Tilak Raj Sharma
Rupesh Deshmukh
Humira Sonah *Editors*

Advances in Agri-Food Biotechnology

Springer

Advances in Agri-Food Biotechnology

Tilak Raj Sharma • Rupesh Deshmukh •
Humira Sonah
Editors

Advances in Agri-Food Biotechnology

Springer

Editors
Tilak Raj Sharma
Department of Agriculture Biotechnology
National Agri-Food Biotechnology
Mohali, Punjab, India

Division of Crop Science
Indian Council of Agricultural Research
New Delhi, India

Humira Sonah
Department of Agriculture Biotechnology
National Agri-Food Biotechnology
Mohali, Punjab, India

Rupesh Deshmukh
Department of Agriculture Biotechnology
National Agri-Food Biotechnology
Mohali, Punjab, India

ISBN 978-981-15-2873-6 ISBN 978-981-15-2874-3 (eBook)
https://doi.org/10.1007/978-981-15-2874-3

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Recent years have witnessed tremendous advancement in the field of Agri-Food Biotechnology. The technological advances have provided numerous new opportunities to address food security-related issues. Notable progress has been made in techniques like next-generation sequencing, genetic engineering, genome-editing, and nanotechnology, directly or indirectly affecting agri-food biotechnology. Considering the current affairs in food biotechnology, we decided to provide a compilation of advances in various aspects defining adequate food production, nutritional quality, storage, and optimal processing.

Almost all the countries have intensified the research efforts to improve the nutritional quality of the agri-food produce. Developing and underdeveloped countries have a dual challenge to improve overall food production while ensuring the nutritional quality. Hidden hunger is a global problem and it can be equally witnessed also in the developing world. Most of the world is able to produce adequate food, but with an increasing population, everyone has the same challenge to cope-up food production with population growth. Therefore, efficient exploration of technological advances and available knowledge is essential. Interdisciplinary efforts are needed to make food more accessible and to preserve nutritional quality during storage and processing. Even after ensuring the nutritional quality of food products, most of the time, it cannot fulfill the need of everyone. Therefore, food products need to be developed considering the requirements, more particularly for the vulnerable groups of the population.

The book covers biofortification efforts, genetic engineering approaches, diagnostic tools, novel nutraceuticals, food coating materials, food safety, and system biology approaches. A global perspective on agri-food security and nutrition is discussed with emphasis on minerals and vitamin deficiencies. Biofortification efforts to combat such deficiencies are discussed in different crop categories like cereals, pulses, vegetables, and fruits. To ensure the health of cattle and derived food products, biofortification of fodder crops has great significance, but very little is talked about the fodder quality. Here we have tried to highlight such areas where more intensified efforts are needed. Over the last couple of decades, many programs exploring transgenic technology have been accomplished. Several of those

succeeded in developing the desired products, but such products have never reached the market and the consumers due to regulatory issues. However, such transgenic efforts also helped to better understand the genetic regulation and pathways. Here such transgenic efforts have been discussed in detail. The book also addresses challenges for the application of microbes for the improvement of the nutritional quality of food and nutraceuticals. Designer microbes for nutraceutical application is a relatively new area but has great potential. Technological advances, more particularly in synthetic and system biology and genetic engineering, make it possible to engineer microbes to produce desired nutraceuticals in a sustainable, economical, and eco-friendly manner. With advancing genomics, control of metabolic diseases and designing personalized nutrition became possible. Nutrigenomics is emerging as an applied field, which brings hope to millions suffering from dietary issues. Here the chapters are illustrated to provide detailed information on nutrigenomics, gut microbiome, and system biology advances. Similarly, a dedicated section discussing nanotechnology applications for food and associated regulatory issues is provided. Nanotechnology application in food is relatively new, and the research community and consumers are not yet educated to accept these products. Many such challenges for the nanotechnology-based food products and nutraceuticals are thoroughly addressed. Edible food coating is another such advancement but has fewer issues with acceptability. Different approaches and materials being used as edible food coating are discussed. These coatings not only improve the storage duration but also preserve the nutritional value of the food. Such coatings can also be considered for the fortification of the food.

Here we tried to make a logistic link between agriculture advances to enhance the production of nutritive food, industrial applications of biological components, food storage and processing, and finally, the food digestibility related knowledge. We believe the book will serve as a reference for the researchers as well as graduate students.

Mohali, Punjab, India — Tilak Raj Sharma
Mohali, Punjab, India — Rupesh Deshmukh
Mohali, Punjab, India — Humira Sonah

Contents

About the Editors

Tilak Raj Sharma is currently working as Deputy Director General (Crop Science) of the Indian Council of Agricultural Research, New Delhi, India. Ph.D. Agriculture, M.Sc. Agriculture. He has published over 150 research articles and one book on agricultural topics, and holds five related patents.

Rupesh Deshmukh is currently working as a Ramalingaswami Fellow at the National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India. Ph.D. Plant Biotechnology, M.Sc. Agriculture (Plant Biotechnology). He has published over seventy research articles and holds one patent.

Humira Sonah is currently working as a Ramalingaswami Fellow at NABI, Mohali, India. Ph.D. Plant Biotechnology, M.Sc. Agriculture (Plant Biotechnology). She has published over fifty research articles in the areas of plant science and biotechnology.

Global Perspectives on Agriculture: Food Security and Nutrition

1

Nitika Rana, Ruchi Bansal, Shiwani Sharma, Yogesh Sharma, Humira Sonah, Rupesh Deshmukh, and Tilak Raj Sharma

Abstract

Increasing global demands for food have necessitated a comprehensive and informed approach to meet food and nutritional security. Agricultural policies framed a few decades back were aimed at the production and availability of surplus staple food crops. Green Revolution, as an outcome of the successful implementation of science, technology, strategy, and distribution, helped the world to feed the people. The rising monotony in agricultural staple food crops leads us to the trap of malnourishment and hidden pangs of hunger. Agricultural challenges and mitigation strategies need to be reframed for integrated and planned efforts spanning the complete agricultural cycle ranging from production to the distribution of agricultural products without neglecting effective waste management. Current scenario of increasing global populations, dwindling resources, and scarce reuse/recycle practices highlights the need to employ such frugal steps actively throughout the world. In addition to critical innovations in scientific methods and techniques, effective management of infrastructure, distribution of farm products, waste treatment, skill development of the workforce, and engaging collaborations between public and private sectors would help equip farmers and producers to meet the increasing food security challenges. Moreover,

N. Rana · R. Bansal
Department of Agriculture Biotechnology, National Agri-Food Biotechnology Institute (NABI), Mohali, India

Department of Biotechnology, Panjab University, Chandigarh, India

S. Sharma · Y. Sharma · H. Sonah (✉) · R. Deshmukh
Department of Agriculture Biotechnology, National Agri-Food Biotechnology Institute (NABI), Mohali, India

T. R. Sharma
Department of Agriculture Biotechnology, National Agri-Food Biotechnology Institute (NABI), Mohali, India

Division of Crop Science, Indian Council of Agricultural Research, New Delhi, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_1

effective and dynamic policies need to be tailored and implemented, which would cater to a wide array of economic, geographical, and resource availability for different regions of the world. This chapter briefly explains the building blocks of agriculture and efficient integration among them in terms of global food and nutritional security.

Keywords

Agriculture · Nutrition · Green revolution · Biofortification · Global hunger

1.1 Introduction to Agriculture

Agriculture, being an art as well as the science of cultivating plants and livestock, is a unique and challenging venture. One of the primary reasons behind the advent of a sedentary human lifestyle over the earlier practiced nomadic culture was the key development of agriculture, which provides reliable availability of food. Modern-day crop breeding techniques, use of pesticides and fertilizers, and highly efficient tools and machines have enormously increased the yield to cater to the food requirements of billions of people. In addition to the food supply, the agriculture sector provides the highest employment in the world. In a broader sense, agriculture can be defined as a process of producing commodities that supports life, including fiber, food, horticultural crops, and forest products, with the use of natural resources. Agriculture is a comprehensive term and includes a diverse form of products and activities. Any agricultural product or commodity in raw or processed forms or any product or commodity derived from livestock when marketed for human or livestock consumption can be considered as an agricultural product, for example, flowers, plants, textiles, food, seed, and feed.

In the production of agricultural products, activities like cultivating soil, raising and harvesting of crops, feeding, and rearing and taking care of livestock are included, while in the case of aquaculture, raising and managing of aquatic animals is included. Similarly, floriculture and horticulture include the cultivation of flowering plants and fruits and vegetables, respectively. Harvesting of Maple Syrup and fresh trees are also included in the production of agricultural products, whereas other agricultural derived activities like storing and preservation of raw materials, storing, and processing of dairy raw or finished products or grains are not included in agricultural products. Agricultural products can broadly categorize into four headings, food, fuel, fibers, and raw material. On the basis of economic value and consumption, food crops can be classified into cash crops or staple crops.

Staple Crops: Staple crop or staple food is the food which comprises the significant portion of a meal or human diet and is often consumed in large quantities by a community or a given set of people. It is also responsible for contributing to the necessary nutrients as well. Generally, staple food provides the majority of the energy needs and macronutrients which are essential for the survival and health of an individual. Mostly, the staple diet is consumed very frequently by a group of

Table 1.1 Types and details of major crops involved in agriculture

Staple crops	Cereals	Rice, wheat, maize, millet, and sorghum
	Starchy tubers	Potatoes, cassava, sweet potatoes, yams
	Pulses	Dried legumes
	Sago	
	Misc	Sugar, coconut oil, olive oil
Cash crops	Sugar crops	Sugarcane & beet root
	Oil seeds	Soybean, groundnuts, castor beans, sunflower seed, rapeseed, linseeds and safflower seed
	Fiber crops	Cotton, flax, hemp, sisal, and jute and jute-type fibers-and tobacco
	Vegetables	Cabbage, artichoke, tomato, cauliflower, pumpkin, squash, gourds, cucumber, eggplant, chili, pepper, onion, garlic, watermelon, cantaloupe and similar melons, and several minor vegetables
	Beverage crops	Coffee, cocoa, and tea
	Tree crops	Fruit, oil palm, and rubber

people on an everyday basis for every meal basis. Staple food crops vary from place to place, and most people depend on a very few numbers of staple food crops. Grains, tubers, legumes, seeds, and roots are the typical staple food items. Over time, those food crops which provide a good source of nutrients, higher availability and are easy to store and have significantly high shelf lives were selected as staple food crops by earlier civilizations.

Cash Crops: Cash crops, as per their name, are the crops that are grown in fields for its commercial value rather than for daily consumption or livestock consumption. They constitute an essential role in framing national food security policies for many agriculture-based countries with low-income brackets in order to promote or push forward the domestic market and exports and hence the foreign exchange for the economy. Many critics, although denied the direct benefits of cultivating cash crops to poor or small farm holders but cash crops, do have a catalytic effect on agriculture, and they do add value to productivity in rural produce. The cash crops are broadly categorized on the basis of the requirement of post-harvest processing, export, or domestic produce into six categories, i.e., sugar crops, oilseeds, fiber crops, vegetables, beverages, and tree crops (Table 1.1).

Fisheries: Fisheries or fish farming deals with raising fishes commercially in a tank or ponds for human consumption. It is one of the imperative segments of the economy of any country and for the coastal communities. Besides providing food security, it ensures employment on a vast scale. With the growing demands of fish protein and fish, fish culture is popularly practiced in non-coastal areas as well. According to FAO reports, the global production of fish attained more than 170 million tonnes mark in 2016. Mostly, tilapia, catfish, carp, and salmon fishes are cultivated in fish farming. On the basis of photosynthetic feed or external feed,

fish farming is practiced under two major headings, i.e., extensive and intensive farming, respectively. Extensive farming is usually conducted in the small to medium-sized pool under natural photosynthetic conditions without much external help. The investments and costs are comparatively minimal, and production directly depends on the natural productivity of the pond in a specific area. The availability of food is the major limiting factor in these types of farming. Those aquatic species which feed directly on phytoplanktons constitute the major farm. The photosynthetic activity is usually increased by the addition of artificial fertilizers (potash, phosphorous, and nitrogen) in the pond water. In contrast to the most basic type of fish farming, intensive farming is technologically well equipped.

Intensive farming is a technologically, highly advanced form of farming. The density of production is maintained at the highest possible levels in the artificial tanks. The tanks are coupled with a large number of regulators to maintain the optimum nutrient levels and the steady-state as well as the removal of toxins from the water source. The nutrient level in the tanks is maintained by the addition of continuous supply of external feeds, and the toxins and waste are also removed from the water. Oxygen supply and quality of water are maintained to improve the growth of fishes in the tank. A high level of costs and investments are required in intensive farming as compared to extensive ones.

1.1.1 Effects of Global Hunger and Nutrition Demands on Agriculture

One of the most basic human requirements is food. To have access to a proper nutritional value, food is/must be the fundamental objective of all the governments. Despite the backbreaking efforts of national, sub-national governments and international forums, hunger is still persistent in many parts of the world. It is always a significant concern in many third world countries. Acute hunger is the most extreme form of hunger. It occurs in the scenario of no or very minimum amount of food consumption (Fig. 1.1). It mainly occurs during the period of natural calamities like famine, drought, disasters, and even wars, etc. Approximately 8% of the total hunger-stricken population suffers from an extreme form of hunger.

The most widespread form of hunger is chronic hunger. When the lack of insufficient food prevails for a long term of time, then it gives rise to chronic hunger. It designates undernourishment over a considerable period of time. Poverty is the main contributor to this type of hunger. According to various reports, the primary reason for hunger in some areas is not the unavailability of food, but it is the inability of the purchasing capacity of food in some families, classes, or even for some countries. The third form of hunger is hidden hunger. It is a much-diversified form of chronic hunger that rises due to the intake of unbalanced diets, which culminates into lack of iron, zinc, vitamin A, and iodine. It affects both poor and rich people due to different reasons. Poor people consume only staple diet over legumes, cereals, nuts, and fruits which is rich in macronutrients but lack micronutrients due to the lack of purchasing power, whereas people in higher-income groups consume too

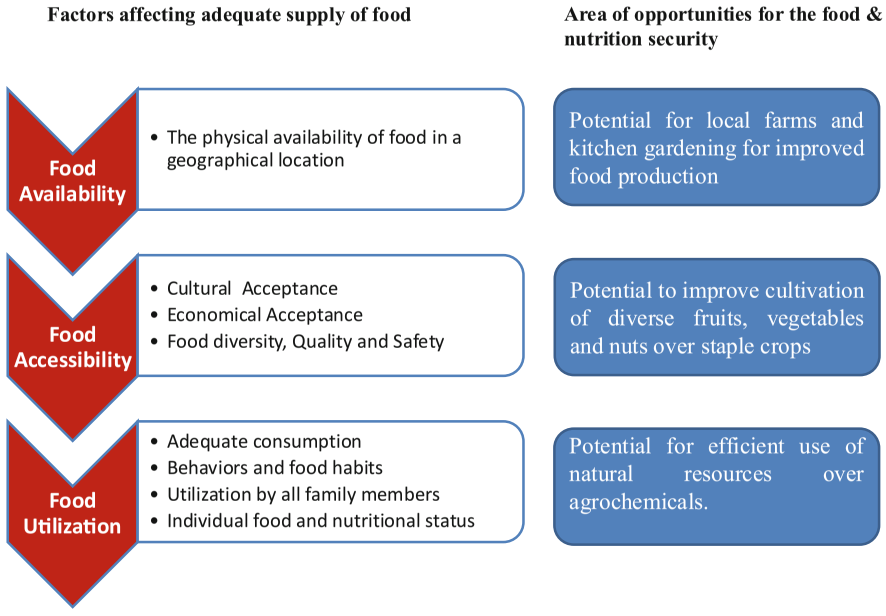

Fig. 1.1 Factors affecting the nutritious diet for sound health: adequate supply and accessibility to nutritious food helps in maintaining body weight and decreasing susceptibility to diseases

much junk food which is high energy diets rich in carbohydrates and fats but lacks micronutrients resulting in obesity and hidden hunger. The intentional/unintentional inability to diversify our diet with fruits, vegetables, or animal-derived food will lead to malnutrition, and it is inevitable. According to the Global hunger index report (GHI report), one out of three or two billion human population all across the world suffer from hidden hunger. More than twice, the population, which feels hunger in the stomach (due to poverty), actually suffers from hunger, which is not felt in the stomach but strike at the core of the health and vitality of a human body.

The deprivation of essential vitamins and minerals leads to the weakening of the immune system, which leads to stunted physical and intellectual growth and, in extreme cases, can even lead to death. Among these groups, the hunger impacts children and women most intensively. In micronutrients intake, 19 vitamins and minerals (Vitamins A, B4, B12, C, D, E, and K; thiamine, riboflavin, niacin, pantothenic acid and biotin, folate and folic acid, calcium, iodine, iron, magnesium, and zinc) are vital for the efficient physical and mental growth, efficient functioning of immune system, and other metabolic processes (Kennedy et al. 2003). Iron, Iodine, and Vitamin A deficiencies are most prevalent across the globe.

Agriculture practices have a vital role to play in the current issue of hunger and hidden hunger on a global scale. Traditionally agriculture practices are aimed at calorie based approaches or protein-energy deficiencies, only to increase yield and productivity to meet the food requirements both in developing and developed countries. Agriculture practices were never targeted for promoting human health. In the 1970s, in developing countries like India, Green Revolution was launched with a vision of providing enough food for the population by the use of fertilizers, pesticides, and improved seeds. It was a myth that if we have enough food, we will have good health. Green Revolution was a successful initiative in a way, but it promoted the production of staple food crops like wheat, rice, and paddy, which leads to simplified diets. Due to the consequences of hidden hunger and malnutrition in the 1990s, the attention was drifted to nutrition with production rather than production only. Research and technological advancements were considered fundamental tools for the improvement of crop species. Supplements and bio-fortified crops are considered as the best possible ways to improve health in vulnerable groups so far. The fortified staple crops with various micronutrients help drastically to eradicate malnutrition in the poverty-stricken groups. Many works have been done to treat such deficiencies like Vitamin A fortified rice to curb night blindness and supplementation with iodine to treat goiter (Burchi et al. 2011). Moreover, the food must be absorbed efficiently in the gut to cure hunger and malfunctioning efficiently. Hence, the research in agriculture plays a vital role in accumulating the nutrients in food crops in the form which is rapidly absorbed in the human gut under the “Farm to Fork to Gut” mission.

1.2 Challenges in Agriculture

It has been projected by the United Nations and many other sources that the world population would be around 8.5 billion by the year 2025 and 11 billion by the end of this century. The production of food, fiber, and feed should be increased at the same pace globally to feed and clothe the growing population. The ever-growing population and urbanization lead to shrinkage in the land area available for agricultural production. The exceeding usage of earth's sustainable resources, increasing population, reducing available agricultural lands has forced us to find ways to improve agricultural yield with a low impact on the environment (Davis et al. 2016). Moreover, the production of yield in agriculture is very much at the mercy of the climatic conditions. The instances of weather (drought or floods) driven yield losses are well evident in all parts of the world from the centuries. Weather also indirectly affects the crop species as well. The increased temperature and humidity conditions can favor the outbreak of a deadly disease (Capinera and Horton 1989; Coyne et al. 1974). There are many examples of an epidemic of a disease reducing crop yields due to the weather. Climate is a very powerful detriment to agriculture. The increasing concentrations of greenhouse gases (CO_2, CH_4, CFCs, N_2O) are though not of much trouble immediately. Still, their impact on the global climate is detrimental by causing the alteration in the distribution of precipitation has tremendous consequences. Moreover, climate change indirectly affects all the other components of agriculture like soil, water distribution, and quality, etc. (Rosenberg 1992).

One of the significant challenges in agriculture despite all the changing scenarios regarding climate change and rising population is the impact of abiotic or biotic stress on the crop species. The productivity of crops is drastically affected by both biotic and abiotic stresses (Sonah et al. 2017; Vishwakarma et al. 2019). The outbreak of a disease can cause the complete loss of the crop in an area in a short time. Similar to biotic stresses, abiotic stresses also drastically reduces crop yield each year across the globe (Bhat et al. 2019; Deshmukh et al. 2014). Abiotic stresses include light (low or high), temperature (low, high), water (drought or flood), and chemicals (salts, pesticides, fertilizers). These abiotic stresses affect the plant at every stage of its growth cycle like inhibition in the germination of the seeds, premature senescence, or reduction in growth or productivity. At physiological levels also, these stresses have a significant effect at the respiration or the transpiration rates of the plant. Some plants accumulate toxic metabolites or growth inhibitors in response to such exposure of light or temperature, and the uptake of water and nitrogen is also severely affected.

The increasing usage of pesticides and fertilizers over the years leads to the increase of soluble salts like sulfates, nitrates, carbonates, chlorides, and calcium and magnesium in the soil causing salinity or sodicity in the soil. The increased salts in the soil interfere with the reduced ability of the plant to uptake the water, which culminates into the slow plant growth (Ahmad et al. 2019; Zargar et al. 2017). Excess amount of soluble salts in the plant cell also causes cellular injury and even chlorophyll degradation. There was a study in which a group of researchers analyzed

the impact of increased salt concentrations on seed germination, a number of spikelets per panicle, tiller per plant, and overall yield. They reported the survival of seedling was drastically reduced when the concentration of salt increased higher than 3.4dS/m (Zeng and Shannon 2000). Similarly, another study was conducted to study the effect of salinity on four varieties of 3-week old rice plantlets at different salt concentrations for a week. Afterward, the rate of growth, Na+ uptake, and antioxidant capacities were studied. They concluded that salt-sensitive stress varieties exhibit increased peroxidase activity and lipid peroxidation. The accumulation of Na^+ was also increased along with the electrolyte leakage (Dionisio-Sese and Tobita 1998). These all factors, whether biotic, abiotic, availability of water and nutrients, and climate very strongly affect the plant growth and yield, and to attain them in the optimized state is the major challenge in the successful agricultural practices.

1.3 Work Done During the Past Few Decades

The challenges posed by a growing population, depleting resources, and inefficient management; to global agricultural security necessitate a multi-faceted approach to combat nutritional inequality. Global coherent efforts are aimed at simultaneously achieving food security, improving nutrient availability, minimizing wastage, and prudent distribution of agricultural resources among all sectors of producers and consumers. Several policies have been formulated during the past few decades, which have highlighted stark interdependence of scientific endeavors in agriculture to economic dynamics and resource management across varying population hierarchies.

1.3.1 Successful Strategies

1.3.1.1 Improvement in Agricultural Produce and Utilization by Consumers

Several government organizations, farming community, and components of food industries including production, supply, and marketing have great role in developing novel strategies to eradicate malnutrition (Fig. 1.2). Genetic improvement of crops for nutritional quality is one of the most significant strategies being used worldwide. Domestication of crops and animals started around 10,000 years BC, wherein humans would select for the best seeds or livestock for farming in the subsequent seasons, which is termed as "selective breeding." Eventually, it was realized that the selection of high yielding crops and animals for specific traits/features viz. yield and production decreased their genetic diversity. As genetic variations for many important traits such as resistance to diseases and abiotic stress were being lost (Learn. Genetics GSLC 2018). Selective breeding or traditional breeding was followed by marker-assisted breeding, transgenic technology, and gene-editing methods (Rana et al. 2019). Similarly, mutation breeding is being used to create novel variations that

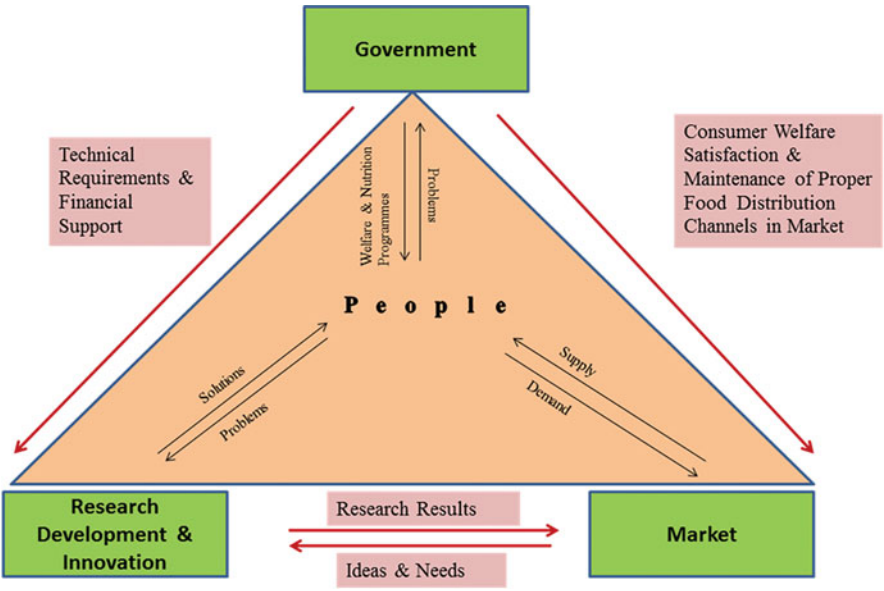

Fig. 1.2 An integrated network depicting roles of different organizations for efficient supply of food to consumers

can directly improve the trait or may help to improve trait with breeding exercise (Bansal et al. 2019; Chaudhary et al. 2019a; Kumawat et al. 2019). Conventional breeding combines desirable traits from elite parents to obtain offspring without information about genes that influence these traits. Conventional breeding is not amenable for traits governed by multiple genes and is often time-consuming (de Ronne et al. 2019). For instance, modern maize with exposed kernels (American Association for the Advancement of Science 2003) and Belgian Blue cow with improved lean muscles (McPherron and Lee 1997) are examples of decades of selective breeding. Marker-assisted breeding, on the other hand, requires knowledge about genes coding for traits of interest, and results can be obtained in a shorter period. This technique focusses on inclusion or exclusion of segments of DNA called markers associated with desired phenotypes (Ram et al. 2019; Yadav et al. 2019). However, this technique is dependent on naturally breeding plant populations and is more fruitful in plants with shorter reproductive cycles only. Several marker-assisted breeding studies have been conducted in maize for corn borer resistance and yield (Bouchez et al. 2002; Willcox et al. 2002), in rice for bacterial blight (Chen et al. 2000), in barley for stripe rust (Toojinda et al. 1998), and in soybean for nematodes and rust (de Ronne et al. 2019; Rasoolizadeh et al. 2018; Vuong et al. 2015). Similarly, several such studies have been undertaken for combating mastitis infection in dairy cattle, double muscling, control of milk fat, etc. (Williams 2005). A more precise and faster method of introduction of the desired gene into crops is transgenic technology (Chaudhary et al. 2019b). Although an advanced technique, it

requires comprehensive information about the gene of interest and its functional impact on host plants (Shivaraj et al. 2019). Several transgenic crops with modified stress resistance such as in corn and soybean, starch metabolism in maize and tomato, and yield increase rice have been undertaken in the past decades (Dunwell 2000). Similarly, livestock with enhanced milk, disease resistance, and enhanced growth have been developed for human consumption (Wheeler 2013). Rapid investment in genome editing has enabled rewriting of the genome sequence of organisms (Mushtaq et al. 2020). Multiple techniques such as TALEN (Transcription activator-like effector nucleases) and CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats) are fore-runners in crop improvement methods (Learn.Genetics GSLC 2018; Vats et al. 2019). Several genome editing efforts have been made for increased grain yield in maize, blast resistance in rice, iron content in wheat, and herbicide resistance in potato (Jaganathan et al. 2018; Mushtaq et al. 2020). In addition, similar efforts in livestock improvement have been aimed at increasing milk production, egg yield, litter size, and lean pork meat, among other traits (Tait-Burkard et al. 2018). Biofortification of crop plants is yet another technique that aims at increasing the micronutrient content of edible plant parts. Several methods, such as the utilization of fertilizers, increasing the absorption of minerals from the soil and genetic biofortification strategies, have aimed towards achieving nutrient security. In addition to biofortification of cereals (Garg et al. 2018; Lyons 2018), methods for enhancement of nutrient content have been utilized in vegetables (Junior et al. 2017; O'Hare 2014), pulses (Ghosh et al. 2019; Singh et al. 2015), fruits (Amah et al. 2019; Das et al. 2018), and fodder as well (Novoselec et al. 2018). Moreover, transgenic approaches are being frequently used to increase the phytoavailability of micronutrients, their uptake by the plant, and finally, transport to edible/economically important plant parts (White and Broadley 2009). Such products are termed as functional foods that have been fortified or enhanced with additional nutrients. In order to successfully combat global hunger and malnutrition, nutrient bioavailability also needs to be explored. Nutrient bioavailability is defined as the efficient absorption of nutrients from the diet (Hurrell and Egli 2010). The utilization of absorbed nutrients for body growth and development is further dependent on several factors, one among them being the microbiome of human body. The microbiome is the plethora of micro-organisms that live primarily in the human gut, and it affects immunity, body development, and nutrition (Rogers and Zhang 2016). Nutrigenomics is yet another approach to gauge the effect of diet on individuals and populations (Sales et al. 2014). This method has opened new arenas of personalized nutritional intervention wherein dietary impacts on metabolic diseases have also been studied (Afman and Müller 2006; Phillips 2013). At the consumer level, there is a necessity for a comprehensive study related to the impact of improved products on the health of individuals. For instance, food safety and related diagnostic tools are one such area that needs critical assessment before consumption (Jacxsens et al. 2011). Detection of harmful compounds in food has also been enabled due to developments in other fields of science such as nanotechnology (Yu et al. 2018). Development and improvement in pioneering scientific techniques and practices have played a prominent role to usher in an era of agronomic security.

Concurrently, efficient agricultural practices have played a pivotal role in reaping maximized benefits from improved agricultural production.

1.3.1.2 Development and Adoption of Efficient Agricultural Practices

The first step towards starting agriculture was adopting a settled lifestyle as compared to the nomadic life. With experiences gained by successive generations, human-kind shifted from cultivating only crops like wheat, barley, flax to rearing livestock. Initially, animals were used for plowing of fields as compared to today, where tractors and harvesters are used to achieve agricultural tasks. Crop rotation, wherein different crops are grown on a piece of land according to varying seasons, is utilized even today. Throughout history, humanity has evolved to improve agricultural strategies for obtaining the highest outputs. The widespread use of fertilizers, herbicides, pesticides, and farm machines has enabled increased food production and affordability. Although with increasing awareness about the negative impacts of chemical fertilizers and pesticides on our environment, alternative methods for crop improvement are being introduced to farmers. In addition to better seed selection and efficient water management, integrated farming methods which aim at growing crops with close-knit rearing of livestock and agroforestry has paved the way for efficient utilization of resources and multiple sources of income for farmers. Moreover, the use of vermicompost, biofertilizers, and multi-cropping (growing vegetables alongside cereal crops) has enabled a sustainable increase in crop production. Several studies have emphasized the advantages of sustainable and organic agricultural practices over conventional practices. For instance, total phenolic contents of agricultural products grown using organic and sustainable farming practices were found to be higher in the case of strawberries, corn, and marionberries (Asami et al. 2003). Similarly, steps should be adopted to increase the soil organic carbon using methods such as reduced tillage, growing trees, and grasses and incorporation of farm remnants back into soil (Antle and Diagana 2003). Similarly, instead of using fungicides and pesticides, biological agents can be utilized for pest control. Hydroponics is another new segment of agriculture, which reduces dependence on excessive tilling areas for agriculture. Several governments have made efforts to encourage efficient use of resources, for example, Government of India initiated the Integrated Scheme of Oilseeds, Pulses, Oil Palm and Maize (ISOPOM) under which public funds could be used by small farmers throughout the agricultural process (Ministry of Agriculture and Farmers Welfare 2013). Another such scheme benefiting the farmers has been the "National Agriculture Development Programme," which enables states to comprehensively design plans for their agriculture sector considering the resources and agricultural conditions specific to their region (Callithen and Matthew 2007). In addition to efficient practices of agricultural production, an improved framework is needed for the storage of agricultural produce.

1.3.1.3 Storage of Agricultural Produce

Storage implies post-harvest methods for deferred utilization of agricultural products. It is an integral part of agriculture since the efficient distribution of final

products is often hampered due to inept storage. Systematic storage is a significant factor responsible for maintaining the supply and demand in agricultural markets. It is mainly targeted at protecting the produce from fluctuating temperatures, moisture, oxygen content, micro-organisms, insects, and rodents (FAO 2018). For example, contamination of maize by mycotoxin is a major cause of grain damage across the world (Kumar and Kalita 2017). Various storage structures include underground and surface storage units along with cold storage systems. Ideally, agricultural products can be packed in bags or stored in bulk as loose storages. In addition, warehousing facilities improve scientific storage for the quantity and quality of products. Various storage units may be owned by private individuals or maintained by public undertakings. Also, bonded warehouses are maintained by the governments at key places of transport such as seaport and airports. Moreover, storage is not only defined in terms of agricultural products but also in terms of fertilizers, herbicides, and pesticides as well. Several products such as cereals and fertilizers can be stored in general storage units, whereas custom warehouses are required for specific products such as cotton, wool, etc. Refrigerated storage is temperature-controlled for dairy, eggs, vegetables, etc. (Agro Processing 2015). Storage planning and structures are also dependent on duration, scale, and method of storing particular products. Assorting, as well as the grading of commodities, are important factors influencing storage strategies. Various methods, such as physical, chemical, and biological, are key ways for efficiently maintaining reservoirs of products. One such example studying the effect of air-drying and freeze-drying strawberries, corn, and marionberries suggested better preservation of total phenolic content in the case of freeze-drying (Asami et al. 2003). Food fortification has also been employed to decrease nutrition deficiency in populations, vitamin A enrichment of vegetable oil has been one such example (Keding et al. 2013). Storage of grains with natural insecticides such as extracts from *Chenopodium ambrosioides* aids prevents insecticidal damage to grains (Kumar and Kalita 2017). Similarly, edible oils such as soybean and palm kernel oil can be used as fumigants against insects in the case of grains (Kumar and Kalita 2017). Edible food coating is another technique that helps in the preservation of food products. Food coatings allow long-term storage of fruits and vegetables. Next-generation food storage methods are being developed rapidly to reduce product loss due to deterioration. Closely following various storage techniques, the efficient distribution of agricultural products is one of the final steps for successful delivery to consumers.

