender 2009). Most extensively consumed cereals include triticale, rice, oat, maize, sorghum, barley, millet, and amaranth. The massive use of cereals as functional food can be attributed to large-scale cultivation, higher harvest index, ease of storage, greater mean life, and substantial nutritional and calorific contribution worldwide.

12.3.1 Cereals and Cereal-Based Food

Cereal grains apart from being the primary source for obtaining daily energy requirements are also part of many cultures in many countries and serve as the raw material of many foods and beverages. Apart from lineage and genomic constituents, nutritional abundance of cereal crops depends on the degree of postharvest processing like hulling, husking, and milling resulting in the removal of outer bran layer which is a rich source of vitamins, fibre, and minerals. Cereal-derived/cereal-based foods are primarily made from diverse grains comprising wheat, maize, rice, oats, rye, barley, millet, and sorghum, with rice, maize, and wheat together accounting for two-thirds of human dietary consumption (Sarwar 2008). Examples of cereal-based products majorly include cornmeal, corn grits, wheat, oat, rice and rye flour-based dough and bread, snack foods, tortillas, cakes, dry mixes, etc. Moreover, cereal-based products are also used as basal material for coating, batter, sweeteners, thickeners, baby food, bakery products, and alcoholic beverages such as wine and beer. Cereal-derived food products can broadly be majorly grouped into four categories:

1. Flour-based baked products like bread, cakes, pastries, cookies, dough, and cakes.
2. Processed or value-enhanced grains (milled, hulled, and polished), cornmeal, pearled barley, hominy, farina, corn grits, soup, and thickening agents rich in starch.
3. Whole-grain products like black rice, rolled oats, puffed and shredded grains.
4. Fermented, roasted, and boiled grains-derived beverages.

12.3.1.1 Bread

Bread making can be traced back to 450 BC in Rome with the advent of water milling. Various types of bread are prepared using different cereals as basal material, and breads using a mixture of different cereal flours mixed in definite proportion to be used as a functional food with superior nutritive significance were used since ancient times in South Asian regions, particularly in the Indian sub-continent which is nowadays being marketed under multigrain flour-based bread. High consumption

of bread across the world can also be credited to its high nutritive value with 40% carbohydrate, 8–9% protein, and fat <3 g/100 g.

12.3.1.2 Breakfast Cereals

Breakfast cereals are the key source of daily nutrition and calorific significance. Deficiency of nutrients like riboflavin, niacin, folate, and vitamin B12 was more profound in infants not consuming fortified breakfast cereals (Patient and Ainsworth 1994). Research on adults exhibited that fibre- and carbohydrate-rich breakfast was associated with the highest post-breakfast alertness (McNulty et al. 1996). In another study, positive correlation between cereal consumption in breakfast and effect on health showed that adults having cereals daily in breakfast reported superior physical and mental wellbeing compared to the ones consuming in lesser frequency (Holt et al. 1999).

12.3.1.3 Bakery Products

Cereal foods contribute 5% of daily fat intake in infants and 7% amongst adults, despite low account of fat in cereal-based foods (Smith 1999). Bakery products like biscuits, pancakes, cookies, and buns constitute foods comprising sugar and fats. Therefore, under the umbrella of "Balance of Good Health", they do not fall into the same category as the cereal products discussed above.

12.4 What Is Nutrigenomics?

Nutrigenomics is a rapidly evolving realm of science that bridges the understanding of the effect of genetic diversity in response to food and its constituents. It focuses on unravelling the underlying mechanism of signalling cascade triggered as a result of the interlinking of dietary bioactives with the genome at the molecular level. It also helps in understanding how a particular nutrient or an anti-nutrient regime present in our diet configures human health. Nutrigenomics teaches us what is the specific nutrient requirement of our body which ultimately determines the genetic messages our body receives.

12.4.1 Origin of Nutrigenomics

Nutrigenomics might sound like a new domain of science to some, but its advent can be dated back to the genesis of the role of genes in shaping the architecture of all the living beings on the planet. The term "nutrigenomics" was coined by Peregrin in the year 2001 (Gregory et al. 2000). In the mid-1970s, the field of nutritional research gained momentum with the advancements in the field of genetics where genes were characterized based on functionality, particularly their role in human health. Later nutrient components were considered as "signalling molecules" which relay the signals to the cells and ultimately to the nucleus to trigger the change in expression

of a particular gene or sets of the gene leading to alteration in protein or a metabolite turnover (Public Health Nutrition n.d.).

12.4.2 Genesis and Components of Nutrigenomics

The postulation about the effect of food on human health has been ages old. Policies were formulated and amendments concerned were made perpetually worldwide to emphasize the prominence of diet and nutrients present therein in preventing and combating chronic diseases. But the major breakthrough came with the publishing of the draft of human genome that eventually led to the interlinking of nutrients and genome. Nutrigenomics is an amalgamation of multidisciplinary sciences under one umbrella, encompassing genetics, proteomics, metabolomics, transcriptomics, and epigenomics (Fig. 12.1) which helps in designing a "genome-tailored diet" for each individual leading to the stabilized genome. Genome stability implies to minimizing damage to DNA, epigenetic alterations (acetylation and methylation during chromatin remodelling), transcriptome (RNA and non-coding miRNAs during post-transcriptional gene regulation), and proteomics (alterations in turnover of a protein).

12.4.3 Modern Nutrigenomics: Personalized Nutrition

Scientists are deciphering diverse interplay between the genes, nutrition, and disease. Modern-day nutrigenomics takes advantage of advanced high-throughput techniques for the identification and prevention of nutritionally based diseases. The role of genes and its association with disease, primarily, the role of dietary and bioactive components affecting the regulation of these genes is also being explored. Different types of diets have been shown to alter body weight, metabolic rate, blood pressure, cholesterol, and other health variables distinctively relying on the genetic constituent of an individual (Bayless and Rosensweig 1966). These findings have put forth resolutely the concept of personalized nutrition, which can provide dietary propositions based on the health and genetic profile of a person.

12.5 Nutrigenomic Diseases and Molecular Diagnosis

Research pertaining to nutrigenomics has relied on the doctrine of nutrition-gene-disease interactions and thereby preventing mankind from so-called modern-day diseases like obesity, cardiovascular diseases, respiratory disorders, diabetes, and different forms of cancers. Collectively such diseases come under the umbrella of non-communicable diseases (NCDs) which are largely mediated by overexposure to a particular type of diet or precisely junk food. Upon overexposure to a certain type of unhealthy diet, our body responds to it by abnormal or disturbed metabolism leading to the synthesis of certain biomolecules which act as biomarker for the identification and diagnosis of diseases. On molecular basis damage or mutation in

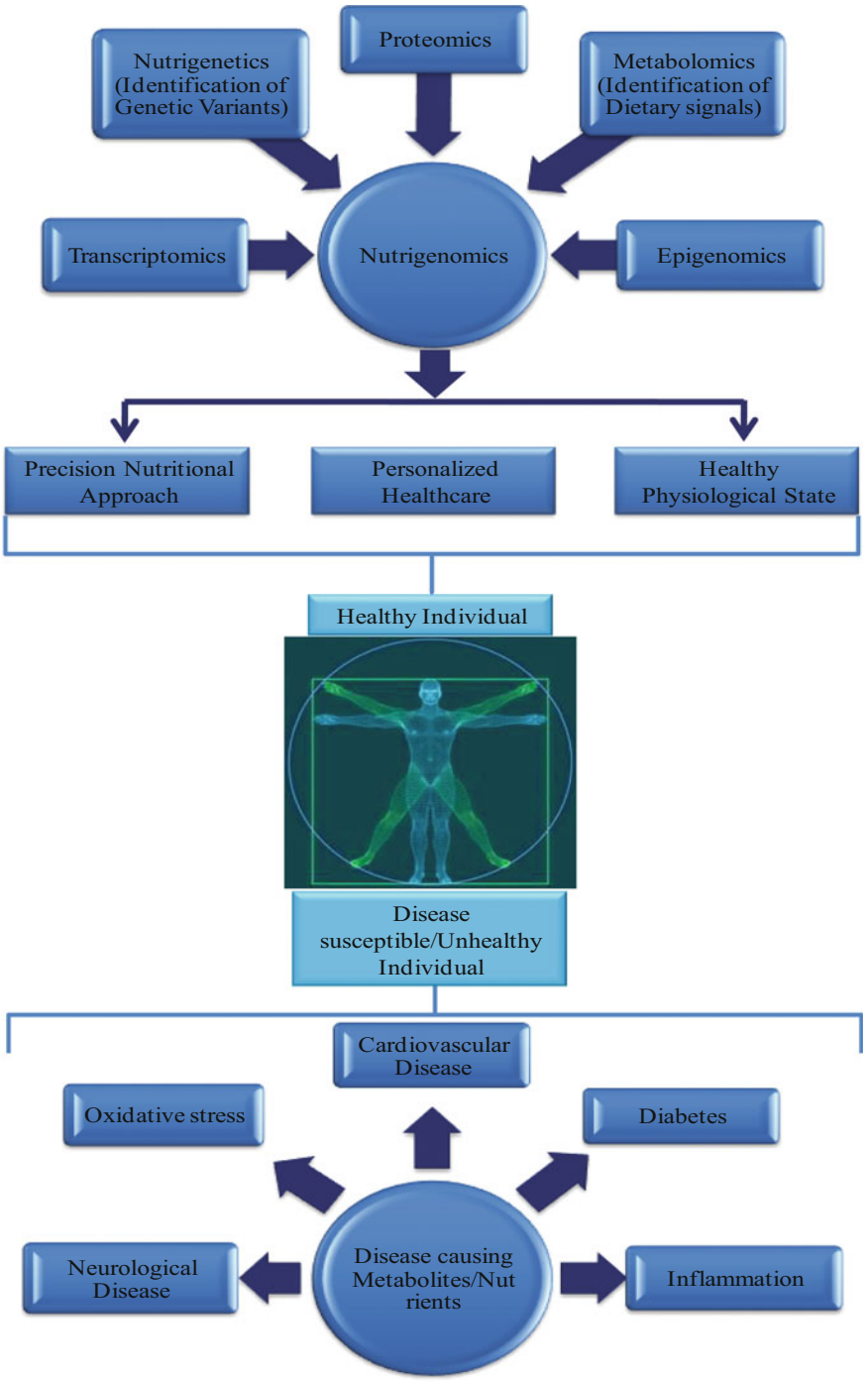

Fig. 12.1 Several components of nutrigenomics and its role in disease prevention

DNA or a portion of DNA fragment can lead to certain genetic or hereditary disorders which can be diagnosed by molecular techniques, for example, chip-based diagnosis, hybridization assays, PCR-based assays, SNP genotyping, etc. In addition, a widely exploited nutrigenomics tool is transcriptomics, which makes use of microarray assay to quantify mRNA copies of all transcribed genes in a spatio-temporal manner.