1.3.1.4 Efficient Distribution of Agricultural Products

An efficient distribution system includes high monetary gain for farmers, low transportation charges, and reasonable prices to be paid by consumers. Previously, the distribution of agricultural commodities was focused on a limited immediate region to a farmer. This has now been replaced with national as well as international exports even for small scale farmers. Distribution is dependent upon the geography, producer–consumer relationship, commodity, technologies utilized throughout the process of agricultural production as well as global conditions prevalent concurrently. Effective prediction of demand and supply through reliable channels helps in

making realistic forecasts. A well-organized distribution system helps curb losses incurred through the supply and demand chain. It also paves the way for effective expansion of the market based on multiple commodities and influences the overall economy of a nation. A single effective nation-wide marketing and distribution scheme reduces instability due to regional fluctuations. An environment of competitiveness created as a result of this leads to cost-effectiveness and increases transparency in markets. An efficient marketing and distribution system promotes alternate marketing and selling channels. An unprecedented increase in the price of an agricultural product leads to decreased demand, which in turn triggers shrunken production during the next season. Several models have been suggested for the efficient distribution of agricultural products after harvesting. One such example suggested a stochastic model for fresh farm produce and showed an increase in profit due to planned distribution systems (Ahumada et al. 2012). Similar studies have devised effective models for the management of agricultural products post-harvest and weighing the balance between the freshness of food and labor or transportation charges (Ahumada and Villalobos 2011). With increasing weather uncertainties such as droughts, floods, and extreme rainfall, efficient product distribution demands dynamic updates to various methods of storage and transportation (Ziska et al. 2016). Hence, the distribution system of agricultural products is frail and sensitive to fluctuations at any level of the hierarchy, wherein careful planning can result in decreased losses.

1.3.1.5 Waste Management

In addition to the factors mentioned above, wastage is prevalent and should be prevented at every facet of agricultural production. An efficient waste management system demands sustainable utilization of resources while minimizing waste generation starting from production until the consumption of agricultural produce. Agricultural waste during production varies in type, consistency (solid, liquid, semisolid, and slurry), amount, etc. (United States Department of Agriculture 2011). Also, there is variation in waste production during different seasons and varying crops or livestock. For instance, the majority of water wastage happens in the form of run-off from farms and fields. Such wastage can be managed by maintaining records of facilities, equipment, and waste production. Prior information regarding utilization of resources such as seeds, fertilizers, and pesticides; in case of crops, and proper feed, housing facilities, and waste handling; in case of livestock; helps in minimizing wastage and efficient recycling of resources. In addition to efforts for reducing wastage during production and setting up of agricultural farms; the waste collected during subsequent stages needs to be sorted and transported to treatment plants. Sorting depends upon the consistency, hazardousness, and recycling efficiency of waste, among other factors. Treatment includes physical, chemical, and biological processing for minimizing the pollution potential of natural, animal, and plant waste. Several waste management techniques utilize discarded products from agriculture as the production of biofuels and recycled products. For example, where at industrial levels, wastewater is recycled and reused to decrease water wastage, at smaller levels excreta from farms can be utilized as manure in fields. Another such

example has been the production of biogas from waste material generated on farms (Hansen and Cheong 2019). Single-cell proteins (SCPs) have also been produced as a result of the oxidation of agricultural wastes. SCPs are high in protein contents and can be used as a protein-rich supplement in human food (Spalvins et al. 2018). In addition to traditional bioprocessing methods, several methods have been developed for the production of biofuels by various research groups (Champagne 2008). Within India, one basic example is using excreta from milch animals such as cows and buffaloes for fuel in the form of dung cakes. Bioremediation is another approach that is based on the utilization of microbes and their products for combating environmental pollution and contamination. Removal of heavy metal contamination is one such example where bioremediation is used (Perpetuo et al. 2011). An efficient agricultural waste management system minimizes environmental impact and maximizes the recycling and re-utilization of agricultural products.

1.3.1.6 Models and Metrics for Measuring Global Food and Nutritional Security

Several models and metrics need to be developed to efficiently gauging worldwide food and nutritional security. Factors that dominate different world populations such as a number of people, economic status, literacy, and availability of resources across developed, developing, and underdeveloped regions should be considered while formulating models of food security. To address the aforementioned issues, several frameworks have been developed globally during the past decades (Table 1.2). Key features of such global models aim at providing country-specific frameworks such that development programs are tailor-made according to their requirements and resources. Effective policies comprise of private as well as public sector stakeholders as well as encourage the participation of women for increasing productivity. In addition, literacy, development of advanced and sustainable technology, and sound investment in infrastructure and labor training have been few of the salient features for the effective formulation of international policies.

1.4 Steps to Achieve Global Food and Nutritional Food Security

Various steps have been taken at national and international levels to combat global food and nutrition insecurity (Fig. 1.3). From past experiences, it has been evident that an integrated approach aimed at social, economic, and sustainable agricultural development is paramount for achieving safe and nutrient-rich food security (Fig. 1.1). In addition to changing population size, economic status, dietary preferences, and consumer awareness, climate change, resource depletion also affect the sustainability of efforts to curb malnutrition and hunger. Several steps that have paved the way for achieving global food and nutrition security have been enumerated in the following section.

Table 1.2 Various frameworks adopted globally for global food and security

Framework	Details	Reference
International covenant on economic, social, and cultural rights (ICESCR)	Recognized "right to health" wherein a person has a right to food and nutrition; among others	Alston and Quinn (1987)
World food summit plan of action and the Rome declaration on world food security	Aimed at the availability of nutritious food and freedom from hunger	FAO (1996)
The voluntary guidelines to support the progressive realization of the right to adequate food in the context of national food security (VGRtF)	Right to food guidelines by food and agriculture organization (FAO) aims at global food security and nutrition	FAO Council (2004)
The final declaration of the 2009 world summit on food security	Discussed eradication of hunger by agricultural improvements in developing nations	FAO UN (2009)
The voluntary guidelines on the responsible governance of tenure of land, fisheries, and forests in the context of national food security (VGGT)	Aims eradication of hunger and utilization of land, fisheries, and forests for the same	FAO UN (2012)
The principles for responsible investment in agriculture and food systems (RAI)	Globally applicable ten principles aiming at food security	FAO (2014)
The framework for action for food security and nutrition in protracted crises (FFA)	Targets mitigating risks posed towards food security and nutrition	FAO (2015)
2030 agenda for sustainable development	Signed by 193 nations, aims at economic, social, and environmental development	Nations (2015)

1.4.1 Research on Improving Crop Varieties, Techniques, Improvement in Agricultural Practices

Numerous steps and strategies have been taken over the years to ensure global food and nutritional security. One such initiative was the Green Revolution, which began in the 1960s with an aim to increase the agricultural production many folds by the use of high yielding varieties (dwarf rice and wheat) in the presence of fertilizers and reliable water supply. On the verge of famine in the 1960s, India, in the guidance of Norman Borlaug and MS Swaminathan, imported wheat seeds from the Internal Maize and Wheat Improvement Centre (CIMMYT). Similarly, India adopted IR8 rice variety, which is a semi-dwarf rice variety produced more grains in association with fertilizers and irrigation. With the adaption of such high yielding varieties along with the advent of modern agricultural practices, India became a food surplus country.

Various researches were conducted over the years to improve the crop plants with different mineral components to combat malnutrition. Major biofortification strategies are undertaken to fortify the crop species with iron, zinc, copper, selenium,

Fig. 1.3 Integrated efforts to achieve global agricultural and nutrient security

iodine, magnesium, and calcium. Among all the adopted strategies to enhance the mineral concentrations in the crop species, two of them are the most widely accepted approaches. In the first approach, the concentration of mineral element is increased by the application of fertilizers during cultivation of crops with an aim to improve the mobilization as well as solubilization of minerals in the soil, whereas in the second approach the capacity of a crop plant to accumulate the mineral substances in the edible tissues in non-toxic forms is enhanced with the help of various biotechnological approaches and reducing the anti-nutrients concentration (oxalate, polyphenolics, and phytate) in the crop products which reduces the bioavailability of nutrients.

Plants absorb minerals from the soil in the specific chemical forms. For instance, the roots of plants can absorb Iron, Zinc, Copper, Calcium, and Magnesium in their cationic forms, whereas the members of graminaceous species can uptake iron, zinc, and copper as metal-chelates (Marschner 1974). Iodine is taken up by the plants in the form of iodide/iodate (Mackowiak et al. 2005), and selenium is absorbed by the plant's roots as selenate, organoselenium or selenite (White et al. 2007). The presence of these minerals in different chemical forms as free ions or as ions absorbed to the mineral or organic surfaces or precipitates or as compounds in the rhizosphere defines the physicochemical and biological properties of the soil. The

pH of the soil, cations exchange capacity, microbial activity of the soil and redox reactions, organic matter and moisture ultimately determines the availability of the elements to the plant (Shuman 1998). An element like silicon has recently been considered as a beneficial element for plant growth even though the reports from the last couple of decades confirmed the numerous benefits (Deshmukh and Bélanger 2016; Deshmukh et al. 2017a). Besides, having plenty of silicon-based fertilizers limited is known about crop-specific silicon requirement, preference for the forms of silicon compounds, and genetic predisposition to uptake the element (Bokor et al. 2019; Deshmukh et al. 2017b; Guerriero et al. 2019). Despite the high availability of minerals in some soils, the phytoavailability of minerals is often restricted due to soil properties.

Plants acquire iron from the soil via two different mechanisms (Grotz and Guerinot 2006; Marschner 1974). The roots of non-graminaceous species acidify the rhizosphere by releasing organic acids and phenolic compounds in order to increase the concentration of Fe^{+3} in the soil. The acids and other compounds chelates with Fe^{+3}. The oxidized form of iron is reduced to Fe^{+2} by the ferric reductases present in the root epidermal cells. These reductases are encoded by the ferric reductase oxidase (FRO) gene family (Robinson et al. 1999; Wu et al. 2005). Numerous other proteins mediate the influx of Fe^{+2} to root cells (Ishimaru et al. 2006; Vert et al. 2002). The second mechanism of absorption of iron by soil is employed by grasses and cereals. In this mechanism, instead of organic acids and phenolic compounds, phytosiderophores are released into the rhizosphere in order to chelate Fe^{+3}, and the complex is taken up by the root cells. The structure and the capacity to acquire Fe^{+3} are different for different crop species (Bashir et al. 2006; Marschner 1974). A number of genes families like multidrug and toxin efflux family (root pericycle to shoot), ZIP family (uptake by shoot cells), natural resistance-associated macrophage protein (iron homeostasis) participate in mineral uptake and redistribution of the minerals to the edible plant parts in the non-toxic forms (Gross et al. 2003; Thomine et al. 2003). It was observed in many studies that the expression of FROs, ZIPs, NRAMPs genes and genes coding for the biosynthesis of Nicotianamine synthase and phytosiderophores was upregulated (Gross et al. 2003; Ishimaru et al. 2006; Robinson et al. 1999; Wintz et al. 2003). The expression of iron accumulation by the first mechanism is regulated by transcription factor LeFER in tomato (basic helix-loop-helix, bHLH) (Ling et al. 2002). However, when a group of researchers overexpressed LeFER in tomato and AtFIT1 in Arabidopsis, it does not lead to constitutive uptake of iron, indicating the involvement of other regulatory cascades (Colangelo and Guerinot 2004; Yuan et al. 2005). In another study conducted in rice ABI3/VP1 transcription factor OsIDEF1 seems to regulate the expression responses through OsIRO2 bHLH transcription factor (Kobayashi et al. 2007; Ogo et al. 2007). Currently, Fe-chelates are used as inorganic fertilizers. In addition to it, acidification of soil with elemental sulfur also enhances the iron uptake. Zinc in the form of Zn^{+2} or Zn-phytosiderophores has majorly transported symplastically and minorly apoplastically across the root to the xylem (White et al. 2002). Similar to iron uptake and distribution, a large number of proteins ZIPs, YSLs, CDF, MHX, ZIF1, NRAMPs are involved in the uptake and distribution of

zinc. It was observed that the plants in which hyper accumulate zinc have a high constitutive expression of ZIPs, MTPs, NRAMPs, HMAs, FRD3, and YSL proteins. Similar to Iron, zinc is supplied to plants in the form of $ZnSO_4$ or zinc chelates, and copper is supplied in the form of calcium oxide and calcium carbonates, calcium nitrates, and calcium phosphates.

1.4.2 Adequate Distribution of Food After Production, Effective Management of Financial Resources

It is said that a hungry man is not a free man. Inadequate distribution of food affects the motive of food security badly. Food security is depended on food availability, food access, and food use. Imbalance in any of these three pillars can aggravate the problem of food security. The problem of unequal distribution of food is so grave and contrasting in both developed and underdeveloped regions of the world that in around every 15 min, one person is reported to die of starvation in many parts of Asia and Africa. Contrary to it, millions of people die in the western world due to lifestyle disorders due to obesity. Both extreme cases led to death or improper health in global populations. The major reason that can be linked to this problem is the unequal distribution of wealth or purchasing capacity. When we talk on global levels, it is directly linked to the nation's power on a global scale or on its economy. Although enough food is produced on earth to feed all the population yet due to harmful economic scenarios, some nations are more privileged than others and have better access to resources. Similar economic divisions prevail within the countries also, where some individuals have more access to food than others.

Extraordinary infrastructure and a reliable interconnected transportation network, technological advanced refrigerated storage houses for perishable food have enormously helped to distribute food to a greater extent. In the USA, with the advent of warehouses, factories, commercial retail shops, the infrastructure, it is estimated that the food travels thousands of miles from producers to consumers. Perishable food like meat, milk, and fruits are transported in different fashions. In the USA, many federal agencies, for example, the Food and Drug Administration and US Department of Agriculture, look after the productivity and distribution of the food. Whereas, in India, Food Corporation of India (FCI) maintains the supply of food crops. Statistically, it is probably the most significant supply chain in Asia. The primary mandate of FCI is to provide price support to the poor farmers of the country by determining the minimum support price and to distribute the food grains throughout the country for public distribution system and to maintain the market price for consumers as well. Moreover, FCI maintains operational buffer stocks of food grains to ensure food security in times of crisis. FCI purchases the food crops like rice or wheat from farmers through Mandis and stores the food crops in depots, buffer storage complexes or equity godowns. Later the food crops are transported throughout the country by means of different transport systems like railways, roadways, or waterways. Despite initiatives taken, a lot of developing and underdeveloped countries still struggle to find an adequate means to feed their population in the era

of economic crisis. Similarly, in India, to provide a safety net to the underprivileged group of people, certain types of operations or schemes like Jawahar Rozgar Yojana, Integrated Child Development Scheme, and Integrated Rural Development Programme and Public Distribution System are carried out. Despite few leakages and inconsistencies, public distribution system in India is by now the most penetrating, expensive, and successful program which runs parallel to free market to ensure the availability of rice, wheat, sugar, oil, etc. to the weaker sections at the subsidized prices (Ahluwalia 1993). In the absence of proper financial security and food distribution system, it is impossible to eradicate hunger and starvation from the world. Various initiatives have been taken by the national, sub-national, and international governments to frame the more effective policies to eradicate hunger. Green, White revolutions are exemplary examples of such initiatives. There is still a lot of population left with low or no food availability. We need to adopt more sensitive and efficient methods to feed humanity.

1.4.3 International Climate Change Policies and Effect on Agriculture, Multinational Efforts to Manage Agricultural Products Trade Policies

With changes in temperature, humidity, precipitation, CO_2 levels, increasing sea levels, and extreme weather conditions, climate change directly affects agriculture. On the other hand, agricultural practices also contribute to the emission of greenhouse gases. Due to increasing consumer demands, it is imperative that agricultural policies mitigate environmental impacts in addition to supplying calories (Fig. 1.2). Where on the one hand, utilization of renewable sources of energy has gained momentum, organic agriculture is also being practiced on the horizon. Concerted efforts are being targeted towards decreasing reliance on chemical fertilizers and pesticides. Several scientific bodies have been formulated which provide comprehensive data related to climate change. For instance, "The Intergovernmental Panel on Climate Change" (IPCC) assesses climate change and can be utilized by policymakers (https://www.ipcc.ch/). Similarly, "The United Nations Framework Convention on Climate Change" (UNFCCC) aims at reducing the impact of greenhouse gases from human activities on the natural climate system (Hickmann et al. 2019). In addition, "The Kyoto Protocol" was aimed at limiting the emission of greenhouse gases (Maamoun 2019). After the first convention ended, "The Kyoto Protocol" was modified to the "Doha Amendment to the Kyoto Protocol" with a commitment period of 2012–2020 (Amendment 2012). "The Paris Agreement" similarly was drafted with the target of limiting the global temperatures below 2° as compared to pre-industrial levels (https://unfccc.int/process-and-meetings/the-paris-agreement/the-paris-agreement). Climate change affects agricultural crops in developing nations and small scale farmers the most. Therefore, effective steps need to be taken to mitigate the threats associated with climate change on agricultural products (Fig. 1.4).

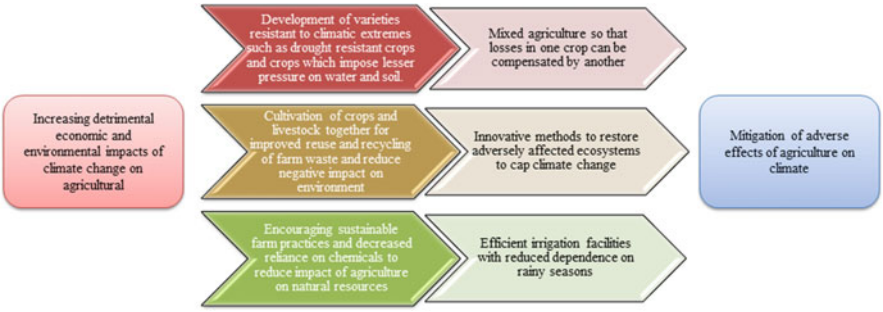

Fig. 1.4 Significant steps towards sustainable agriculture under changing climatic conditions

1.4.4 Skill Development for Production and Management of Agricultural Crops and Practices

Owing to changes in consumer preferences, resources, technologies, agricultural practices, and dynamic climate impacts, it is imperative to foster an environment of constant learning at every level of agricultural production. Globalization necessitates constantly updating the skills of the workforce to combat changing consumer requirements. For instance, farmers can be trained in livestock rearing and utilization of agricultural products according to changing market demands. Similarly, with an increase in pest infestation, several preventive measures must be incorporated in the agricultural chain to minimize losses. Integrated agriculture wherein cash crops are grown alongside staple food is another such example. Management of waste generated from livestock farms and fields sustainably either in the form of recycled goods or reused products demands skill development. Also, the introduction of improved crops and livestock varieties necessitates better cultivation and nutrient management techniques. Training of personnel in subsidiary occupations such as horticulture, beekeeping will ensure better farm management. With an increase in awareness and independent work ethics, entrepreneur-friendly policies must be devised, which encourages innovation in the agricultural sector. Global demand and supply of agricultural produce also invite skilled labor involved in effective storage and transportation. Improvement in agricultural infrastructure calls for the establishment of evolving learning arenas for effective skill development. Hence, it is imperative that the increasing demands for improved agricultural products are simultaneously met with a skilled workforce equipped to meet the changing global requirements. A few government initiatives aimed at the development of skills for farmers, wage workers, etc. For example, "Agriculture Skill Council Of India (ASCI)" under "Ministry of Skill Development and Entrepreneurship (MSDE)" has helped develop programs covering a range of sectors, viz. dairy, poultry, fisheries, forestry, animal husbandry farm management; mechanization, landscaping, and entrepreneurship development (Bhattacharyya and Mukherjee 2019). Similarly, the "Green Skill Development Programme (GSDP)" under the "Ministry of Environment, Forest and Climate Change" suggests youth skill development in

forestry, the environment in alignment with climate change, and effects on wildlife (http://www.gsdp-envis.gov.in/). Another such initiative is the "Skill Training of Rural Youth" (STRY), as the name suggests helped rural youth hone their skills in agricultural and allied sections (STRY 2019). Similar initiatives have been adopted throughout the world for securing global food and nutritional security through efficient workforce skill management.

1.4.5 Integrated Public and Private Sector Initiatives

Consolidated partnerships between public and private communities strengthen the framework of any sector. Public and private entities complement diverse agricultural needs. Increasing requirements for agricultural products necessitates either expansion of area under agriculture or production from a defined region. Since an increase in the former is limited; there is a need to enhance the latter. This has been achieved by the utilization of improved technologies and breeds of crops and livestock. Investments in agriculture have been made both at public and private levels. An enhanced production requires an amalgamation of both enabling synchronous efforts to address the changing demands of consumers. On the one hand, the public sector can benefit from innovations and employment opportunities generated by the private sector. On the other hand, private beneficiaries can achieve reduced costs and increased productivity. Both can complement each other in terms of funding and competitive market innovations (Hermans et al. 2019). Public–private partnership (PPP) is one such intervention for improved agricultural output. For a successful partnership, agricultural growth observed should exceed outputs obtained from individual undertakings. Each participant must understand its responsibilities and expertise and achievements must be incentivized. Both the partners must comprehend not only its own but also the vulnerabilities of its counterpart and deploy measures to minimize risks from either side (FAO 2016). Several countries across the globe have benefitted from such partnerships, for example; in Thailand, disease-free okra varieties were developed with the aim of export; in Indonesia, joint public-private efforts have promoted use of renewable energy in industries; in the Philippines, aquaculture has been promoted through development of infrastructure and effective investment options (FAO 2016). Hence, increasing requirements of sufficient and nutritious food supply have paved the way for integrated public and private sector efforts to meet the increasing demands.

1.4.6 Awareness Drives Among Producers (Farmers) and Consumers

The primary objective of awareness drives among producers/farmers is to educate them regarding the improved crop varieties and livestock, technological advances, marketing, storage, and successful risk management. The obstacles faced by farmers, whether in procuring hybrid seeds or adopting better farming methods such as

organic farming, need to be addressed for successful agricultural revolutions. Farmers consolidated in a certain area should be provided exposure to producers in other regions for the exchange of ideas and solutions to common problems faced in agriculture. In addition, detailed information regarding novel farming practices curbs reluctance among farmers to adopt innovative farming techniques and resources. Governments across the globe have framed various policies aimed at improving agricultural production and enabling farmers to increase their profits. Subsidized technologies and various training programs explicitly organized to bridge the gap between the scientific community and producer's aim towards achieving food and nutritional security. Moreover, in addition to awareness among producers, consumer knowledge should also be enhanced for a holistic utilization of resources. Whether the benefits of organic produce or bio-fortified foods, the successful management of lacunae in information regarding agricultural products among consumers is imperative for maximizing buyer satisfaction and minimizing wastage and adverse environmental impacts. Awareness about the safety and availability of bio-fortified foods, for instance, can aid the alleviation of global food and nutrition insecurity. Similarly, knowledge about a wide variety of staple and cash crops facilitates meeting the dynamic dietary preferences among consumers. In India itself, several programs have been commenced to enhance agricultural awareness. "National Mission of Agricultural Extension and Technology (NMAET)," for instance, has enabled upgrading of farms with the newest technologies and efficient agricultural practices.

References

Afman L, Müller M (2006) Nutrigenomics: from molecular nutrition to prevention of disease. J Am Diet Assoc 106(4):569–576

Agro Processing (2015) Agricultural marketing and agri-business. TNAU Agritech Portal

Ahluwalia D (1993) Public distribution of food in India: coverage, targeting and leakages. Food Policy 18:33–54

Ahmad P, Tripathi DK, Deshmukh R, Singh VP, Corpas FJ (2019) Revisiting the role of ROS and RNS in plants under changing environment. Environ Exp Bot 161:3

Ahumada O, Villalobos JR (2011) Operational model for planning the harvest and distribution of perishable agricultural products. Int J Prod Econ 133:677–687

Ahumada O, Villalobos JR, Mason AN (2012) Tactical planning of the production and distribution of fresh agricultural products under uncertainty. Agric Syst 112:17–26

Alston P, Quinn G (1987) The nature and scope of states parties' obligations under the International Covenant on Economic, Social and Cultural Rights. Hum Rights Q 9:156

Amah D, van Biljon A, Brown A, Perkins-Veazie P, Swennen R, Labuschagne M (2019) Recent advances in banana (musa spp.) biofortification to alleviate vitamin A deficiency. Crit Rev Food Sci Nutr 59(21):3498–3510

Amendment D (2012) Doha amendment to the Kyoto protocol. Annex B to the Kyoto protocol. https://unfccc.int/files/kyoto_protocol/application/pdf/kp_doha_amendment_english.pdf

American Association for the Advancement of Science (2003) Selective breeding of maize by early farmers. Science 302:1111–1111

Antle JM, Diagana B (2003) Creating incentives for the adoption of sustainable agricultural practices in developing countries: the role of soil carbon sequestration. Am J Agric Econ 85:1178–1184

Asami DK, Hong Y-J, Barrett DM, Mitchell AE (2003) Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J Agric Food Chem 51:1237–1241

Bansal R, Rana N, Kumawat S, Kumar N, Preeti P, Bhatt V, Waqar A, Altaf N, Katara JL, Samantaray S et al (2019) Advances in induced mutagenesis and mutation mapping approaches in rice. Oryza 56(Special issue):8

Bashir K, Inoue H, Nagasaka S, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2006) Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. J Biol Chem 281:32395–32402

Bhat JA, Shivaraj S, Singh P, Navadagi DB, Tripathi DK, Dash PK, Solanke AU, Sonah H, Deshmukh R (2019) Role of silicon in mitigation of heavy metal stresses in crop plants. Plan Theory 8:71

Bhattacharyya S, Mukherjee A (2019) Importance of skill development in Indian agriculture. In: ICT and social media for skill development in agriculture. Today & Tomorrow's Printers and Publishers, New Delhi, pp 47–62

Bokor B, Soukup M, Vaculík M, Vďačný P, Weidinger M, Lichtscheidl I, Vávrová S, Šoltys K, Sonah H, Deshmukh R (2019) Silicon uptake and localisation in date palm (Phoenix dactylifera)-a unique association with sclerenchyma. Front Plant Sci 10:988

Bouchez A, Causse M, Gallais A, Charcosset A (2002) Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genetics 162:1945–1959

Burchi F, Fanzo J, Frison E (2011) The role of food and nutrition system approaches in tackling hidden hunger. Int J Environ Res Public Health 8:358–373

Callithen N, Matthew N (2007) United Nations Development Programme (UNDP).

Capinera JL, Horton DR (1989) Geographic variation in effects of weather on grasshopper infestation. Environ Entomol 18:8–14

Champagne P (2008) Bioethanol from agricultural waste residues. Environ Prog 27:51–57

Chaudhary J, Alisha A, Bhatt V, Chandanshive S, Kumar N, Mir Z, Kumar A, Yadav SK, Shivaraj S, Sonah H (2019a) Mutation breeding in tomato: advances, applicability and challenges. Plan Theory 8:128

Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari A, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H (2019b) Advances in Omics approaches for abiotic stress tolerance in tomato. Biology 8:90

Chen S, Lin X, Xu C, Zhang Q (2000) Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Sci 40:239–244

Colangelo EP, Guerinot ML (2004) The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. Plant Cell 16:3400–3412

Coyne D, Steadman J, Anderson F (1974) Effect of modified plant architecture of great northern dry bean varieties (Phaseolus vulgaris) on white mold severity, and components of yield. Plant Dis Rep 58:379–382

Das A, Laha S, Mandal S, Pal S, Siddiqui MW (2018) Preharvest biofortification of horticultural crops. In: Preharvest modulation of postharvest fruit and vegetable quality. Elsevier, Cambridge, pp 381–434

Davis KF, Gephart JA, Emery KA, Leach AM, Galloway JN, D'Odorico P (2016) Meeting future food demand with current agricultural resources. Glob Environ Chang 39:125–132

de Ronne M, Labbé C, Lebreton A, Sonah H, Deshmukh R, Jean M, Belzile F, O'Donoughue L, Bélanger R (2019) Integrated QTL mapping, gene expression and nucleotide variation analyses to investigate complex quantitative traits: a case study with the soybean–Phytophthora sojae interaction. Plant Biotechnology Journal:3. https://doi.org/10.1111/pbi.13301

Deshmukh R, Bélanger RR (2016) Molecular evolution of aquaporins and silicon influx in plants. Funct Ecol 30:1277–1285
Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R, Vuong T, Valliyodan B, Nguyen HT (2014) Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci 5:244
Deshmukh RK, Ma JF, Bélanger RR (2017a) Role of silicon in plants. Front Plant Sci 8:1858
Deshmukh RK, Nguyen HT, Belanger RR (2017b) Aquaporins: dynamic role and regulation. Front Plant Sci 8:1420
Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. Plant Sci 135:1–9
Dunwell JM (2000) Transgenic approaches to crop improvement. J Exp Bot 51:487–496
FAO (1996) World food summit: Rome declaration on world food security and world food summit plan of action (FAO). FAO, Rome
FAO (2014) Responsible investment in agriculture and food systems. Committee on World Food Security, Rome
FAO (2015) Framework for action for food security and nutrition in protracted crises (FFA). Committee on World Food Security, Rome
FAO (2016) Public-private partnerships for agribusiness development. FAO, Rome
FAO (2018) Agricultural engineering, storage. FAO, Rome
FAO Council (2004) Voluntary guidelines to support the progressive realization of the right to adequate food in the context of national food security. FAO Council, Rome. http://www.fao.org/3/y7937e/y7937e00.htm
FAO UN (2009) World summit on food security. FAO, Rome
FAO UN (2012) Voluntary guidelines on the responsible governance of tenure of land, fisheries and forests in the context of national food security. FAO Viale delle Terme di Caracalla 153:74
Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Front Nutr 5:12
Ghosh A, Reja MH, Nalia A, Kanthal S, Maji S, Venugopalan VK, Nath R (2019) Micronutrient biofortification in pulses: an agricultural approach. Curr J Appl Sci Technol 35:1–12
Gross J, Stein RJ, Fett-Neto AG, Fett JP (2003) Iron homeostasis related genes in rice. Genet Mol Biol 26:477–497
Grotz N, Guerinot ML (2006) Molecular aspects of Cu, Fe and Zn homeostasis in plants. Biochim Biophys Acta Mol Cell Res 1763:595–608
Guerriero G, Deshmukh R, Sonah H, Sergeant K, Hausman J-F, Lentzen E, Valle N, Siddiqui KS, Exley C (2019) Identification of the aquaporin gene family in Cannabis sativa and evidence for the accumulation of silicon in its tissues. Plant Sci 287:110167
Hansen CL, Cheong DY (2019) Agricultural waste management in food processing. In: Handbook of farm, dairy and food machinery engineering. Elsevier, Amsterdam, pp 673–716
Hermans F, Geerling-Eiff F, Potters J, Klerkx L (2019) Public-private partnerships as systemic agricultural innovation policy instruments–assessing their contribution to innovation system function dynamics. NJAS-Wagening J Life Sci 88:76–95
Hickmann T, Widerberg O, Lederer M, Pattberg P (2019). The United Nations framework convention on climate change secretariat as an orchestrator in global climate policymaking. Int Rev Adm Sci 0020852319840425.
Hurrell R, Egli I (2010) Iron bioavailability and dietary reference values. Am J Clin Nutr 91:1461S–1467S
Ishimaru Y, Suzuki M, Tsukamoto T, Suzuki K, Nakazono M, Kobayashi T, Wada Y, Watanabe S, Matsuhashi S, Takahashi M (2006) Rice plants take up iron as an Fe3+-phytosiderophore and as Fe2+. Plant J 45:335–346
Jacxsens L, Luning P, Marcelis W, van Boekel T, Rovira J, Oses S, Kousta M, Drosinos E, Jasson V, Uyttendaele M et al (2011) Tools for the performance assessment and improvement of food safety management systems. Trends Food Sci Technol 22:S80–S89

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G (2018) CRISPR for crop improvement: an update review. Front Plant Sci 9:985

Junior RM, Gomes RS, de Almeida CF, Alves FM, Delazari FT, Laurindo RDF, Fernandes RH, da Silva DJ (2017) Vegetable breeding as a strategy of biofortification in carotenoids and prevention of vitamin A deficiency. Afr J Agric Res 12:1059–1066

Keding GB, Schneider K, Jordan I (2013) Production and processing of foods as core aspects of nutrition-sensitive agriculture and sustainable diets. Food Secur 5:825–846

Kennedy G, Nantel G, Shetty P (2003) The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. Food Nutr Agric 32:8–16

Kobayashi T, Ogo Y, Itai RN, Nakanishi H, Takahashi M, Mori S, Nishizawa NK (2007) The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants. Proc Natl Acad Sci 104:19150–19155

Kumar D, Kalita P (2017) Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods 6:8

Kumawat S, Rana N, Bansal R, Vishwakarma G, Mehetre ST, Das BK, Kumar M, Kumar Yadav S, Sonah H, Sharma TR (2019) Expanding avenue of fast neutron mediated mutagenesis for crop improvement. Plan Theory 8:164

Learn. Genetics GSLC (2018) Crop improvement methods. GSLC, Salt Lake City

Ling H-Q, Bauer P, Bereczky Z, Keller B, Ganal M (2002) The tomato fer gene encoding a bHLH protein controls iron-uptake responses in roots. Proc Natl Acad Sci 99:13938–13943

Lyons G (2018) Biofortification of cereals with foliar selenium and iodine could reduce hypothyroidism. Front Plant Sci 9:730

Maamoun, N. (2019). The Kyoto protocol: Empirical evidence of a hidden success. J Environ Econ Manag 95:227–256.

Mackowiak C, Grossl P, Cook K (2005) Iodine toxicity in a plant-solution system with and without humic acid. Plant Soil 269:141–150

Marschner H (1974) Mechanisms of regulation of mineral nutrition in higher plants. Bull R Soc NZ Apr:99–109

McPherron AC, Lee S-J (1997) Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci 94:12457–12461

Ministry of Agriculture and Farmers Welfare (2013). ISOPOM, Govt. of India

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Nations U (2015) The millennium development goals report. New York: United Nations.

Novoselec J, Klir Ž, Domaćinović M, Lončarić Z, Antunović ZJP (2018) Biofortification of feedstuffs with microelements in animal nutrition. Agriculture 24:25

Ogo Y, Nakanishi Itai R, Nakanishi H, Kobayashi T, Takahashi M, Mori S, Nishizawa NK (2007) The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. Plant J 51:366–377

O'Hare T (2014) Biofortification of vegetables for the developed world. In: XXIX international horticultural congress on horticulture: sustaining lives, livelihoods and landscapes (IHC2014): VI 1106. ISHS, Leuven, pp 1–8

Perpetuo EA, Souza CB, Nascimento CAO (2011) Engineering bacteria for bioremediation. In: Progress in molecular and environmental bioengineering-from analysis and modeling to technology applications. IntechOpen, London

Phillips CM (2013) Nutrigenetics and metabolic disease: current status and implications for personalised nutrition. Nutrients 5:32–57

Ram H, Singh BP, Katara J, Chopra R, Kumar P, Jaiswal R, Geetika RD, Sonah H (2019) A genome-wide resource of intron spanning primers compatible for quantitative PCR and intron length polymorphism in rice. Indian J Genet 79:499–502

Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). Crit Rev Food Sci Nutr 13:1–17

Rasoolizadeh A, Labbé C, Sonah H, Deshmukh RK, Belzile F, Menzies JG, Bélanger RR (2018) Silicon protects soybean plants against Phytophthora sojae by interfering with effector-receptor expression. BMC Plant Biol 18:97

Robinson NJ, Procter CM, Connolly EL, Guerinot ML (1999) A ferric-chelate reductase for iron uptake from soils. Nature 397:694

Rogers Y-H, Zhang C (2016) Genomic technologies in medicine and health: past, present, and future. In: Medical and health genomics. Elsevier, Amsterdam, pp 15–28

Rosenberg NJ (1992) Adaptation of agriculture to climate change. Clim Chang 21:385–405

Sales N, Pelegrini P, Goersch M (2014) Nutrigenomics: definitions and advances of this new science. J Nutr Metab 2014:6

Shivaraj S, Dhakate P, Sonah H, Vuong T, Nguyen HT, Deshmukh R (2019) Progress toward development of climate-smart flax: a perspective on Omics-assisted breeding. In: Genomic designing of climate-smart oilseed crops. Springer, Berlin, pp 239–274

Shuman LM (1998) Micronutrient fertilizers. J Crop Prod 1:165–195

Singh U, Praharaj C, Singh S, Bohra A, Shivay Y (2015) Biofortification of pulses: strategies and challenges. In: Proceedings of the second international conference on bio-resource and stress management, Hyderabad, India. Puspa Publishing House, Kolkata, pp 50–55

Sonah H, Deshmukh RK, Labbé C, Bélanger RR (2017) Analysis of aquaporins in Brassicaceae species reveals high-level of conservation and dynamic role against biotic and abiotic stress in canola. Sci Rep 7:2771

Spalvins K, Zihare L, Blumberga D (2018) Single cell protein production from waste biomass: comparison of various industrial by-products. Energy Procedia 147:409–418

STRY (2019) Skill training for rural youth. National Institute of Agricultural Extension Management, Hyderabad

Tait-Burkard C, Doeschl-Wilson A, McGrew MJ, Archibald AL, Sang HM, Houston RD, Whitelaw CB, Watson M (2018) Livestock 2.0–genome editing for fitter, healthier, and more productive farmed animals. Genome Biol 19:204

Thomine S, Lelièvre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H (2003) AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. Plant J 34:685–695

Toojinda T, Baird E, Booth A, Broers L, Hayes P, Powell W, Thomas W, Vivar H, Young G (1998) Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. Theor Appl Genet 96:123–131

United States Department of Agriculture (2011) Agricultural waste management systems. USDA, Washington, DC

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8:1386

Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat J-F, Curie C (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell 14:1223–1233

Vishwakarma K, Mishra M, Patil G, Mulkey S, Ramawat N, Pratap Singh V, Deshmukh R, Kumar Tripathi D, Nguyen HT, Sharma S (2019) Avenues of the membrane transport system in adaptation of plants to abiotic stresses. Crit Rev Biotechnol 39:861–883

Vuong T, Sonah H, Meinhardt C, Deshmukh R, Kadam S, Nelson RL, Shannon J, Nguyen H (2015) Genetic architecture of cyst nematode resistance revealed by genome-wide association study in soybean. BMC Genomics 16:593

Wheeler MB (2013) Transgenic animals in agriculture. Nat Educ Knowl 4(11):1

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

White PJ, Whiting SN, Baker AJ, Broadley MR (2002) Does zinc move apoplastically to the xylem in roots of Thlaspi caerulescens? New Phytol 153:201–207

White PJ, Bowen HC, Marshall B, Broadley MR (2007) Extraordinarily high leaf selenium to sulfur ratios define 'Se-accumulator' plants. Ann Bot 100:111–118

Willcox M, Khairallah M, Bergvinson D, Crossa J, Deutsch J, Edmeades G, Gonzalez-de-Leon D, Jiang C, Jewell D, Mihm J (2002) Selection for resistance to southwestern corn borer using marker-assisted and conventional backcrossing. Crop Sci 42:1516–1528

Williams J (2005) The use of marker-assisted selection in animal breeding and biotechnology. Rev Sci Tech Off Int Epizoot 24:379

Wintz H, Fox T, Wu Y-Y, Feng V, Chen W, Chang H-S, Zhu T, Vulpe C (2003) Expression profiles of Arabidopsis thaliana in mineral deficiencies reveal novel transporters involved in metal homeostasis. J Biol Chem 278:47644–47653

Wu H, Li L, Du J, Yuan Y, Cheng X, Ling H-Q (2005) Molecular and biochemical characterization of the Fe (III) chelate reductase gene family in Arabidopsis thaliana. Plant Cell Physiol 46:1505–1514

Yadav MK, Aravindan S, Raghu S, Prabhukarthikeyan S, Keerthana U, Ngangkham U, Pramesh D, Banerjee A, Adak T, Kar MK (2019) Assessment of genetic diversity and population structure of Magnaporthe oryzae causing rice blast disease using SSR markers. Physiol Mol Plant Pathol 106:157–165

Yu H, Park J-Y, Kwon CW, Hong S-C, Park K-M, Chang P-S (2018) An overview of nanotechnology in food Science: preparative methods, practical applications, and safety. J Chem 2018:10

Yuan YX, Zhang J, Wang DW, Ling HQ (2005) AtbHLH29 of Arabidopsis thaliana is a functional ortholog of tomato FER involved in controlling iron acquisition in strategy I plants. Cell Res 15:613

Zargar SM, Nagar P, Deshmukh R, Nazir M, Wani AA, Masoodi KZ, Agrawal GK, Rakwal R (2017) Aquaporins as potential drought tolerance inducing proteins: towards instigating stress tolerance. J Proteome 169:233–238

Zeng L, Shannon MC (2000) Salinity effects on seedling growth and yield components of rice. Crop Sci 40:996

Ziska L, Crimmins A, Auclair A, DeGrasse S, Garofalo J, Khan A, Loladze I, de León AP, Showler A, Thurston J (2016) Ch. 7: Food safety, nutrition, and distribution. In: The impacts of climate change on human health in the United States: a scientific assessment. U.S. Global Change Research Program, Washington, DC, pp 189–216

Advanced Approaches for Biofortification

2

Kanti Kiran

Abstract

Biofortification can be defined as the enhancement of minerals and vitamins in order to elevate the nutritional value of any food crop. This can be achieved by increasing the content of provitamin A, carotenoids, zinc, and iron. Around 50% of the world's populations suffer from micronutrient deficiency of zinc, iron, selenium, calcium, and iodine. Among the many interventions, like dietary modification and diversification, supplementary nutrient supply, etc., biofortification is presently one of the best mediation to combat micronutrient malnutrition. Deficiencies of iron, iodine, vitamin A, vitamin B12, vitamin D, calcium, and magnesium are incredibly common worldwide. Besides, there also exist other nutrient deficiencies that come under the category of macronutrients, including carbohydrates, proteins (essential amino acids), fats (essential fatty acids), macrominerals, and water. Plant crops are an indispensable source of nutrition. Therefore a couple of years back, the idea of enhancing the nutrient qualities of plants emerged as a vital strategy to combat the prevailing nutrient deficiencies. Though, agronomic methods involved in developing crops with enhanced micronutrients have been traditionally practiced for years but are not sufficient. Later on, the conventional breeding approach was commonly followed and considered the best means of biofortification in crops. Decades later, transgene methods were developed and significantly gained popularity as a means to develop biofortified crops. Recently genome editing methods like TALENS, ZNFs, and CRISPR based technologies have also shown a huge advancement in the revolution of biofortifying plants. While there are other struggles related to acceptance and approval of such crops from both regulatory bodies as well as consumers and farmers. Nevertheless, recent years have seen the development and release of many micronutrient biofortified crops, for example, rice, maize,

K. Kiran (✉)
National Institute of Plant Biotechnology, New Delhi, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_2

wheat, etc. and have proven to have a bright future as a nobel strategy essentially required for the health of humans worldwide.

Keywords

Biofortification · Agronomic practices · Conventional breeding · Genome editing

2.1 Introduction

Worldwide around 792.5 million people are malnourished, out of which 780 million people live in developing countries (McGuire 2015). Besides, about two billion people across the world suffer from a lesser-known "hidden hunger" (Hodge 2016; Muthayya et al. 2013). The extremely poor condition of people around the globe, specifically in developing and underdeveloped countries, usually has been affected by this. Though, always not among the poor people does this condition persist. The condition of undernutrition where the body lacks essential vitamins and minerals that keep people healthy is termed as hidden hunger (www.harvestplus.org/biofortification-nutrition-revolution-now). Consumption of single staple crops results in diminished vitamins and minerals essentially required for a healthy human body. Health problems like mental retardation, stunted growth, blindness, learning disabilities, premature death, and less work capacity are some of the irreparable damages caused to the human body due to deficiencies of micronutrients such as zinc, iron, and vitamin A (www.harvestplus.org/biofortification-nutrition-revolution-now). Most of the nutrition and food consumed by humans come from plants threrefore, a link was set up between agriculture and nutrition in order to improve human health. The idea of enhancing crop plants with essential micronutrients through biological means was then termed as Biofortification. The international multidisciplinary HarvestPlus (Biofortification Challenge) program is one of the main reasons behind the expansion of biofortification. It was launched in 2004 by the Consultative Group on International Agricultural Research (CGIAR) and the International Food Policy Research Institute (IFPRI) (Brooks 2010; HarvestPlus 2009). The project was the key project for the development and circulation of the crops rich in iron, zinc, and vitamin A to overcome these deficiencies in Africa and Asia. Before 1999 when golden rice was first biofortified as a staple crop by genetic engineering to tackle vitamin A deficiency, only conventional biofortified crops were investigated (Beyer et al. 2002; Potrykus 2008). Since then, developing plants with enhanced micronutrient levels through transgene methods widely became popular.

There persist three basic approaches to achieve biofortification in crops: Production of staple crops with desirable agronomic traits or nutrients by conventional crossbreeding methods of the plants bearing naturally higher amounts of the desired micronutrient called conventional biofortification; Through agronomic biofortification by using sprays or fertilizers that are rich in micronutrient and are absorbed by the edible parts of plants; And by introducing new genes or existing

Table 2.1 Essential nutrients required by the human body

Type of nutrients	Requirement
Macronutrients	
Carbohydrates	
Fiber	To reduce the risk of noncommunicable diseases
Sugars	Low intake for childhood overweight and obesity, low intake for unhealthy weight gain in adults, low intake to reduce the risk of noncommunicable diseases in children and adults
Fats and fatty acids	Long-chain polyunsaturated fatty acids during pregnancy
Protein	During pregnancy
Micronutrients	
Vitamins and minerals	
Calcium	
Foliate	During pregnancy, in malaria endemic areas, in adult women and adolescent girls, for anemic children and adults
Iodine	In pregnant and lactating women, preventing iodine deficiency diseases
Iron	During pregnancy, in malaria endemic areas, in adult women and adolescent girls, for anemic children and adults
Zinc	For respiratory infections and growth in children, during pregnancy, for the management of diarrhea in children
Sodium	For iodization of salt for the prevention and control of iodine deficiency disorders, low consumption of sodium intake to control blood pressure in children, low consumption of sodium intake to reduce blood pressure and risk of cardiovascular diseases in adults
Potassium	To control blood pressure in children, to reduce blood pressure and risk of cardiovascular diseases in adults
Vitamin A	During pregnancy, for the cure of severe acute malnutrition in children, HIV infected adults, and children
Vitamin B6	During pregnancy
Vitamin C	During pregnancy
Vitamin D	During pregnancy, for respiratory infections in children and infants
Vitamin E	During pregnancy, for preventing morbidity and mortality in premature infants

genes with minimal bioavailability that are responsible for increasing the levels of micronutrients into crops, known as transgenic biofortification. The decade around 2009 to 2013 played a crucial role when the breeding of the biofortified crops was in full swing. During this period releasing the biofortified crops by national release committees and setting up rules for approval were developed after the trials of nutritional efficacy were tested.

To carry out a productive and healthy lifestyle, an average human requires almost 40 known nutrients in a sufficient amount (Garg et al. 2018). Table 2.1 enlists the macronutrients and micronutrients required by the human body. Some essential nutrients like sodium, potassium, calcium, magnesium, phosphorous, chlorine, and sulfur are required more in amount than micronutrients such as iron, zinc, copper,

Table 2.2 List of crops/plants targeted for micronutrient enrichment

Crop/plant	Micronutrient	Released/available to farmers	Under testing in the country	Year respectively
Banana/ plantain	Vitamin A		Nigeria, South Africa	2018
Cassava		Nigeria, Democratic Republic of Congo		2008, 2011
Maize		Zambia, Nigeria, Ghana, China	India	2012, 2012, 2012, 2015
Sweet potato		China, Uganda		2001, 2004
Cauliflower		India	India	2016
Beans	Iron	Rwanda, Democratic Republic of Congo		2010, 2011
Pearl millet		India		2015
Sorghum			India	
Cow pea		India		2008
Irish potato				
Lentils		India, Nepal, Bangladesh		2012, 2013, 2013
Sorghum	Zinc		India	2012
Cow pea		India		
Irish potato				
Lentil		India		2012
Maize				
Rice		Bangladesh		2013
Sorghum			India	
Wheat		India		2014
Lettuce	Iodine	Poland		2011
Tomato		Italy		2011
Onion	Selenium	Norway		2012
Brinjal	Anthocyanin	India		2016

manganese, iodine, selenium, molybdenum, cobalt, nickel, and vitamin A (Prashanth et al. 2015). All these nutrients are very crucial for the physical and mental development of humans (White and Broadley 2005).

Overall, generating biofortified food crops with improved nutrient contents like increase in iron, Selenium, zinc, and provitamin A content shall provide appropriate levels of nutrition to humans living in developing and developed countries (Table 2.2). Global initiatives, such as the HarvestPlus program and national initiatives are serving as a large source to accomplish these targets. Staple cereal crops like maize, wheat, beans, cassava, sweet potatoes, and millets have been developed with a higher content as well as bioavailability of essential mineral elements in the human diet through these initiatives. Biofortification is a difficult task and has quite many challenges. Though, there are shreds of evidence of several

countries that have shown consumer acceptance among Africa, Asia, and Latin America (Birol et al. 2015). Presently there is a greater emphasis on breeding based approaches mediated through transgenic means due to their higher success rates. The development of transgenically fortified crop is time-consuming because of its regulatory approval processes and the expensive implimentation make it more difficult to be accepted by consumers. Apart from these, producing biofortified crops is quite promising and has a very bright future since these have the potential to fully scratch away micronutrient malnutrition among billions of poor people, especially in developing countries and improve the human health conditions worldwide.

2.2 Biofortification Pathway Includes Several Approaches

Biofortification arose with a promising aim to produce adequate and viable, safe, nutritious foods. Crop plants are biofortified with the essential micronutrients by conventional, agronomic, and transgenic approaches by means of crop breeding, fertilization strategies, and biotechnology methods, respectively. There persist many advantages and drawbacks of one approach versus the other. Genetic diversity among individual crops plays a vital role in deciding the method of biofortification. Breeding methods are more common and successful, where the gene pool of the targeted crop is hugely genetically diverse. The transgenic-based approach has an advantage over this and can be utilized as a better approach for crops with less or no genetic diversity available, moreover targeting multiple crops with just one beneficial gene discovered can be performed in multiple events. Therefore transgenic-based approach has taken over the breeding technology and other conventional approaches in the present era. Though cereals, legumes, and vegetables have been targeted by all three approaches. For example, staple crops like wheat, rice, maize, lupine, sorghum, common bean, tomato, sweet potato, and potato are among the most common to be addressed by conventional breeding, transgenic and agronomical approaches. On the other hand, certain plants have been biofortified by a combination of both transgenic and breeding approaches (banana, cauliflower, and cassava) while some others like barley, soybean, lettuce, carrot, canola, and mustard have been biofortified with transgene and agronomic approaches (Garg et al. 2018).

2.3 Biofortification Through Agronomic Practices

Agronomic based biofortification approach involves micronutrients uptake from the surrounding soil and translocation into edible parts of the plants in a natural biological process. This process is traditionally used to enrich crop grains with essential micronutrients. The entire pathway of micronutrient uptake from soil to plant, food, and into the human body is a complex process and depends on their bioavailability. Various factors are responsible to finally decide the entire effectiveness of agronomic biofortification since there is a potential nutrient loss during the

transition stages from soil to the plants, plants to food, and finally to humans (Daud et al. 2016; Daud et al. 2017). Soil management strategies to enhance the properties of soil have been practiced for decades and older times. Application of organic wastes like plants residual matter and animal manure not only improve the soil properties but also contribute to various factors including water holding capacity, nutrient bioavailability by releasing the nutrients constantly and slowly, cation exchange capacity, etc. (Zingore et al. 2008).

For example, iron-fortified rice crop through agronomic method includes various ways like spraying the fertilizer on to the plants, by applying the fertilizer to the soil, and application of other macronutrients to the soil which is responsible for an increased level of iron in plants. Though there are some drawbacks involved in the methods of applying iron fertilizers in soil or as sprays directly on plants as compared to applying macronutrients along with the iron fertilizers into the soil. Foliar feeding methods require repeated spraying, which is costly and harmful to the environment, while iron uptake through soil is minimal due to the strong bond of iron with soil (García-bañuelos and Sida-arreola 2014; Petry et al. 2015). Several studies have shown the positive effects of macronutrients like nitrogen, phosphorous, and potassium mostly in the form of NPK fertilizer, in increasing the iron content in rice (Sperotto et al. 2012; Prasad et al. 2014; Petry et al. 2015; García-bañuelos and Sida-arreola 2014). Therefore applying micronutrient-enriched fertilizers in combinations with macronutrient fertilizers could be effective in increasing the nutritional qualities of certain crops like Zn and Se on wheat and maize and also no serious negative environmental effect has been reported when used at appropriate rates.

Studies associated with human health improved through micronutrient fertilizer applications are very limited. Therefore, the pilot-scale program should be implemented to explore the knowledge gap of the direct link between micronutrient-enriched fertilizer applications to crops and know the health benefits of the dietary micronutrient uptake by humans. Meanwhile, several successful experiments to enrich rice, wheat, and maize crops by agronomic biofortification has been observed from over a decade now (Oikeh et al. 2003; Banziger and Long 2000; Maqsood et al. 2009) supporting agronomic biofortification as an effective mode in increasing yields and nutritional quality of certain crops.

2.4 Biofortification Through Conventional Breeding

Conventional plant breeding strategy is centuries old and is practiced with an aim to improve the features of food crops by crossing plants (parents either developed or identified) with desired characteristics followed by selecting offspring with desired agronomic traits inherited from both parent plants (Saltzman et al. 2017). Biofortified crops developed by traditional breeding methods require plenty of genetic variation in crop populations for the chosen traits, e.g., high protein content

or essential elements like iron or zinc, etc. A conventional breeding strategy in staple grains usually is difficult; for example, no rice varieties are rich in vitamin A; therefore, improvement of vitamin A is not possible using this technique. Provitamin is produced only in the green organs of all plants, but not in the starch-storing part of the seed. Deficiency of vitamin A causes ~4500 child deaths, which are preventable, and the golden rice enriched with provitamin A, therefore, is a boon to regions where rice is a staple crop (Dubock 2017).

Though, all methods of plant breeding are safe and acceptable. For example, Via breeding approach, opaque2 gene was introduced into maize, which resulted in the doubling of lysine and tryptophan. In several countries, QPM maize varieties have been released and grown successfully in a million hectares (Pray et al. 2007). With the possibility of undesirable traits inherited from parents during the selection process based on phenotypes and involvement of extensive backcrossing (Bhullar and Gruissem 2013) which consumes a lot of time, the approach of conventional breeding alone is not as efficient as when combined with approaches of genetic engineering and agronomic practices, for example, enhancing iron content in grains (Virmani and Ilyas-Ahmed 2008; Jeng et al. 2012). Best examples of developing iron biofortified crops are the rice semi-dwarf IR68144 (Peng et al. 1999) and low phytic acid maize mutant lpa241, which was achieved by a combination approach. The reason for HarvestPlus (www.harvestplus.org) being successful since when it was implemented in 2003 in developing several crops and raising extensive operational funding was based on the plan of the "no-GMO-crop" strategy. This helped them to avoid the barriers and criticism that persists with the GMO crops (Levitt 2011). While they could achieve success in developing rice crops with increased iron and zinc by using conventional breeding along with the transgenic strategy. Figure 2.1 represents the crops and plants developed by HarvestPlus enriched with respective nutrients.

2.5 Biofortification Through Transgenes

Genetic engineering is more preferred these days as an alternative for biofortification in grains. Introduction of gene/s (desired) from other related organisms, overexpression of the desired gene, gene silencing interference (RNAi), and gene knockout through genome editing methods are utilized to characterize the function of the genes through these transgene methods. Moreover, genetic engineering technologies in comparison to agronomic and conventional plant breeding are more efficient and reliable to study the genotypic and phenotypic relationships of plants (Gaj et al. 2013; Yin et al. 2017) and are less time-consuming. Therefore, a different combination of transgenic approaches has been attempted and used to enhance the micronutrient content in cereal grains, for example, iron biofortification in rice has been performed through various approaches (Kok et al. 2018).

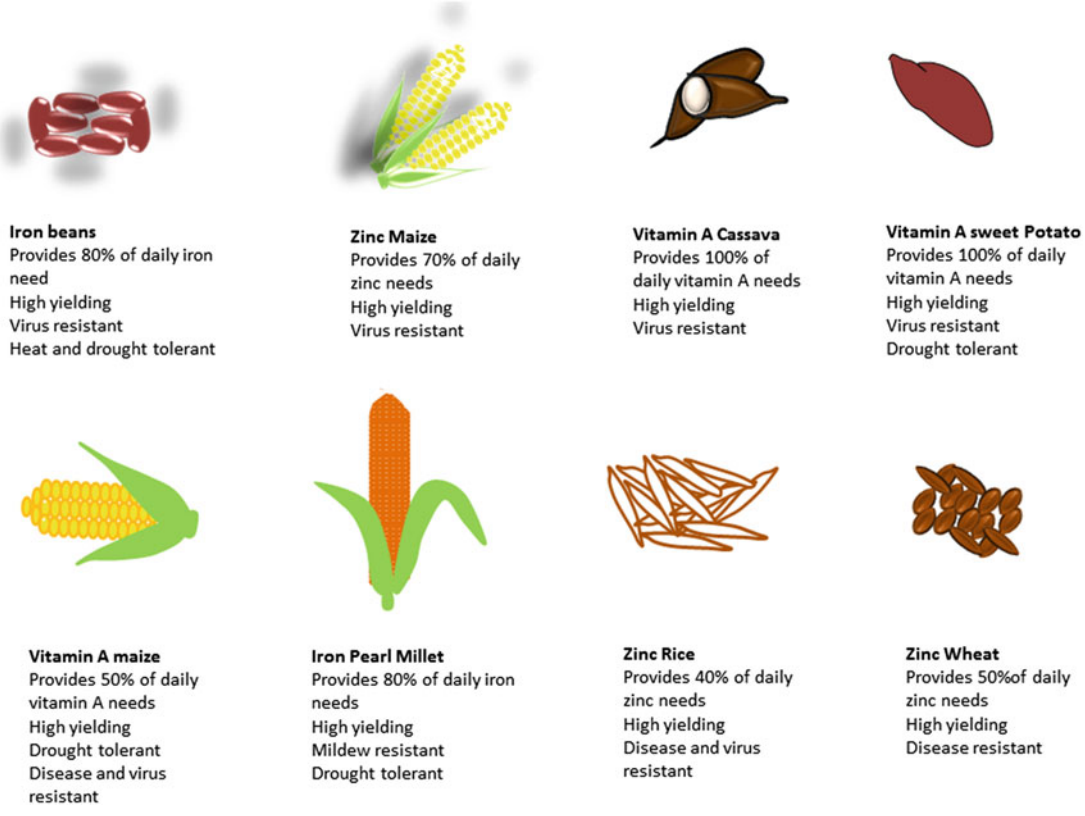

Fig. 2.1 Biofortified plants with specific nutrients. Eight major plants biofortified by Harvest Plus with iron, zinc, and vitamin A. Their beneficial characteristics are described as shown. The information was gathered from the site www.harvestplus.org

2.6 Genome Editing Methods Utilized for Biofortification

The advancement in genome editing technologies, such as zinc-finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) and Cas9 are potential and promising approach for biofortification practices in crops and plants which allows efficient and effective gene-editing without affecting the plant as a whole (Mushtaq et al. 2020; Vats et al. 2019). Moreover, they have less stringent regulations and monitoring from both government and nongovernment bodies as compared to genetically modified crops. Therefore, gene-edited crops have higher consumer acceptance all through. These techniques have been widely implied in crop improvement for various vital objectives like disease resistance, enhanced yield, nutritional value, and other traits. The past 5 years have seen its intense application in many plant systems for functional studies and combating biotic and abiotic stresses as well as to improve other important agronomic traits and biofortification (Jaganathan et al. 2018). In this regard, the latest CRISPR/Cas9 based genome editing is a breakthrough technique in the area of developing new, improved crop varieties worldwide (Table 2.3). The first generation genome editing tools like ZNFs and TALENs are presently less preferred to the second generation CRISPR/Cas and its derivatives, for example, ZFNs show low target specificity, are labor-intensive in nature, show many off-targets cleavages, and possess a limited number of available target sites (Chen and Gao 2013). Likewise, TALENs require a thymidine base at the starting position, possess the large size, and are repetitive in nature. While as compared to ZFNs, TALENs show higher target binding specificity due to their length. As compared to ZFNs and TALENs, CRISPR/Cas based genome editing is much simpler, convenient, faster, and precise. The CRISPR/Cas based genome editing technique is evolving rapidly, and numerous applications have already been developed (Fig. 2.2).

2.7 Advantages of Genome Editing Approaches for Biofortification

The application of genome editing in biofortification of staple crops and plants to improve human health could be more advantageous than the other prevailing methods like conventional breeding and through transgenic. Accumulation of micronutrients in the edible parts of the plants does not occur due to the absence of certain pathways, while certain existing pathways do not allow the accumulation to occur. In order to make these mechanism work precisely, possible changes in the genome of the plants is a requisite. Using conventional breeding in such cases would not be possible due to too much complexity involved. Genome editing by altering the endogenous pathways where multiple genes are involved can provide a better micronutrient accumulation (Scheben et al. 2017). Several examples like altering the carotenoid pathway in golden rice by transgenes *psy* and *crtI* (Ye et al. 2000) and many other crops such as *Brassica napus* (Ravanello et al. 2003), maize

Table 2.3 CRISPR systems used for biofortification

Plant species	Application perspectives	Targeted sequence(s)	Molecular functions	Delivery method//main strategy	Transgene-free plants studied (yes/no)	Publication
Camelina sativa	Enhancement of seed oil (fatty acid) composition in seeds	Fatty acid desaturase 2 (FAD2) genes	Key gene involved in the synthesis of polyunsaturated fatty acids (insertion of a double bond at the delta-12 (omega-6) position of oleic acid to obtain linoleic acid)	Agrobacterium-mediated transformation with Cas9/gRNA plasmid vectors (floral dipping)/ gene knockout with Cas9/gRNA	No	Jiang et al. (2017)
	Reduced levels of polyunsaturated fatty acids and increased accumulation of oleic acid in the oil	Fatty acid desaturase 2 (FAD2)	Key gene involved in the synthesis of polyunsaturated fatty acids (insertion of a double bond at the delta-12 (omega-6) position of oleic acid to obtain linoleic acid)	Agrobacterium-mediated transformation with Cas9/gRNA plasmid vectors (floral dipping)/ gene knockout with Cas9/gRNA	No	Morineau et al. (2016)
	Seed oil biosynthesis	CsDGAT1 or CsPDAT1 homeologous genes	Involved in triacylglycerol (TAG) synthesis in developing seeds	Agrobacterium-mediated floral vacuum infiltration method/CRISPR– Cas9-mediated multiplex genome editing	No	Aznar-Moreno and Durrett (2017)

Hordeum vulgare	N-glycans modification in cereal grains	The putative endogenous barley ENGase gene	Involved in N-glycans biosynthesis	Co-bombarding selected combinations of sgRNA with wild-type cas9 using separate plasmids, or by co-infection with separate Agrobacterium tumefaciens cultures/ CRISPR–Cas9-mediated multiplex genome editing	No	Kapusi et al. (2017)
Oryza sativa	High amylose content	SBEI, SBEIIb		CRISPR–Cas9-mediated		Sun et al. (2017)
Oryza sativa	Increased fragrance content	*OsBADH2*[2]		TALEN		Shan et al. (2015)
Solanum tuberosum	High-amylopectin starch	*GBSS*		CRISPR–Cas9-mediated		Andersson et al. (2017)
Zea mays	Reduced phytic acid content	*ZmIPK*		CRISPR– Cas9-mediated		Liang et al. (2014)
Soybean	High oleic acid contents	*FAD2-1A, FAD2-1B*		TALENs		Demorest et al. (2016)
		FAD2-1A, FAD2-1B, FAD3A		TALENs		
Salvia miltiorrhiza	Knockout the committed diterpene synthase gene	*Diterpene synthase gene SmCPS1*	Involved in tanshinone biosynthesis	Agrobacterium rhizogenes-mediated transformation/gene knockout with Cas9/ gRNA	No	Li et al. (2017)

(continued)

Table 2.3 (continued)

Plant species	Application perspectives	Targeted sequence(s)	Molecular functions	Delivery method//main strategy	Transgene-free plants studied (yes/no)	Publication
Solanum tuberosum	Starch quality (amylopectin potato starch)	Three different regions of the gene encoding granule-bound starch synthase (GBSS)	Enzyme responsible for the synthesis of amylose (encoded by a single locus)	PEG-mediated protoplast transfection with CRISPR-Cas9 expression plasmid constructs/gene knockout with Cas9/gRNA	Yes	Andersson et al. (2017)
	Starch quality (amylopectin potato starch)	four different target regions of the gene encoding granule-bound starch synthase (*StGBSS1*)	Enzyme responsible for the synthesis of amylose	PEG-mediated protoplast transfection with CRISPR-Cas9 expression plasmid constructs/ Agrobacterium rhizogenes-mediated transformation/gene knockout with Cas9/gRNA	yes	Veillet et al. (2019)
Papaver somniferum	Biosynthesis of Benzylisoquinoline alkaloids (BIAs): medical biomolecules	*3′-hydroxyl-N-methylcoclaurine 4′-O-methyltransferase* isoform 2 (*4′ OMT2*) gene	Implicated in the regulation of the biosythesis of benzylisoquinoline alkaloids (BIAs, e.g. morphine, thebaine)	*Agrobacterium*-mediated transformation of leaves with TRV-based synthetic plasmids expressing gRNA and a Cas9-encoding synthetic vector/**gene knockout with Cas9/gRNA**	No	Alagoz et al. (2016)

Nicotiana tabacum	Production of biotherapeutic proteins	XylT gene FucT gene	Involved in glycans biosynthesis	*Agrobacterium*-mediated transformation/**gene knockout with Cas9/ gRNA**	No	Hanania et al. (2017)
	Production of biotherapeutic proteins	Beta(1,2)-xylosyltransferase (XylT) and alpha(1,3) fucosyltransferase (FucT)	Involved in glycans biosynthesis	Agrobacterium-mediated transformation/ **CRISPR-Cas9-mediated multiplex genome editing**	No	Mercx et al. (2017)

Fig. 2.2 Different research areas addressing improvement and applications of CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein) based genome editing in plants. The figure is reproduced from Vats et al. (2019) (CC BY 4.0)

(Naqvi et al. 2009), and wheat (Wang et al. 2014). The glucoraphanin pathway by genes *LSU1 and BAT5* in tobacco (Crocoll et al. 2016) could possibly be achieved by using genome editing of the endogenously occurring specific pathways. The transgenic approach to achieve such biofortified crops has been more popular over conventional breeding, but then the general disapproval of the genetically modified crop plants with respect to public concern as well as the unstable and non-specific integration of transgenes into the plant genomes further adds as a limitation to this approach.