12.5.1 Phenylketonuria (PKU)

Phenylketonuria (PKU) is a metabolic genetic disorder characterized by a mutation in a single gene coding for hepatic enzyme phenylalanine hydroxylase (PAH) causing the fatal build-up of phenylalanine in the blood. PAH enzyme plays a key role in metabolizing aromatic amino acid phenylalanine, and affected individual is prohibited from consuming phenylalanine-rich foods like milk, cheese, chicken, fish, egg white, etc. (Enattah et al. 2002). Infants and young-aged children are more prone to phenylketonuria (PKU), but nowadays infants are routinely screened for PKU, and if found affected are recommended a special diet lacking phenylalanine.

12.5.2 Identification of the Gene(s) for Lactose Intolerance (LI)

Lactose intolerance (LI) or hypolactasia is a genetic disorder faced by the majority of adults worldwide with an estimate of 68% of people facing issues digesting lactose. In the early 1960s, researchers unravelled that inability to digest milk is primarily a genetic disorder (Moraes and Pereira 2009). Loktionov (2003) identified that mutation in *lactase* (*LCT*) gene, encoding for lactase enzyme, causes incompetence to break down lactose. Further, the advent of lactose intolerance was traced back to 10,000–12,000 years ago in Europe, where polymorphism in a single nucleotide (SNP) of a gene resulted in constitutive expression of lactase gene in adults. The person with lactose intolerance could utilize nutrition-rich dairy products much more efficiently than normal persons. The most commonly recommended treatment focuses on the avoidance of dairy products.

12.5.3 Galactosemia

Galactosemia is a rare inherited metabolic disorder in which the affected person is unable to utilize galactose. In other words, it's the inability of body to convert galactose into glucose for generating energy in the form of ATP. The molecular basis of galactosemia has been found to be a defect in *galactose 1-phosphate uridylyltransferase* (GALT) gene, the product of which is involved in the catabolism of galactose (Tucker et al. 2013). Alterations in dietary intake can prove to be fruitful; further in-depth knowledge is imperative where a single nutrient may affect our biological system.

12.6 Malnutrition: The Genesis of Chronic Diseases

A diet rich in all essential nutrients is a decisive factor for the promotion and maintenance of sound health throughout the life of an individual. Malnutrition is found to be the key factor for chronic-related disorders in approximately 40% of patients (Ilnytska and Argyropoulos 2008). Malnutrition can be correlated with complication rates, disability, need for care, as well as mortality. Malnutrition can be primarily attributed to two factors: inadequate feeding and anomalous nutrient uptake or metabolism by the body. Some of the nutrition-dependent chronic diseases that pose a serious threat to public health include cardiovascular diseases, obesity, cancer, diabetes, and osteoporosis.

12.6.1 Obesity

Obesity is the most prevalent multifactorial disease that forms the core of numerous metabolic disorders affected by variable environmental and genetic factors. Nearly one-third, i.e. 30% of the world population, is either obese or overweight, leading to a rise in chronic diseases [WHO 2018]. Excessive food intake has been found to be associated with polymorphism in genes encoding several sensory or taste receptors including leptin, insulin, ghrelin, and cholecystokinin (Ferguson 2006). Nutrigenomics assists in the formulation of novel functional diets for restraining obesity by exploiting scientific and molecular mechanisms of effect of bioactive components on basal metabolic rate (BMR) and body weight. Obesity has been found to drastically augment the peril of various chronic diseases including CVD and cancer (Kopelman 2000; Bianchini et al. 2002). Increasing physical activity is complemented with the shift from fat-rich foods and beverages to food rich in anti-inflammatory bioactive, such as caffeic acid, tyrosol, quercetin, and seeds of *Salvia hispanica*. These bioactive compounds have been found to modulate gene expression via activation/inactivation of transcription factors (Cozzolino and Cominetti 2013; Costa and Rosa 2011).

12.6.2 Cancers

Cancer can be defined as anomalous and unconstrained cellular growth with the potential to spread to distinct cells and tissues. Being a multifactorial disease, cancer is characterized by mutations in several oncogenes and alteration in gene expression, transcriptome, and eventually metabolic operations. Mutation in tumour suppressor gene p53 resulting in mutated or non-function p53 protein has been found to be the primary cause of cancer in >50% of different types of human tumours. Apart from being genetically governed, natural and dietary components heterogeneously manifest tumour stimulation, invasion, and progression by targeting and altering several pathways. The majority of cancers (35–40%) are primarily influenced by diet. However, numerous dietary components having nutraceutical and phytochemical

significance help in the prevention of cancer through the genetic and epigenetic mechanism, for example, certain nutrients play a key role in the maintenance of normal cellular methylation. Numerous preclinical studies have stipulated the role of nutrients in cancer prevention. Deciphering the crosstalk between nutrigenomics in the regulation of cancer is already ascertained. Another prominent cause of cancer is irreversible damage to DNA and the inability of cellular machinery to repair the genetic material because of overexposure to certain genotoxins and deficiency of nutrients required as cofactors for DNA repair enzymes. Apart from macronutrients, micronutrient plays an indispensable role in shaping up individual's health depending upon genetic makeup, physical state, and age (Costa and Rosa 2011; Almendro and Gascan 2012). Studies have highlighted that deficiency of micronutrients, such as vitamins, selenium, niacin, and zinc, can lead to mutation in DNA in a similar manner as observed during radiation exposure. These mutations can lead to degeneration of the helical structure of DNA and oxidative lesions leading to cancer (Cozzolino and Cominetti 2013; Costa and Rosa 2011; Almendro and Gascan 2012). Unhealthy eating practices lead to the cellular build-up of obnoxious metabolites which can interact and alter DNA structure causing mutation at nucleotide residue. For example, aflatoxin B1 present in fungi (*Aspergillus flavus*)-contaminated food causes apurination of DNA causing acute damage to liver including cirrhosis, necrosis, and carcinoma (Moraes and Pereira 2009; Mahan and Scott Stump 2005). Methionine, an essential amino acid, is synthesized from its precursor 5-methyltetrahydrofolate which in turn is synthesized from folate present in food. Insufficient dietary uptake of folate can lead to hindrance of DNA mutilation leading to increased risk of cancer (Cozzolino and Cominetti 2013; Costa and Rosa 2011; Almendro and Gascan 2012; Cozzolino 2012). Several bioactives such as selenium, prostacyclins, zinc, ascorbic acid, etc. act as antioxidants for coping with imbalanced levels of reactive oxygen species (ROS), which cause irreversible oxidative damage to cell membrane and biomolecules such as DNA, lipid, and lipoproteins.

12.6.3 Type 2 Diabetes

Diabetes alone accounts for >90% of all diseases afflicting ~200 million people worldwide (Surh 2003). Rapid transition from traditional diets to excessive uptake of food rich in carbohydrates and saturated fats is one of the primary causative factors of type 2 diabetes. Mutation in the gene causes alteration in the metabolic function of insulin, the key hormone which regulates glucose and lipid metabolism which is rendered non-functional. Diabetes acts as a genesis for other life-threatening diseases such as cardiovascular disease, stroke, and renal dysfunction (Calder et al. 1998). On the genomic level, a total of 65 SNPs has been identified to be associated with the precarious level of developing diabetes. Reliable and economically cheap genome-based rapid detection has helped early detection of genetic predisposition of SNPs related to cause type 2 diabetes (Chang et al. 1998). Early detection of diabetes helps

in tailoring lifestyle, particularly dietary intake to minimize any obnoxious increase in blood sugar without hampering the health of a person.

12.6.4 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are alone the principal cause of a high mortality rate accounting for 17.9 million deaths (representing ~31% worldwide) in 2017 [WHO 2019]. CVDs are primarily a multifactorial condition involving other health complications such as thrombosis, obesity, and hypertension which can be attributed to environmental factors. The interrelation between dietary factors and peril for CVD can be rooted back to the 1950s, when heart diseases were linked to the consumption of saturated fatty acids. Through advanced technologies, relationship between nutrients and CVD risk has now been well established. Studies have focused on the identification of genes and genetic variation associated with CVDs and related health complications. Atherosclerosis is one of the substituent elements of CVD pathogenesis causing metabolic disorder and lipid transport with chronic inflammation (Cozzolino and Cominetti 2013). Studies have shown that polymorphism in E4 gene coding for apolipoprotein E displayed higher LDL cholesterol. Similarly, allelic variation in the gene encoding for angiotensinogen (AGT), angiotensin-converting enzyme (ACE), and aldosterone synthase (CYP11B2) has been accounted for blood pressure regulation and thus hypertension (Hooper et al. 2001; Schaefer 2002). Several loci have been identified which regulate the progression of CVD: e.g. FTO and 9p21 have been characterized to be associated with obesity and CVD, respectively, which in turn are regulated by gene-diet interaction (Corella and Ordovas 2009; Ye and Kwiterovich 2000). Several biomarkers have been characterized for the detection of CVD; one of such biomarkers is an elevated level of homocysteine which is regulated by dietary folate and alcohol consumption (Mahley and Rall 2000; Luft and Weinberger 1997). Furthermore, reports have fortified the role of macro- and micronutrients in epigenetic regulation of cardiometabolic risks. Some of primary nutrients include methionine, choline, vitamin B12, zinc, and folate which are required for methylation of histones in DNA. Thus, the role of personalized or precision dietary nutrients has become the need of the hour with accentuating CVD worldwide.

12.7 Global Status of Nutrigenomics Research

12.7.1 Global Health Scenario

Despite exceptional advancements being made in the realm of improving public health and disease prevention, a large proportion of the population worldwide suffers from chronic diseases and remains astoundingly overlooked in the global health agenda. Chronic diseases can largely be attributed to the transition in dietary and lifestyle habits which is a direct consequence of rapid globalization. Developing

countries account for the largest proportion of people suffering from non-communicable and infectious diseases. Therefore, a lot of emphasis and efforts need to be made to combat chronic diseases with utmost priority at various international and national levels. Amongst non-communicable diseases (NCDs), cancer, respiratory disorders, cardiovascular diseases, and diabetes attribute 71% of death globally. Every year more than 15 million people die from NCDs in age group of 30–69 years. Such a large percentage lays a considerable strain on health budgets, particularly of developing and underdeveloped economies. Thus, the significance of nutrigenomics in the present era has grown by leaps and bounds to tackle global health issues, particularly NCDs. This is evident from the fact that the global nutrigenomics market is expected to grow by approximately 11% (USD 425.61 million) in the next 5 years from 2021 to 2026 [WHO 2020].

12.7.2 India's Health Scenario

Compared to western countries and other developed nations, the rate of fatal diseases in India is lower. However, India is witnessing an expeditious epidemiological, socio-economic, health, and nutritional transformation over the last two to three decades which can be accredited to the rapid rate of urbanization and changes in lifestyle, particularly eating habits (Franco and Reitsma 2001; Godard and Hurlimann 2009; Rao 2001). Transition in eating habits from consumption of coarse grains to processed rice and wheat has led to an increased rate of cardiovascular and cancers in the Indian population (Shetty 2002). Malnutrition, particularly undernutrition-related disorders, has a deleterious impact on major sections of the population. Contrarily, overnutrition has led to a steep increase in the number of people suffering from obesity and chronic lifestyle disorders, primarily CVD, diabetes, and cancer (Anderson et al. 2001; Sharma and Majumdar 2009; Sinha et al. 2003). Taking such conditions into account, a common consensus needs to be made pertaining to diet-related disorders leading to alteration at molecular and metabolic levels; therefore, nutrigenomics should be employed efficiently and effectively.