2.8 Challenges of Using Genome Editing Technology

The prominent obstacles for the establishment of the latest genome editing process for crop improvement are, collection of huge pangenome references, the presumption of right candidate editing sites, a proper delivery method for genome editing system with reduced off-target editing.

One of the major hurdles using CRISPR/Cas9 is the off-target effect. Cas9 specifically recognizes 20 nucleotide base pair SgRNA adjacent to protospacer adjacent motif (PAM) immediately after the target sequence in the gene. In some cases, potential off-target cleavage activity could still occur (50% chance) on DNA sequence with even 3–5 base pair mismatches. To avoid the use of GMO's CRISPR/Cas9 can be used with legal regulation (Omodamilola and Ibrahim 2018). Another approach is mutation breeding which is not new but with recent advances in sequencing technology getting more efficient. Numerous genes regulating nutritional traits have been identified through mutagenesis approach and further utilized for the crop improvement. Some of the novel methods like MutMap and MutMap plus look promising to dissect loci governing mutant traits (Bansal et al. 2019; Chaudhary et al. 2019a). However, the induced mutagenesis approaches are random and uncertain to get desired phenotypic improvements. Therefore, genome editing which can be used as site directed mutagenesis are more assured.

2.9 Biofortified Crops/Plants by Genome Editing

2.9.1 Soybean

The fatty acid composition of soybean oil is lesser than desirable. Approximately 24% monounsaturated fatty acids, which is 61% to 75% less than canola and olive, respectively. While, consumption of oils high in monounsaturated fats are considered healthier for consumption and have a longer shelf life and enhanced oxidative stability, Table 2.3 (Clemente and Cahoon 2009). Polyunsaturated fats can be decreased by the process of hydrogenation to improve the fatty acid content in oil, but this process increases the trans fatty acids in the oil and raises the LDL cholesterol levels on consumption with a risk to heart disease (Ascherio et al. 1999). Soybean oil with high oleic acid oil by reducing the activity of the FAD2-1A and FAD2-1B genes has been the objective of different approaches since these two genes convert oleic acid to linoleic acid (Chaudhary et al. 2019b, d). The knockdown of *FAD2-1A and FAD2-1B* gene expression using RNA interference were one of the methods (Clemente and Cahoon 2009; Wagner et al. 2011). But due to variability in transgene expression, this approach requires tremendous screening events to identify the stable lead lines over multiple generations with desired phenotypes. Moreover, the regulation process for the release of the transgenic lines to the farmer is far too lengthy and costly. Yet another approach is through the breeding process by using X-ray or EMS-induced or even naturally occurring mutations within the two genes (*FAD2-1A and FAD2-1B*) and introgressing into elite germplasm (Chaudhary et al. 2019a; Pham et al. 2010). However, mutation breeding is time-consuming and requires years of backcrossing (Chaudhary et al. 2019c; Kumawat et al. 2019). To overcome these problems, Haun et al., in the year 2014, created targeted mutations in both FAD2-1A and FAD2-1B coding sequences using TALENs producing a high oleic acid soybean line in a single generation. Further, another soybean line with high oleic acid by directly delivering mutated

fatty acid desaturase 3A (*FAD3A*) into *fad2-1a fad2-1b* soybean plants was developed by Demorest et al. (2016). The final high oleic acid soybean lines created by both groups using TALENs made all the downstream in vivo assays quite convenient and time-saving. These lines also did not show any foreign DNA integrated into the genome.

2.9.2 Rice (*Oryza sativa*)

High amylose content (AC) diet is highly desirable and considered as a healthy food for humans with heart disease, diabetes, and certain colon and rectum cancers. A cereal grain higher in AC is always a good source of resistant starch (RS) (Jiang et al. 2010). RS products are not digestible and absorbed in the body and are passed on directly to the large intestine (Asp and Björck 1992). For the betterment of human health, cereals with higher RS are preferred. Moreover, it reduces the risk of non-infectious diseases (Regina et al. 2006). Cereal crops do not generally contain high AC content (Zhu et al. 2012). To overcome challenges in nutrition for humans, there is a constant demand to develop cereal crops with higher RS content (Regina et al. 2006; Li and Gilbert 2016). Rice, being one of the major staple food crops, is consumed by proximately half of the world population. Sun et al. (2017) demonstrated the benefits of CRISPR/Cas9-mediated genome editing in rice improvement (Table 2.3). In this study, specific gRNAs targeting the *SBEI* and *SBEIIb* in rice were designed, and finally, transgene-free homozygous *SBEIIb* mutants were achieved with a significantly increased AC and RS contents and thus conferred a better alternative approach to breed for high amylose rice to fulfil the increasing demand of people suffering from diet-related non-infectious chronic diseases.

Their results not only demonstrated in the germ-line with increased oleic acid content but also suggested that the presence of *FAD2* knockout mutations in somatic cells also may contribute to the increased levels of oleic acid concentrations within the somatic cells as well. These results suggest that to modify fatty acid composition in Arabidopsis and Camelina gene-editing techniques like RNAi and CRISPR/Cas9 can also be used. In the present study, data reveals that there was an absence of over off-target effects in the phenotypes of plants containing Cas9/SgRNA-mediated gene mutation.

2.9.3 Potato (*Solanum tuberosum)*

Breeding through traditional crossbreeding in cultivated potato is a challenge due to its tetrasomic inheritance property as the crop is tetraploid and mostly heterozygous in nature (Muthoni et al. 2015). In recent years there have been few successful attempts to develop commercially important potato with new traits through genome editing techniques like TALEN or CRISPR-Cas9 to mainly avoid the crossbreeding strategy (Clasen et al. 2016, Andersson et al. 2017). However, when using DNA

transfection of protoplasts, unintended inserts have been detected that originated from the plasmid DNA used (Clasen et al. 2016, Andersson et al. 2017). In order to overcome the problem, the CRISPR-Cas9 system was developed for the potato breeding method by using ribonucleoprotein (RNP) delivery in protoplasts. Native starch comprises of a mixture of amylopectin and amylase, by knocking out the enzyme "granule bound starch synthase" (GBSS) responsible for the synthesis of amylose, amylopectin starch potatoes were developed. The significantly high number of GBSS knockout genotypes developed by CRISPR-Cas9 RNP technology makes it a promising tool for further potato breeding methods (Andersson et al. 2018). The study proposed using RNP instead of DNA into developed protoplast isolation, transfection, and regeneration method since it yields a high frequency of transgene-free mutated lines, further simplifying the analysis and selection of lines. Previously, the same group reported the use of breeding technology to develop a trait of commercial interest (amylopectin potato starch) without disrupting the valuable overall heterozygous genetic context by avoiding additional sexual crosses. Three different regions of the same gene (*GBSS*) were targeted, and two different promoters were used to drive the guide sequences and three different transfection conditions. A higher mutation rate in all transgenics proved the robustness of the CRISPR-Cas9 technique for potato breeding as well as research (Andersson et al. 2017) (Table 2.3).

2.9.4 Maize (*Zea mays*)

Maize is an important crop and cultivated worldwide for consumption. Phytic acid (PA), inositol 1, 2, 3, 4, 5, 6-hexakisphosphate, is a natural product present in maize seeds. PA is an anti-nutritional compound since it cannot be digested by omnivores. Maize seeds naturally contain PA, which represents about 75% of the total seed phosphorus content. Therefore, it is important to reduce the PA content of maize seeds. Liang et al. (2014) for the first time designed and constructed TALENs and a gRNA: Cas9 constructs that targeted the *ZmIPK1A* (Sun et al. 2007), *ZmIPK* (Shi et al. 2003), and *ZmMRP4* (Shi et al. 2007) genes encoding enzymes that catalyze three steps in the phytic acid biosynthetic pathway. Transgenic maize raised by introducing mutations at the target sites of the specific genes by both TALEN and CRISPR systems was first of its kind. This much-anticipated study has been an inspirational work to follow genome editing technologies to make targeted gene modification a routine practice in maize as well as various other economically important crops and substantially increase the potential for molecular breeding for biofortification.

2.9.5 Barley (*Hordeum vulgare*)

For the development of the Cas9-mediated knockout in the ENGase gene of barley, five SgRNAs were designed by Kapusi et al. 2017. Generally, plant synthesize two

kinds of N-glycans, namely oligomannoside-type glycans carrying only core N-acetylglucosamine (GlcNAc) and mannosyl residues, and complex-type glycans harboring other residues such as galactose, xylose, and fucose (Lerouge et al. 1998). N-glycans can be removed by two enzymes: endo-*N*-acetyl-β-D-glucosaminidase (ENGase) and peptide-N(4)-(*N*-acetyl-β-D-glucosaminyl) asparagine amidase (PNGase). Hydrolysis of the bond between two GlcNAc residues is catalyzed by ENGase with peptide chain containing the proximal GlcNAc still linked to the asparagine residue, while PNGase converts asparagine to aspartic acid residue by completely releasing the entire N-glycan. Both these activities are observed in mature barley seeds. A possible endogenous ENGase activity is seen in the recombinant glycoproteins produced in cereal grains since they often carry a single GlcNAc linked to the asparagine residue (Rademacher et al. 2008; Hensel et al. 2015; Vamvaka et al. 2016). Kapusi et al. 2017 showed the benefit of the CRISPR/Cas9 system allowing the use of multiplexing and avoiding the protein engineering steps (Table 2.3). They designed five SgRNAs to target the DNA strand and accomplished the chromosomal fragment deletion in target sites and also induced indels. Moreover, the T1 plants showed the presence of homozygous mutants.

2.9.6 Sage (*Salvia miltiorrhiza*)

Salvia miltiorrhiza belonging to family Lamiaceae is native to some regions of China and is a diploid species. The presence of diterpene compounds like tanshinones (lipid-soluble compounds) along with rosmarinic acid, salvianolic acid, and lithospermic acid (Luo et al. 2014) (water-soluble phenolic acids) has made this plant highly prized in the Chinese herbal medicine sector. These compounds and acids have demonstrated efficacy as a treatment for improved blood circulation and antiphlogosis as well as for some cardiovascular ailments and protectants against ischemia-reperfusion injury to the brain, respectively (Zhou et al. 2005). *S. miltiorrhiza* have tanshinones, particularly accumulated in the periderm of the reddish roots (Cui et al. 2015; Xu et al. 2016). The emphasis lies here that GGPP precursor is required in both taxol biosynthesis and tanshinones biosynthesis pathway; in the tanshinones biosynthesis pathway accumulation of the substrate for taxol synthesis can be achieved by silencing the post-GGPP synthesis step. That could be utilized by adding a group of genes involved in the taxol pathway to roots. The biosynthesis of tanshinones begins from the diterpenoid precursor (E,E,E)-geranylgeranyl diphosphate by sequential reactions catalyzed by copalyl diphosphate synthase (CPS) and kaurene synthase-like cyclase (KSL) (Gao et al. 2009). By using RNAi approach, earlier studies have demonstrated the involvement of SmCPS1in the tanshinone biosynthetic pathway (Cui et al. 2015; Cheng et al. 2014). First of all, Li et al. (2017) reported knockout of SmCPS1 in *S. miltiorrhiza* using the CRISPR/Cas9 technique (Table 2.3). They also demonstrated the efficiency of the strategy to block the metabolic flux through GGPP to tanshinone and underlined the capacity for directing GGPP to valuable diterpenes like taxol.

2.9.7 Flax (*Camelina sativa*)

The oilseed crop Camelina sativa has grabbed attention for its short growing season, productivity in geographic areas with less rainfall, and soil fertility (Iskandarov et al. 2014; Pilgeram et al. 2007; Zubr 1997). The seed contains a higher amount of polyunsaturated fatty acids, especially linolenic acid, that accounts for around 30–40% of seed oil from most Camelina cultivars (Iskandarov et al. 2014), and therefore there is limited use of its seed oil in biofuels, lubricants, and food applications. Food products developed from Camelina oil are more prone to rancidity since the oil is rich in polyunsaturated fatty acids and undergo incomplete oxidation and hydrolysis of fats (Frolich and Rice 2005). The oil quality of Camelina is improved by the genetic modification of *FAD2* gene suppression, leading to an increase in the more oxidative stable oleic acid and a decrease in polyunsaturated fatty acid of seed oils (Hutcheon et al. 2010; Kang et al. 2011; Nguyen et al. 2013). Earlier several approaches for the silencing of *FAD2* in Camelina and other crops have been reported (Clemente and Cahoon 2009, Graef et al. 2009; Jung et al. 2011, Nguyen et al. 2013, Belide et al. 2012, Haun et al. 2014, Kang et al. 2011, Pham et al. 2012, Thambugala et al. 2013; Wells et al. 2014). Jiang et al. 2016 firstly developed *FAD2* genes knockout using the Cas9/sgRNA gene-editing system in Arabidopsis and Camelina leaves and seeds. They demonstrated in their studies that knockout of *FAD2* genes increased oleic acid content from ~16% to >50% and the total monounsaturated fatty acid (18:1, 20:1, 22:1) from ~32% to >70% while decreasing the existing linoleic and linolenic fatty acid content (Table 2.3).

2.10 Production of Biopharmaceuticals in Plants

The role of plants in human health is indispensable and way too complicated, recently generating biopharmaceuticals in plants has added to another layer of complexity altogether. Presently plants producing vaccines and other therapeutic proteins, including monoclonal antibodies, have already been generated and are in the early stages of commercialization (Hefferon 2015). The idea of molecular farming as a strategy originally emerged from the need for safe and inexpensive biopharmaceuticals, especially in developing countries. For the implementation, vaccines that could be easily transported and did not require refrigeration with surety to be accessible to remote regions worldwide were considered. A wide range of different variety of therapeutic proteins can be produced in plants, like human monoclonal antibodies against HIV to vaccine proteins against smallpox, to a mixture of anti-cancer therapeutic agents for the newly emerging field of personalized medicine and other potential biological warfare threats (Hefferon 2015, Jansing et al. 2019, Mercx et al. 2017). The highest infant mortality in developing countries is observed through diseases like cholera, rotavirus, and Norwalk virus (Mugode et al. 2014); therefore, the earliest plant-made pharmaceuticals through research and development were focused on these diarrheal infectious diseases. Vaccines produced in food crops like banana, tomato, and

soybean can be directly consumed and effectively elicit an immune response to a particular pathogen, for example, bananas producing therapeutic proteins in the form of dried chips, and tomatoes lyophilized into a powder to reconstituted as a juice (Hefferon 2013) is a direct and convenient mode of consumption. While some therapeutic proteins are generated in transgenic plants, an increasing number of these therapeutic proteins are generated from plant virus expression vector systems (Lai et al. 2012). Though, transgenic plants still remain a preferable choice since they can generate transgenic seed considered a stable storage system. Some other production systems do exist, which focus on chloroplasts and hairy roots of plants (Davoodi-Semiromi et al. 2010; Talano et al. 2012). At present clinical trials to examine the potential of plant-derived therapeutic proteins to treat challenging diseases such as HIV and Ebola virus are in the process (Richter et al. 2000).

2.11 Challenges for the Adoption of Biotech Biofortified Crops

Golden rice was made available to farmers free of cost after clearing 72 intellectual property issues and 16 patents filed/granted (http://www.goldenrice.org/). A general approval by public and boundless acceptance of the crops by farmers and consumers is a prerequisite for a successful strategy of biofortification, and adequate information programs need to be implemented for this (Powell 2007). Also, this involves various challenges like perception among the public about the changes the modified crops with new traits possess like color, taste, etc. Biofortification through genetic transformations is considered as GMO plants that are generally either patented or are owned by a company. Therefore challenges regarding intellectual property issues need to be cleared. Another major problem persisting is the cost involved in developing the biofortified crops through GM technology and dealing with the severe regulatory consent for their approval and release. The very fewer profit margins of private technology developers and the scarcity of public funds collectively worsen the whole problem of dealing with biofortified crops (Powell 2007). Poor agricultural infrastructure in developing countries also is a serious challenge for the adoption of new biofortified varieties. Overall major steps like adequate educational programs at low areas of developing countries with a wide circulation of the advanced technologies, better market networks both at local and global levels, and precise means for the promotion of agricultural information is a requisite for the success of accepting and adoption of the developed biofortified crops.

2.12 Conclusions

Continuous climatic changes and consumer preferences are the two forces responsible for the essential ongoing process of important crop improvement plans. Improvement of multiple prevailing biofortification approaches shall be beneficial to achieve target levels of macro or micronutrients within the desired crops. Based on survey studies, the rates of micronutrient deficiency are too high; therefore, there is

constraining evidence of biofortification to be one of the most important objectives for plant breeders along with the traditional objectives of disease resistance, yield, drought tolerance, etc. Conventional seed breeding techniques for increased yield need to very rapidly be reached by plant breeders in order to enhance micronutrient biofortification in crops. To increase the yields of crops in difficult growing conditions, to adapt yields to climate change and for macronutrients yield, there are more advanced and accurate agriculture methods, including the GMO crops.

There are numerous developing countries where micronutrient deficiency is very common; biofortification has been very successful in improving the nutrient intake and status of such areas by installing the process into local nutrition strategies. In developed countries where the public health condition is strong enough not to consider biofortification as a central plank of nutrition policy, there are other possible benefits of using biofortified crops. For example, the use of biofortified crops and products can gear up the standards and regulations and increase global consumer demand for biofortified foods. It could also help in making the consumption of such foods as additional alternative ingredients to improve the nutritional quality of the already existing food products of developed countries and make use of biofortified foods popular by associating with the influence of developed markets on the global food chain.

In the last few years, scientific evidence has demonstrated that biofortification is technically achievable. Crops like sweet potato, wheat, and maize with increased beneficial micronutrient concentration without compromising their agronomic traits are some examples of successful events of biofortification. The main challenge is to get acceptance for biofortified crops through which the intake of the target nutrients could be increased. And development of advanced global markets and products, the onset of better seed production systems, and creating demand could make biofortified food a reality in the coming years.

References

Alagoz Y, Gurkok T, Zhang B, Unver T (2016) Manipulating the biosynthesis of bioactive compound alkaloids for next-generation metabolic engineering in opium poppy using CRISPR-Cas 9 genome editing technology. Sci Rep 6:30910. https://doi.org/10.1038/srep30910

Andersson M, Turesson H, Nicolia A, Fält A-S, Samuelsson M, Hofvander P (2017) Efficient targeted multiallelic mutagenesis in tetraploid potato (Solanum tuberosum) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Rep 36(1):117–128

Andersson M, Turesson H, Olsson N, Fält A-S, Ohlsson P, Gonzalez MN, Samuelsson M, Hofvander P (2018) Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. Physiol Plant 164(4):378–384

Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC (1999) Trans fatty acids and coronary heart disease. N Engl J Med 340(25):1994–1998

Asp N-G, Björck I (1992) Resistant starch. Trends Food Sci Technol 3:111–114

Aznar-Moreno JA, Durrett TP (2017) Simultaneous targeting of multiple gene homeologs to alter seed oil production in Camelina sativa. Plant Cell Physiol 58:1260–1267. https://doi.org/10.1093/pcp/pcx058

Bansal R, Rana N, Kumawat S, Kumar N, Preeti P, Bhatt V, Waqar A, Altaf N, Katara JL, Samantaray S et al (2019) Advances in induced mutagenesis and mutation mapping approaches in rice. Oryza 56(Special issue):8

Banziger M, Long J (2000) The potential for increasing the iron and zinc density of maize through plant-breeding. Food Nutr Bull 21:397–400

Belide S, Petrie JR, Shrestha P, Singh SP (2012) Modification of seedoil composition in Arabidopsis by artificial microRNA-mediated genesilencing. Front Plant Sci 3:168–178

Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R et al (2002) Golden Rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering. J Nutr 132:S506–S510

Bhullar NK, Gruissem W (2013) Nutritional enhancement of rice for human health: the contribution of biotechnology. Biotechnol Adv 31:50–57

Birol E, Meenakshi JV, Oparinde A, Perez S, Tomlins K (2015) Developing country consumers' acceptance of biofortified foods: a synthesis. Food Secur 7(3):555–568

Brooks S (2010) Rice biofortification: lessons for global science and development. Earthscan, London

Chaudhary J, Alisha A, Bhatt V, Chandanshive S, Kumar N, Mir Z, Kumar A, Yadav SK, Shivaraj S, Sonah H (2019a) Mutation breeding in tomato: advances, applicability and challenges. Plan Theory 8:128

Chaudhary J, Deshmukh R, Mir ZA, Bhat JA (2019b) Metabolomics: an emerging technology for soybean improvement. In: Biotechnology products in everyday life. Springer, New York, pp 175–186

Chaudhary J, Deshmukh R, Sonah H (2019c) Mutagenesis approaches and their role in crop improvement. Plan Theory 8:467

Chaudhary J, Shivaraj S, Khatri P, Ye H, Zhou L, Klepadlo M, Dhakate P, Kumawat G, Patil G, Sonah H (2019d) Approaches, applicability, and challenges for development of climate-smart soybean. In: Genomic designing of climate-smart oilseed crops. Springer, New York, pp 1–74

Chen K, Gao C (2013) TALENs: customizable molecular DNA scissors for genome engineering of plants. J Genet Genomics 40(6):271–279

Cheng L, Gilbert RG (2016) Progress in controlling starch structure by modifying starch-branching enzymes. Planta 243(1):13–22

Cheng Q, Su P, Hu Y, He Y, Gao W, Huang L (2014) RNA interference-mediated repression of SmCPS (copalyl diphosphate synthase) expression in hairy roots of Salvia miltiorrhiza causes a decrease of tanshinones and sheds light on the functional role of SmCPS. Biotechnol Lett 36:363–369

Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebu R, Davison S, Ray EE, Daulhac A, Coffman A, Yabandith A, Retterath A, Haun W, Baltes NJ, Mathis L, Voytas DF, Zhang F (2016) Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnol J 14(1):169–176

Clemente TE, Cahoon EB (2009) Soybean oil: genetic approaches for modification of functionality and total content. Plant Physiol 151:1030–1040

Crocoll C, Mirza N, Reichelt M, Gershenzon J, Halkier BA (2016) Optimization of engineered production of the glucoraphanin precursor dihomomethionine in Nicotiana benthamiana. Front Bioeng Biotechnol 4:14

Cui G, Duan L, Jin B, Qian J, Xue Z, Shen G et al (2015) Functional divergence of diterpene syntheses in the medicinal plant Salvia miltiorrhiza Bunge. Plant Physiol 169:1607–1618

Daud NSM, Zaidel DNA, Lai KS, Muhamad II, YM MJ (2016) Antioxidant properties of rice bran oil from different varieties extracted by solvent extraction methods. J Teknologi 78 (6–12):107–110

Daud NSM, Zaidel DNA, Lai KS, Jusoh YMM, Muhamad II, Ya'akob H (2017) Microwave assisted stabilisation and storage stability study of rice bran oil from different varieties. Chem Eng Trans 56:1285–1290

Davoodi-Semiromi A, Schreiber M, Nalapalli S, Verma D, Singh ND, Banks RK et al (2010) Chloroplast-derived vaccine antigens confer dual immunity against cholera and malaria by oral or injectable delivery. Plant Biotechnol J 8:223–242

Demorest ZL, Coffman A, Baltes NJ, Stoddard TJ, Clasen BM, Luo S, Retterath A, Yabandith A, Gamo ME, Bissen J, Mathis L, Voytas DF, Zhang F (2016) Direct stacking of sequence-specific nuclease-induced mutations to produce high oleic and low linolenic soybean oil. BMC Plant Biol 16(1):225

Dubock A (2017) An overview of agriculture, nutrition and fortification, supplementation and biofortification: Golden Rice as an example for enhancing micronutrient intake. Dubock Agric Food Secur 6:59. https://doi.org/10.1186/s40066-017-0135-3

Frolich A, Rice B (2005) Evaluation of Camelina sativa oil as a feedstock for biodiesel production. Ind Crop Prod 21:25–31

Gaj T, Gersbach CA, Barbas CF (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31(7):397–405

Gao W, Hillwig ML, Huang L, Cui G, Wang X, Kong J et al (2009) A functional genomics approach to tanshinone biosynthesis provides stereochemical insights. Org Lett 11:5170–5173

García-bañuelos ML, Sida-arreola JP (2014) Biofortification – Promising approach to increasing the content of iron and zinc in staple food crops. J Elem 19(3):865–888

Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Front Nutr 5:12. https://doi.org/10.3389/fnut.2018.00012

Graef G, LaVallee BJ, Tenopir P, Tat M, Schweiger B, Kinney AJ et al (2009) A high-oleic-acid and low-palmitic-acid soybean: agronomic performance and evaluation as a feedstock for biodiesel. Plant Biotechnol J 7:411–421

Hanania U, Ariel T, Tekoah Y, Fux L, Sheva M, Gubbay Y et al (2017) Establishment of a tobacco BY2 cell line devoid of plant-specific xylose and fucose as a platform for the production of biotherapeutic proteins. Plant Biotechnol J 15:1120–1129. https://doi.org/10.1111/pbi.12702

HarvestPlus (2009) Breeding crops for better nutrition. International Food Policy Research Institute (IFPRI), Washington, DC

Haun W, Coffman A, Clasen BM, Demorest ZL, Lowy A, Ray E et al (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnol J 12:934–940

Hefferon K (2013) Plant-derived pharmaceuticals for the developing world. Biotechnol J 8:1193–1202

Hefferon KL (2015) Nutritionally enhanced food crops; progress and perspectives. Int J Mol Sci 16:3895–3914. https://doi.org/10.3390/ijms16023895

Hensel G, Floss DM, Arcalis E, Sack M, Melnik S, Altmann F et al (2015) Transgenic production of an anti HIV antibody in the barley endosperm. PLoS One 10:e0140476. https://doi.org/10.1371/journal.pone.0140476

Hodge J (2016) Hidden hunger: approaches to tackling micronutrient deficiencies. In: Gillespie S, Hodge J, Yosef S, Pandya-Lorch R (eds) Nourishing millions: stories of change in nutrition. International Food Policy Research Institute (IFPRI), Washington, DC, pp 35–43

Hutcheon C, Ditt RF, Beilstein M, Comai L, Schroeder J, Goldstein E, Shewmaker CK et al (2010) Polyploid genome of Camelina sativa revealed by isolation of fatty acid synthesis genes. BMC Plant Biol 10:233–248

Iskandarov U, Kim HJ, Cahoon EB (2014) Chapter 8: Camelina: an emerging oilseed platform for improved biofuels and bio-based materials. In: Carpita N, McCann M, Buckeridge MS (eds) Plants and BioEnergy. Springer, New York, pp 131–140

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G (2018) CRISPR for crop improvement: an update review. Front Plant Sci 9:985

Jansing J, Sack M, Augustine SM, Fischer R, Bortesi L (2019) CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in Nicotiana benthamiana for the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose. Plant Biotechnol J 17(2):350–361

Jeng TL, Lin YW, Wang CS, Sung JM (2012) Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the indica type cultivar IR64. J Food Compos Anal 28:149–154

Jiang H, Horner HT, Pepper TM, Blanco M, Campbell M, Jane J-l (2010) Formation of elongated starch granules in high-amylose maize. Carbohydr Polym 80(2):533–538

Jiang WZ, Henry IM, Lynagh PG, Comai L, Cahoon EB, Donald P (2017) Weeks. Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing. Plant Biotechnol J 15:648–657

Jung JH, Kim H, Go YS, Lee SB, Hur CG, Kim HU et al (2011) Identification of functional BrFAD2-1 gene encoding microsomaldelta-12 fatty acid desaturase from Brassica rapa and development of Brassica napus containing high oleic acid contents. Plant Cell Rep 30:1881–1892

Kang J, Snapp AR, Lu C (2011) Identification of three genes encoding microsomal oleate desaturases (FAD2) from the oilseed crop Camelina sativa. Plant Physiol Biochem 49:223–229

Kapusi E, Corcuera-Gómez M, Melnik S, Stoger E (2017) Heritable genomic fragment deletions and small indels in the putative ENGase gene induced by CRISPR/Cas9 in barley. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00540

Kok AD, Yoon LL, Sekeli R, Yeong WC, Yusof ZNB, Song LK (2018) Iron biofortification of rice: progress and prospects. https://doi.org/10.5772/intechopen.73572

Kumawat S, Rana N, Bansal R, Vishwakarma G, Mehetre ST, Das BK, Kumar M, Kumar Yadav S, Sonah H, Sharma TR (2019) Expanding avenue of fast neutron mediated mutagenesis for crop improvement. Plan Theory 8:164

Lai H, He J, Engle M, Diamond MS, Chen Q (2012) Robust production of virus-like particles and monoclonal antibodies with geminiviral replicon vectors in lettuce. Plant Biotechnol J 10:95–104

Lerouge P, Cabanes-Macheteau M, Rayon C, Fischette-Lainé AC, Gomord V, Faye L (1998) N-glycoprotein biosynthesis in plants: recent developments and future trends. Plant Mol Biol 38:31–48. https://doi.org/10.1023/A:1006012005654

Levitt T (2011) Can GM-free biofortifed crops succeed after Golden Rice controversy? Ecologist. http://www.theecologist.org/News/news_analysis/1159571/can_gmfree_biofortifed_crop

Li B, Cui G, Shen G, Zhan Z, Huang L, Chen J, Qi X (2017) Targeted mutagenesis in the medicinal plant Salvia miltiorrhiza. Sci Rep 7(1):43320

Liang Z, Zhang K, Chen K, Gao C (2014) Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. J Genet Genomics 41(2):63–68

Luo H, Zhu Y, Song J, Xu L, Sun C, Zhang X et al (2014) Transcriptional data mining of Salvia miltiorrhiza in response to methyl jasmonate to examine the mechanism of bioactive compound biosynthesis and regulation. Physiol Plant 152:241–255

Maqsood MA, Kanwal RS, Aziz T, Ashraf M (2009) Evaluation of Zn distribution among grain and straw of twelve indigenous wheat (Triticumaestivum L.) genotypes. Pak J Bot 41:225–231

McGuire S (2015) FAO, IFAD, WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO. Adv Nutr 6 (5):623–624. https://doi.org/10.3945/an.115.009936

Mercx S, Smargiasso N, Chaumont F, Pauw ED, Boutry M, Navarre C (2017) Inactivation of the β(1,2)-xylosyltransferase and the α(1,3)-fucosyltransferase genes in Nicotiana tabacum BY-2 cells by a multiplex CRISPR/Cas9 strategy results in glycoproteins without plant-specific glycans. Front Plant Sci 8:403. https://doi.org/10.3389/fpls.2017.00403

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogué F et al (2016) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. Plant Biotechnol 15:729–739. https://doi.org/10.1111/pbi.12671

Mugode L, Há B, Kaunda A, Sikombe T, Phiri S, Mutale R et al (2014) Carotenoid retention of biofortified provitamin a maize (*Zea mays* L.) after Zambian traditional methods of milling, cooking and storage. J Agric Food Chem 62:6317–6325

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Muthayya A, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE (2013) The global hidden hunger indices and maps: an advocacy tool for action. PLoS One 8(6):e67860. https://doi.org/10.1371/journal.pone.0067860

Muthoni J, Kabira J, Shimelis H, Melis R (2015) Tetrasomic inheritance in cultivated potato and implications in conventional breeding [online]. Aust J Crop Sci 9(3):185–190

Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, Perez Conesa D, Ros G, Sandmann G, Capell T, Christou P (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci 106(19):7762–7767

Nguyen HT, Silva JE, Podicheti R, Macrander J, Yang W, Nazarenus TJ et al (2013) Camelina seed transcriptome: a tool for meal and oil improvement and translational research. Plant Biotechnol J 11:759–769

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003) Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. J Plant Nutr 26:2307–2319

Omodamilola OI, Ibrahim AU (2018) CRISPR technology: advantages, limitations and future direction. J Biomed Pharm Sci 1:115

Peng S, Cassman KG, Virmani SS, Sheehy J, Khush GS (1999) Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci 39 (6):1552–1559

Petry N, Boy E, Wirth JP, Hurrell RF (2015) Review: the potential of the common bean (Phaseolus vulgaris) as a vehicle for iron biofortification. Nutrients 7(2):1144–1173

Pham A-T, Lee J-D, Shannon JG, Bilyeu KD (2010) Mutant alleles of FAD2-1A and FAD2-1B combine to produce soybeans with the high oleic acid seed oil trait. BMC Plant Biol 10:195

Pham AT, Shannon JG, Bilyeu KD (2012) Combinations of mutant FAD2 and FAD3 genes to produce high oleic acid and low linolenic acid soybean oil. Theor Appl Genet 125:503–515

Pilgeram AL, Smith DC, Boss D, Dale N, Wichman S, Lamb P et al (2007) Camelina sativa: a Montana omega-3 and fuel crop. In: Janik J, Whipkey A (eds) Issues in new crops and new uses. ASHS Press, Alexandra, VA, pp 129–131

Potrykus I (2008) Golden Rice – from idea to reality. In: Foundation Bertebos (ed) Golden Rice and other biofortified food crops for developing countries challenges and potential. KSLA, Slöige, pp 11–16