12.8 Role of Nutrigenomics for Better, Healthier, and Longer Life

Health is a barometer for measuring the quality of life and prosperity of an individual or a society. The health of a person is also reflected by the ability to adapt and self-acclimatize to adverse conditions. In recent times a radical change in the pattern of diseases has been observed which can be attributed largely to eating habits, rapid urbanization, environment, and lifestyle (Hossain et al. 2007). The interaction between genomic and environmental cues plays a decisive role in the buildout and progression of numerous life-threatening diseases. Diet is one such environmental factor that not only meets the daily metabolic energy requirement of the body but

also regulates epigenetic changes associated with aging (Kaput et al. 2007; Neeha and Kinth 2013). Physical and mental stresses along with lifestyle considerably contribute to the regulation of gene expression in response to the dynamic environment. Bioactive and chemopreventive molecules present in food help in preventing the risk of chronic diseases through regulation at the molecular level (World Health Organization 1990; Bacalini et al. 2014; Jeffery and O'Toole 2013). Traditionally it was believed that nutritional requirements for all the beings are the same, but with the advancement of nutrigenomics, the role of personalized diet or functional foods gained recognition. Every age group and sex has a specific recommended dietary allowance (RDA), specifying the daily nutrient requirement for a healthy and disease-free life. Nutrigenomics is creating a power shift, from an unhealthy disease-prone lifestyle to a healthy disease-free lifestyle. Incurable genetic disorders like cardiovascular disease, obesity, diabetes, cancer, and Alzheimer's cannot be avoided, but following genome-tailored personalized functional foods, one can lead a healthy lifestyle with minimal complications and illness.

12.9 Nutrigenomics Research Tools

The remarkable advancements being made in the field of nutrigenomics can be attributed to various "omic" technologies such as genomics (e.g. SNP genotyping for polymorphism analysis), transcriptomics (e.g. gene expression arrays), metabolomics (e.g. assessment of active metabolites and bioactive compounds), and proteomics (e.g. abundance of a particular bioactive peptide and proteins). These technologies are substantially used to explore the molecular mechanism underlying the role of dietary components in diseases pertaining to nutrition such as cancer, diabetes, inflammatory disease, cardiovascular disease, and obesity (Chae et al. 2007). These technologies will pave way for genome-wide transcript and protein analyses, coupled with bioinformatic tools and databases which can prove to be handy in the identification of the novel gene, proteins, and other bioactive components for unravelling mechanisms underlying disease development and progression.

12.9.1 Genomics

The sequencing of human genome has opened floodgates of immense information and opportunities in the field of nutrigenomics and disease prevention. Novel genes and polymorphism amongst a susceptible and tolerant population in response to disease are now being functionally characterized. Reliable and rapid sequencing technologies have helped in the identification of mutation in a particular gene or several genes at early stages in life which can prove instrumental in minimizing the risk of disease progression.

12.9.1.1 Single Nucleotide Polymorphism

With the advancements in the realm of molecular biology, scientists have been able to identify genes coding for nutritionally imperative proteins primarily digestive enzymes, hormones regulating metabolic pathways, and molecules responsible for the intercellular and intracellular transport of nutrients and cofactors at the site of metabolism. Numerous common SNPs have been reported to alter nutrient requirements. One such example is SNP methylenetetrahydrofolate dehydrogenase (MTHFD1-G1958A) related to organ impairment when a person gets choline low or has deficient diet, most prominent in premenopausal women. Infected mother with such polymorphism has fourfold high risk of having a child born with neural tube dysfunction in comparison to the women on a choline-rich diet (Arts et al. 2001). A new preventive medicine approach is alternative therapies utilizing nutritional approach for disease prevention; one such technology is the development of SNP array which helps in the identification of haplotypes amongst a population. The role of SNPs in relation to diseases like cancer, diabetes, cardiovascular disorders, leukaemia, Down syndrome, and neural tube defect (NTD) has been investigated. SNP profiling for intracellular folate metabolic pathway has been investigated in the Indian population (Najafian et al. 2012). Similarly, the role of vitamin B12 (cobalamine), homocysteine, and folate in acute lymphoblastic leukaemia (ALL) fortifies the imperativeness of gene-environment-nutrition interaction in the development and progression of cancer (Trujillo et al. 2006).

12.9.2 Transcriptomics and RNA-Seq Technology

Transcriptomics is the most extensively used technique for real-time expression profiling of genes in a spatio-temporal manner as well as in response to the defined nutritional state of a being. Variation in intracellular RNA transcript abundance in response to dietary interventions forms the elemental basis for deciphering the complex interrelation between genes and nutrients (Wickramasinghe et al. 2012; O'Brien et al. 2012). In-depth diligent transcriptomic analysis pools all forms of RNA (coding and non-coding) for understanding the post-transcriptional modification, particularly mRNA splicing of a gene. Several types of chips or microarrays are now available which are extensively being used for gene expression and genotyping pertaining to SNPs, point mutations, and short tandem repeats (SSR). Compared to microarray-based transcriptional profiling, a much more efficient and less error-prone technique is RNA-Seq to study nutrient-gene interactions with high unparalleled accuracy and reliability. RNA-seq has led to the identification of novel genes that are upregulated in response to dietary factors, nutritional and physiological state of an individual, micro- and macronutrient deficiencies, and diseases (Wickramasinghe et al. 2012; Swanson et al. 2003; Külahoglu and Bräutigam 2014). High-throughput RNA-seq has become the most preferable choice for enabling inexpensive and routine comprehensive analysis of human transcriptomes or genomes (Shendure and Ji 2008; Chen et al. 2015). This technology is primarily used for quantitative expression analysis of known and unknown transcripts and

mRNA splice variants and for the analysis of SNPs which can be used as potential biomarkers pertaining to a trait (Külahoglu and Bräutigam 2014; Han et al. 2015; Piskol et al. 2013).

12.9.3 Proteomics

Proteins are the key component of the central dogma of inevitably all biological processes; thus, proteomics acquires centre stage as one of the key tools in nutrigenomics research. The role of post-translational modification (e.g. acetylation, ubiquitylation, phosphorylation, etc.) of proteins will help in deciphering the regulation mechanism in response to nutrients and the environment. For example, protein phosphorylation of extracellular signal-regulated protein kinase (ERK) is altered upon exposure to diallyl disulphide, a compound present in processed garlic causing cell cycle arrest (Ghodke et al. 2011). Qualitative and quantitative profiling of proteins for the development of peptide and protein-based markers can be instrumental in addressing questions pertaining to nutritional competency of various bioactive dietary components (Adiga et al. 2008; Ordovas and Corella 2004). Proteomics, structural dynamism, and crystallographic studies of peptide and complex protein can help in the identification of aberrant protein structures and their effect in response to diet. For example, effects of dietary fish oil, trans fat elaidic acid, or conjugated linoleic acid on lipoprotein metabolism and insulin levels have been well characterized in animal models. Fish oil supplement and elaidic acid have been reported to lower plasma and liver cholesterol. Proteomics coupled with physiologic analysis has proved to be instrumental in unravelling the mechanism underlying the regulatory role of dietary fatty acids in lipid metabolism (Knowles and Milner 2003).

12.9.3.1 Applications of Proteomics in Nutrigenomics

Researchers world over are employing proteomics as a tool to decipher the kinetics of transcript/protein abundance in disease progression in response to nutritional intervention. Proteomics in nutritional studies helps in the identification and quantification of dietary proteins and peptides and assesses their potent nutritional bio-efficacy. The inhibitory effect of sodium butyrate on the succession of human HT-29 cancer cells was assessed in vitro using 2DMS-based proteomic tool. Butyrate has been found to be involved in regulating the expression of genes involved in ubiquitin-mediated proteosomal degradation of proteins involved in regulation of apoptosis and cell cycle and differentiation (Kussmann and Affolter 2006). Combinatorial use of DNA microarrays and proteomics has been exploited to characterize numerous bioactives for their anti-colorectal cancer properties and their role in the progression of cancer in vivo using colon epithelial cell lines (Kussmann et al. 2005).

12.9.4 Metabolomics

One of the contemporary tools of nutrition biology is metabolomics, which attributes to the quantification and extensive analysis of macro and micro biomolecules, within cells, tissues, or organism, and their temporal changes in response to the external environment. Metabolomics is a key tool for the identification of food-derived biomarkers and their variability for metabolizing the same in a healthy and diseased individual. For example, biochemical profiling occurring after soy-rich dietary intervention in humans led to the analysis of changes that occur in plasma components, like alterations in amino acid, plasma lipoprotein, and carbohydrate metabolism (Solanky et al. 2003). Foods contain myriad of non-nutrient molecules that are absorbed, metabolized, and released into body fluid which variably alters the metabolome of an individual. Nuclear magnetic resonance (NMR) spectroscopy pattern recognition and other spectroscopic technologies along with high-throughput chromatographic techniques are being used for metabolic profiling of blood resulting from different dietary treatments. The new technologies for the analysis of metabolites together with the bioinformatic tools and data processing are proving instrumental for researchers and healthcare professionals to detect predispositions to disease and its management to improve individual health. Metabolomics will help in the characterization of the population on the basis of metabolic activities and thereby designing personalized food for maximizing health benefits (Table 12.2).

12.10 Nutrigenomics and Public Awareness

Despite growing scientific evidences and expanding horizons in the realm of nutrigenomics as a panacea for improving individual and community health, debate pertaining to the spread and public acceptance of nutrigenomics still remains the primary daunting task. Some of the key issues that needs to be addressed from public health perspective are highlighted herein as follows: Firstly, availability of scientific evidences to fortify the advancements and co-relation of dietary components with genome and its impact on individual health. Secondly, privacy issue pertaining to sharing genetic information and health profile used to generate a nutritional prescription. It is imperative for the consumers to know who has the access to their personal information and up to what extent it will be used to make a public database. Thirdly, the regulation and presence of a concerned authority to supervise test, health claims, and how the services will be provided keeping the risk assessment into the equation. Much of the public concerns are associated with scientific know-how and techniques involved therein. Fourth and lastly, public fear related to genetically modified organisms (GMO) and genome editing (CRISPR technology) needs to be addressed keeping scientific and social, health, and ethical issues in mind. Bridging the trench between nutrition and genomics will help in overcoming these challenges, create more awareness, and build trust and confidence amongst the public.