Powell K (2007) Functional foods from biotech—an unappetizing prospect? Nat Biotechnol 25 (5):525–531

Prasad R, Shivay YS, Kumar D (2014) Agronomic biofortification of cereal grains with iron and zinc. Adv Agron 125(1):55–91

Prashanth L, Kattapagari KK, Chitturi RT, Baddam VR, Prasad LK (2015) A review on role of essential trace elements in health and disease. J NTR Univ Health Sci 4:75–85. https://doi.org/10.4103/2277-8632.158577

Pray C, Paaarlberg R, Unnevehr L (2007) Patterns of political response to biofortified varieties of crops produced with different breeding techniques and agronomic traits. J Agrobiotechnol Manag Econ 10(3):Article 2

Rademacher T, Sack M, Arcalis E, Stadlmann J, Balzer S, Altmann F et al (2008) Recombinant antibody 2G12 produced in maize endosperm efficiently neutralizes HIV-1 and contains predominantly single-GlcNAc N-glycans. Plant Biotechnol J 6:189–201. https://doi.org/10.1111/j.1467-7652.2007.00306.x

Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, Kosar-Hashemi B, Li Z, Rahman S, Morell M (2006) High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. Proc Natl Acad Sci 103(10):3546–3551

Ravanello MP, Ke D, Alvarez J, Huang B, Shewmaker CK (2003) Coordinate expression of multiple bacterial carotenoid genes in canola leading to altered carotenoid production. Metab Eng 5(4):255–263
Richter LJ, Thanavala Y, Arntzen CJ, Mason HS (2000) Production of hepatitis B surface antigen in transgenic plants for oral immunization. Nat Biotechnol 18:1167–1171
Saltzman A, Birol E, Oparinde A, Andersson MS, Asare-Marfo D, Diressie MT et al (2017) Availability, production, and consumption of crops biofortified by plant breeding: Current evidence and future potential. Ann N Y Acad Sci 1390:104–114
Scheben A, Wolter F, Batley J, Puchta H, Edwards D (2017) Towards CRISPR/Cas crops – bringing together genomics and genome editing. New Phytol 216(3):682–698
Shan Q, Zhang Y, Chen K, Zhang K, Gao C (2015) Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. Plant Biotechnol J 13(6):791–800
Shi J, Wang H, Wu Y, Hazebroek J, Meeley RB, Ertl DS (2003) The maize low-phytic acid mutant is caused by mutation in an inositol phosphate kinase gene. Plant Physiol 131(2):507–515
Shi J, Wang H, Schellin K, Li B, Faller M, Stoop JM, Meeley RB, Ertl DS, Ranch JP, Glassman K (2007) Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. Nat Biotechnol 25(8):930–937
Sperotto RA, Ricachenevsky FK, Waldow V, Fett JP (2012) Iron biofortification in rice: it's a long way to the top. Plant Sci 190:24–39
Sun Y, Thompson M, Lin G, Butler H, Gao Z, Thornburgh S, Yau K, Smith DA, Shukla VK (2007) Inositol 1,3,4,5,6-pentakisphosphate 2-kinase from maize: molecular and biochemical characterization. Plant Physiol 144(3):1278–1291
Sun Y, Jiao G, Liu Z, Zhang X, Li J, Guo X, Wenming D, Jinlu D, Francis F, Zhao Y, Xia L (2017) Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Front Plant Sci 8:298
Talano MA, Oller AL, González PS, Agostini E (2012) Hairy roots, their multiple applications and recent patents. Recent Pat Biotechnol 6:115–133
Thambugala D, Duguid S, Loewen E, Rowland G, Booker H, You FM et al (2013) Genetic variation of six desaturase genes in flax and their impact on fatty acid composition. Theor Appl Gene 126:2627–2641
Vamvaka E, Twyman RM, Murad AM, Melnik S, Teh AYH, Arcalis E et al (2016) Rice endosperm produces an underglycosylated and potent form of the HIV-neutralizing monoclonal antibody 2G12. Plant Biotechnol J 14:97–108. https://doi.org/10.1111/pbi.12360
Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8:1386
Veillet F, Chauvin L, Kermarrec M-P, Sevestre F, Merrer M, Terret Z, Szydlowski N, Devaux P, Gallois J-L, Chauvin J-E (2019) The Solanum tuberosum GBSSI gene: a target for assessing gene and base editing in tetraploid potato. Plant Cell Rep 38(9):1065–1080
Virmani SS, Ilyas-Ahmed M (2008) Rice breeding for sustainable production. In: Breeding major food staples. Blackwell Publishing Ltd., Oxford, pp 141–191
Wagner N, Mroczka A, Roberts PD, Schreckengost W, Voelker T (2011) RNAi trigger fragment truncation attenuates soybean FAD2-1 transcript suppression and yields intermediate oil phenotypes. Plant Biotechnol J 9:723–728
Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu J-L (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32:947–951
Wells R, Trick M, Soumpourou E, Clissold L, Morgan C, Werner P et al (2014) The control of seed oil polyunsaturate content in the polyploid crop species *Brassica napus*. Mol Breed 33:349–362
White J, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593. https://doi.org/10.1016/j.tplants.2005.10.001
Xu H, Song J, Luo H, Zhang Y, Li Q, Zhu Y et al (2016) Analysis of the genome sequence of the medicinal plant Salvia miltiorrhiza. Mol Plant 9:949–952

Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P et al (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287 (5451):303–305

Yin X, Biswal AK, Dionora J, Perdigon KM, Balahadia CP, Mazumdar S, Chater C, Lin H-C, Coe RA, Kretzschmar T, Gray JE, Quick PW, Bandyopadhyay A (2017) CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene EPFL9 in rice. Plant Cell Rep 36(5):745–757

Zhou L, Zuo Z, Chow MS (2005) Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 45:1345–1359

Zhu L, Minghong G, Meng X, Cheung SCK, Yu H, Huang J, Sun Y, Shi Y, Liu Q (2012) High-amylose rice improves indices of animal health in normal and diabetic rats. Plant Biotechnol J 10(3):353–362

Zingore S, Delve RJ, Nyamangara J, Giller KE (2008) Multiple benefits of manure: The key to maintenance of soil fertility and restoration of depleted sandy soils on African smallholder farms. Nutr Cycl Agroecosyst 80(3):267–282

Zubr J (1997) Oil seed crop: Camelina sativa. Ind Crop Prod 6:113–119

Biofortification for Nutrient Content and Aroma Enrichment in Rice (*Oryza sativa* L.)

3

Sonali Chandanshive, Yasmin Shaikh, Gaurav Raturi, Atul Prakash Sathe, Sandhya Sanand, and Altafhusain Nadaf

Abstract

Biofortification is the process of enhancing the content and density of vitamins and minerals that are improving nutritional quality in a staple crop through conventional plant breeding or agronomic practices or using transgenic approaches. Rice (*Oryza sativa* L.) ranks second in most widely consumed cereals in the world and used as a staple food for more than 60% of the world population. Micronutrients are essential for plant growth and development as well as for animal and human health. In the last two decades, the concept of hidden hunger arises in which one-sixth of the world's population suffers from hunger that is a deficiency of micronutrients, vitamins, and nutrients. Rice grain has large genetic variability in the concentration of micronutrients; hence, it is included in the biofortification program, and breeding of new rice cultivars with an enhanced level of grain micronutrients is one of the most sustainable and cost-effective strategies for preventing hidden hunger. To overcome the major problem of hidden hunger and looking to the future, the agricultural community has a fundamental responsibility to produce crop enriched with minerals and vitamins to secure national health, and there is a need to increase the nutritional quality of food through various methods like supplementation, fortifications, etc. Aroma acts as a supporting trait for nutrition; therefore, it needs to be considered in biofortification. It has been shown to enhance food appetite in hungry and in satiated states, so it may be used to stimulate meal initiation and appetite in people

S. Chandanshive · Y. Shaikh · A. Nadaf (✉)
Department of Botany, Savitribai Phule Pune University, Pune, India
e-mail: abnadaf@unipune.ac.in

G. Raturi · A. Prakash Sathe
National Agri-Food Biotechnology Institute, Mohali, India

S. Sanand
ICAR- National Institute for Plant Biotechnology, New Delhi, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_3

that are malnourished. Here the process and progress of biofortification with micronutrients have been briefly described, and future prospects to alleviate widespread micronutrient deficiencies in the human population are discussed.

Keywords

Biofortification · Minerals · Vitamins · Transgenic · Aroma · HarvestPlus

3.1 Introduction

3.1.1 Rice

Rice is the staple food of around three billion people, most of them in Asia, which accounts for 90% of global rice consumption. Rice constitutes a major source of nutrition and contributes a significant share of dietary energy in a number of Asian countries. Among 23 species of genus *Oryza, Oryza sativa* L. is cultivated in Asia and *O. glaberrima* Steud. in West Africa (Vaughan et al. 2003). *O. sativa* L. has been further differentiated into *indica* and *japonica* subspecies (Khush 2000). Rice has immense diversity, and it is estimated that more than 100,000 varieties of rice exist in the world (Khush 1997). India has an ancient heritage of rice cultivation and has over 70,000 cultivars of rice germplasm (Siddiq 1992).

Rice is a rich source of proteins, starch, carbohydrates, vitamins, and minerals, which increases the importance of consumption of rice and thus included in a balanced diet. Over two billion people in Asia derive 80% of their energy from rice (Juliano 1985). In addition to common widely consumed white rice, there is some special colored rice like red, brown, and black. These colored rice are due to the deposition of anthocyanin pigment in the outer coat of rice grain (Chaudhary 2003). Black rice is especially rich in anthocyanin, protein, phytochemicals, and vitamins and known for its antioxidant activity as antioxidants are crucial for strengthening the immune system and enhancing the memory (Pengkumsri et al. 2015). According to Ahuja et al. (2007) red and black rice has a high amount of zinc (Zn), iron (Fe), and minerals. These qualities like high mineral content, antioxidant activity, starch quality, and the glycemic index have made rice unique among all the cereals. India is a home for various rice varieties that have medicinal properties and fits in the description of healthy food in terms of modern as well as old concepts (Chaudhari et al. 2018).

Scented rice constitutes small but a special group of rice and considered as of the best quality. Indian sub-continent flourishes with hundreds of indigenous aromatic cultivars and landraces, and the diversity of scented rice of India is highest in the world (Singh and Singh 2003). Scented rice is further classified as basmati and non-basmati type. Basmati type is a long-slender grain; it exhibits kernel length above 6.6 mm, L/B ratio of more than 3, and high kernel elongation after cooking (ratio above 1.8). These unique features of basmati are said to be due to the culmination of centuries of selection and cultivation by farmers that are well preserved and maintained in their purest form as traditional basmati varieties (Siddiq

et al. 2012). In addition to basmati varieties, many indigenous non-basmati scented rice varieties are also locally cultivated. In a compendium published by Singh and Singh (2003), the authors mentioned that the diversity of scented rice from India is the largest in the world. The majority of the indigenous scented rice cultivars are small and medium-grained (Singh et al. 2000).

3.1.2 Importance of Micronutrients

Micronutrients are essential for plant growth and development as well as for human and animal health. Manganese (Mn), iron (Fe), and zinc (Zn) are of special interest among the micronutrients as they are essential for all higher organisms (Bashir et al. 2013). In the last two decades, the concept of hidden hunger arises in which one-sixth of the world's population suffers from hunger that is a deficiency of micronutrients, vitamins, and nutrients. This hidden hunger is due to the quality of food available, and it is related to the fact that many developing countries rely on low nutrient (intake of vitamins and minerals are too low) staple crops that fail to sustain good health and development. Micronutrient malnutrition is unacceptable and has a severe impact on the health of an individual resulting in poor health, low workability, and decreased earning potential (Bailey et al. 2015). Vitamin and mineral malnutrition affect children less than 5 years of age and women of reproductive age. Deficiency of vitamin A, iron, zinc, iodine, and folate is most common in many countries, which ultimately results in anemia, blindness, increased susceptibility to various diseases, lower IQ, and mortality (Nestel et al. 2006). Anemia is most common worldwide which occurs due to deficiency of iron and affects 38% pregnant woman, 29% non-pregnant women, and 43% pre-school children (Organization 2005).

3.1.3 Weapons to Fight against Hidden Hunger and Micronutrients Malnutrition

To overcome the major problem of hidden hunger and looking to the future, the agricultural community has a fundamental responsibility to produce minerals and vitamins rich food to secure national health, and there is a need to increase the nutritional quality of food through various methods like supplementation, dietary diversification, commercial fortification, etc. In supplementation, pharmaceutical manufacturers produce tablets, capsules, and injections supplemented with a high concentration of vitamins and minerals to fill the short-term gap. Dietary diversification includes the cultivation of multifarious staple food crops such as vegetables and fruits with an elevated level of nutrient content that can be produced for better consumer behavior. Commercial fortification is the practice of increasing the content of essential micronutrients in food and fortifies food with essential nutrients at the time of food processing.

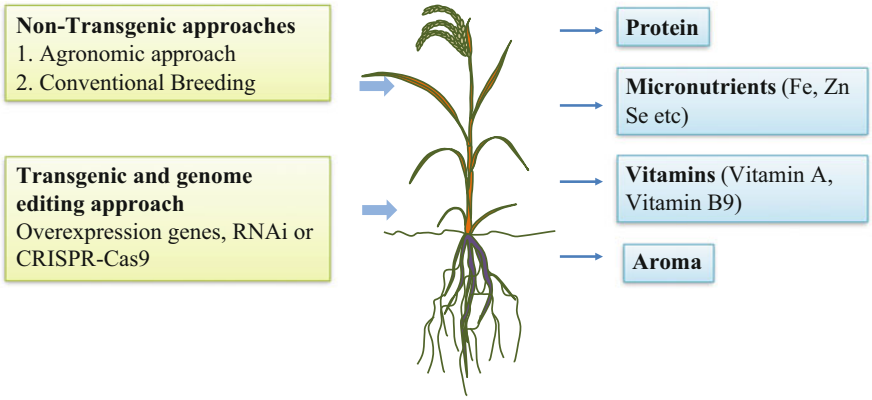

Fig. 3.1 Different approaches being used for the biofortification in rice. The major biofortification aspects include improvement in protein, micronutrient like Fe, Zn, and Se, vitamins like *A* and B9, and aromatic compounds

These approaches cannot efficiently solve the problem of malnutrition because they only have a temporary role in malnutrition and have expensive costs. These methods also fail to reach the poorest areas of the general people who are at a high risk of micronutrient deficiency.

3.1.3.1 Biofortification

A recent approach with high potential to overcome many conventional strategies is the biofortification of staple crops. Biofortification is the process of enhancing the content and density of vitamins and minerals that are improving nutritional quality in a staple crop through conventional plant breeding or agronomic practices or transgenic approaches (Fig. 3.1) (Bouis 2018). Biofortification is a promising and sustainable strategy to target the above-mentioned nutrient deficiencies, especially in a hidden hunger. One of the first biofortified crops under the initiative was rice. Later several varieties of staple crops were developed with an increased level of specific micronutrients (Haas et al. 2005). Biofortification has two key advantages that it is an effective and cheaper alternative to traditional way as these methods are difficult to afford by a large proportion of the world's population especially those who have limited resources and low income and second, it is a feasible means of reaching underserved rural populations that only have limited access to market and healthcare facilities. Once implemented, this strategy will lower the number of micronutrients deficient population, which depends on supplementation and fortification programs (Bouis and Welch 2010).

3.1.4 Need for Biofortification in Rice

In comparison to other cereals, rice is eaten every day by poor malnourished people as a staple food; even the addition of a small amount of micronutrients in the diet is beneficial to improve the health and development of an individual. Rice is a major source of carbohydrates and has other nutritional values. Rice grain has large genetic variability in the concentration of micronutrient; hence, it is included in biofortification program, and breeding of new rice cultivars with an enhanced level of grain micronutrients is one of the most sustainable and cost-effective strategies for preventing hidden hunger (Babu et al. 2013). The rice grain is made up of the outer hull, pericarp layer, and inner starchy endosperm with the embryo. The rice is subjected to various operations like harvesting, transport, processing by drying, or milling from the time it was harvested to convert it into white rice with superior cooking quality (Roy et al. 2011). In the first stage of rice milling, the hull is removed, converting it into brown rice, which consists of bran and endosperm. In the next stage, during polishing, the bran layer is removed, which yields the most commonly consumed white rice. Milling is essential as it decreases cooking time, but over milling can result in high breakage and loss of nutrients. The consumers always prefer well-milled rice, but proteins, minerals, and vitamins are high in outer layers, and removal of these during processing can cause considerable loss of nutrients, hence cannot be ignored (Abbas et al. 2011). Losses of macro and micronutrients have been studied in rice grain. Loss of 29% of protein, 67% of iron, approximately 80% of thiamin and other nutrients like lysine, riboflavin has been reported upon milling (Ramberg and McAnalley 2002). The lower content of lysine is the major amino deficiency (Mandić et al. 2009). The current concern in developing new biofortified rice varieties signalizes a need for genotyping differences in polishing losses and how they relate to the distribution of mineral elements in the rice grain.

3.1.5 Indian Scenario of Malnutrition

Hidden hunger and micronutrient malnutrition is a major problem in India. Intake of an improper daily diet, which has fewer amounts of micronutrients (<50% RDA), is observed in over 70% of the Indian population (Vijayaraghavan et al. 2002).

The following are the major malnutrition related issues in India.

- Deficiency of micronutrients among children, adults, pregnant and lactating women.
- Iron deficiency anemia (IDA) is a major issue; about 62% of pre-school children are suffering from vitamin A deficiency leading to an annual 3.3 lakh child mortality. 69.5% pre-school children, 58.7% pregnant women, 63.2% lactating mothers are anemic.
- About 57% of pre-school children and their mothers have subclinical VAD.

- The Zn deficiency has not been properly investigated, due to a lack of suitable biomarkers.

3.2 Criteria for Biofortification

1. Effective—Micronutrient enhancement level must have an appreciative impact on human health.
2. Stability—Enriched levels of micronutrients must be relatively stable.
3. High yield—For farmers to accept this new concept, crop productivity must be maintained to the guaranteed level.
4. Efficacious—Bioavailability in biofortified lines must be tested in humans so that to ensure that they improve the micronutrient status of people consuming them.
5. Taste and cooking quality—Taste and cooking quality of biofortified crops should be maintained.
6. Consumer acceptance—Acceptability of biofortified crops by the consumer is the major criteria of biofortification.

3.3 Approaches Used for Biofortification in Rice

There are several non-mutually exclusive methods used to develop biofortified crops like agronomic biofortification, conventional plant breeding, and transgenic manipulation, which involves genetic modifications or bio-engineering.

3.3.1 Non-Transgenic Approaches

Non-transgenic approaches have less regulatory concerns compared to transgenic approaches. Such approaches involve the following methods to enhance micronutrients and to improve the nutritional quality of crops, especially in rice.

3.3.1.1 Agronomic Biofortification

Agronomic biofortification is the fastest and easiest way of biofortification in crops with essential micronutrients in developing countries where cereals are a staple food. Micronutrient fertilizers containing both N, P, K, and S and micronutrient fertilizers like Zn, Ni, Co, Mo, and Se can have substantial effects on the accumulation of nutrients in edible plant parts (Allaway 1986). Agronomic biofortification involves the application of nutrient-rich fertilizers to soil or foliage to elevate the micronutrients concentration in edible parts of the crop and thus increase the uptake of essential micronutrients by consumers. From the viewpoint of biofortification, the foliar application is much better and requires fewer amounts of fertilizers than soil application. Using agronomic biofortification, selenium fertilization has been successfully implemented in increasing selenium content in rice up to 35.9% (Chen et al. 2002). 58% enhanced zinc content in the wheat grain and 76% increase in

wheat flour has also been reported (Zhang et al. 2012). This approach is the only way to reach the poorest of the poor rural populations, which cannot afford to improve the components of their diet by incorporating animal products, and it is a win-win approach for developing countries where cereals are a major staple food. However, there are some limitations of agronomic biofortification, like limited access, short-term approach, non-availability in abundance, expenses involved, and failure to reach all individuals, which limit its use and success.

3.3.1.2 Conventional and Marker Assisted Plant Breeding

Plants show genetic variation in micronutrients. Some plants show the high concentration of these essential micronutrients, which allow their use through breeding programs to improve the level of minerals and vitamins in other crops. Plant breeding has been adopted by the farmers for the last hundreds of years. Plant breeders search germplasm bank or seed for the existing population of crops, which are naturally high yielding and high in micronutrients content. These selected varieties were used for breeding. In conventional plant breeding, one of the parents in an initial cross has a high level of target micronutrients, and crossing of these parents results in the progeny with characteristics of both parents (Garcia-Casal et al. 2016). Rice is the best example of conventional plant breeding. In the last decade, biofortification through conventional breeding was focused on increasing the level of three most important micronutrients: zinc, iron, and vitamin A (Ortiz-Monasterio et al. 2007). Besides these, breeders have also developed hybrids with increased content of other micronutrients. Progeny with a high level of micronutrients and high yield was developed by crossing rice variety, which contains a high level of iron and zinc with high yielding rice variety (Khush et al. 2012). This approach has several significant disadvantages when compared with transgenic approaches such as this strategy rely of limited genetic variation present in the gene pool. In some cases, these limitations can be overcome by crossing to distinctly related crops. Conventional plant breeding is prevalent at present as it is cheaper, quicker, and less controversial than genetically engineered crops. Table 3.1 gives an account of the

Table 3.1 Rice varieties improved for nutritional quality through conventional breeding

Variety released	Nutrient targeted	Country	Reference/source
CR Dhan 310, hybrid (*O.nivara* × IR 64)	Protein	India, Columbia	Mahender et al. (2016) and Mahmoud et al. (2007)
BRRI Dhan 62, BRRI Dhan 72, BRRI Dhan 64, DRR Dhan 45 (IET 23832)	Zinc	Bangladesh	HarvestPlus
IR68144-3B-2-2-3	Iron	India, Philippines	Gregorio et al. (2000)
Jalmagna	Iron and Zinc	India	Gregorio et al. (2000)
BRRI Dhan 62, BRRI Dhan 72, BRRI Dhan 64	Zinc Iron	Bangladesh	HarvestPlus

nutrient rice hybrid rice varieties developed through a conventional breeding program.

3.3.2 Transgenic Approach

In some crops, where the target nutrient does not naturally exist at the required levels, and crops that are very difficult to breed, transgenic approach is a feasible way to produce biofortified crops with desired agronomic traits and nutrients.

3.3.2.1 Genetic Engineering Via Overexpression of Genes, RNAi, CRISPR/Cas9

In contrast to plant breeding nowadays, the availability of modern techniques to identify and characterize desirable gene function has been a driving force in recent biofortification efforts to transfer these heritable traits between completely unrelated species through genetic engineering. This was made achievable by the rapid development of high throughput whole-genome sequencing techniques, metabolite profiling, physical mapping, and global gene expression analysis in a variety of plant species. The methodology involves molecular techniques or genetic modifications to transfer specific traits or introduce a gene from novel sources for desirable traits to a recipient organism. This approach has benefits like a rapid and direct application by introduction into popular varieties, unlimited access to the gene of interest, and targeted expression in tissues of interest. Genetic modification has two advantages over conventional plant breeding that it allows the transfer of specific genes and takes a short duration to produce a crop with a trait of interest expressed in a stable way.

Several transgenic experiments have been successfully done in many agriculturally important crops to target proteins and micronutrient accumulation in specific tissues. For the efficient manipulation using transgenic technology appropriate integration of omics scale data is useful (Deshmukh et al. 2014; Chaudhary et al. 2019b, c). In-depth understanding about the transgene effect ensures the success of such program aiming for the release of transgenic product for the cultivation. Golden rice is a popular example of genetically modified biofortified crops (Ye et al. 2000). Rice, with high zinc and iron content has been developed through a transgenic approach (Trijatmiko et al. 2016). Currently, significant progress is being made to develop transgenic plants with an increased level of iron and zinc and also increased phyto-availability of mineral elements in soil, their uptake, translocation to the shoot, and accumulation in inedible parts (Zhu et al. 2007; Rana et al. 2019) (Fig. 3.2). These transgenic varieties have tremendous nutritional potential, but limited progress for release has been made so far due to constraints of intellectual property and approval through national biosafety and regulatory processes. In this regard, genome editing approaches more particularly the CRISPR/Cas mediated genome editing looks promising to achieve desirable changes bypassing the regulatory concerns (Vats et al. 2019; Mushtaq et al. 2020). Numerous studies using different CRISPR/Cas9 based tools have been performed in rice (Vats et al. 2019). Most of the initial studies

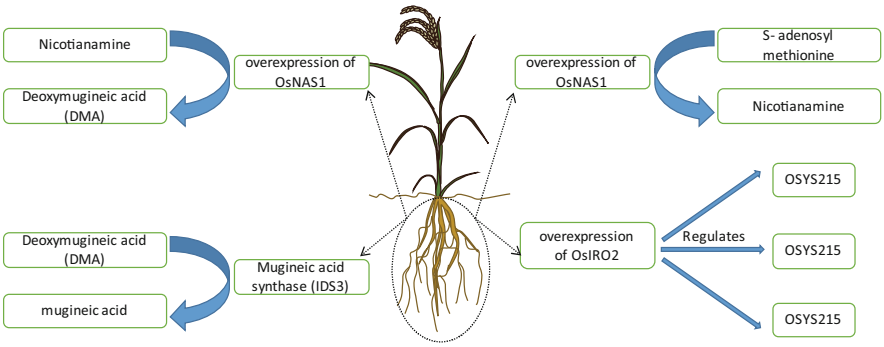

Fig. 3.2 Set of genes regulating molecular mechanism involved in the iron uptake in rice. These are the major four genes which has been widely targeted through transgenic and other approaches for the iron biofortification in rice

have performed with genes where sufficient information about molecular mechanism is known. Up to now few studies aiming for biofortification through genome editing have been performed in rice. Such studies are discussed here in subsequent sections. Similarly, traditional approach like mutation breeding is more cheaper and convenient way to get desired trait improvement (Chaudhary et al. 2019a, d). However, mutation breeding is more depends on chance to get the desired modification (Bansal et al. 2019; Kumawat et al. 2019). In case of biofortification related traits governed by single gene, chances are much higher to get desired improvements. Another limitation with mutation breeding is the chances getting improvements in the traits governed by positive regulation are much lesser than the traits governed by negative regulation. Recently, mutants selected from the set of 87,000 EMS induced rice mutants have been screened for the improved iron and zinc content in the grains. The study showed a wide range of variation for different elements (Sevanthi et al. 2018). There are numerous studies where mutants with improved iron and zinc have been identified. For instance, a study by Cheng et al. (2007) identified a rice mutant having sequence variation in nicotianamine aminotransferase which found to stimulated the Fe(II) acquisition system and resulted into increased iron accumulation in rice. Similarly, another study where several mutants having high iron and zinc content in polished grains have been identified by screening a set of mutant lines derived from IR-64 variety (Jeng et al. 2012).

3.4 Biofortification in Rice

3.4.1 Protein

It is estimated that milled rice grain contains 6–7% of protein, representing second abundant constituent in rice (Juliano 1985). It is one of the cereals which are naturally gluten-free with highly digestible protein, therefore ideal for people with

celiac disease, babies, and older people. On the basis of solubility, rice proteins are classified into four classes, glutelin (alkali/acid-soluble), a prolamin (alcohol soluble), globulin (salt soluble), and albumin (water-soluble). Despite being the staple food of the world, rice has the lowest protein content as compared to other cereals, so it is essential to produce rice with higher protein content. An inbreed "Frontiere" rice cultivar with high protein was developed by Utomoa Professor and his team at LSU (*Louisiana State* University) and was released in 2015. Another protein-rich rice varieties CR Dhan 310 and CR Dhan 3119 Mukul was developed by ICAR-NRRI, by improving the popular high yielding variety Naveen through conventional breeding and released in 2016 (Mahender et al. 2016). Both varieties have 11% protein content, which is 53% higher than its original parents. Mahmoud et al. (2007) reported that interspecific hybrid between IR64 and *Oryza nivara* showed 12.4% the higher protein. Rice proteins are deficient in some essential amino acids (Lee et al. 2001). Therefore, rice with increased levels of glycinins, lysine, tryptophan, and sulfur-rich storage seed protein such as cysteine has been produced by transgenic approach (Wu et al. 2003; Katsube et al. 1999; Tozawa et al. 2001; Hagan et al. 2003). More recently, the WBLRP lysine-rich protein from winged bean seeds (WBLRP) was transferred into rice (Gao et al. 2001; Liu 2002) and 12% of total soluble seed protein content was observed. Two transgenic rice lines HFL1 and HFL2 (high free lysine) were obtained by a transgenic approach that showed a 25-fold increase in lysine content (Yang et al. 2016).

3.4.2 Minerals

Minerals like zinc, iron, selenium, manganese, and copper play an important role in plants and animals. It is estimated that half of the world population is affected by a mineral deficiency, and it is the greatest health concern in the human population (Pfeiffer and McClafferty 2007). It is estimated more than two billion people of the world suffer from minerals deficiency (IFAD W. FAO 2013). Therefore, the crop plants need to biofortified with these minerals.

3.4.2.1 Zinc

In comparison to other cereals, rice (*Oryza sativa* L.) contains low Zn concentration (10–22 mg/kg) with less bioavailability (Welch 1993; Myers et al. 2014). Therefore, it is necessary to increase Zn concentration in rice. Pooniya and Shivay (2013) reported that the application of Zn (as zinc sulfate heptahydrate) in Zn deficient soil significantly increased grain yield of rice as well as Zn concentration in rice grain. Zn content in rice can be enhanced through foliar or soil application of fertilizers or $ZnSO_4$ (zinc sulfate). They further reported that combined application of fertilizer with green manures and Zn increases Zn content in grains and yield in basmati rice. Agronomic biofortification of rice by the triple foliar spray of Fe, Zn, and pesticide is a cost-effective strategy to increase Fe and Zn content in rice (Zhang et al. 2018). Biofortification with Zn fertilizer in rice can increase zinc content (Welch 1986). It is reported that Zn content in rice was increased by overexpression of

OsIRT1 (Lee et al. 2009) and the introduction of mugineic acid family phytosiderophores (MAs) synthesis genes from barley (Masuda et al. 2008). "BRRI dhan-62" is the world's first Zn enriched rice variety developed by Bangladesh Rice Research Institute (BRRI) in 2013 and contains 19–20 parts per million (ppm) zinc. The variety has been developed by conventional breeding by crossing with local variety. DRR Dhan 45 (IET 23832)' is India's first zinc enriched (22.6 ppm) and high yielding rice variety produced by ICAR-IIRR and released in 2015. It was developed by a cross between IR 77080-B-34-3 and IR 73707-45-3-2-3.

3.4.2.2 Iron

Iron (Fe) deficiency is one of the most serious micronutrient deficiency problems in the human population. Iron content in plants can be enhanced by changing soil conditions such as pH, moisture content, and aeration and through fertilizer application. Wei et al. (2012) and Yuan et al. (2013) reported that Fe fertilizer through foliar application increases iron uptake and efficient translocation into the rice as compared to soil fertilizer. Macronutrient content in the soil also plays a crucial role in the enhancement of iron content in plants. For crop improvement, landraces and wild relatives are considered as an important source of donor genes (Hoisington et al. 1999). So, with this goal, IRRI evaluated 939 rice genotypes for the variability of iron content in grains. The range in Fe concentration was from 7.5 to 24.4 mg kg^{-}(Graham et al. 1999). This suggests that sufficient potential of genetic diversity exists to increase the concentration of Fe in the grain. Biofortified high iron rice was developed by conventional breeding at IRRI that has 4–5 times more iron than commercially available rice (Haas et al. 2005). Besides this, through the transgenic approach, the ferritin gene from *Phaseolus vulgaris* in rice endosperm under the control of the glutelin promoter was transferred and recorded twofold increase in the iron content (Lucca et al. 2001). Through*Agrobacterium*-mediated transformation Goto et al. (1999) transferred the soybean ferritin gene, SoyferH1, into the endosperm of rice under the control of endosperm-specific GluB-1 promoter; the transgenic rice showed three-fold more iron content than untransformed rice. Qu et al. (2005) generated transgenic rice by introducing soybean ferritin gene SoyferH-1in the endosperm under the control of the rice seed storage glutelin gene promoter, GluB-1, and the rice seed storage globulin gene promoter, Glb-1, and by introducing the SoyferH-1gene under the control of Glb-1promoter alone. They observed three-fold more iron content than non-transformed rice. Further, for the production of iron-biofortified rice was produced by Masuda et al. (2012) using combined three transgenic approaches. First, through the expression of the Fe storage protein ferritin under the control of endosperm-specific promoters to enhance Fe storage in grains. Second, by overproduction of the metal chelator nicotianamine to enhanced Fe translocation. Third, through the expression of Fe (II)–NA transporter OsYSL2 (metal nicotianamine transporter) under the control of an endosperm-specific promoter and sucrose transporter promoter to enhance Fe flux into the rice endosperm. They observed 4.4-fold increases in Fe concentration when cultivated in the field and six-fold in the greenhouse condition and concluded that for

iron biofortification, the introduction of multiple iron homeostasis genes is more effective than the introduction of an individual gene. Tan et al. (2015) generated transgenic rice by overexpression of MxIRT1 (*Malus xiaoginenses iron-regulated transporter 1*) gene from apple trees. The transgenic rice exhibited three-fold higher levels of iron and zinc content.