Table 12.2 List of nutrients and related deficiency disorder along with preventive food sources

Nutrient	Deficiency/disease and symptoms	Preventive food sources
Vitamin A (retinol)	Poor vision/night blindness	Spinach, carrot, butter, mangoes, green leafy vegetables, apricot
Vitamin B1 (thiamine)	Extreme weakness, beriberi	Yeast, eggs, meat, whole grain, cereals, dried beans
Vitamin B2 (riboflavin)	Retarded growth, bad skin	Green leafy vegetables, beans, peas, milk
Vitamin B3 (niacin)	Pellagra, diarrhoea, dermatitis, and dementia	Mushroom, brown rice, green leafy vegetables, peanut, sweet potato
Vitamin B5 (pantothenic acid)	Fatigue; malaise; apathy; restlessness; insomnia; cramps; gastrointestinal disorders	Sunflower seed, milk, whole grain, meat, lentils, oats
Vitamin B6 (pyridoxine)	Seborrheic dermatitis, microcytic anaemia, epileptiform convulsions, weakened immune function	Poultry products, beans, oats, banana, potato, avocado, soya bean
Vitamin B9 (folic acid)	Megaloblastic anaemia, neural tube defects	Beans, peanuts, sunflower seeds, fruits, whole grains, seafood
Vitamin B12 (cyanocobalamin)	Anaemia	Pomegranate, beetroot, spinach, seafood
Vitamin C (ascorbic acid)	Scurvy, swollen gums, loose teeth	Lemon, oranges, Indian gooseberry
Vitamin D (calciferol)	Rickets, brittle bones in children	Milk, fish, liver oil, sun exposure
Vitamin E (tocopherol)	Chronic pancreatitis, cholestasis, cystic fibrosis, neuromuscular dysfunction	Wheat germ oil, nuts, sunflower, safflower, rapeseed, corn, and soybean and olive oils
Vitamin K (phylloquinone)	Excessive bleeding due to injury	Green leafy vegetables, dairy products
Biotin	Unhealthy skin, hair fall, neurological abnormalities	Egg yolk, beans, legumes, nuts, liver oil, linseed, mushroom, banana
Calcium	Brittle bones, excessive bleeding, bad muscular movement	Milk, green leafy vegetables
Phosphorus	Bad teeth and bones	Pulses, cereals, milk
Iron	Anaemia, lack of red blood cells	Green leafy vegetables, drumstick, pulses
Iodine	Goitre, enlarged thyroid gland	Iodine-fortified salt, fish
Copper	Low appetite, retarded growth	Pulses and leafy vegetables
Zinc	Undeveloped genitals, skin lesions, poor appetite, impotency	Seafood, nuts, whole grain, dairy products, fortified cereals
Magnesium	Hypocalcaemia; hypokalaemia; cardiac and neuromuscular manifestation; latent tetany; osteoporosis; insulin resistance and impaired insulin secretion	Pumpkin seeds, almond, legumes, whole grains, green leafy vegetables
Manganese	Dermatitis, hypocholesterolaemia	Whole grains, nuts, chickpea, clams, mussels, soybeans, brown

(continued)

Table 12.2 (continued)

Nutrient	Deficiency/disease and symptoms	Preventive food sources
		rice, sweet potato, leafy vegetables, coffee, tea
Protein	Kwashiorkor, extremely underweight	Sprouted beans, cereals, fish, milk, soya, milk, poultry
Carbohydrate	Non-insulin-dependent diabetes mellitus (NIDDM), ketosis, cardiovascular disease, stunted or poor growth in children	Whole grains, milk, potato, fruits, vegetables, legumes, cereals
Essential fatty acids	Improper fat absorption, scaly dermatitis, alopecia, thrombocytopenia (impaired wound healing), stunted growth in infants	Meat, oil-rich beans, linseed, walnut, almond

12.10.1 Public Awareness Events

The relationship between rapidly changing lifestyle and nutritional status quo of the Indian population has been investigated extensively for the formulation of policies and programme to spread knowledge and public awareness (Acharya et al. 2004). Community health programmes are being organized to make common people understand that the genetic makeup of an individual or community cannot be altered, but the effect of environment (dietary) component on genes can be regulated to attain desired health benefits. An executive course on genomic policies was conducted to provide a platform for stakeholders to deliberate the significance of nutrigenomics for health improvement in India (Krishnaswamy 2008). The course aimed to enlighten participants with the significance of genomics for improving health. A common multidisciplinary consensus is being devised for issues related to policy drafting and ethics. Therefore, food-based dietary guideline (FBDG) was developed by a multidisciplinary group comprising nutritionists, molecular biologists, agriculturists, statisticians, technologists, dieticians, etc. to overpower diet-related disorders and promote health has been emphasized (Fafournoux et al. 1990). The objective of FBDG is the prevention of both under- and overnutrition and to promote a healthier lifestyle. Routine conferences and workshops are being organized where people from different realms involved in public health spread awareness and address queries related to nutrigenomic-based personalized nutrition service amongst people.

12.11 Nutrition and Gene Interactions

Recent advancements in the realm of genomics, proteomics, and metabolomics have propelled genome-wide studies on the association of diseases and health in response to dietary nutrients. The nutrient-gene interaction at cellular, individual, and community extent is now being investigated comprehensively for the identification of novel genes and pathways. The response to a nutrient implies to be genotype-

specific, where the nutrition status of a person alters gene expression. Bioactive molecules, carbohydrates, fatty acids, minerals, and vitamins, are invariably involved in the regulation of gene expression. In eukaryotes, the regulation of gene expression involves intricate interaction of neural, hormonal, and environmental factors. Although genetics is the central driving force governing phenotypic or visible traits of an organism, a smaller proportion of overall phenotype including health profile is a result of metabolic and environmental effect it exerts. In the era of post-genomics, technological advancements have led to the identification of several biomarkers related to nutrient-gene interactions for genomic and metabolic profiling in diseased and healthy individuals.

12.11.1 Nutrition and Gene Regulation

Gene regulation is a very complex mechanism where dietary intervention impacts successive steps of signal transduction from transcription, translation, to post-translational modifications (Trayhurn 2000). Using nutrigenomics tools such as genomics, proteomics, and metabolomics, researchers are able to elucidate the genotypic and phenotypic effect of dietary constituents on cellular and intermediary functioning simultaneously (Hinds et al. 2005). Transcriptional regulation via activation and deactivation of transcription factors is the most prominent mechanism for gene control. Nuclear receptors are one of the most abundant classes of transcriptional regulators in animals which act as sensors to nutritional, pathophysiological, developmental, and endocrine dynamism and thereby trigger adaptive response via gene regulation. Ligand binding to the receptor induces a conformational change, resulting in segregation of corepressor and recruitment of coactivator proteins, triggering downstream effector targets. Nuclear receptors in metabolically active organs like the intestine, liver, and adipose tissues are induced by the nutritional state of an individual, thereby regulating transcription factors to alter several nutrient-responsive genes. Nuclear receptors thus act as a sensory receptor in regulation of numerous nutrition and metabolic responsive genes to further regulate various cellular development and differentiation processes.

12.11.2 Synergism of Nutrients and Gene Expression

Predominantly, three types of nutrient-gene interaction can lead to altered phenotype, which are summarized as follows:

1. **Genetic variations**: These include polymorphism at genetic level, e.g. single nucleotide polymorphisms (SNPs) leading to altered functionality of genes. Degree of variability is high across human genome, accounting for 50,000 SNPs in genes (Gibney and Gibney 2004), which results in altered gene expression and ultimately change in protein which might be structurally and functionally distinct from the native one. A number of SNPs have been identified known

to affect nutrient requirement. For example, mutation in *MTHFR* gene encoding for methylenetetrahydrofolate reductase enzyme, required for metabolizing one form of vitamin B, and folate into another, results in elevated plasma homocysteine levels which may increase the risk of cardiovascular diseases, formation of blood clots (thrombosis), and stroke. Two primary MTHFR SNP variants called C677T and A1298C are the most prevalent forms, comprising 15–30% of the affected individuals (da Costa et al. 2006). Similarly, *phosphatidylethanolamine N-Methyltransferase (PEMT)* gene encodes for protein involved in the endogenous synthesis of choline, an imperative nutrient involved in cellular growth and metabolism, but is rendered non-functional because of SNP in the promoter of *PEMT* gene (rs12325817). Such variants are associated with susceptibility to choline deficiency, which causes fatty acid liver and organ dysfunction (Oommen et al. 2005).

2. **Direct interactions**: Sometimes nutrients interact with a receptor which acts as a transcription factor by binding to DNA and induces expression of a gene. For example, vitamin A (retinol) interacts with retinoic acid receptor which further activates or represses transcription by binding to DNA motifs (e.g. retinoic acid response elements) in promoter regions (Muller and Kersten 2003). Likewise, vitamin D interacts with vitamin D receptor, calcium with calcineurin, and zinc with metal-responsive transcription factor-1 (Hsu and Huang 2006). Dietary fatty acids interact with peroxisome proliferators-activated receptors (PPARs), which then bind to DNA and alter gene expression (Davis and Uthus 2004). Nutrients that are components in single-carbon metabolism provide the most substantial evidence of synergy between nutrients and DNA methylation because they alter the availability of methyl groups and therefore the biochemical pathways of methylation processes (Steinmetz et al. 1998). Such single-carbon metabolites include vitamin B6, folate, vitamin B12, methionine, and choline, deficiencies of which in combination cause global hypomethylation, cirrhosis, hepatic steatosis, and ultimately hepatic tumorigenesis in rodents in the absence of carcinogen treatment (Steinmetz et al. 1998; Christou and Twyman 2004).
3. **Epigenetic interactions**: Sometimes nutrients alter the structure of DNA through chromatin remodelling and alter gene regulation by acetylation, methylation, or biotinylation of histones. Such expression can occur in a spatio-temporal manner or can even persist through generations. Epigenetic interaction with histones leads to the uncoiling of chromatin structure, creating grooves through which transcription factors can intercalate and activate gene promoters (Lane and Bailey 2005).

12.12 The Possibilities of Transgenic Foods for Malnutrition Eradication Through Cereals

Plants are the paramount source of energy and nutrients for all humans and livestock. Despite occupying an indispensable role, the majority of our food crops lack certain essential nutrients, although a mixed diet assists in overcoming diet-based

malnutrition. More than 50% of the world's population suffers from undernutrition or malnutrition which has become a global health concern (Zhu et al. 2007). The majority of global population notably across the developing nations relies on cereal crops primarily rice, wheat, and maize which lack the full complement of essential nutrients (Al-Babili and Beyer 2005). Great advancement has been made to address the issue through the application of plant biotechnology via engineering of cereal food crops for nutrition enhancement and improving public health. Nutritionally enhanced genetically modified cereal crops have consistently shown efficacy in providing safe and available nutrients to combat malnutrition. Research on up-scaling essential nutrients such as minerals, fats, vitamins, and amino acids with a simultaneous reduction in anti-nutrient factors in cereal crops has become a daunting task for researchers worldwide. Biofortification of key cereal food crops is now being achieved fundamentally through two main approaches: transgene introgression (genetic engineering) and conventional breeding approach. The selection of approach relies predominantly on whether the bioactive compound is synthesized de novo by the plant or acquired from the surrounding environment. In de novo approach, enhancing the nutritional content requires engineering of metabolites or the existing metabolic pathway with the intent to accentuate the basal level of the bioactive compound or downturning the antagonistic compound to generate a novel product with phyto-nutritive significance.

12.12.1 Genetically Engineered Rice (*Oryza sativa*)

Rice has been one of the most imperative food crops worldwide and has acquired central stage for enhancement of its nutritive content in a way to combat the global malnutrition challenge. In this realm provitamin A (beta-carotene)-fortified golden rice has been marked as a major breakthrough in confronting vitamin A deficiency (VAD) in underprivileged economically compromised populations, particularly infants and children less than 5 years old. Genes encoding maize *phytoene synthase* (*ZmPSY*) gene and *carotene desaturase* were overexpressed in rice resulting in enhancement of beta-carotene precursor, i.e. phytoene by up to 23-fold in transgenic rice (Paine et al. 2005; Tang et al. 2009, 2012; Shumskaya and Wurtzel 2013; Tanumihardjo et al. 2010). In a similar approach, enhanced folic acid (vitamin B9) content was achieved in genetically engineered rice through overexpression of *Arabidopsis GTP-cyclohydrolase I* (*AtGTPCHI*) and *aminodeoxy-chorismate synthase* gene, thereby increasing folate content (up to 150-fold) (Haskell 2012; Moghissi et al. 2015). Rice has also been targeted to enhance iron content in order to combat anaemia. Several reports have shown that overexpression of genes encoding iron transporter *OsIRT1* (Xudong et al. 2000), *nicotianamine aminotransferase* (*NAAT*) (Zheng et al. 2010), *nicotianamine synthase* 1 (*OsNAS1*) and 2 (*OsNAS2*) (Trijatmiko et al. 2016; Lee et al. 2009), soybean *ferritin* (Goto et al. 1999; Vasconcelos et al. 2003; Lucca et al. 2002), and common bean ferritin (Hurrell and Egli 2010) resulted in increased iron content. Silencing genes responsible for the synthesis of anti-nutrient compounds like phytic acid have shown to increase iron

bioavailability (Lee and An 2009). Similarly, overexpression of metal homeostasis gene *OsIRT1* (Masuda et al. 2008) and *mugineic acid synthesis* genes from barley (*HvNAS1*, *HvNAS1*, *HvNAAT-A*, *HvNAAT-B*, *IDS3* (Anai et al. 2003)) leads to increased iron and zinc content in genetically modified rice. Overexpression of soybean *omega-3 fatty acid desaturase* (*GmFAD3*) gene (Crawford et al. 2000) in rice leads to enhanced polyunsaturated fatty acid (PUFA) content in seed which can help in reduction of bad cholesterol levels in the body (Zheng et al. 1995). Seed-specific overexpression of bean *β-phaseolin* (Lee et al. 2003); sesame 2S albumin (Katsube et al. 1999); soybean glycinin (Sindhu et al. 1997); pea legumin (Yang et al. 2016); *dihydrodipicolinate synthase* (*DHPS*) (Lee et al. 2001); maize *DHPS* (Wakasa et al. 2006); rice *anthranilate synthase* α-subunit (Zhou et al. 2009); and *E. coli aspartate aminotransferase* (Shin et al. 2006) has been reported to enrich protein quality in transgenic rice. Antioxidant activity has been increased in rice by overexpressing maize C1 and R-S regulatory genes (*ZmMYB*-HLH transcription factor) leading to increased flavonoid content (Wang et al. 2014).

12.12.2 Genetically Engineered Wheat (*Triticum aestivum*)

Wheat is one of the key staple food crops with an annual consumption of 733.2 million metric tons globally [FAOStat 2020]. In terms of nutritive significance, wheat is a rich source of energy in the form of carbohydrates and starch and a substantial amount of protein, dietary fibres, and phytochemicals. Through genetic engineering, overexpression of bacterial *phytoene synthase* (*PSY*) and *carotene desaturase* genes (*CrtB*, *CrtI*) (Cong et al. 2009; Xiaoyan et al. 2012) and provitamin A content has been increased in transgenic wheat. Overexpressing the ferritin gene from soybean and wheat (TaFer1-A) has been reported to increase iron content in transgenic wheat (Borg et al. 2012; Brinch-Pederson et al. 2000). The bioavailability of iron has been increased either by overexpressing the phytochrome gene (*phyA*) (Bhati et al. 2016) to accentuate phytase activity or by silencing ABCC13 transporter gene to decrease phytic acid content (Tamas et al. 2009). Protein content and primarily essential amino acids, cysteine, lysine, methionine, and tyrosine, content in wheat grain have been enhanced by overexpressing *Amaranthus albumin* gene (*ama1*) (Doshi et al. 2006). Further overexpression of maize regulatory genes (C1, B-peru) involved in anthocyanin production has been reported to enhance antioxidant activity in transgenic wheat (Ramesh et al. 2004).

12.12.3 Genetically Engineered Barley (*Hordeum vulgare*)

Despite nutrimental significance, barley lacks certain micronutrients. Therefore, zinc content has been improved by overexpression of zinc transporters in transgenic barley (Holme et al. 2012). Similarly, the bioavailability of iron and zinc has been fortified by enhancing phytase activity by overexpressing the barley *phytase* gene (*HvPAPhy*_a) (Ohnoutkova et al. 2012). Lysine content has been increased by

overexpressing the DHPS gene (*dapA*) (Dikeman and Fahey 2006) in transgenic barley. Overexpression of *cellulose synthase-like* gene (*HvCslF*) (Carciofi et al. 2012) has been reported to increase β-glucan in transgenic barley which plays a key role in combating cardiovascular disease and type 2 diabetes in humans (Burton et al. 2011). Overexpression of *delta-6-desaturase* (*D6D*) gene has been reported to enhance polyunsaturated fatty acids (PUFA), γ-linolenic acid, and stearidonic acid (STA) in transgenic barley (Mihalik et al. 2014).

12.12.4 Genetically Engineered Maize (*Zea mays*)

Maize is one of the globally imperative cereal crops that has been addressed to increase its vitamin, protein, mineral, and fibre content with simultaneous lowering of its anti-nutrient components by means of genetic modification. Enrichment of endosperm-specific provitamin A and other carotenoids was accomplished by overexpressing bacterial *crtB* (Decourcelle et al. 2015) and other carotenoid-encoding genes (Zhu et al. 2008; Cahoon et al. 2003). Overexpression of *homogentisic acid geranylgeranyl transferase* (*HGGT*) gene has been reported to upscale tocotrienol and tocopherol content in maize (Chen et al. 2003). Vitamin C (L-ascorbic acid) content has been increased by nearly 100-fold in transgenic maize by expression of *dehydroascorbate reductase* (DHAR gene) (Naqvi et al. 2009). Multivitamin corn, containing a higher percentage of beta-carotene, double-fold folate, and sixfold the normal ascorbate content, has been developed by engineering three distinct metabolic pathways (Drakakaki et al. 2005). Overexpressing soybean ferritin (Chen et al. 2008) and *phyA2* gene from *Aspergillus niger* (Shi et al. 2007) has been successfully reported to increase iron bioavailability in transgenic maize. Maize kernels have destitute nutritional quality particularly essential amino acids like lysine and tryptophan. Several molecular approaches have been devised for improving protein profiles. Lysine-rich protein has been increased by overexpressing the *sb401* gene from potato (Tang et al. 2013; Huang et al. 2006), while lysine and tryptophan content has been increased by silencing alpha-zeins in transgenic maize (Yang et al. 2002). Methionine content has been enhanced in maize via alteration in *cis*-acting site for *Dzs10* gene (Lipkie et al. 2013). Further, overexpression of milk α-lactalbumin has been reported to balance amino acid content in maize (Lai and Messing 2002).

12.12.5 Genetically Engineered Sorghum (*Sorghum Bicolor*)

Sorghum bicolor is a multipurpose cereal crop is also popularly known as "food of the poor" feeding millions of poverty-ridden populations around the world as it can be grown anywhere and withstand harsh environmental conditions. Provitamin A (beta-carotene) content in transgenic sorghum has been enhanced by overexpressing *Homo188-A* (Zhao et al. 2002). Overexpressing the high-lysine protein (*HT12*) gene has been reported to enhance lysine content in transgenic sorghum (Elkonin et al.

2016). Sorghum is difficult to digest when compared to other cereal crops because of the presence of seed storage protein γ-kafirin, which is inert to protease digestion. Therefore, RNAi-mediated silencing of gene encoding *γ-kafirin* along with combined suppression of its isoform genes (*γ-kafirin-1*, *γ-kafirin-2*, and α-*kafirin A1*) has been reported to improve the digestibility index of transgenic sorghum (Grootboom et al. 2014; Malzahn et al. 2017).

12.13 Genome Editing Approaches for Nutrient Enrichment in Cereals

With the advent of high-throughput genome sequencing, numerous crop species have been sequenced, enabling genome editing approaches to tailor genomes for optimizing desirable traits irrespective of genetic barriers across species. Several genome editing approaches such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR-Cas) system are being extensively used to precisely target and edit any gene of interest in several crops such as wheat, rice, maize, and barley. Genome editing involves precise and regulated changes in the genome employing site-specific nucleases (SSNs), generating double-stranded breaks (DSBs) at specific loci. Contrary to the transgenic approach which leads to the generation of random insertion events and off-target mutations resulting in undesirable and random phenotype, genome editing methods generate mutants with minimal chances of having off-target phenotype. Using chimeric DNA/RNA oligonucleotides (ONDs), *acetohydroxyacid synthase* genes (*AHAS108* and *AHAS109*) were edited in maize for generating herbicide-tolerant lines (Gao et al. 2010). Likewise, heritable targeted mutagenesis was induced in maize using engineered meganuclease I-CreI to target specific mutation in *LIGULELESS1* (LG1) gene promoter (Kim et al. 1996). These molecular approaches will prove to be instrumental in functional genomics studies and crop improvement breeding programmes resulting in varieties with mass acceptability and relatively lesser regulatory concerns in comparison to the conventional genetically modified (GM) crops (Zhu et al. 1999).

12.13.1 Zinc Finger Nucleases (ZFNs)

In recent years several novel approaches and techniques have enabled precision modification of plant genomes. Amongst these zinc finger nucleases (ZFNs) are engineered multidomain endonucleases, harbouring zinc finger DNA-binding domain fused with a nuclease, primarily a *Fok*I endonuclease. These nuclease functions as a dimer, where pairs of zinc finger domain binds to upstream and downstream target sites, thereby generating double-stranded nicks in the targeted DNA. Zinc finger nucleases are assembled from C2H2 zinc finger domains where each finger recognizes three nucleotides; thereby three-finger adheres to nine

nucleotides of a DNA sequence. One of the major negative aspects in employing ZFNs is low specificity resulting from off-target dimerization leading to genotoxicity (Porteus and Baltimore 2003; Szczepek et al. 2007; Porteus 2006; Ramirez et al. 2008). Despite certain drawbacks, ZFNs have been applied across many cereal crops. *Inositol-pentakisphosphate 2-kinase* (*IPK1*) gene catalysing terminal step in phytic acid biosynthesis pathway has been edited in maize by generating 66 ZFNs against 5 intragenic positions (Cantos et al. 2014). In a recent study, non-coding genomic regions were identified in the rice genome for site-specific integration and higher gene expression using ZFNs which resulted in localization of 28 genomic regions including only 1 non-coding discovered for the safe integration of ZFN constructs carrying a *β-glucuronidase* gene (Christian et al. 2010).