3.4.2.3 Selenium

Selenium is an essential element for humans and plays an important role in many metabolic pathways, production of selenoproteins, thyroid hormone metabolism, and immune functions (Malagoli et al. 2015). Selenium deficiency leads to many diseases such as Keshan disease, cardiovascular diseases, hyperthyroidism, enhanced susceptibility to infections, and cancer (Malagoli et al. 2015; Brown and Arthur 2001). Selenium content in rice is usually low, so it limits the nutritional requirement of populations that depend on rice consumption for their dietary selenium intake (Williams et al. 2009). Mangueze et al. (2018) reported that foliar Zn and Se application increases the accumulation of these minerals in the IR grains. Boldrin et al. (2013) reported that soil selenate application was more effective for Se accumulation in grain than selenite. Foliar application of both selenite and selenate enhances grain yield. Both soil and foliar Se application could be useful for increasing Se content in rice. Premarathna et al. (2012) reported that broadcast application of SeO_4^{2-} (selenate ion) enriched urea granules to flood water at the heading stage in rice was extremely effective as an agronomic biofortification strategy.

3.4.3 Vitamins

Vitamins are essential micronutrients; they cannot provide energy but play an important role in many metabolic processes. The human body cannot synthesize all vitamins, so it is necessary to get them from the diet. Rice is a poor source of vitamins. So far, through the transgenic approach, rice with vitamin A and vitamin B9 has been produced.

3.4.3.1 Vitamin A

Vitamin A deficiency can result in night blindness and xerophthalmia (Sommer 1982). Rice plants produce β-carotene, the precursor of vitamin A in green tissues, but rice grains are devoid of β-carotene. Since rice did not have any cultivar with carotenoids in the grain (Beyer 2010), transgenic approaches have been used for increasing vitamin A content in rice.

Golden Rice Golden rice was developed by Prof. Ingo Potrykus of Swiss federal institute of technology and Prof. Peter Beyer of the University of Freiburg. For the development of Golden rice, two genes encode the enzymes phytoene synthase (PSY) from daffodil and phytoene desaturase (CRTI) from the soil bacterium *Erwinia uredovora* naturally involved in carotene biosynthesis were inserted into

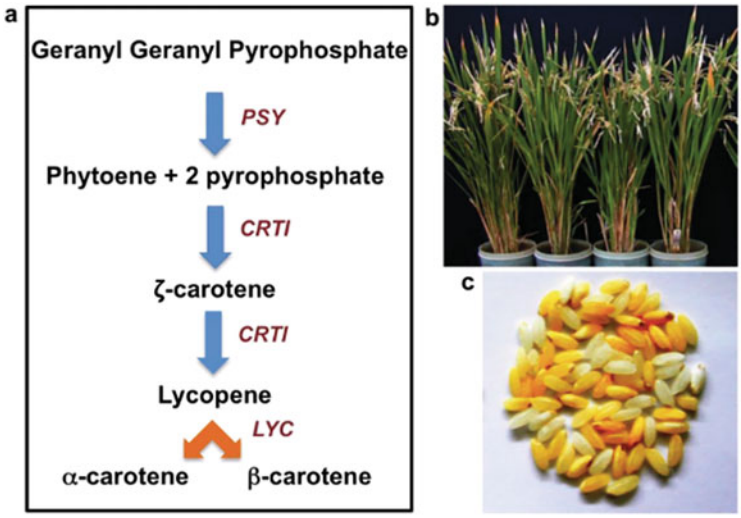

Fig. 3.3 Development of golden rice by introducing carotenoid biosynthesis pathway through transgenic approach, (**a**) Carotenoid biosynthesis pathway with genes involved in different steps; (**b**) Uniform morphology of transgenic and control rice plants; (**c**) similarly polished rice grains from golden rice (yellow and orange) mixed with control (white). The figure is reproduced from Majumder et al. (2019) available with Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)

the rice genome under the control of endosperm-specific promoter to restart the carotenoid biosynthetic pathway that is normally inactive in rice leads to the production and accumulation of β-carotene in the grains (Fig. 3.3). The new version of golden rice GR2 (31 μg/g and more β-carotene) was produced by Syngenta scientists (Paine et al. 2005), which contains a PSY gene from maize.

3.4.3.2 Vitamin B9 (Folates)

Vitamin B9 is an essential water-soluble vitamin and synthesized de novo in bacteria, fungi, and plants and but the human body does not synthesize and store vitamin B9. It plays an important role in growth and several metabolic processes. Through the transgenic approach, folate concentration in rice has been increased by overexpression of genes encoding *Arabidopsis* GTP-cyclohydrolase I (GTPCHI) and aminodeoxy- chorismate synthase (ADCS) (Storozhenko et al. 2007). They reported that folate concentration increases up to 100 times (38.3 nmol/g) over wild-type rice. Vitamins are unstable molecules; they can degrade easily. So, it is important to stabilize folate in rice. Blancquaert et al. (2015) used two strategies to stabilize folate upon storage, by complexing folates with folate binding proteins and by extending the tail of the folate molecules. They found up to 150-fold higher folate concentration than those in wild-type rice.

3.4.4 Oil Quality and Flavonoids

Rice bran is an important oil source, contains antioxidants such as flavonoids, γ-oryzanol, tocopherols and tocotrienols and phytosterol (Park et al. 2017; Sharif et al. 2014) and fatty acids such as palmitic acid, oleic acid, and linoleic acid (Taira et al. 1988) but lacks high levels of α-linolenic acid, which is beneficial for health. α-Linolenic acid content in rice has been increased by introducing the soybean microsomal omega-3 fatty acid desaturase cDNA, the enzyme of which converts linoleic acid to α-linolenic acid under the control of maize ubiquitin-1 promoter (Anai et al. 2003). Transgenic rice seed oil showed enhanced levels of α-linolenic acid content. Oleic acid is oxidatively stable in comparison to linoleic acid (Lopez-Huertas 2010). So, to produce high oleic/low linoleic in rice bran oil, Abe et al. (2018) attempted to disrupt the OsFAD2-1 (fatty acid desaturase) gene, which catalyzes the conversion of oleic acid to linoleic acid by CRISPR/Cas9-mediated targeted mutagenesis. The content of oleic acid increased up to more than twice over wild type. *Japonica* rice varieties possess more antioxidant compounds compared *to indica types* (Goufo and Trindade 2014). Flavonoid content in rice has been increased by expressing maize C1 and R-S regulatory genes (Shin et al. 2006) and phenylalanine ammonia-lyase and chalcone synthase (CHS) genes (Ogo et al. 2013).

3.4.5 Aroma and Nutrition

Proper diet is important to the health; hence aroma and taste are essential for proper diet because these are the key determining factor in acceptance and palatability of food. The aroma in the food determines the taste and has many functions such as appetite enhancement, salivation (Rogers and Hill 1989; Engelen et al. 2003; Yeomans 2006), and pleasantness during eating, stimulation, and release of insulin (Johnson and Wildman 1983) and gastric acid (Feldman and Richardson 1986). Besides these, it protects from foul and hazardous food. The aroma in food has been shown to enhance food appetite in hungry as well as in satiated states, so it may be used to stimulate meal initiation and appetite in people that are malnourished (Zoon et al. 2016). It acts as a supporting trait for nutrition; therefore, it needs to be considered in biofortification. Flavor is a combination of smell, taste, and appearance, and it is estimated that 75–95% of flavors of the food we taste actually come from what is smelled (Spence 2015). Taste buds in humans can identify five basic tastes, sweet, sour, bitter, salty, and umami and other remaining tastes are actually identified by the nose through aroma (Choi and Han 2015). Most of the aromatic molecules are volatile such as ether, alcohol, amines, ester, aldehyde, and essential oil, etc. A very minute amount of these molecules can change the flavor of food, and can directly affect the business of food industries.

3.4.5.1 How Is Aroma Perceived?

Richard Axel from Howard Hughes Medical Institute at Columbia University, New York, and from Buck Fred Hutchinson Cancer Research Center in Seattle,

Washington. They discovered a gene family consisting of 1000 different genes (3% of human genes) that code for the different olfactory receptors. Compared with the other sensory receptor genes, these are the largest number of genes for one particular system; the olfactory system has been shown to have an important influence. The aroma perception takes place in two ways, orthonasal and retronasal (Landis et al. 2005). The first way, orthonasal is the detection of an aromatic molecule through the nose by sniffing. And the second way, retronasal, is the detection of aromatic molecules that are released from food during eating and drinking. During eating, aromatic molecules are pumped into the nasopharynx, then detected by the same olfactory receptors which are responsible for orthonasal olfaction (Mozell et al. 1969; Halpern 2003). Aromatic molecules are detected by the G protein receptors, which are present on the surface of olfactory cells of the olfactory epithelium. After binding, the receptor triggers a sequence of signals in an olfactory system. The olfactory epithelium passes the signal to the olfactory bulb, which is situated in the brain. Through the olfactory bulb, signals are transferred to the cortex, and response is generated. The proper perception of aroma in the brain is still not fully understood.

3.4.5.2 Aromatic Rice

The aroma is one of the most precious characteristics of rice. Nowadays consumers prefer fragrant rice due to their characteristic and pleasant odor. The aroma is rated the highest desired trait, followed by taste and elongation after cooking by Indian consumers (Bhattacharjee et al. 2002). Asian consumers from the USA consider appearance and aroma as the most important acceptance factors of cooked rice (Meullenet et al. 2001). Hence the demand for aromatic rice is increasing in both domestic and international markets. Several reports show that aromatic rice varieties exhibited higher nutrient content (Gregorio et al. 2000; Renuka et al. 2016).

2-Acetyl-1-Pyrroline (2AP) as a Potent Aroma Molecule and Other Aroma Volatiles

The aroma is a result of more than 250 volatile and non-volatile compounds. 2-Acetyle-1-pyrroline (2AP) has been considered as primary contributor in imparting unique popcorn-like aroma in rice (Buttery et al. 1982). It has been reported that 2AP also gives "roasted flavor" in different food products viz. popcorn (Schieberle 1991), cooked beef, wheat, and rye bread (Schieberle and Grosch 1985), wetted ground pearl millets (Seitz et al. 1993), etc. It has been reported that in addition to rice, 2AP is found in many other plants (Pandan, bread flower, Soybean etc.), animals, fungi, and bacteria also (Wakte et al. 2017). Besides being naturally found in plants, the 2-AP molecule can be synthesized by Maillard reaction, a nonenzymatic reaction between sugar and the amino acid at high temperature (Fuganti et al. 2007)). It is reported that the concentration of 2AP also depends on storage duration and post-harvest treatment (Widjaja et al. 1996; Hashemi et al. 2013; Goufo et al. 2010). Hien et al. (2006) reported that the concentration of 2-AP in the plant is affected by environmental and genetic factors. There are lots of environmental factors that affect the concentration of aroma in plants viz. water stress (Bradbury et al. 2005), abiotic stress (Goufo et al. 2010).

Compounds like alk-2-enals, alka(E)-2, alkanals, 4-dienals, 2-pentylfurans, 2-phenylethanol and 2-acetyl-1-pyroline have been reported as major contributors in total aroma profiling of rice (Gaur et al. 2016).

Inactivation of BADH2 Gene Leads to Aroma

Any mutational event in functional BADH2 (*Betaine aldehyde dehydrogenase 2*) results in premature termination in the gene producing a truncated protein that results in nullification of the function of the enzyme BADH2 and leads to the synthesis of 2AP. These mutations include 8 bp deletion and 3 single nucleotide polymorphism sites (SNPs) in the seventh exon of Badh2 gene (Bradbury et al. 2005), 806 bp deletion in exon 4–5 (Shao et al. 2013), 7 bp deletion in exon 2 (Shi et al. 2008), and 2 bp deletion in exon 1 (Kovach et al. 2009); these mutations revealed that the aroma is a result of multiple mutational events in BADH2 causing defective BADH2 protein.

Aroma and Biofortification

Indian sub-continent is considered as a home for aromatic rice diversity (Bisne and Sarawgi 2008). Major attention was given on the improvement of basmati rice type. The improvement of indigenous aromatic non-basmati rice, which showed outstanding quality like aroma, taste, and kernel elongation after cooking and were somewhat neglected because they lacked market value; therefore it needs to be focused on the improvement of indigenous aromatic non-basmati rice type along with basmati type.

There are several examples of improved aromatic rice varieties that are developed by conventional breeding (Table 3.2). India has become the first country for the development of a hybrid variety of basmati rice, Pusa Rice Hybrid-10 (RH-10). Indian Agricultural Research Institute (IARI) released this variety for commercial seed production; apart from this IARI had also developed high yielding basmati varieties viz. Pusa Basmati 1, Pusa Sugandh 2, and Pusa Sugandh 3. Pusa Basmati 1121 released by ICAR-IARI for commercial cultivation, having pleasant aroma, extra-long slender milled grains (9.00 mm) and high cookedkernel elongation ratio of 2.5 with a cooked kernel length of up to 22 mm, volume expansion more thanfour times, good tasteand easy digestibility (Singh et al. 2018). LSU Agriculture Rice Research Station developed the Jazzman rice variety, first US-bred Jasmine-type aromatic rice by cross breeding between the Arkansas variety Ahrent and the Chinese aromatic rice line 96a-8Jazzman has a strong aroma, extremely translucent grains and soft sweet on cooking.

Lei et al. (2017) reported that exogenous application of mixed micronutrients improves yield, quality, and 2-acetyl-1-pyrroline contents in fragrant rice. In vitro study of detached aromatic rice panicles reported that the application of 2-AP, Zn, and La significantly increases 2-AP concentration, Zn and La might be helpful for aroma improvement in rice. Deshmukh et al. (2016) and Mo et al. (2016) reported that the inoculation of rhizobacteria in the basmati rice-growing area at regular intervals might play a role in the enhancement of aroma.

In addition to the non-transgenic approach, the advance transgenic approaches are also promoted and applied for the improvement of aromatic rice varieties (Shan et al. 2015; Niu et al. 2008; Chen et al. 2012)). It is reported that overexpression of the P5CS gene in aromatic rice cultivars results in a more than

Table 3.2 List of some improved aromatic rice varieties through conventional breeding

Sr. no	Name of variety	Parentages	Salient features	Reference/ source
1	Pawana	Pusa-33 × IR-28	Semi-dwarf, grains: Long slender, resistant to blast, moderately resistant to leaf scald, tolerant to major pests	http://drdpat.bih.nic.in/
2	Himalaya-2	Sabarmati × Ratna	Semi-dwarf, grains: Long bold resistant to blast, susceptible to glume blotch	http://drdpat.bih.nic.in/
3	Type-3	A selection from basmati of Dehradun	Tall, grains: Long slender, white, very fine	http://drdpat.bih.nic.in/
4	Punjab Basmati-1	Sona × Basmati-370	Medium tall, spikelet awned, grains: Long slender	http://drdpat.bih.nic.in/
5	Prabhavati (PBN-1)	Mutant of Ambemohar local variety	Dwarf, grains: Coarse, tolerant to iron chlorosis	http://drdpat.bih.nic.in/
6	SYE-ER-1 (IET-9296)	Sona × SYE-44-3	Grains: Short slender, moderately resistant to blast	http://drdpat.bih.nic.in/
7	Indrayani (IET-12897)	Amb-157 × IR-8	Semi dwarf, grains: Long slender, moderately susceptible to leaf scald, resistant to blast	http://drdpat.bih.nic.in/
8	Mahi Sugandha	BK-79 × Basmati-370	Semi-dwarf, grains: Long slender, strongly scented	http://drdpat.bih.nic.in/
9	Karjat-3 (IET-12481)	IR-36 × Karjat 35-3	Grains: Short bold	http://drdpat.bih.nic.in/
10	Taraori Basmati	Pure line selection from local Basmati	Tall, grains: Long slender	http://drdpat.bih.nic.in/
11	Ranbir Basmati (IET-11348)	Pure line selection from Basmati-370-90-95	Grains: Long slender	http://drdpat.bih.ni5n/
12	Pusa Basmati-1	Pusa150 × Karnal local	Extra elongation upon cooking, fine texture, grains: Long slender	Siddiq et al. (2009)
13	Basmati 385	TN1 × Basmati370	Grains: Long slender	Nagaraju et al. (2002)
14	Pusa 1121	Sister line of Pusa Basmati-1	Grains: Extra-long slender, high cooked kernel elongation ratio	Singh et al. (2018)
15	Pusa RH 10	Pusa 6A × PRR 78	Superfine grain, awnless	Siddiq et al. (2009)

(continued)

Table 3.2 (continued)

Sr. no	Name of variety	Parentages	Salient features	Reference/ source
16	Vasumathi	PR 109 × Pak. Basmati	Semi-dwarf high yielding	Shobha Rani and Singh (2003)
17	Bhogavati	Selection from Basmati composite	High yielding, resistant to leaf and neck blast	Kumbhar and Sarawate (2010)
18	Pusa Basmati 6	Pusa Basmati 1 × PB 1121	Cooked rice uniform in shape, strong aroma	Siddiq et al. (2012)
19	Jazzman	Arkansas variety Ahrent × Chinese aromatic rice line 96a-8	Soft-cooking, grains: Long slender, no seed dormancy, high yielding, moderately early-maturing, strong aroma	Sha et al. (2011)
20	Jasmine 85	IR262 × Khao Dawk Mali 105	Disease resistant, soft texture	Marchetti et al. (1998)

two-fold increase in 2AP content (Kaikavoosi et al. 2015). Niu et al. (2008) reported that RNAi-based downregulation of OsBADH2 expression influences aroma accumulation in rice. Transcription activator-like effector nucleases (TALENs) method was used by Shan, Zhang et al. (2015) to target and disrupt the OsBADH2 gene for development of aromatic rice, their result revealed significant increased in the 2AP content in transgenic line, this method provides rice breeders with a new approach to breed fragrant rice. A single customized sgRNA (single guide RNA) was used to bring target mutations in three rice genes, OsBADH2, OsMPK2, and Os02g2382 used by Shan et al. (2013) and their result revealed that CRISPR/Cas9 system has higher mutation frequency as compared to TALENs. All these studies will give us important information about the development of biofortified aromatic rice varieties.

3.5 Advantages of Biofortification

Biofortification can be a supportable approach for culminating nutritional security along with other strategies like dietary supplementation, diversification, and commercial fortification. The ideal advantages of biofortification are:

1. From an economic viewpoint, it is a one-time investment to develop biofortified seeds, thus cost-effective, and it is beneficial for controlling micronutrient deficiencies, which ultimately improves human health.
2. The most important advantage of biofortification is it does not compromise with yield; therefore, it is economically sustainable to farmers.

3. It capitalizes on the regular daily consumption of large and consistent amounts of staple food across populations regardless of gender, age, and economic status.
4. Constant monitoring is not needed for biofortified crops as it is highly sustainable.

3.6 Challenges

For biofortification to be successful and acceptable by consumers, many broad questions must be addressed.

Can biofortification increase the micronutrient density in food to a target level that makes a significant and measurable impact on nutritional status?

Will farmers accept to grow the biofortified varieties of staple crops, and will consumers eat/buy them in sufficient quantities? To answer these questions, research must be carried out.

Getting consumers to accept biofortified crops will be a challenge, but with the help of good seed systems, development of products and markets, and demand creation, this can be solved (Nestel et al. 2006). A successful biofortification strategy requires the adaptation of fortified crops by farmers and consumers.

The success of biofortified crops also relies on good market networks and channels for the distribution of agricultural information. In developing countries, lack of agricultural infrastructure leads to a significant challenge for the adaptation of biofortified crops.

Though the several crops were developed as a proof of concept for biofortification, precise studies of their nutritional impact are needed so that the demand for biofortified food will increase.

Adequate information programs are needed to create public awareness and will play an essential role in ensuring acceptance by the public and farmers.

Strong policy interventions are needed to create interlinking between biofortified producers and various national programs like Rashtriya Krishi Vikas Yojana. High profit would make farmers interested to grow these improved cultivars. Several government-sponsored programs such as the National Food Security Mission and integrated Child Development Program and integration of biofortified grains in mid-day meal scheme would provide an impetus for its popularization.

3.7 Organizations

The HarvestPlus program is an international organization that aims to develop micronutrient-rich staple foods to address human micronutrient malnutrition (Singh et al. 2017). The program has targeted grain Zn levels of brown and polished rice. A combination of agronomic and genetic strategies is required to raise grain Zn concentration as many rice fields have low availability of Zn. The transgenic approaches can be advanced through germplasm screening of traditional varieties, old landraces, and wild species to create novel genetic tools to enhance the Zn level in rice grain (Nakandalage et al. 2016). Other organizations viz. DBT, ICMR, and

ICAR, along with international organizations like HarvestPlus, IRRI are now giving their research efforts to biofortification for product development validation and testing. With a proper idea, planning, implementation, and execution, biofortified crops will help to reduce micronutrients malnutrition with a minimum investment in research in India and have a measurable impact on the lives and health of millions of people in the country. In 2004, DBT initiated the India biofortification program for wheat, maize, and rice biofortified with Zn, Fe, and provitamin A. In XI plan, DBT funded biofortification of pigeon pea and groundnut for enhancing vitamin A and sorghum for high Zn and Fe content.

3.8 Future Prospects

To increase micronutrient concentrations in staple/edible crops, future research should focus on the identification of the mechanisms involved in micronutrient uptake, mineral-homeostasis in plant cells, integration of agronomic and transgenic approaches to develop a novel strategy to increase mineral transport to tissues. There is a need to refine the planning and monitoring of biofortification programs, considering the biofortification technologies and stakeholders/NGOs that fund biofortification programs. Although the HarvestPlus program is performing a great job, it is required to set priorities and indicators to evaluate the performance of biofortification programs. Regarding the production and consumption of biofortified crops, there is a need to develop marketing and communication strategies considering all issues. Quality assurance and food safety are vital; possible risks of excessive intake must be addressed. Considering environmental changes, possible toxicities, and allergies related to enhanced micronutrient intake still require explosive work. National policies and international standards on food content information and health claims must be highlighted. The transgenic approach requires a convenient regulatory framework for its adaptation. To reach as many as world's population by 2030, with biofortified crops, policymakers must give high priority to the role of agriculture to refine health. National governments and other institutions must ensure that biofortification is included or based on the nutrition agenda. For further strengthening, research coordination between the agriculture and nutrition specialists requires to decide the target level of micronutrients and proteins, their storage, processing, cooking, and potential levels of consumption by the consumer. Food processors and other factors, along with the value chain, must incorporate biofortified crops in their products. Only through a collaborative effort that reaches across the value chain, biofortification will become successful in upcoming years to overcome the problem of micronutrient malnutrition and hidden hunger.

3.9 Conclusion

Recent research reports and developments conclude that an increase in the concentration of micronutrients can be retained in the edible parts after processing, and nutrients are available after consumption by humans. Biofortification is now a proven technology to fight against micronutrient malnutrition and hidden hunger, especially in developing countries where most people rely on staple food crops, which are inherently low in micronutrient concentrations. Enhanced use of fertilizers with required micronutrients, conventional breeding, and genetic engineering are used to develop biofortified crops, and it is being introduced in many countries as a strategy to improve human health. No single type of intervention can, by itself, solve the problem of micronutrient malnutrition. Therefore, biofortification is one of the several evidence-based interventions which can complement the existing interventions with its own implications in improving the overall quality of the diet achieving nutritional security. In recent years biofortification has been shown as a feasible, cost-effective, and promising approach of delivering micronutrients to populations who may have limited access to diverse diets and other inventions and efforts are going on to improve global nutrition by its use further. Although biofortification has many advantages, to ensure sustained impact, it requires continued investment and interest by governments for monitoring the delivery, and in addition, investments by donors for both existing and new programs can further improve impact and footprint. Large scale biofortification should be integrated into nutrition-sensitive and nutrition-specific efforts to control and prevent micronutrient malnutrition.

References

Abe K, Araki E, Suzuki Y, Toki S, Saika H (2018) Production of high oleic/low linoleic rice by genome editing. Plant Physiol Biochem 131:58–62

Abbas A, Murtaza S, Aslam F, Khawar A, Rafique S, Naheed S (2011) Effect of processing on nutritional value of rice (Oryza sativa). World J Med Sci 6(2):68–73

Ahuja U, Ahuja S, Chaudhary N, Thakrar R (2007) Red Rices–past, present and future. Asian Agri-History 11(4):291–304

Allaway W (1986) Soil-plant-animal and human interrelationships in trace element nutrition. Trace elements in human and animal nutrition. Academic Press, Orlando

Anai T, Koga M, Tanaka H, Kinoshita T, Rahman S, Takagi Y (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

Babu R, Rojas NP, Gao S, Yan J, Pixley K (2013) Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on provitamin A concentrations for 26 tropical maize populations. Theor Appl Genet 126(2):389–399

Bailey RL, West KP Jr, Black RE (2015) The epidemiology of global micronutrient deficiencies. Ann Nutr Metab 66(Suppl. 2):22–33

Bansal R, Rana N, Kumawat S, Kumar N, Preeti P, Bhatt V, Waqar A, Altaf N, Katara JL, Samantaray S, Deshmukh R (2019) Advances in induced mutagenesis and mutation mapping approaches in rice. Oryza 56(Special issue):8

Bashir K, Takahashi R, Nakanishi H, Nishizawa NK (2013) The road to micronutrient biofortification of rice: progress and prospects. Front Plant Sci 4:15

Beyer P (2010) Golden Rice and 'Golden' crops for human nutrition. New Biotechnol 27 (5):478–481

Bhattacharjee P, Singhal RS, Kulkarni PR (2002) Basmati rice: a review. Int J Food Sci Technol 37 (1):1–12

Bisne R, Sarawgi A (2008) Agro-morphological and quality characterization of Badshah bhog group from aromatic rice germplasm of Chhattisgarh. Bangladesh J Agric Res 33(3):479–492

Blancquaert D, Van Daele J, Strobbe S, Kiekens F, Storozhenko S, De Steur H, Gellynck X, Lambert W, Stove C, Van Der Straeten D (2015) Improving folate (vitamin B 9) stability in biofortified rice through metabolic engineering. Nat Biotechnol 33(10):1076

Boldrin PF, Faquin V, Ramos SJ, Boldrin KVF, Ávila FW, Guilherme LRG (2013) Soil and foliar application of selenium in rice biofortification. J Food Compos Anal 31(2):238–244

Bouis HE (2018) Biofortification: an agricultural tool to address mineral and vitamin deficiencies. In: Food fortification in a globalized world. Elsevier, San Diego, pp 69–81

Bouis HE, Welch RM (2010) Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50(Supplement_1):S-20–S-32

Bradbury LM, Henry RJ, Jin Q, Reinke RF, Waters DL (2005) A perfect marker for fragrance genotyping in rice. Mol Breed 16(4):279–283

Brown KM, Arthur J (2001) Selenium, selenoproteins and human health: a review. Public Health Nutr 4(2b):593–599

Buttery RG, Ling LC, Juliano BO (1982) 2-Acetyl-1-pyrroline: an important aroma component of cooked rice. Chem Ind 23:958–959

Chaudhari PR, Tamrakar N, Singh L, Tandon A, Sharma D (2018) Rice nutritional and medicinal properties: A. J Pharmacogn Phytochem 7(2):150–156

Chaudhary R (2003) Speciality rices of the world: effect of WTO and IPR on its production trend and marketing

Chaudhary J, Deshmukh R, Sonah H (2019a) Mutagenesis approaches and their role in crop improvement. Plan Theory 8(11):467

Chaudhary J, Deshmukh R, Mir ZA, Bhat JA (2019b) Metabolomics: an emerging technology for soybean improvement. In: Biotechnology products in everyday life. Springer, Cham, pp 175–186

Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari A, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H (2019c) Advances in omics approaches for abiotic stress tolerance in tomato. Biology 8(4):90

Chaudhary J, Alisha A, Bhatt V, Chandanshive S, Kumar N, Mir Z, Kumar A, Yadav SK, Shivaraj S, Sonah H (2019d) Mutation breeding in tomato: advances, applicability and challenges. Plan Theory 8(5):128

Chen L, Yang F, Xu J, Hu Y, Hu Q, Zhang Y, Pan G (2002) Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. J Agric Food Chem 50(18):5128–5130

Chen M, Wei X, Shao G, Tang S, Luo J, Hu P (2012) Fragrance of the rice grain achieved via artificial microRNA-induced down-regulation of OsBADH2. Plant Breed 131(5):584–590

Cheng L, Wang F, Shou H, Huang F, Zheng L, He F, Li J, Zhao F-J, Ueno D, Ma JF (2007) Mutation in nicotianamine aminotransferase stimulated the Fe (II) acquisition system and led to iron accumulation in rice. Plant Physiol 145(4):1647–1657

Choi N-E, Han JH (2015) How flavor works: the science of taste and aroma. Wiley, Chichester

Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R, Vuong T, Valliyodan B, Nguyen HT (2014) Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci 5:244

Deshmukh Y, Khare P, Patra D (2016) Rhizobacteria elevate principal basmati aroma compound accumulation in rice variety. Rhizosphere 1:53–57

Engelen L, de Wijk RA, Prinz JF, van der Bilt A, Bosman F (2003) The relation between saliva flow after different stimulations and the perception of flavor and texture attributes in custard desserts. Physiol Behav 78(1):165–169

Feldman M, Richardson CT (1986) Role of thought, sight, smell, and taste of food in the cephalic phase of gastric acid secretion in humans. Gastroenterology 90(2):428–433

Fuganti C, Gatti FG, Serra S (2007) A general method for the synthesis of the most powerful naturally occurring Maillard flavors. Tetrahedron 63(22):4762–4767

Gao Y, Jing Y, Shen S, Tian S, Kuang T, Samuel SS (2001) Transfer of lysine-rich protein gene into rice and production of fertile transgenic plants. Acta Bot Sin 43(5):506–511

Garcia-Casal MN, Peña-Rosas JP, Pachón H, De-Regil LM, Tablante EC, Flores-Urrutia MC (2016) Staple crops biofortified with increased micronutrient content: effects on vitamin and mineral status, as well as health and cognitive function in the general population. Cochrane Database Syst Rev(8). https://doi.org/10.1002/14651858.cd012311

Gaur A, Wani S, Deepika P, Bharti N, Malav A, Shikari A, Bhat A (2016) Understanding the fragrance in rice. Rice Res 4. https://doi.org/10.4172/2375-4338.1000e125

Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) Iron fortification of rice seed by the soybean ferritin gene. Nat Biotechnol 17(3):282

Goufo P, Trindade H (2014) Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Sci Nutr 2 (2):75–104

Goufo P, Duan M, Wongpornchai S, Tang X (2010) Some factors affecting the concentration of the aroma compound 2-acetyl-1-pyrroline in two fragrant rice cultivars grown in South China. Front Agric China 4(1):1–9

Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I (1999) Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crop Res 60 (1–2):57–80

Gregorio GB, Senadhira D, Htut H, Graham RD (2000) Breeding for trace mineral density in rice. Food Nutr Bull 21(4):382–386

Haas JD, Beard JL, Murray-Kolb LE, Del Mundo AM, Felix A, Gregorio GB (2005) Iron-biofortified rice improves the iron stores of nonanemic Filipino women. J Nutr 135 (12):2823–2830

Hagan N, Upadhyaya N, Tabe L, Higgins T (2003) The redistribution of protein sulfur in transgenic rice expressing a gene for a foreign, sulfur-rich protein. Plant J 34(1):1–11

Halpern BP (2003) When are oral cavity odorants available for retronasal olfaction? In: Handbook of flavor characterization. CRC Press, Boca Raton, pp 69–79

Hashemi FG, Rafii M, Ismail M, Mahmud T, Rahim H, Asfaliza R, Malek M, Latif M (2013) Biochemical, genetic and molecular advances of fragrance characteristics in rice. Crit Rev Plant Sci 32(6):445–457

Hien NL, Yoshihashi T, Sarhadi WA, Hirata Y (2006) Sensory test for aroma and quantitative analysis of 2-acetyl-1-pyrroline in Asian aromatic rice varieties. Plant Prod Sci 9(3):294–297

Hoisington D, Khairallah M, Reeves T, Ribaut J-M, Skovmand B, Taba S, Warburton M (1999) Plant genetic resources: what can they contribute toward increased crop productivity? Proc Natl Acad Sci 96(11):5937–5943

IFAD W. FAO (2013) The state of food insecurity in the world: the multiple dimensions of food security

Jeng TL, Lin YW, Wang CS, Sung JM (2012) Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the indica type cultivar IR64. J Food Compos Anal 28(2):149–154

Johnson WG, Wildman HE (1983) Influence of external and covert food stimuli on insulin secretion in obese and normal persons. Behav Neurosci 97(6):1025

Juliano B (1985) Criteria and tests for rice grain qualities

Kaikavoosi K, Kad TD, Zanan RL, Nadaf AB (2015) 2-Acetyl-1-pyrroline augmentation in scented indica rice (Oryza sativa L.) varieties through Δ 1-pyrroline-5-carboxylate synthetase (P5CS) gene transformation. Appl Biochem Biotechnol 177(7):1466–1479

Katsube T, Kurisaka N, Ogawa M, Maruyama N, Ohtsuka R, Utsumi S, Takaiwa F (1999) Accumulation of soybean glycinin and its assembly with the glutelins in rice. Plant Physiol 120(4):1063–1074

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35(1–2):25–34

Khush GS (2000) Rice germplasm enhancement at IRRI. Phillipp J Crop Sci 25:45–51

Khush GS, Lee S, Cho J-I, Jeon J-S (2012) Biofortification of crops for reducing malnutrition. Plant Biotechnol Rep 6(3):195–202

Kovach MJ, Calingacion MN, Fitzgerald MA, McCouch SR (2009) The origin and evolution of fragrance in rice (Oryza sativa L.). Proc Natl Acad Sci 106(34):14444–14449