12.13.2 Transcription Activator-Like Effector Nucleases (TALENs)

Transcription activator-like effector nucleases (TALENs) are protein-based DNA targeting systems that leverage engineered or synthetic restriction enzymes generated through the fusion of TAL effector DNA-binding domain with DNA-cleaving nuclease (*Fok*I) subunit. TALENs introduce highly specific targeted mutations via repair of double-stranded breaks (DSBs) either by homology-directed repair (HDR) or by non-homologous end joining (NHEJ). The TAL DNA-binding domain is composed of repeats which may vary in number from 16 to 30 constituting a protein encoded by ~3.8 kb CDS. TALENs have evolved from the *Xanthomonas* type III AvrBs3 superfamily effectors which function as transcription factors *in planta* (Boch and Bonas 2010; Li et al. 2012). Each protein constituting AvrBs3 superfamily has different DNA-binding motif repeats that regulate host-pathogen diversity. A higher frequency of repeats enables TALENs to have higher target specificity and affinity resulting in escalated genome editing rates with low genotoxicity. TALEN-based genome editing proves to be one of the most accurate systems with a high mutability rate, but the system possesses certain drawbacks. Similar to ZFNs, TALENs also employ an engineered *Fok*I nuclease subunit and thereby require designing two monomers for individual genome target resulting in large ORF length which cannot be used in viral-based vectors. Moreover, length and the need to synthesize novel pairs of enzymes for each target may constrain the ability to edit multiple targets.

12.13.3 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-CAS System)

CRISPR or clustered regularly interspaced short palindromic repeats were discovered in bacteria *E. coli* as a host defence mechanism against foreign genetic elements such as those present within plasmids and phage DNA by inducing RNA-guided DNA cleavage. The CRISPR-CAS system incorporates fragments of foreign DNA known as spacers into CRISPR cassettes followed by transcription of CRISPR

arrays and spacers to make a guide crRNA (CRISPR RNA) which then specifically cleaves the targeted genome (Wiedenheft et al. 2012; Van der Oost et al. 2009; Makarova et al. 2011; Barrangou 2013). The key factor for high specificity and efficiency is (protospacer adjacent motif) PAM recognition that distinguishes between bacterial encoding RNA from the bacteriophage target sequence. Cas9 binds to the PAM sequence, resulting in the unfolding of DNA, allowing RNA/DNA hybridization or R-loop formation followed by cleavage of both DNA/RNA and ssDNA strands. CRISPR/Cas9 employs binding of sgRNA at conserved 20 bp complementary sequence and cleaving the target sequence through the action of Cas9 nuclease protein (HNH domain cleaves the complementary DNA strand, while RuvC domain cleaves the non-complementary DNA strand), thereby generating double-stranded break (DSB). Plants' endogenous repair mechanism then repairs DSB generated by NHEJ (non-homologous end joining) or by HR (homologous recombination) which introduces small indels resulting in frame-shift mutations or premature stop codon (Szczelkun et al. 2014; Tsui and Li 2015; Shan et al. 2013). The CRISPR-CAS9 system has been successfully used for genome editing in rice using codon-optimized *sp*Cas9 for targeting the *phytoene desaturase* (*OsPDS*) gene causing non-functionality of carotenoid biosynthetic pathway resulting in white kernels and albino seedlings (Sun et al. 2017). Furthermore, amylose content in rice has been successfully increased by targeting two starch branching enzymes (SBE) SBEIIb (Tang et al. 2017). CAS9-based editing has also been used to decrease heavy metal toxicity in the engineered rice. For example, cadmium (Cd) is a highly toxic heavy metal causing negative health effects upon consuming high-Cd content rice. CRISPR/Cas9 editing system has been employed to knock down metal transporter gene *OsNramp5* to generate mutant *indica* rice lines having low cadmium content (Connorton et al. 2017). Biofortification of wheat to alleviate the intrinsic iron content has been achieved by genome engineering of vacuolar iron transporter (Liang et al. 2014). Similarly, anti-nutritional phytic acid content has been decreased in maize by knocking down genes encoding for phytic acid synthesis (*ZmIPK1A*, *ZmIPK*, and *ZmMRP4*) in maize protoplast by designing two gRNAs targeting respective genes at frequencies of 16.4% and 19.1%, respectively (Zhu et al. 2016).

12.14 Nutrigenomics Future

The imperativeness of nutrition in disease prevention and treatment has gained a central stage with the emergence of high-precision next-generation sequence (NGS) technologies allowing full-genome sequencing in a time- and cost-effective manner. NGS has revolutionized the way we think about daily nutrition, health, illness, and disease prevention and has exhibited that the present modern-day diet and eating habits of human beings are different from its original food niche. Genome-tailored or personalized nutritional counselling can be explored not just for changing eating habits for improving lifestyle but also assisting in the early and precise diagnosis of diseases, thereby retarding the progression of chronic illness and also assisting in the treatment of others. Proponents believe that health care can be improved if

nutritionists and practitioners recommend and promote personalized rather community guidelines based upon genetic profile, health status, diet preference, phenotype, and environmental factors.

The realm of nutrigenomics is still in its infancy, and some uncertainties related to its public acceptance and technological advancements still pose a bigger challenge. Ethical issues pertaining to consumer confidentiality and rights need to be thoroughly addressed. Instilling public awareness and trust in terms of knowledge, on how the genes interact with their environment and dietary and lifestyle choices that affect their health status, needs to be prioritized across different public health policies and nutrigenomic based programs at community as well as national levels. Identification of genes and subsidiary pathways underlying combating and progression of disease will help in using "food as medicine". Furthermore, it will also help in escalating levels of essential micronutrients in staple food crops and thus will have a significant impact on the improvement of the nutritional status of populations worldwide. Diseases related to heredity and malnutrition, which are prevalent across various ethnic population groups throughout the world, could be reduced by a personalized and nutrition-rich food supplementation, particularly if efforts are channelized on staple food crops including rice, wheat, beans, maize, and cassava. Synergism between healthcare practice and nutritionally enhanced food product development will enable a swift uptake and translation of novel knowledge in the realm of nutrigenomics; however steps in this direction must be taken with utter discretion and foresightedness. In terms of scientific perspective, focus on cheap and easily accessible knowledge and technological advancement that can largely benefit population particularly socio-economic weaker sections of the world need to occupy centre stage. Novel and affordable technologies can effectively coordinate alongside clinical practices; the information generated thereby must be overlaid onto the integrated metabolic pathway matrix that health professionals already understand. An in-depth understanding of human intermediary metabolism by nutrition scientists is a valuable asset required for the advancement of nutrigenomics which further could fortify a place for nutrition clinicians. Future development in the field of nutrigenomics undoubtedly will place its seemingly huge potential in a better perspective.

References

Acharya T, Kumar NK, Muthuswamy V et al (2004) Harnessing genomics to improve health in India—an executive course to support genomics policy. Health Res Policy Syst 2:1–13

Adiga MNS, Chandy S, Ramaswamy G et al (2008) Homocysteine, vitamin B12 and folate status in pediatric acute lymphoblastic leukemia. Indian J Pediatr 7:235–238

Al-Babili S, Beyer P (2005) Golden Rice—five years on the road—five years to go? Trends Plant Sci 10:565–573

Almendro V, Gascan P (2012) Nutrigenomics and cancer. http://www.fundacionmhm.org/pdf/Mono9/Articulos/articulo9

Anai T, Koga M, Tanaka H et al (2003) Improvement of rice (Oryza sativa L.) seed oil quality through introduction of a soybean microsomal omega-3 fatty acid desaturase gene. Plant Cell Rep 21(10):988–992

Anderson RA, Jones CJ, Goodfellow J (2001) Is the fatty meal a trigger for acute coronary syndromes. Atherosclerosis 159:9–15

Arts IC, Hollman PC, Feskens EJ et al (2001) Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen elderly study. Am J Clin Nutr 74:227–232

Bacalini MG, Friso S, Olivieri F et al (2014) Present and future of anti-ageing epigenetic diets. Mech Ageing Dev 136–137:101–115, 138

Barrangou R (2013) CRISPR-Cas systems and RNA-guided interference. Wiley Interdiscip Rev RNA 4:267–278

Bayless TM, Rosensweig NS (1966) A racial difference in incidence of lactase deficiency. A survey of milk intolerance and lactase deficiency in healthy adult males. JAMA 197(12):968–972

Bender DA, Bender AE (2009) Benders' dictionary of nutrition and food technology, 7th edn. Woodhead Publishing, Abington

Bhati KK, Alok A, Kumar A et al (2016) Silencing of ABCC13 transporter in wheat reveals its involvement in grain development, phytic acid accumulation and lateral root formation. J Exp Bot 67(14):4379–4389

Bianchini F, Kaaks R, Vainio H (2002) Overweight, obesity, and cancer risk. Lancet Oncol 3:565–574

Boch J, Bonas U (2010) *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. Annu Rev Phytopathol 48:419–436

Borg S, Brinch-Pedersen H, Tauris B et al (2012) Wheat ferritins: improving the iron content of the wheat grain. J Cereal Sci 56:204–213

Brinch-Pederson H, Olesen A, Rasmussen SK et al (2000) Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus phytase*. Mol Breed 6:195–206

Burton RA, Collins HM, Kibble NA et al (2011) Over-expression of specific *HvCslF* cellulose synthase-like genes in transgenic barley increases the levels of cell wall (1,3;1,4)-β-d-glucans and alters their fine structure. Plant Biotechnol J 9(2):117–135

Cahoon EB, Hall SE, Ripp KG et al (2003) Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat Biotechnol 21:1082–1087

Calder PC, Davis J, Yaqoob P et al (1998) Dietary fish oil suppresses human colon tumour growth in athymic mice. Clin Sci (London) 94:303–311

Cantos C, Francisco P, Trijatmiko KR et al (2014) Identification of "safe harbor" loci in indica rice genome by harnessing the property of zinc-finger nucleases to induce DNA damage and repair. Front Plant Sci 5:302

Carciofi M, Blennow A, Jensen SL et al (2012) Concerted suppression of all starch branching enzyme genes in barley produces amylose-only starch granules. BMC Plant Biol 12(1):223

Chae YJ, Kim CH, Ha TS, Hescheler J, Ahn HY, Sachinidis A (2007) Epigallocatechin-3-O-gallate inhibits the angiotensin-II induced adhesion molecule expression in human umbilical vein endothelial cell via inhibition of MAPK pathways. Cell Physiol Biochem 20:859–866

Chang WL, Chapkin RS, Lupton JR (1998) Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. J Nutr 128:491–497

Chen Z, Young TE, Ling J et al (2003) Increasing vitamin C content of plants through enhanced ascorbate recycling. Proc Natl Acad Sci U S A 100:3525–3530

Chen R, Xue G, Chen P et al (2008) Transgenic maize plants expressing a fungal phytase gene. Transgenic Res 17(4):633–643

Chen D, Li W, Du M et al (2015) Sequencing and characterization of divergent marbling levels in the beef cattle (longissimus dorsi muscle) transcriptome. Asian Australas J Anim Sci 28:158–165

Christian M, Cermak T, Doyle EL et al (2010) Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186(2):757–761
Christou P, Twyman RM (2004) The potential of genetically enhanced plants to address food insecurity. Nutr Res Rev 17:23–42
Cong L, Wang C, Chen L et al (2009) Expression of phytoene synthase1 and carotene desaturase crtI genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). J Agric Food Chem 57(18):8652–8660
Connorton JM, Jones ER, Rodríguez-Ramiro I et al (2017) Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. Plant Physiol 174(4):2434–2444
Corella D, Ordovas JM (2009) Advances in genetics. Nutrigenomics in cardiovascular medicine. Nutrition and Genomics Laboratory, JM-USDA Human Nutrition Research Center on aging at Tufts University, Boston
Costa NMB, Rosa COB (2011) Functional foods: bioactive components and physiological effects. 1 Reprint, R'ubio, Rio de Janeiro
Costabile A, Klinder A, Fava F, Napolitano A et al (2008) Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br J Nutr 99:110–120
Cozzolino SMF (2012) The bioavailability of nutrients, 4th edn. Monole, São Paulo
Cozzolino SMF, Cominetti C (2013) Biochemical and physiological bases of nutrition in different stages of life in health and disease, 1st edn. Monole, São Paulo
Crawford M, Galli C, Visioli F et al (2000) Role of plant-derived omega-3 fatty acids in human nutrition. Ann Nutr Metab 44:263–265
da Costa K, Kozyreva OG, Song J et al (2006) Common genetic polymorphisms have major effects on the human requirement for the nutrient choline. FASEB J 20:1336–1344
Davis CD, Uthus EO (2004) DNA methylation, cancer susceptibility and nutrient interactions. Exp Biol Med 229:988–995
Decourcelle M, Perez-Fons L, Baulande S et al (2015) Combined transcript, proteome, and metabolite analysis of transgenic maize seeds engineered for enhanced carotenoid synthesis reveals pleotropic effects in core metabolism. J Exp Bot 66(11):3141–3150
Dietitians Association of Australia (DAA) (n.d.) The Australian Dietary Guidelines. https://daa.asn.au/smart-eating-for-you/smart-eating-fast-facts/healthy-eating/the-australian-dietary-guidelines
Dikeman CL, Fahey GC (2006) Viscosity as related to dietary fiber: a review. Crit Rev Food Sci Nutr 46:649–663
Doshi KM, Eudes F, Laroche A et al (2006) Transient embryo specific expression of anthocyanin in wheat. In Vitro Cell Dev Biol Plant 42:432–438
Drakakaki G, Marcel S, Glahn RP et al (2005) Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus phytase* in maize results in significant increases in the levels of bioavailable iron. Plant Mol 59(6):869–880
Elkonin LA, ItalianskayaI JV, Domanina VN et al (2016) Transgenic sorghum with improved digestibility of storage proteins obtained by Agrobacterium-mediated transformation. Russ J Plant Physiol 63:678–689
Enattah NS, Sahi T, Savilahti E et al (2002) Identification of a variant associated with adult-type hypolactasia. Nat Genet 30(2):233–237
EU Science Hub (n.d.) Food-based Dietary Guidelines in Europe. https://ec.europa.eu/jrc/en/health-knowledge-gateway/promotion-prevention/nutrition/food-based-dietary-guidelines
Fafournoux P, Remesy C, Demigne C (1990) Fluxes and membrane transport of amino acids in rat liver under different protein diets. Am J Physiol Endocrinol Metab 259:E614–E625
FAO (2002) Food outlook, no 4, october 2002 (fao.org)
FAO (2020) World food and agriculture – statistical yearbook 2020 (fao.org)
FAOStat (2020) https://www.fao.org/3/cb1329en/CB1329EN.pdf
Ferguson LR (2006) Nutrigenomics: integrating genomic approaches into nutrition research. Mol Diagn Ther 10(2):101–108

Franco RF, Reitsma PH (2001) Gene polymorphisms of the haemostatic system and the risk of arterial thrombotic disease. Br J Haematol 115:491–506
Gao H, Smith J, Yang M et al (2010) Heritable targeted mutagenesis in maize using a designed endonuclease. Plant J 61(1):176–187
Ghodke Y, Chopra A, Shintre P et al (2011) Profiling single nucleotide polymorphisms (SNPs) across intracellular folate metabolic pathway in healthy Indians. Indian J Med Res 133:274–279
Gibney MJ, Gibney ER (2004) Diet, genes and disease: implications for nutrition policy. Proc Nutr Soc 63(3):491–500
Godard B, Hurlimann T (2009) Nutrigenomics for global health: ethical challenges for underserved populations. Curr Pharmacogenomics Person Med 7:205
Goto F, Yoshihara T, Shigemoto N et al (1999) Iron fortification of rice seed by the soybean ferritin gene. Nat Biotechnol 17:282–286
Gregory J, Lowe S, Bates CJ et al (2000) National Diet and nutrition survey: young people aged 4 to 18 years, Report of the diet and nutrition survey, vol 1. The Stationary Office, London
Grootboom AW, Mkhonza NL, Mbambo Z et al (2014) Co-suppression of synthesis of major α-kafirin sub-class together with γ-kafirin-1 and γ-kafirin-2 required for substantially improved protein digestibility in transgenic sorghum. Plant Cell Rep 33(3):521–537
Hall C, Hillen C, Garden Robinson J (2017) Composition, nutritional value and health benefits of pulses. Cereal Chem 94:11–31
Han Y, Gao S, Muegge K et al (2015) Advanced applications of RNA sequencing and challenges. Bioinform Biol Insights 9(Suppl 1):29–46
Haskell MJ (2012) The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion—evidence in humans. Am J Clin Nutr 96:1193S–1203S
Hinds DA, Stuve LL, Nilsen GB et al (2005) Whole-genome patterns of common DNA variation in three human populations. Science 307:1072–1079
Holme IB, Dionisio G, Brinch-Pedersen H et al (2012) Cisgenic barley with improved phytase activity. Plant Biotechnol J 10(2):237–247
Holt SH, Delargy HJ, Lawton CL et al (1999) The effects of high-carbohydrate vs high-fat breakfasts on feelings of fullness and alertness, and subsequent food intake. Int J Food Sci Nutr 50(1):13–28
Hooper L, Summerbell CD, Higgins JPT et al (2001) Dietary fat intake and prevention of cardiovascular disease: systemic review. Br Med J 322:757–763
Hossain P, Kawar B, Nahas ME et al (2007) Obesity and diabetics in the developing world—a growing challenge. N Engl J Med 356:213–215
Hsu SC, Huang CJ (2006) Reduced fat mass in rats fed a high oleic acid-rich safflower oil diet is associated with changes in expression of hepatic PPARa and adipose SREBP-1c-regulated genes. J Nutr 136:1779–1785
Huang S, Frizzi A, Florida CA et al (2006) High lysine and high tryptophan transgenic maize resulting from the reduction of both 19- and 22-kD alpha-zeins. Plant Mol Biol 61(3):525–535
Hurrell R, Egli I (2010) Iron bioavailability and dietary reference values. Am J Clin Nutr 91:1461S–1467S
Ilnytska O, Argyropoulos G (2008) The role of the agouti-related protein in energy balance regulation. Cell Mol Life Sci 65(17):2721–2731
Jeffery IB, O'Toole PW (2013) Diet microbiota interactions and their implications for healthy living. Nutrients 5:234–252
Kaput J, Noble J, Hatipoglu B et al (2007) Application of nutrigenomic concepts to type 2 diabetes mellitus. Nutr Metab Cardiovasc 17:89–103
Katsube T, Kurisaka N, Ogawa M et al (1999) Accumulation of soybean glycinin and its assembly with the glutelins in rice. Plant Physiol 120:1063–1073
Kim YG, Cha J, Chandrasegaran S (1996) Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A 93(3):1156–1160
Knowles LM, Milner JA (2003) Diallyl disulfide induces ERK phosphorylation and alters gene expression profiles in human colon tumor cells. J Nutr 133:2901–2906

Kopelman PG (2000) Obesity as a medical problem. Nature 404:635–643
Krishnaswamy K (2008) Developing and implementing dietary guidelines in India. Asia Pac J Clin Nutr 17(Suppl 1):66–69
Külahoglu C, Bräutigam A (2014) Quantitative transcriptome analysis using RNA-seq. In: Staiger D (ed) Plant circadian networks. Humana, New York, pp 71–91
Kussmann M, Affolter M (2006) Proteomic methods in nutrition. Curr Opin Clin Nutr Metab Care 9(5):575–583
Kussmann M, Affolter M, Fay LB (2005) Proteomics in nutrition and health. Comb Chem High Throughput Screen 8(8):679–696
Lai JS, Messing J (2002) Increasing maize seed methionine by mRNA stability. Plant J 30:395–402
Lane MA, Bailey SJ (2005) Role of retinoid signalling in the adult brain. Prog Neurobiol 75:275–293
Lee S, An G (2009) Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. Plant Cell Environ 32:408–416
Lee SI, Kim HU, Lee YH et al (2001) Constitutive and seed-specific expression of a maize lysine-feedback-insensitive dihydrodipicolinate synthase gene leads to increased free lysine levels in rice seeds. Mol Breed 8:75–84
Lee TTT, Wang MMC, Hou RCW et al (2003) Enhanced methionine and cysteine levels in transgenic rice seeds by the accumulation of sesame 2S albumin. Biosci Biotechnol Biochem 67:1699–1705
Lee S, Jeon US, Lee SJ et al (2009) Iron fortification of rice seeds through activation of the nicotianamine synthase gene. Proc Natl Acad Sci U S A 106:22014–22019
Li T, Liu B, Spalding MH et al (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30(5):390–392
Liang Z, Zhang K, Chen K et al (2014) Targeted mutagenesis in *Zea mays* using TALENs and the CRISPR/Cas system. J Genet Genomics 41(2):63–68
Lipkie TE, De Moura FF, Zhao ZY et al (2013) Bioaccessibility of carotenoids from transgenic provitamin a biofortified sorghum. J Agric Food Chem 61(24):5764–5771
Loktionov A (2003) Common gene polymorphisms and nutrition: emerging links with pathogenesis of multifactorial chronic diseases. J Nutr Biochem 14:426–451
Lucca P, Hurrell R, Potrykus I (2002) Fighting iron deficiency anemia with iron-rich rice. J Am Coll Nutr 21:184S–190S
Luft FC, Weinberger MH (1997) Heterogeneous responses to changes in dietary salt intake: the salt-sensitivity paradigm. Am J Clin Nutr 65(Suppl 2):612S–617S
Mahan LK, Scott Stump S (2005) Food, nutrition & diet therapy, 6th edn. Roca, Sao Paulo
Mahley RW, Rall SC (2000) Apolipoprotein E: far more than a lipid transport protein. Ann Rev Genomics Hum Genet 1:507–537
Makarova KS, Haft DH, Barrangou R et al (2011) Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol 9:467–477
Malzahn A, Lowder L, Qi Y (2017) Plant genome editing with TALEN and CRISPR. Cell Biosci 24(7):21
Masuda H, Suzuki M, Morikawa KC et al (2008) Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. Rice 1:100–108
McNulty H, Eaton-Evans J, Cran G et al (1996) Nutrient intakes and impact of fortified breakfast cereals in schoolchildren. Arch Dis Child 75(6):474–481
Mihalik D, Gubisova M, Klempova T et al (2014) Transgenic barley producing essential polyunsaturated fatty acids. Biol Plant 58(2):348–354
Moghissi AA, Pei S, Liu Y (2015) Golden rice: scientific, regulatory and public information processes of a genetically modified organism. Crit Rev Biotechnol 21:1–7
Moraes AAC, Pereira FEL (2009) Nutrigenomics of the soy. In: Waitzberg D, Enteral L (eds) Parenteral nutrition in clinical practice, 4th edn. Atheneu, São Paulo, pp 2039–2047
Muller M, Kersten S (2003) Nutrigenomics: goals and strategies. Nat Rev Genet 4:315–322