Kumawat S, Rana N, Bansal R, Vishwakarma G, Mehetre ST, Das BK, Kumar M, Kumar Yadav S, Sonah H, Sharma TR (2019) Expanding avenue of fast neutron mediated mutagenesis for crop improvement. Plan Theory 8(6):164

Kumbhar S, Sarawate C (2010) Bhogavati (1ET 13549)-a new scented rice variety for Maharashtra. J Maharashtra Agric Univ 35(2):229–232

Landis BN, Frasnelli J, Reden J, Lacroix JS, Hummel T (2005) Differences between orthonasal and retronasal olfactory functions in patients with loss of the sense of smell. Arch Otolaryngol Head Neck Surg 131(11):977–981

Lee SI, Kim HU, Lee Y-H, Suh S-C, Lim YP, Lee H-Y, Kim H-I (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(1):75–84

Lee S, Jeon US, Lee SJ, Kim Y-K, Persson DP, Husted S, Schjørring JK, Kakei Y, Masuda H, Nishizawa NK (2009) Iron fortification of rice seeds through activation of the nicotianamine synthase gene. Proc Natl Acad Sci 106(51):22014–22019

Lei S, Wang C, Ashraf U, Mo Z, Nawaz M, Ashraf I, Muzaffar W, Liu S, Tang X (2017) Exogenous application of mixed micro-nutrients improves yield, quality, and 2-acetyl-1-pyrroline. Appl Ecol Environ Res 15(3):1097–1109

Liu Q (2002) Genetically engineering rice for increased lysine. PhD dissertation, Yangzhou University and the Chinese University of Hong Kong

Lopez-Huertas E (2010) Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmacol Res 61(3):200–207

Lucca P, Hurrell R, Potrykus I (2001) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor Appl Genet 102(2–3):392–397

Mahender A, Anandan A, Pradhan SK, Pandit E (2016) Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. Springerplus 5 (1):2086

Mahmoud AA, Sukumar S, Krishnan HB (2007) Interspecific rice hybrid of Oryza sativa× Oryza nivara reveals a significant increase in seed protein content. J Agric Food Chem 56(2):476–482

Majumder S, Datta K, Datta SK (2019) Rice biofortification: high iron, zinc, and vitamin-A to fight against "hidden hunger". Agronomy 9(12):803

Malagoli M, Schiavon M, Pilon-Smits EA (2015) Effects of selenium biofortification on crop nutritional quality. Front Plant Sci 6:280

Mandić ML, Kenjerić D, Pirički AP (2009) Intake of some minerals in healthy adult volunteers from eastern Croatia. Int J Food Sci Nutr 60(suppl 5):77–87

Mangueze AVJ, Pessoa MF, Silva MJ, Ndayiragije A, Magaia HE, Cossa VS, Reboredo FH, Carvalho ML, Santos JP, Guerra M (2018) Simultaneous Zinc and selenium biofortification in rice. Accumulation, localization and implications on the overall mineral content of the flour. J Cereal Sci 82:34–41

Marchetti M, Bollich C, Webb B, Jackson B, McClung A, Scott J, Hung H (1998) Registration of 'Jasmine 85' rice. Crop Sci 38:896

Masuda H, Suzuki M, Morikawa KC, Kobayashi T, Nakanishi H, Takahashi M, Saigusa M, Mori S, Nishizawa NK (2008) Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. Rice 1(1):100–108

Masuda H, Ishimaru Y, Aung MS, Kobayashi T, Kakei Y, Takahashi M, Higuchi K, Nakanishi H, Nishizawa NK (2012) Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. Sci Rep 2:543

Meullenet JF, Griffin V, Carson K, Davis G, Davis S, Gross J, Hankins J, Sailer E, Sitakalin C, Suwansri S (2001) Rice external preference mapping for Asian consumers living in the United States. J Sens Stud 16(1):73–94

Mo Z, Huang J, Xiao D, Ashraf U, Duan M, Pan S, Tian H, Xiao L, Zhong K, Tang X (2016) Supplementation of 2-Ap, Zn and La improves 2-acetyl-1-pyrroline concentrations in detached aromatic rice panicles in vitro. PLoS One 11(2):e0149523

Mozell MM, Smith BP, Smith PE, Sullivan RL, Swender P (1969) Nasal chemoreception in flavor identification. Arch Otolaryngol 90(3):367–373

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ, Carlisle E, Dietterich LH, Fitzgerald G, Hasegawa T (2014) Increasing CO_2 threatens human nutrition. Nature 510 (7503):139

Nagaraju J, Kathirvel M, Kumar RR, Siddiq E, Hasnain SE (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc Natl Acad Sci 99(9):5836–5841

Nakandalage N, Nicolas M, Norton RM, Hirotsu N, Milham PJ, Seneweera S (2016) Improving rice zinc biofortification success rates through genetic and crop management approaches in a changing environment. Front Plant Sci 7:764

Nestel P, Bouis HE, Meenakshi J, Pfeiffer W (2006) Biofortification of staple food crops. J Nutr 136 (4):1064–1067

Niu X, Tang W, Huang W, Ren G, Wang Q, Luo D, Xiao Y, Yang S, Wang F, Lu B-R (2008) RNAi-directed downregulation of OsBADH2 results in aroma (2-acetyl-1-pyrroline) production in rice (Oryza sativa L.). BMC Plant Biol 8(1):100

Ogo Y, Ozawa K, Ishimaru T, Murayama T, Takaiwa F (2013) Transgenic rice seed synthesizing diverse flavonoids at high levels: a new platform for flavonoid production with associated health benefits. Plant Biotechnol J 11(6):734–746

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Pena R (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J Cereal Sci 46 (3):293–307

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23(4):482

Park H-Y, Lee K-W, Choi H-D (2017) Rice bran constituents: immunomodulatory and therapeutic activities. Food Funct 8(3):935–943

Pengkumsri N, Chaiyasut C, Saenjum C, Sirilun S, Peerajan S, Suwannalert P, Sirisattha S, Sivamaruthi BS (2015) Physicochemical and antioxidative properties of black, brown and red rice varieties of northern Thailand. Food Sci Technol 35(2):331–338

Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47 (Supplement_3):S-88–S-105

Pooniya V, Shivay YS (2013) Enrichment of basmati rice grain and straw with zinc and nitrogen through ferti-fortification and summer green manuring under Indo-Gangetic plains of India. J Plant Nutr 36(1):91–117

Premarathna L, McLaughlin MJ, Kirby JK, Hettiarachchi GM, Stacey S, Chittleborough DJ (2012) Selenate-enriched urea granules are a highly effective fertilizer for selenium biofortification of paddy rice grain. J Agric Food Chem 60(23):6037–6044

Qu LQ, Yoshihara T, Ooyama A, Goto F, Takaiwa F (2005) Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. Planta 222(2):225–233
Ramberg J, McAnalley B (2002) From the farm to the kitchen table: a review of the nutrient losses in foods. GlycoScience Nutr 3(5):1–12
Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). Crit Rev Food Sci Nutr: 1–17. https://doi.org/10.1080/10408398.2019.1685454
Renuka N, Mathure SV, Zanan RL, Thengane RJ, Nadaf AB (2016) Determination of some minerals and β-carotene contents in aromatic indica rice (Oryza sativa L.) germplasm. Food Chem 191:2–6
Rogers PJ, Hill AJ (1989) Breakdown of dietary restraint following mere exposure to food stimuli: interrelationships between restraint, hunger, salivation, and food intake. Addict Behav 14 (4):387–397
Roy P, Orikasa T, Okadome H, Nakamura N, Shiina T (2011) Processing conditions, rice properties, health and environment. Int J Environ Res Public Health 8(6):1957–1976
Schieberle P (1991) Primary odorants in popcorn. J Agric Food Chem 39(6):1141–1144
Schieberle P, Grosch W (1985) Identification of the volatile flavour compounds of wheat bread crust—comparison with rye bread crust. Z Lebensm Unters Forsch 180(6):474–478
Seitz LM, Wright RL, Waniska RD, Rooney LW (1993) Contribution of 2-acetyl-1-pyrroline to odors from wetted ground pearl millet. J Agric Food Chem 41(6):955–958
Sevanthi A, Kandwal P, Kale PB, Prakash C, Ramkumar M, Yadav N, Mahato AK, Sureshkumar V, Behera M, Deshmukh RK (2018) Whole genome characterization of a few EMS-induced mutants of upland rice variety Nagina 22 reveals a staggeringly high frequency of SNPs which show high phenotypic plasticity towards the wild-type. Front Plant Sci 9:1179
Sha X, Linscombe S, Jodari F, Chu Q, Groth D, Blanche S, Harrell D, White L, Oard J, Chen M (2011) Registration of 'Jazzman' aromatic long-grain rice. J Plant Registr 5(3):304–308
Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu J-L (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol 31(8):686
Shan Q, Zhang Y, Chen K, Zhang K, Gao C (2015) Creation of fragrant rice by targeted knockout of the Os BADH 2 gene using TALEN technology. Plant Biotechnol J 13(6):791–800
Shao G, Tang S, Chen M, Wei X, He J, Luo J, Jiao G, Hu Y, Xie L, Hu P (2013) Haplotype variation at Badh2, the gene determining fragrance in rice. Genomics 101(2):157–162
Sharif MK, Butt MS, Anjum FM, Khan SH (2014) Rice bran: a novel functional ingredient. Crit Rev Food Sci Nutr 54(6):807–816
Shi W, Yang Y, Chen S, Xu M (2008) Discovery of a new fragrance allele and the development of functional markers for the breeding of fragrant rice varieties. Mol Breed 22(2):185–192
Shin YM, Park HJ, Yim SD, Baek NI, Lee CH, An G, Woo YM (2006) Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. Plant Biotechnol J 4(3):303–315
Shobha Rani N, Singh R (2003) Efforts on aromatic rice improvement in India. In: A treatise on the scented rices of India. Kalyani Publishers, New Delhi, pp 23–72
Siddiq EA (1992) Rice in India potential areas for research and development. Oryza (India)
Siddiq E, Singh V, Zaman F, Sadananda A, Abraham M, Hari Prasad A, Mahendru A, Natrajan U, Nagarajan M, Atwal S (2009) Development of high yielding Basmati quality rice varieties: a success story. Indian Farming 59(1):13–17
Siddiq E, Vemireddy L, Nagaraju J (2012) Basmati Rices: genetics, breeding and trade. Agric Res 1 (1):25–36
Singh RK, Singh US (2003) Treatise on the scented rices of India. Kalyani Publishers, New Delhi
Singh RK, Singh US, Khush GS, Rohilla R, Singh JP, Singh G, Shekhar KS (2000) Small and medium grained aromatic rices of India. Aromatic rices. Oxford & IBH, New Delhi, pp 155–177

Singh V, Yadav R, Yadav N, Yadav R, Malik R, Singh J (2017) Identification of genomic regions/genes for high iron and zinc content and cross transferability of SSR markers in mungbean (Vigna radiata L.). Legume Research: An International Journal 40(6):1004

Singh V, Singh AK, Mohapatra T, Ellur RK (2018) Pusa Basmati 1121–a rice variety with exceptional kernel elongation and volume expansion after cooking. Rice 11(1):19

Sommer A (1982) Nutritional blindness. Xerophthalmia and keratomalacia. Oxford University Press, New York

Spence C (2015) Just how much of what we taste derives from the sense of smell? Flavour 4(1):30

Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang G-F, Lambert W, Van Der Straeten D (2007) Folate fortification of rice by metabolic engineering. Nat Biotechnol 25(11):1277

Taira H, Nakagahra M, Nagamine T (1988) Fatty acid composition of Indica, Sinica, Javanica, japonica groups of nonglutinous brown rice. J Agric Food Chem 36(1):45–47

Tan S, Han R, Li P, Yang G, Li S, Zhang P, Wang W-B, Zhao W-Z, Yin L-P (2015) Over-expression of the MxIRT1 gene increases iron and zinc content in rice seeds. Transgenic Res 24 (1):109–122

Tozawa Y, Hasegawa H, Terakawa T, Wakasa K (2001) Characterization of rice anthranilate synthase α-subunit GenesOASA1 and OASA2. Tryptophan accumulation in transgenic Rice expressing a feedback-insensitive mutant of OASA1. Plant Physiol 126(4):1493–1506

Trijatmiko KR, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C, Balindong J, Oliva N, Sapasap MV, Borrero J (2016) Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. Sci Rep 6:19792

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8 (11):1386

Vaughan DA, Morishima H, Kadowaki K (2003) Diversity in the Oryza genus. Curr Opin Plant Biol 6(2):139–146

Vijayaraghavan K, Venkaiah K, Damayanthi K, Nayak MU (2002) National nutrition monitoring bureau. National Institute of Nutrition, Hyderabad

Wakte K, Zanan R, Hinge V, Khandagale K, Nadaf A, Henry R (2017) Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (Oryza sativa L.): a status review. J Sci Food Agric 97(2):384–395

Wei Y, Shohag M, Yang X, Yibin Z (2012) Effects of foliar iron application on iron concentration in polished rice grain and its bioavailability. J Agric Food Chem 60(45):11433–11439

Welch RM (1986) Effects of nutrient deficiencies on seed production and quality. Adv Plant Nutr 2:205–247

Welch RM (1993) Zinc concentrations and forms in plants for humans and animals. In: Zinc in soils and plants. Springer, Dordrecht, pp 183–195

Widjaja R, Craske JD, Wootton M (1996) Comparative studies on volatile components of non-fragrant and fragrant rices. J Sci Food Agric 70(2):151–161

Williams PN, Lombi E, Sun G-X, Scheckel K, Zhu Y-G, Feng X, Zhu J, Carey A-M, Adomako E, Lawgali Y (2009) Selenium characterization in the global rice supply chain. Environ Sci Technol 43(15):6024–6030

World Health Organization (2005) Modern food biotechnology, human health and development: an evidence-based study, World Health Organization

Wu XR, Chen ZH, Folk WR (2003) Enrichment of cereal protein lysine content by altered tRNAlys coding during protein synthesis. Plant Biotechnol J 1(3):187–194

Yang Q-q, Zhang C-q, Chan M-l, Zhao D-s, Chen J-z, Wang Q, Li Q-f, Yu H-x, Gu M-h, Sun SS-m (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 X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451):303–305

Yeomans MR (2006) Olfactory influences on appetite and satiety in humans. Physiol Behav 89 (1):10–14
Yuan L, Wu L, Yang C, Lv Q (2013) Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. J Sci Food Agric 93(2):254–261
Zhang Y-Q, Sun Y-X, Ye Y-L, Karim MR, Xue Y-F, Yan P, Meng Q-F, Cui Z-L, Cakmak I, Zhang F-S (2012) Zinc biofortification of wheat through fertilizer applications in different locations of China. Field Crop Res 125:1–7
Zhang C-M, Zhao W-Y, Gao A-X, Su T-T, Wang Y-K, Zhang Y-Q, Zhou X-B, He X-H (2018) How could agronomic biofortification of rice be an alternative strategy with higher cost-effectiveness for human iron and zinc deficiency in China? Food Nutr Bull 39(2):246–259
Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci 12(12):548–555
Zoon H, de Graaf C, Boesveldt S (2016) Food odours direct specific appetite. Foods 5(1):12

Biofortification in Pulses

4

Satveer Kaur, Anita Kumari, Pargat Singh, Lovenpreet Kaur, Natasha Sharma, and Monika Garg

Abstract

Biofortification is a sustainable and cost-effective method of delivering micronutrients to the population. Unfortunately, major food crops are poor sources of one or more micronutrients which are essential for normal growth and development of humans. The present manuscript describes the biofortification of pulses by breeding, agronomy, microbiology, and genetic modification based approaches. There are some accomplishments by traditional breeding approaches, mutation breeding, and foliar/root application of respective fertilizers in pulses. But research on marker development and their utilization in marker-assisted breeding is still in initial phases. Pulses are mostly non-staple legumes; therefore, these have received little attention from the scientific community. But considering their richness in protein content and dietary fibers these have the potential to be nutraceutical foods. Therefore, the biofortification of pulses with increased nutritional quality is expected to gain attention in the future.

Keywords

Biofortification · Micronutrients · Pulses · Health benefits · Breeding · Dietary fibers

4.1 Introduction

World agricultural practices aim to provide a sufficient amount of food energy and nutrients for the well-being and health of human beings. Over time, agricultural development has been one of the greatest achievements of mankind. Green revolution has expanded the global production of staple food crops, especially cereals. The major concern of the green revolution was to increase the crop yield and minimum

S. Kaur · A. Kumari · P. Singh · L. Kaur · N. Sharma · M. Garg (✉)
National Agri-Food Biotechnology Institute, Mohali, Punjab, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_4

effort was laid to improve the quality of these food products. This led to the displacement of traditional food crops providing greater levels of essential micronutrients with less nutritious but high yielding crops. Micronutrient malnutrition is known to affect more than half of the world's population and considered to be among the most serious global challenges to humankind. Like cereals, pulses have a long history of cultivation and have been a significant constituent in human diets since around 10,000 BC (Fuller and Harvey 2006). Pulses are the plants belonging to family *Fabaceae* and recognized as the second most important food source after cereals which play an important role due to their nutritional and health-related benefits. Pulses are the dried edible seeds of certain plants in the legume family. Pulses are very high in protein and fiber and are low in fat. Pulses are also nitrogen-fixing crops that improve the environmental sustainability of annual cropping systems. Pulses come in a variety of shapes, sizes, and colors and can be consumed in many forms including whole or split, ground into flours, or separated into fractions such as protein, fiber, and starch. Other foods in the legume family like fresh beans and peas are not considered pulses; the term "pulse" only refers to the dried seed. Soybeans and peanuts are also not considered pulses because they have a much higher fat content, whereas pulses contain virtually no fat. The most important pulses include chickpea (*Cicer arietinum*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), mungbean (*Vigna radiata*), urdbean (*Vigna mungo*), cowpea (*V. unguiculata*), lentil (*Lens culinaris ssp. culinaris*), lathyrus (*Lathyrus sativus* L.), etc. (Singh et al. 2015; Fig. 4.1).

Fig. 4.1 Major pulses being consumed worldwide as a protein-rich nutritive food

Table 4.1 Recommended daily allowance of different micronutrients

Micronutrients	Recommended daily allowance (mg day^{-1})	References
Iron (Fe)	8–18 mg	Russell et al. 2001 Trumbo et al. 2001
Zinc (Zn)	8–11 mg	
Selenium (Se)	55 mcg	
Vitamin A	700–900 mcg	
Magnesium (Mg)	310–420 mg	

Table 4.2 Major constituents of different pulses

Pulses	Carbohydrate (%)	Protein (%)	Fat (%)	References
Chickpea	52–71	19–27	2–7	Singh and Jambunathan (1982) and Massod et al. (2014)
Kidney bean	63–74	17–27	1–5	Wang et al. (2010), Fan et al. (2014), and Caprioli et al. (2016)
Peas	55–72	14–31	1–4	Fan et al. (2014), Jha et al. (2015), Yoshida et al. (2007a, b), and Ray et al. (2014)
Lentil	42–72	23–31	1–3	Ghumman et al. (2016), Fouad and Rehab (2015), and Ray et al. (2014)

4.2 Role of Micronutrients on Human Health

Pulses provide protein, fibers, and significant micronutrients such as iron, zinc, selenium, and vitamins. There is a recommended daily allowance of each micronutrient (Table 4.1). Micronutrients present in pulses play a crucial role in metabolism and the maintenance of various body functions. Micronutrients such as Fe, Zn, and Se help in the nervous system function, healing wounds, and defending cells against damage from stress. Iron helps in the formation of hemoglobin formation and oxygen transport. Deficiency of iron associated with anemia and pregnancy related issues like mortality and low birth weight, etc. Zinc deficiency affects the physical growth and development of human beings. Additionally, selenium acts as a good source of antioxidant which helps in the treatment of cancer, skin and cardiovascular diseases (Elahi et al. 2009).

4.3 Nutritional Composition of Pulses

Pulses are a part of the daily diet of many vegetarians as well as people in developing countries. Pulses are rich in protein (20–30%) and an excellent source of dietary fiber, low molecular weight carbohydrates, essential amino acids, polyunsaturated fatty acids, minerals, and vitamins which may play a role in the functioning of the immune and digestive system (Table 4.2). Pulse crop development may provide a

Table 4.3 Health benefits of various pulses

Type of pulses	Health benefits	References
Bean extract	Anti-hypertensive	Eide (2011)
Chickpea and lentils	Low GI	Hartman et al. (2009)
Red, brown, and black beans	Anti-inflammatory and anti-carcinogenic	Hartman et al. (2009) and Adebamowo et al. (2005)
Canned kidney, pinto beans, chick peas and green lentils	Hypocholesterolemic	Iqbal et al. (2006) and Winham et al. (2008)

whole food solution to developing country micronutrient deficiencies as well as a means to reduce the prevalence of diseases of higher-income populations related to high caloric (Iriti and Varoni 2017). Pulses are also rich in micronutrients like folate, thiamine, riboflavin, niacin, calcium (Ca), magnesium, iron (Fe), and zinc (Zn) (Iqbal et al. 2006). The given below table elucidates the major constituents of pulses.

4.4 Health Benefits of Pulses

Pulses are recognized as a healthy food which is associated with the reduction of several lifestyle disorders such as obesity (Curran 2012), osteoporosis (Mollard et al. 2011), cardiovascular diseases (CVD), diabetes (Iqbal et al. 2006; Winham et al. 2008), and improvement in brain health which have a beneficial effect on physiological functions (Table 4.3). Currently, the World Cancer Research Fund (WCRF), the United States Food and Drug Administration, and Canadian Cancer Society have commended the consumption of health promoting pulses in order to lessen the cancer risk (Venter and van Eyssen 2001). Various publications have also been reported on general as well as tissue-specific cancer reduction, e.g. colorectal cancer (WCRF 2010; Campos-Vega et al. 2013) and breast cancer (Adebamowo et al. 2005). However, the WCRF 2010 also mentions about non-conclusiveness of the hypothesis of the positive effect of pulses on the reduction of cancer. The dietary fibers are the chief constituents in pulses and play an important role in cancer reduction. Pulses also contain micronutrients Zn and Fe, that help in the reduction of oxidative stress in cells (Eide 2011) and ultimately maintain a better immune system (Ibs and Rink 2003) and its depletion has been reported to cause DNA damage (Song et al. 2009). Additionally, selenium (Se) has been suggested to help in cancer reduction (Ellis and salt 2003). On the other hand, bioactive compounds such as saponins, protease inhibitors, phytic acid, and tannins occur naturally in pulse crops which possess antioxidant capacity, but these components also act as anti-nutrients because these are capable of chelating several available minerals (Parca et al. 2018).

The low GI and high-fiber content of pulses make it suitable for consumption by general population as well as diabetics (Jenkins et al. 1981). The resistant starch in pulses is not/less digested by the human digestive system and thus contributes to the

improvement of glucose tolerance, insulin sensitivity, and diabetes control (Jenkins et al. 2002). Meta-analysis has also indicated a similar effect of pulses (Sievenpiper et al. 2009). However, contrasting observation of increase rather than decrease of blood glucose after consumption of pulses has also been reported (Hartman et al. 2009).

4.5 Biofortification of Pulses

Although several positive effects have been associated with pulse composition, these need further improvement in terms of major and minor nutrients. The development of micronutrient-enriched staple food crops may be an effective and sustainable means to increase micronutrient intake to support general human health. Biofortification is a process of increasing the density of nutrients in a crop. It is a feasible, upcoming, and cost-effective technique that includes various strategies such as classical breeding, mapping population, and genetic selection to get the superior lines of pulse crops as shown in Fig. 4.1. Therefore, biofortification of various pulses including lentils (*Lens culinaris* L.), field pea (*Pisum sativum* L.), and chickpea (*Cicer arietinum* L.) with highly bioavailable Fe, Zn, Se, and I is urgently required to tackle with chronic diseases linked to micronutrient malnutrition around the world. Recently, the genetic potential for biofortification of bioavailable Fe, Zn, and provitamin A has been reported for the edible portions of several staple food crops, including rice (*Oryza sativa* L.), wheat (*Triticum sp*.), maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.), sweet potato (*Ipomoea batatas* L.), and cassava (*Manihot esculenta* C.). Until now, our agricultural system has not been designed to promote human health; instead, it only concentrates on increasing grain yield and crop productivity. This approach has led to an increase in micronutrient deficiency in legumes, thereby increasing micronutrient malnutrition among consumers. Now, agriculture is undergoing a move from producing more quantity of food crops to producing nutrient-rich food crops in sufficient quantities to improve the nutritional quality of various foods (Singh et al. 2015). Biofortified pulses have a huge potential to combat hidden hunger as the edible portions are denser in bioavailable micronutrients, minerals, and vitamins. Thus, biofortification will emerge as an agricultural-based cheaper strategy in mitigating nutritional needs. Biofortified staple crops, when consumed regularly, will generate measurable improvements in human health and nutrition. This will facilitate fighting against "hidden hunger" or "micronutrient malnutrition" particularly in poor and developing countries (Garg et al. 2018).

4.5.1 Interventions for Biofortification of Pulses

There are various ways through which we can enrich the pulse crops with nutrients such as agronomic interventions, breeding, genetic modification, and microbiological interventions (Fig. 4.2).

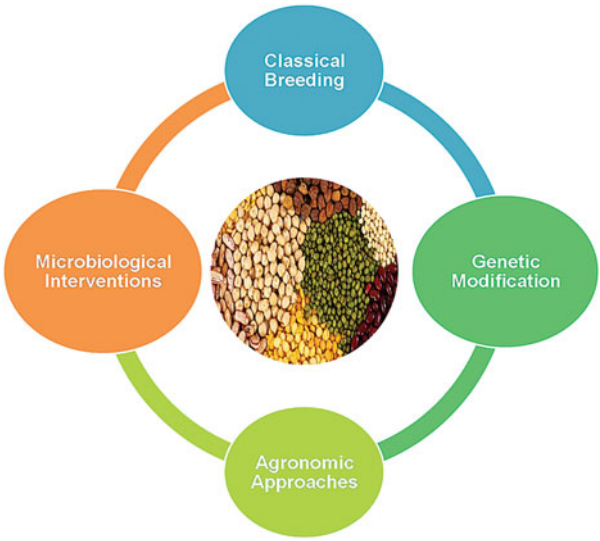

Fig. 4.2 Various biofortification approaches for nutritional enrichment of pulse crops

4.5.1.1 Breeding

Plant breeding programs such as conventional, mutation, and molecular breeding approaches focus on improving the level and bioavailability of minerals in staple crops mostly by using their natural genetic variation. Breeding approaches include the discovery of genetic variation affecting heritable mineral traits, checking their stability under different conditions, and the feasibility of breeding for increasing mineral content in edible tissues without affecting yields or other quality traits (Welch and Graham 2005). Once a suitable genetic variation is present, traditional breeding relies on effective selection dependent upon additive genetic effects, the phenomenon of heterosis in F1 progeny, and transgressive segregation in later generations. Genotypes with distant ancestry and intermediate values are breed to produce superior transgressive segregants and introgression of genes from wild relatives with higher micronutrient content are common practices in wheat biofortification for micronutrients. Molecular breeding or marker-assisted breeding (MAS) utilizes molecular markers that are tightly linked to the trait of interest. The genetic analysis of markers associated with the target QTL (quantitative trait loci) is accomplished using QTL mapping. For QTL mapping, various mapping populations are used, which are either mortal (segregating) or immortal (non-segregating) lines (Fig. 4.3). The mortal lines consist of F2, F2:3, and back cross (BC), while double haploid (DH), recombinant inbred lines (RIL) attained after 6–8 cycles of single seed descent method (SSD) are covered under immortal lines. However, QTLs are not stable across different environments and show additive and epistatic nature. The method of meta-QTL has been proposed to identify a few robust and reproducible markers, which will be present across diverse environments. Moreover, the latest development in genome-wide association studies (GWAS), which involves variation

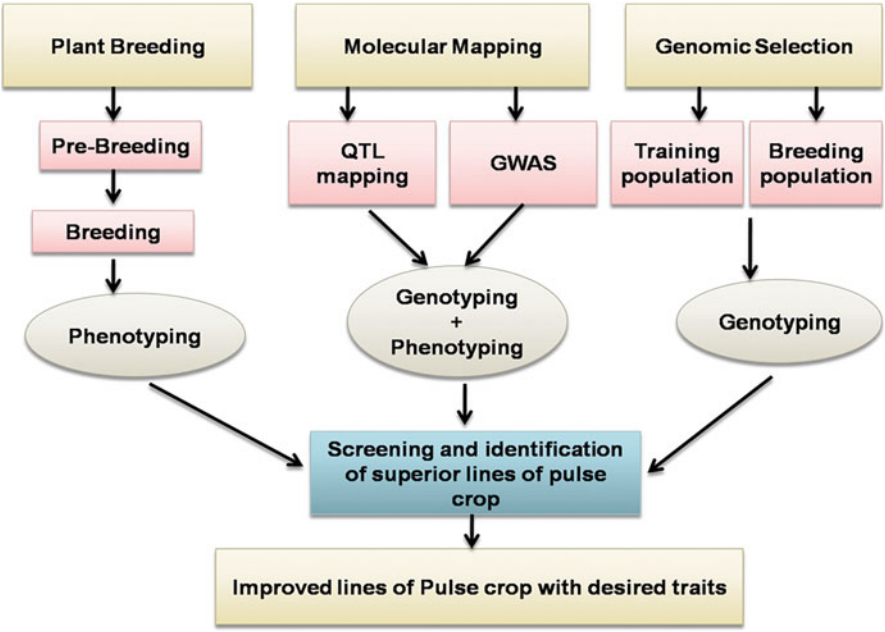

Fig. 4.3 The schematic representation of breeding based strategies of biofortification for pulse crops

present in naturally diverse lines (DL) or elite lines (EL), helps in the production of dense linkage maps (Fig. 4.3).

4.5.1.2 Genetic Modification

Genetic engineering is the advanced biotechnology technique to introduce genes directly into breeding varieties. The genes can come from any source (including animals and microbes) and are designed to improve the efficiency with which minerals are mobilized in the soil, reduce the level of anti-nutritional compounds, and increase the level of nutritional enhancer compounds such as inulin (Zhu et al. 2013).

4.5.1.3 Agronomic Approaches

Agronomic strategies rely on the application of mineral fertilizers in order to increase the mineral concentration in edible tissues (White and Broadley 2009). The mineral fertilizers have been applied to soil for hundreds of years to improve the health of their plants, but within certain limits, the same strategy can also be used to increase mineral accumulation within cereal grains for nutritional purposes (Gomez-Galera et al. 2010).

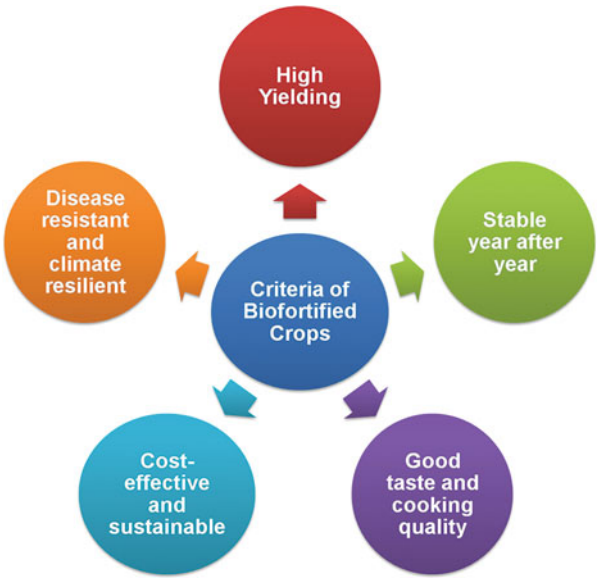

Fig. 4.4 The figure depicts the different criteria that are required for biofortified crops

4.5.1.4 Microbiological Interventions

Biofortification of crops through application of PGPR considered as a supplementary measure which helps to increase micronutrient concentrations in staple crop, besides improving yield and soil fertility. These include beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture, as it offers an attractive way to reduce the use of chemical fertilizers, pesticides, and related agrochemicals (Rana et al. 2012b).

4.6 Criteria of Biofortified Crop

The main objective of biofortification is to develop micronutrient-rich staple crops to reduce micronutrient malnutrition which helps in food security, productivity, and quality of life in developing countries (Fig. 4.4).

- Effective: The increased level of micronutrients must have a beneficial impact on human health.
- Stable: Increased level of micronutrients crops must be stable year after year.
- High yielding: Crop productivity must be maintained.
- Quality: Possessed good taste and cooking quality.
- Cost-effective and sustainable: The technique must be cost-effective and sustainable to develop micronutrient dense staple crops to achieve provitamin A, iron, and zinc concentrations that can have a measurable impact on nutritional status.
- To develop disease-resistant and climate-resilient varieties (Singh et al. 2015).

Table 4.4 The given table illustrates the nutrients, research status, and concerned publication of pulses through agronomic and transgenic approaches

Type of pulses	Type of biofortification	Type of approach	Status	Papers
Pea	Zinc	Agronomic approaches	Research	Poblaciones and Rengel (2016)
Common bean	Zinc, N, P, K, Copper, Manganese		Research	Ram et al. (2016), Ibrahim and Ramadan (2015), and Westermann et al. (2011)
Chickpea	Zn, Se		Research	Shivay et al. (2015) and Poblaciones et al. (2013)
Common beans	Methionine	Transgenic approach	Research	Arago et al. (1999)
Lupines	Methionine		Research	Molvig et al. (1997)

4.7 Nutritional Enrichment in Pulse Crops through Biofortification

4.7.1 Pulses and Essential Amino Acids

Pulses are a rich source of proteins that are made of 20 different amino acids. Plants can synthesize all 20 amino acids, but human beings can only synthesize 11 amino acids, while other 9 essential amino acids must be obtained from the diet. Pulse proteins are not balanced and are deficient in sulfur (S) containing essential amino acids methionine and cysteine (Robinson et al. 2019) (Table 4.4).