Najafian M, Jahromi MZ, Nowroznejhad MJ et al (2012) Phloridzin reduces blood glucose levels and improves lipids metabolism in streptozotocin induced diabetic rats. Mol Biol Rep 39:5299–5306

Naqvi S, Zhu C, Farre G et al (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci U S A 106(19):7762–7767

Neeha VS, Kinth P (2013) Nutrigenomics research: a review. J Food Sci Technol 50:415–428

O'Brien MA, Costin BN, Miles MF (2012) Using genome-wide expression profiling to define gene networks relevant to the study of complex traits: from RNA integrity to network topology. In: Chesler EJ, Haendel MA (eds) International review of neurobiology. Academic, London, pp 91–133

Ohnoutkova L, Zitka O, Mrizova K et al (2012) Electrophoretic and chromatographic evaluation of transgenic barley expressing a bacterial dihydrodipicolinate synthase. Electrophoresis 33(15): 2365–2373

Oommen AM, Griffin JB, Sarath G et al (2005) Roles for nutrients in epigenetic events. J Nutr Biochem 16:74–77

Ordovas JM, Corella D (2004) Nutritional genomics. Annu Rev Genomics Hum Genet 5:71–118. https://doi.org/10.1146/annurev.genom.5.061903.180008

Paine JA, Shipton CA, Chaggar S et al (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23:482–487

Patient D, Ainsworth P (1994) The chemistry of flour and bread. Nutr Food Sci 94(3):22–24

Piskol R, Ramaswami G, Li JB (2013) Reliable identification of genomic variants from RNA-seq data. Am J Hum Genet 93:641–651

Porteus MH (2006) Mammalian gene targeting with designed zinc finger nucleases. Mol Ther 13(2):438–446

Porteus MH, Baltimore D (2003) Chimeric nucleases stimulate gene targeting in human cells. Science 300(5620):763

Public Health Nutrition (n.d.) ISSN: 1475-2727 (electronic), 1368–9800 (paper)

Ramesh SA, Choimes S, Schachtman DP (2004) Over-expression of an *Arabidopsis* zinc transporter in *Hordeum vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. Plant Mol Biol 54(3):373–385

Ramirez CL, Foley JE, Wright DA et al (2008) Unexpected failure rates for modular assembly of engineered zinc fingers. Nat Methods 5(5):374–375

Rao S (2001) Nutritional status of Indian population. J Biosci 26(4):481–489

Rosentrater KA, Evers AD (2018) Feed and industrial uses for cereals. In: Kent's technology of cereals. Woodhead Publishing, Abington, pp 785–837

Sarwar M (2008) Laboratory studies on different wheat genotypes for their resistance against Khapra Beetle Trogoderma granarium everts (Coleoptera: Dermestidae). Pak J Seed Technol 2(11&12):46–53

Schaefer EJ (2002) Lipoproteins, nutrition, and heart disease. Am J Clin Nutr 75:191–212

Shan Q, Wang Y, Li J et al (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol 31(8):686–688

Sharma M, Majumdar PK (2009) Occupational life style diseases: an emerging issue. Indian J Occup Environ Med 13(3):109–112

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26:1135–1145

Shetty PS (2002) Nutrition transition in India. Public Health Nutr 5(1A):175–172

Shi J, Wang H, Schellin K et al (2007) Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. Nat Biotechnol 25(8):930–937

Shin YM, Park HJ, Yim SD et al (2006) Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. Plant Biotechnol J 4:303–315

Shumskaya M, Wurtzel ET (2013) The carotenoid biosynthetic pathway: thinking in all dimensions. Plant Sci 208:58–63

Sindhu AS, Zheng Z, Murai N (1997) The pea seed storage protein legumin was synthesized, processed, and accumulated stably in transgenic rice endosperm. Plant Sci 130:189–196
Sinha R, Anderson DE, McDonal SS et al (2003) Cancer risk and diet in India. J Postgrad Med 49: 222–228
Smith JP (1999) Healthy bodies and thick wallets: the dual relation between health and economic status. J Econ Perspect 13(2):144–166
Solanky KS, Bailey NJ, Beckwith-Hall BM et al (2003) Application of biofluid 1H nuclear magnetic resonance based metabonomic techniques for the analysis of the biochemical effects of dietary isoflavones on human plasma profile. Anal Biochem 323:197–204
Steinmetz KL, Pogribny IP, James SJ et al (1998) Hypomethylation of the rat glutathione S-transferase p (GSTP) promoter region isolated from methyl-deficient livers and GSTP positive liver neoplasms. Carcinogenesis 19(8):1487–1494
Sun Y, Jiao G, Liu Z et al (2017) Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Front Plant Sci 8:298
Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780
Swanson KS, Schook LB, Fahey GC Jr (2003) Nutritional genomics: implications for companion animals. J Nutr 133:3033–3040
Szczelkun MD, Tikhomirova MS, Sinkunas T et al (2014) Direct observation of R-loop formation by single RNA-guided Cas9 and Cascade effector complexes. Proc Natl Acad Sci U S A 111(27):9798–9803
Szczepek M, Brondani V, Büchel J et al (2007) Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. Nat Biotechnol 25(7):786–793
Tamas C, Kisgyorgy BN, Rakszegi M et al (2009) Transgenic approach to improve wheat (*Triticum aestivum* L.) nutritional quality. Plant Cell Rep 28(7):1085–1094
Tang G, Qin J, Dolnikowski GG et al (2009) Golden Rice is an effective source of vitamin A. Am J Clin Nutr 89:1776–1783
Tang G, Hu Y, Yin SA et al (2012) β-carotene in Golden Rice is as good as β-carotene in oil at providing vitamin A to children. Am J Clin Nutr 96:658–664
Tang M, He X, Luo Y et al (2013) Nutritional assessment of transgenic lysine-rich maize compared with conventional quality protein maize. J Sci Food Agric 93:1049–1054
Tang L, Mao B, Li Y et al (2017) Knockout of *OsNramp5* using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. Sci Rep 7(1):14438
Tanumihardjo SA, Palacios N, Pixley KV (2010) Provitamin A carotenoid bioavailability: what really matters? Int J Vitam Nutr Res 80:336–350
Trayhurn P (2000) Proteomics and nutrition—a science for the first decade of the new millennium. Br J Nutr 83:1–2
Trijatmiko K, Duenas C, Tsakirpaloglou N et al (2016) Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. Sci Rep 6:19792
Trujillo E, Davis C, Milner J (2006) Nutrigenomics, proteomics, metabolomics, and the practice of dietetics. J Am Diet Assoc 106:403–413
Tsui TK, Li H (2015) Structure principles of CRISPR-Cas surveillance and effector complexes. Annu Rev Biophys 44:229–255
Tucker KL, Smith CE, Lai CQ et al (2013) Quantifying diet for nutrigenomic studies. Annu Rev Nutr 33:349–371
U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015) 2015–2020 Dietary Guidelines for Americans, 8th Edition. https://health.gov/sites/default/files/2019-09/2015-2020_Dietary_Guidelines.pdf
Van der Oost J, Jore MM, Westra ER et al (2009) CRISPR-based adaptive and heritable immunity in prokaryotes. Trends Biochem Sci 34:401–407
Vasconcelos M, Datta K, Oliva N et al (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci 164:371–378

Wakasa K, Hasegawa H, Nemoto H et al (2006) High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. J Exp Bot 57:3069–3078
Wang C, Zeng J, Li Y et al (2014) Enrichment of provitamin A content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI. J Exp Bot 65(9):2545–2556
WHO (2018) https://apps.who.int/iris/rest/bitstreams/1137482/retrieve
WHO (2019) https://apps.who.int/iris/bitstream/handle/10665/311696/WHO-DAD-2019.1-eng.pdf
WHO (2020) https://www.who.int/data/gho/publications/world-health-statistics
Wickramasinghe S, Rincon G, Islas-Trejo A et al (2012) Transcriptional profiling of bovine milk using RNA sequencing. BMC Genomics 13:45
Wiedenheft B, Sternberg SH, Doudna JA (2012) RNA-guided genetic silencing systems in bacteria and archaea. Nature 482:331–338
World Health Organization (1990) Diet, nutrition and the prevention of chronic diseases. Report of a WHO Study Group (WHO technical report series, no. 797), Geneva
World Health Organization (2003) Diet, nutrition and the prevention of chronic diseases. WHO, Geneva. ISSN 0512-3054
Xiaoyan S, Yan Z, Shubin W (2012) Improvement Fe content of wheat (*Triticum aestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. J Agric Biotechnol 20:766–773
Xudong Y, al-Babili S, Klöti A et al (2000) Engineering the provitamin A (b-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305
Yang SH, Moran DL, Jia HW et al (2002) Expression of a synthetic porcine alpha-lactalbumin gene in the kernels of transgenic maize. Transgenic Res 11:11–20
Yang QQ, Zhang CQ, Chan ML et al (2016) Biofortification of rice with the essential amino acid lysine: molecular characterization, nutritional evaluation, and field performance. J Exp Bot 67(14):4285–4296
Ye SQ, Kwiterovich PO (2000) Influence of genetic polymorphisms on responsiveness to dietary fat and cholesterol. Am J Clin Nutr 52(Suppl 5):1275S–1284S
Zhao ZY, Glassman K, Sewalt V et al (2002) Nutritionally improved transgenic sorghum. In: Plant biotechnology 2002 and beyond. Springer, Dordrecht, pp 413–416
Zheng A, Sumi K, Tanaka K et al (1995) The bean seed storage protein β-phaseolin is synthesized, processed and accumulated in the vacuolar type-II protein bodies of transgenic rice endosperm. Plant Physiol 109:777–786
Zheng L, Cheng Z, Ai C et al (2010) Nicotianamine, a novel enhancer of rice iron bioavailability to humans. PLoS One 5(4):e10190
Zhou Y, Cai H, Xiao J et al (2009) Over-expression of aspartate aminotransferase genes in rice resulted in altered nitrogen metabolism and increased amino acid content in seeds. Theor Appl Genet 118:1381–1390
Zhu T, Peterson DJ, Tagliani L et al (1999) Targeted manipulation of maize genes *in vivo* using chimeric RNA/DNA oligonucleotides. Proc Natl Acad Sci U S A 96(15):8768–8773
Zhu C, Naqvi S, Gomez-Galera S et al (2007) Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci 12:548–555
Zhu C, Naqvi S, Breitenbach J et al (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. Proc Natl Acad Sci U S A 105(47):18232–18237
Zhu J, Song N, Sun S et al (2016) Efficiency and inheritance of targeted mutagenesis in maize using CRISPR-Cas9. J Genet Genom 43:5–36