4.7.1.1 Agronomic/Microbial Strategy

Foliar application of S is useful for rapid/higher translocation and accumulation in seed (Jangir et al. 2017). Improvement in grain protein concentration of chickpea has been reported after the inoculation of plant roots with four microbial strains (Wani et al. 2007).

4.7.1.2 Breeding/Transformation Strategy

Transgenic approaches help to overcome the deficiency of S-containing amino acids by expressing heterologous proteins rich in these amino acids in the pulse seeds. As an example, a maize 27 kDa γ-zein, a cysteine-rich protein has been successfully expressed in several crops including beans to improve the nutritional value of proteins (Li et al. 2005). An increase in grain methionine content by expression of S-rich proteins has been also adopted in narbon bean (*Vicia narbonensis*) (Saalbach et al. 1995) and lupin (Tabe and Droux 2002). Additionally, researchers have used seed-specific promoters to express a Brazil nut 2S albumin storage protein along with the enzyme aspartate kinase to enhance seed methionine levels by up to 2.4 times in comparison to wild type (Demidov et al. 2003; Zhang et al. 2003). The rice

OASA1D transgene was also shown to raise the free tryptophan level when expressed in transgenic adzuki bean (*Vigna angularis*) (Hanafy et al. 2013).

4.7.2 Pulses and Iron (Fe)/Zinc (Zn)

Among the different trace elements, Fe and Zn are essential for a variety of metabolic processes. Fe deficiency is afflicting more than two billion individuals worldwide (WHO 2008). As Fe plays a very important role in hemoglobin formation and oxygen transport, its deficiency brings influence on the immune system (Failla 2003), learning ability, ability to work (Viteri 1974), and cognitive development (Beard and Connor 2003). It has been identified as the greatest contributor to anemia, accounting for 66.2% of cases globally (Alvarez-Uria et al. 2014). Zn also plays a key role in different metabolic processes and development. Zn deficiency curtails the physical growth and development in children (Brown et al. 2002). Gastrointestinal, central nervous, epidermal, immune, skeletal, and reproductive systems are known to be largely affected by Zn deficiency (Hambidge 1991). Plants acquire Fe and Zn from surrounding rhizosphere and immediate environment as these minerals are not synthesized in plants (Bouis and Welch 2010). Collectively, a meta-analysis of trials indicated biofortified crops to be particularly beneficial to individuals suffering from Fe deficiency (Finkelstein et al. 2017).

4.7.2.1 Agronomic/Microbial Strategy

Foliar application has been demonstrated to increase Fe accumulation (Pahlavan Rad and Pessarakli 2009; Cakmak et al. 2010; Zhang et al. 2010; Aciksoz et al. 2011). The use of bacterial inoculants has also been found to be helpful (Rana et al. 2012a, b; Sharma et al. 2013). Application of foliar Zn increased the grain Zn and Fe concentration in cowpea and chickpea (Shivay et al. 2015). Soil application of Zn has also been recommended to increase grain Zn concentration in chickpea (Hidoto et al. 2017). Other biofortification methods like seed priming and seed coating are spotted to give a very infrequent result. Seed priming with both B and Zn has been reported to increase the seed Zn and B content of chickpea and lentil (Johnson et al. 2005).

4.7.2.2 Breeding/Transformation Strategy

Conventional breeding and genetic engineering techniques are the two approaches to biofortify the crops with Fe and Zn (Pfeiffer and McClafferty 2007; Johns and Eyzaguirre 2007; Kumar et al. 2018; Tiwari et al. 2009). Since the uptake and accumulation of Fe and Zn in edible parts of crops are controlled by polygenes having minor effects, thus marker-assisted breeding based biofortification approaches for Fe and Zn have met with only marginal success (Naqvi et al. 2009). Several quantitative trait loci (QTLs) for Fe accumulation have been identified in cowpea (Fernandes and Boiteux 2015) and bean (Blair et al. 2008, 2010, 2016). Moreover, the success achieved by using this approach depends mainly on the natural variation that exists in the gene pool. In the absence of adequate

genetic variability and fixable major gene effects, genetic engineering has been considered as a viable alternative tool for the enhancement of micronutrients in pulses (Bhullar and Gruissem 2013; Dunwell 2014). Mainly, transgenic strategies have focused on to increase the uptake and utilization efficiency of plants for Fe and Zn through variation in transporters expression (Kerkeb et al. 2008) and decreasing various anti-nutritional factors like phytic acid.

Under the Harvest Plus program, different crops have been developed through conventional breeding programs, among them, the notable one is Fe biofortified beans. Petry et al. (2013) also reported increased Fe absorption in biofortified bean meals. International Center for Agricultural Research in Dry Areas (ICARDA) along with regional centers have released several varieties of lentil rich in Fe and Zn (Barimusur-4, Barimusur-5, Barimusur-6, Barimusur-7in Bangladesh; Sisir, Khajurah-2, Khajurah-1, Shekhar in Nepal; Pusa Vaibhav, L4704, IPL220, Pusa Ageti Masoor in India; Idlib-2 and Idlib-3 in Syria and Alemaya in Ethiopia (Ghosh et al. 2019). Several varieties of Fe biofortified beans have been released in Rwanda (RWV 3006, RWV 3316, RWV 3317, RWV 2887, RWV 1129, RWR 2245, RWR 2154, CAB 2, MAC 42, MAC 44) and Democratic Republic of Congo (PVA 1438, COD MLV 059, VCB 81013, COD MLB 001, COD MLB 032, HM 21-7, RWR2245, Nain de Kyondo, Cuarentino, Namulenga) (Garg et al. 2018).

4.7.3 Pulses and Selenium (Se)

Se is an essential element for mammals. Millions of people have Se-deficient diets and Se biofortified crops could prevent such deficiency. It also has a positive effect on plant health. It has been reported to increase grain yield and antioxidant activity (Ekanayake et al. 2015).

4.7.3.1 Breeding/Transformation Strategy

Se concentration varies in different soils and different genotypes. Thus, to increase Se content, first step would be to check genetic diversity and then to identify genes/QTLs associated with it and to follow marker-assisted breeding or transformation strategy. Limited genetic diversity in Se content of lentils has been observed by Ates et al. (2016), while high genetic diversity in cultivated germplasm, landraces, and wild germplasm has been reported in other publications (Diapari et al. 2015; Nair et al. 2015). QTL mapping carried out by Ates et al. (2016) has found out four major QTLs in lentil.

4.7.3.2 Agronomic/Microbial Strategy

Both foliar and soil application of Se are recommended for its increase in the plant (Lyons et al. 2003, 2005; Hartikainen 2005; Broadley et al. 2006; Hawkesford and Zhao 2007; Rayman 2008). Soil applications of Se have been found to increase seed Se content (Lyons et al. 2005). Foliar application of Se provided higher efficiency for increasing the Se content of soybean than soil application (Yang et al. 2003). Among the sources of Se, both sodium selenate (Na_2SeO_4) and potassium selenate (K_2SeO_4)

provide phyto-available Se for immediate uptake by lentils. The foliar application of sodium selenate has been reported to give better results. Inoculation of plant roots with Se solubilizing bacteria and sodium selenate is recommended as a natural way to Se biofortification (Motesharezadeh et al. 2019).

4.7.4 Pulses and Dietary Fiber

Dietary fiber includes soluble and insoluble fibers which may help to lower blood cholesterol and regulate blood glucose levels. Dietary fibers are mostly found in the cell wall of plants. Insoluble fibers such as cellulose, hemicellulose, and lignin play a vital role in the movement of material through the digestive system and ultimately improve laxation. Soluble fibers include the oligosaccharides, pectins, β-glucans, and galactomannan gums (Rodriguez et al. 2006). Pulses contain both insoluble and soluble fibers.

Landraces are a valuable source of genetic variability for breeders to transfer economically useful traits to cultivated species. Lentil lines with higher dietary fiber content have been prepared by utilizing these resources (Liu et al. 2019).

4.7.5 Pulses and Anti-Nutrients

Pulses contain anti-nutritional factors such as phytate, protease inhibitors, saponins, and tannins that can hinder the bioavailability of various micronutrients like Fe, Zn, and other nutrients and eventually reducing their bioavailability in the gut. Some dietary organic acids, amino acids, long chain fatty acids, beta-carotene, and fructo-oligosaccharides promote the bioavailability of Fe and Zn in the presence of anti-nutrients. Modern plant breeding and molecular biology tools now make it possible to reduce anti-nutrients, such as phytic acid or increase the concentrations of promoter substances, such as beta-carotene and ascorbic acid, in plant foods (Banziger and Long 2000; Hurrell and Egli 2010; Lockyer and Nugent 2017). The relationship studies between phytate concentration and Fe bioavailability indicated that lower phytate levels in immature peas correlated with better Fe bioavailability (Moore et al. 2018). Similarly, utilization of low phytate common beans has been shown to boost Fe absorption in young women (Petry et al. 2013). Several approaches have been tried to reduce the phytate content in pulses. Among them, non-transgenic techniques helped in developing low phytic acid (LPA) mutants. Pea cultivars with lower phytate content and higher Fe absorption have been developed with the help of chemical mutagenesis (Warkentin et al. 2012; Liu et al. 2015).

4.8 Advantages of Biofortification

- Except agronomic biofortification, other strategies have an advantage that after the one-time investment is made to develop seeds that fortify themselves, recurrent costs are low and germplasm may be shared internationally. It is this multiplier aspect of plant breeding across time and distance that makes it cost-effective.
- Once in place, the biofortified crop system is highly sustainable. Nutritionally improved varieties will continue to be grown and consumed year after year.
- Enhancement of nutritional quality in daily diets which reduces diseases related dietary deficiency.
- Improvement of plant or crop quality and increment of variability in germplasm (Ghosh et al. 2019).

4.9 Future Challenges in Biofortification

- There is a need to reduce the level of anti-nutritional compounds such as phytic acid, which inhibit the absorption of minerals such as Fe, Zn, and Ca in the gut.
- Intensifying the understanding of the transport and accumulation of minerals from the roots to storage tissues, such as grain.
- Promoting large-scale prospective studies on assessing the effects of nutrient enhancement in major staple crops to reduce malnutrition-related disorders in the future (Singh et al. 2015).

4.10 Conclusion

In developing countries, large portion of the populations are deficient in one or more essential vitamins and minerals, mainly due to low concentrations and reduced bioavailability of essential micronutrients present in commonly eaten foods. Many pulse crops are dietary staples, so researchers are developing biofortified varieties. Pulse crops such as lentils and beans are highly nutritious, but there is an urgent need for their improvement. Biofortified pulses are having huge potential to combat hidden hunger as the edible portions are denser in bioavailable, micronutrient, minerals, and vitamins. Pulses have been targeted for deficient S-containing amino acid improvement, Fe, Zn, and Se improvement and for reducing anti-nutrients like phytic acid. There are some accomplishments by traditional breeding approaches, mutation breeding, and foliar/root application of respective fertilizers. But research on marker development and their utilization in marker-assisted breeding is still in initial phases (Subuola et al. 2012).

References

Aciksoz SB, Yazici A, Ozturk L et al (2011) Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. Plant Soil 349(1–2):215–225. https://doi.org/10.1007/s11104-011-0863-2

Adebamowo CA, Cho E, Sampson L et al (2005) Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. Int J Cancer 114(4):628–633. https://doi.org/10.1002/ijc.20741

Alvarez-Uria G, Naik PK, Midde M et al (2014) Prevalence and severity of anaemia stratified by age and gender in rural India. Anemia 2014:1–5. https://doi.org/10.1155/2014/176182

Arago F, Barros L, Sousa MD et al (1999) Expression of a methionine-rich storage albumin from the Brazil nut (*Bertholletia excelsa* H.B.K., *Lecythidaceae*) in transgenic bean plants (*Phaseolus vulgaris* L., *Fabaceae*). Genet Mol 22(3):445–449. https://doi.org/10.1590/s1415-47571999000300026

Ates D, Sever T, Aldemir S et al (2016) Identification QTLs controlling genes for Se uptake in lentil seeds. PLoS One 11(3):e0149210. https://doi.org/10.1371/journal.pone.0149210

Banziger M, Long J (2000) The potential for increasing the iron and zinc density of maize through plant-breeding. Food Nutr Bull 21(4):397–400. https://doi.org/10.1177/156482650002100410

Beard JL, Connor JR (2003) Iron status and neural functioning. Annu Rev Nutr 23(1):41–58. https://doi.org/10.1146/annurev.nutr.23.020102.075739

Bhullar NK, Gruissem W (2013) Nutritional enhancement of rice for human health: the contribution of biotechnology. Biotechnol Adv 31(1):50–57. https://doi.org/10.1016/j.biotechadv.2012.02.001

Blair MW, Astudillo C, Grusak MA et al (2008) Inheritance of seed iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.). Mol Breed 23(2):197–207. https://doi.org/10.1007/s11032-008-9225-z

Blair MW, Knewtson SJ, Astudillo C et al (2010) Variation and inheritance of iron reductase activity in the roots of common bean (*Phaseolus vulgaris* L.) and association with seed iron accumulation QTL. BMC Plant Biol 10(1):215. https://doi.org/10.1186/1471-2229-10-215

Blair MW, Wu X, Bhandari D et al (2016) Genetic dissection of ICP-detected nutrient accumulation in the whole seed of common bean (*Phaseolus vulgaris* L.). Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00219

Bouis HE, Welch RM (2010) Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50(Supplement_1):S-20. https://doi.org/10.2135/cropsci2009.09.0531

Broadley MR, White PJ, Bryson RJ et al (2006) Biofortification of UK food crops with selenium. Proc Nutr Soc 65(2):169–181. https://doi.org/10.1079/pns2006490

Brown KH, Peerson JM, Rivera J et al (2002) Effect of supplemental zinc on the growth and serum zinc concentrations of pre-pubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr 75(6):1062–1071. https://doi.org/10.1093/ajcn/75.6.1062

Cakmak I, Pfeiffer WH, Mc Clafferty B (2010) REVIEW: biofortification of durum wheat with zinc and iron. Cereal Chem J87(1):10–20. https://doi.org/10.1094/cchem-87-1-0010

Campos-Vega R, Oomah B, Loarca-Pina G et al (2013) Common beans and their non-digestible fraction: cancer inhibitory activity-an overview. Foods 2(3):374–392. https://doi.org/10.3390/foods2030374

Caprioli G, Giusti F, Ballini R et al (2016) Lipid nutritional value of legumes: evaluation of different extraction methods and determination of fatty acid composition. Food Chem 192:965–971. https://doi.org/10.1016/j.foodchem.2015.07.102

Curran J (2012) The nutritional value and health benefits of pulses in relation to obesity, diabetes, heart disease and cancer. Br J Nutr 108(S1):S1–S2. https://doi.org/10.1017/s0007114512003534

Demidov D, Horstmann C, Meixner M et al (2003) Additive effects of the feed-back insensitive bacterial aspartate kinase and the Brazil nut 2S albumin on the methionine content of transgenic

narbon bean (*Vicianar bonensis* L.). Mol Breed 11(3):187–201. https://doi.org/10.1023/a:1022814506153

Diapari M, Sindhu A, Warkentin TD et al (2015) Population structure and marker-trait association studies of iron, zinc and selenium concentrations in seed of field pea (*Pisum sativum* L.). Mol Breed 35(1):30. https://doi.org/10.1007/s11032-015-0252-2

Dunwell JM (2014) Transgenic cereals: current status and future prospects. J Cereal Sci 59 (3):419–434. https://doi.org/10.1016/j.jcs.2013.08.008

Eide DJ (2011) The oxidative stress of zinc deficiency. Metallomics 3(11):1124. https://doi.org/10.1039/c1mt00064k

Ekanayake LJ, Thavarajah D, Vial E et al (2015) Selenium fertilization on lentil (*Lens culinaris* Medikus) grain yield, seed selenium concentration, and antioxidant activity. Field Crops Res 177:9–14. https://doi.org/10.1016/j.fcr.2015.03.002

Elahi MM, Kong YX, Matata BM (2009) Oxidative stress as a mediator of cardiovascular disease. Oxidative Med Cell Longev 2(5):259–269. https://doi.org/10.4161/oxim.2.5.9441

Ellis DR, Salt DE (2003) Plants, selenium and human health. Curr Opin Plant Biol 6(3):273–279. https://doi.org/10.1016/s1369-5266(03)00030-x

Failla ML (2003) Trace elements and host defense: recent advances and continuing challenges. J Nutr 133(5):1443S–1447S. https://doi.org/10.1093/jn/133.5.1443s

Fan P, Zang M, Xing J (2014) Oligosaccharides composition in eight food legumes species as detected by high-resolution mass spectrometry. J Sci Food Agric 95(11):2228–2236. https://doi.org/10.1002/jsfa.6940

Fernandes SC, Boiteux L (2015) Genetic control and transgressive segregation of zinc, iron, potassium, phosphorus, calcium, and sodium accumulation in cowpea (*Vignaun guiculata*) seeds. Genet Mol Res 14(1):259–268. https://doi.org/10.4238/2015.january.16.10

Finkelstein JL, Haas JD, Mehta S (2017) Iron-biofortified staple food crops for improving iron status: a review of the current evidence. Curr Opin Biotechnol 44:138–145. https://doi.org/10.1016/j.copbio.2017.01.003

Fouad AA, Rehab FM (2015) Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris* Medik.) sprouts. Acta Sci Pol Technol Aliment 14(3):233–246. https://doi.org/10.17306/j.afs.2015.3.25

Fuller DQ, Harvey EL (2006) The archaeobotany of Indian pulses: identification, processing and evidence for cultivation. Environ Archaeol 11(2):219–246. https://doi.org/10.1179/174963106x123232

Garg M, Sharma N, Sharma S et al (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Front Nutr. https://doi.org/10.3389/fnut.2018.00012

Ghosh A, Hasim RM, Nalia A et al (2019) Micronutrient biofortification in pulses: an agricultural approach. J Appl Sci Technol:1–12. https://doi.org/10.9734/cjast/2019/v35i430194

Ghumman A, Kaur A, Singh N (2016) Impact of germination on flour, protein and starch characteristics of lentil (*Lens culinari*) and horsegram (*Macrotyloma uniflorum* L.) lines. LWT-Food Sci Technol 65:137–144. https://doi.org/10.1016/j.lwt.2015.07.075

Gomez-Galera S, Rojas E, Sudhakar D et al (2010) Critical evaluation of strategies for mineral fortification of staple food crops. Transgenic Res 19(2):165–180. https://doi.org/10.1007/s11248-009-9311-y

Hambidge KM (1991) Trace minerals. In: Neonatal nutrition and metabolism. Cambridge University Press, Cambridge, pp 273–290. https://doi.org/10.1017/cbo9780511544712.018

Hanafy MS, Rahman SM, Nakamoto Y et al (2013) Differential response of methionine metabolism in two grain legumes, soybean and azuki bean, expressing a mutated form of *Arabidopsis* cystathionine γ-synthase. J Plant Physiol 170(3):338–345. https://doi.org/10.1016/j.jplph.2012.10.018

Hartikainen H (2005) Biogeochemistry of selenium and its impact on food chain quality and human health. J Trace Elem Med Biol 18(4):309–318. https://doi.org/10.1016/j.jtemb.2005.02.009

Hartman TJ, Albert PS, Zhang Z et al (2009) Consumption of a legume-enriched, low-glycemic index diet is associated with biomarkers of insulin resistance and inflammation among men at risk for colorectal cancer. Nutr J 140(1):60–67. https://doi.org/10.3945/jn.109.114249

Hawkesford MJ, Zhao F (2007) Strategies for increasing the selenium content of wheat. J Cereal Sci 46(3):282–292. https://doi.org/10.1016/j.jcs.2007.02.006

Hidoto L, Worku W, Mohammed H et al (2017) Effects of zinc application strategy on zinc content and productivity of chickpea grown under zinc deficient soils. J Soil Sci Plant Nutr 17 (1):112–126. https://doi.org/10.4067/s0718-95162017005000009

Hurrell R, Egli I (2010) Iron bioavailability and dietary reference values. Am J Clin Nutr 91 (5):1461S–1467S. https://doi.org/10.3945/ajcn.2010.28674f

Ibrahim EA, Ramadan WA (2015) Effect of zinc foliar spray alone and combined with humic acid or/and chitosan on growth, nutrient elements content and yield of dry bean (*Phaseolus vulgaris* L.) plants sown at different dates. Sci Hortic 184:101–105. https://doi.org/10.1016/j.scienta.2014.11.010

Ibs K, Rink L (2003) Zinc-altered immune function. Nutr J 133(5):1452S–1456S. https://doi.org/10.1093/jn/133.5.1452s

Iqbal A, Khalil IA, Ateeq N et al (2006) Nutritional quality of important food legumes. Food Chem 97(2):331–335. https://doi.org/10.1016/j.foodchem.2005.05.011

Iriti M, Varoni E (2017) Pulses, healthy, and sustainable food sources for feeding the planet. Int J Mol Sci 18(2):255. https://doi.org/10.3390/ijms18020255

Jangir CK, Kumar S, Lakhran H et al (2017) Towards mitigating malnutrition in pulses through biofortification. Biosci Trends 10(17):0974–8431, 2999-3002.

Jenkins DJ, Wolever TM, Taylor RH et al (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 34(3):362–366. https://doi.org/10.1093/ajcn/34.3.362

Jenkins DJ, Kendall CW, Augustin LS et al (2002) High-complex carbohydrate or lente carbohydrate foods? Am J Med 113(9):30–37. https://doi.org/10.1016/s0002-9343(01)00989-5

Jha AB, Taran B, Diapari M et al (2015) SNP variation within genes associated with amylose, total starch and crude protein concentration in field pea. Euphytica 206(2):459–471. https://doi.org/10.1007/s10681-015-1510-4

Johns T, Eyzaguirre PB (2007) Biofortification, biodiversity and diet: a search for complementary applications against poverty and malnutrition. Food Policy 32(1):1–24. https://doi.org/10.1016/j.foodpol.2006.03.014

Johnson SE, Lauren JG, Welch RM et al (2005) A comparison of the effects of micronutrient seed priming and soil fertilization on the mineral nutrition of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), rice (*Oryza sativa)* and wheat (*Triticum aestivum*) in Nepal. Exp Agric 41 (4):427–448. https://doi.org/10.1017/s0014479705002851

Kerkeb L, Mukherjee I, Chatterjee I et al (2008) Iron-induced turnover of the arabidopsis iron regulated transporter1 metal transporter requires lysine residues. Plant Physiol 146 (4):1964–1973. https://doi.org/10.1104/pp.107.113282

Kumar S, Hash C, Nepolean T et al (2018) Mapping grain iron and zinc content quantitative trait loci in an iniadi-derived immortal population of pearl millet. Genes 9(5):248. https://doi.org/10.3390/genes9050248

Li Z, Meyer S, Essig JS et al (2005) High-level expression of maize γ-zein protein in transgenic soybean (*Glycine max*). Mol Breed 16(1):11–20. https://doi.org/10.1007/s11032-004-7658-6

Liu X, Glahn RP, Arganosa GC et al (2015) Iron bioavailability in low phytate pea. Crop Sci 55 (1):320. https://doi.org/10.2135/cropsci2014.06.0412

Liu K, Blackshaw RE, Johnson EN et al (2019) Lentil enhances the productivity and stability of oilseed-cereal cropping systems across different environments. Eur J Agron 105:24–31. https://doi.org/10.1016/j.eja.2019.02.005

Lockyer S, Nugent AP (2017) Health effects of resistant starch. Nutr Bull 42(1):10–41. https://doi.org/10.1111/nbu.12244

Lyons G, Stangoulis J, Graham R (2003) High-selenium wheat: biofortification for better health. Nutr Res Rev 16(01):45. https://doi.org/10.1079/nrr200255

Lyons GH, Genc Y, Stangoulis JC et al (2005) Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. Biol Trace Elem Res 103(2):155–168. https://doi.org/10.1385/bter:103:2:155

Massod T, Shah HU, Zeb A (2014) Effect of sprouting time on proximate composition and ascorbic acid level of mung bean (*Vigna radiate* L.) and chickpea (*Cicer arietinum* L.) seeds. J Anim Plant Sci 24:850–859

Mollard RC, Zykus A, Luhovyy BL et al (2011) The acute effects of a pulse-containing meal on glycaemic responses and measures of satiety and satiation within and at a later meal. Br J Nutr 108(3):509–517. https://doi.org/10.1017/s0007114511005836

Molvig L, Tabe LM, Eggum BO et al (1997) Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. PNAS 94(16):8393–8398. https://doi.org/10.1073/pnas.94.16.8393

Moore KL, Rodríguez-Ramiro I, Jones ER et al (2018) The stage of seed development influences iron bioavailability in pea (*Pisum sativum* L.). Sci Rep 8(1). https://doi.org/10.1038/s41598-018-25130-3

Motesharezadeh B, Ghorbani S, Alikhani HA (2019) The effect of selenium biofortification in alfalfa (*Medicago sativa*). J Plant Nutr:1–11. https://doi.org/10.1080/01904167.2019.1676900

Nair RM, Thavarajah D, Thavarajah P et al (2015) Mineral and phenolic concentrations of mungbean [*Vigna radiata* (L.) R. Wilczek var. radiate] grown in semi-arid tropical India. J Food Compos Anal 39:23–32. https://doi.org/10.1016/j.jfca.2014.10.009

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 06(19):7762–7767. https://doi.org/10.1073/pnas.0901412106

Pahlavan Rad MR, Pessarakli M (2009) Response of wheat plants to zinc, iron, and manganese applications and uptake and concentration of zinc, iron, and manganese in wheat grains. Commun Soil Sci Plant Anal 40(7–8):1322–1332. https://doi.org/10.1080/00103620902761262

Parca F, Koca YO, Unay A (2018) Nutritional and anti-nutritional factors of some pulses seed and their effects on human health. Int J Second Metab 5:331–342. https://doi.org/10.21448/ijsm.488651

Petry N, Egli I, Campion B et al (2013) Genetic reduction of phytate in common bean (*Phaseolus vulgaris* l.) seeds increases iron absorption in young women. J Nutr 143(8):1219–1224. https://doi.org/10.3945/jn.113.175067

Pfeiffer WH, McClafferty B (2007) Harvest plus: breeding crops for better nutrition. Crop Sci 47 (Supplement_3):S-88. https://doi.org/10.2135/cropsci2007.09.0020ipbs

Poblaciones M, Rengel Z (2016) Soil and foliar zinc biofortification in field pea (*Pisum sativum* L.): grain accumulation and bioavailability in raw and cooked grains. Food Chem 212:427–433. https://doi.org/10.1016/j.foodchem.2016.05.189

Poblaciones MJ, Rodrigo S, Santamaria O et al (2013) Selenium accumulation and speciation in biofortified chickpea (*Cicer arietinum L.*) under Mediterranean conditions. J Sci Food Agric 94 (6):1101–1106. https://doi.org/10.1002/jsfa.6372

Ram H, Rashid A, Zhang W et al (2016) Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. Plant Soil 403 (1–2):389–401. https://doi.org/10.1007/s11104-016-2815-3

Rana A, Joshi M, Prasanna R et al (2012a) Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. Eur J Soil Biol 50:118–126. https://doi.org/10.1016/j.ejsobi.2012.01.005

Rana A, Saharan B, Nain L et al (2012b) Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. J Soil Sci Plant Nutr 58(5):573–582. https://doi.org/10.1080/00380768.2012.716750

Ray H, Bett K, Taran B et al (2014) Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. Crop Sci 54(4):1698. https://doi.org/10.2135/cropsci2013.08.0568

Rayman MP (2008) Food-chain selenium and human health: emphasis on intake. Br J Nutr 100 (2):254–268. https://doi.org/10.1017/s0007114508939830

Robinson GH, Balk J, Domoney C (2019) Improving pulse crops as a source of protein, starch and micronutrients. Nutr Bull 44(3):202–215. https://doi.org/10.1111/nbu.12399

Rodriguez R, Jimenez A, Fernandez-Bolanos J et al (2006) Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci Tech 17(1):3–15. https://doi.org/10.1016/j.tifs.2005.10.002

Russell RM, Beard JL, Cousins RJ et al (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. A report of the panel on micronutrients, subcommittees on upper reference levels of nutrients and of interpretation and uses of dietary reference intakes, and the standing committee on the scientific evaluation of dietary reference intakes food and nutrition board institute of medicine. https://doi.org/10.17226/10026

Saalbach I, Waddell D, Pickardt T et al (1995) Stable expression of the Sulphur-rich 2s albumin gene in transgenic *Vicia narbonensis* increases the methionine content of seeds. J Plant Physiol 145(5–6):674–681. https://doi.org/10.1016/s0176-1617(11)81280-0

Sharma A, Shankhdhar D, Shankhdhar SC (2013) Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. Plant Soil Environ 59(2):89–94. https://doi.org/10.17221/683/2012-pse

Shivay YS, Prasad R, Pal M (2015) Effects of source and method of zinc application on yield, zinc biofortification of grain, and Zn uptake and use efficiency in chickpea (*Cicer arietinum* L.). Commun Soil Sci Plant Anal 46(17):2191–2200. https://doi.org/10.1080/00103624.2015.1069320

Sievenpiper JL, Kendall CW, Esfahani A et al (2009) Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomized controlled experimental trials in people with and without diabetes. Diabetologia 52(8):1479–1495. https://doi.org/10.1007/s00125-009-1395-7

Singh U, Jambunathan R (1982) Changes in starch, oligosaccharides and soluble sugars in developing pod wall and seed of chickpea. Phytochemistry 21(2):297–299. https://doi.org/10.1016/s0031-9422(00)95254-3

Singh U, Praharaj CS, Singh SS et al (2015) Biofortification of pulses: strategies and challenges. In Proceedings of the second international conference on bio-resource and stress management, Hyderabad, India (pp. 50–55)

Song Y, Leonard SW, Traber MG et al (2009) Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. J Nutr 139(9):1626–1631

Subuola F, Widodo Y, Kehinde T (2012) Processing and utilization of legumes in the tropics. Trends Vital Food Control Eng. https://doi.org/10.5772/36496

Tabe LM, Droux M (2002) Limits to sulfur accumulation in transgenic lupin seeds expressing a foreign sulfur-rich protein. Plant Physiol 128:1137–1148

Tiwari VK, Rawat N, Chhuneja P et al (2009) Mapping of quantitative trait loci for grain iron and zinc concentration in diploid a genome wheat. J Hered 100(6):771–776. https://doi.org/10.1093/jhered/esp030

Trumbo P, Yates AA, Schlicker S et al (2001) Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Acad Nutr Diet 101(3):294. https://doi.org/10.17226/10026

Venter CS, Van Eyssen E (2001) More legumes for better overall health. S Afr J Clin Nutr 14 (3 Suppl):S32

Viteri FE (1974) Anemia and physical work capacity. Clin Haematol 3:609

Wang N, Hatcher D, Tyler R et al (2010) Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). Food Res Int 43(2):589–594. https://doi.org/10.1016/j.foodres.2009.07.012

Wani P, Khan M, Zaidi A (2007) Co-inoculation of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. Acta Agronomica Hungarica 55(3):315–323. https://doi.org/10.1556/aagr.55.2007.3.7

Warkentin TD, Delgerjav O, Arganosa G et al (2012) Development and characterization of low-phytate pea. Crop Sci 52(1):74. https://doi.org/10.2135/cropsci2011.05.0285

Welch RM, Graham RD (2005) Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. J Trace Elem Med Biol 18(4):299–307. https://doi.org/10.1016/j.jtemb.2005.03.001

WCRF A (2010) Continuous Update Project Report. Food, nutrition, physical activity, and the prevention of breast cancer. Washington, DC: American Institute for Cancer Research

Westermann D, Teran H, Munoz-Perea C et al (2011) Plant and seed nutrient uptake in common bean in seven organic and conventional production systems. Can J Plant Sci 91(6):1089–1099. https://doi.org/10.4141/cjps10114

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182 (1):49–84. https://doi.org/10.1111/j.1469-8137.2008.02738.x

WHO (2008) Worldwide prevalence of anaemia1993–2005. World Health Organisation

Winham D, Webb D, Barr A (2008) Beans and good health. Nutr Today 43(5):201–209. https://doi.org/10.1097/01.nt.0000303354.21347.45

Yang F, Chen L, Hu Q et al (2003) Effect of the application of selenium on selenium content of soybean and its products. Biol Trace Elem Res 93(1–3):249–256. https://doi.org/10.1385/bter:93:1-3:249

Yoshida H, Tomiyama Y, Saiki M et al (2007a) Tocopherol content and fatty acid distribution of peas (*Pisum sativum* L.). J Am Oil Chem Soc 84(11):1031–1038. https://doi.org/10.1007/s11746-007-1134-5

Yoshida H, Tomiyama Y, Tanaka M et al (2007b) Distribution of fatty acids in triacylglycerols and phospholipids from peas (*Pisum sativum* L.). J Sci Food Agric 87(14):2709–2714. https://doi.org/10.1002/jsfa.3035

Zhang P, Jaynes JM, Potrykus I et al (2003) Transfer and expression of an artificial storage protein (ASP1) gene in cassava: towards improving nutritive value of storage roots. In: Plant biotechnology 2002 and beyond. Springer, Dordrecht, pp 425–427. https://doi.org/10.1007/978-94-017-2679-5_88

Zhang Y, Shi R, Rezaul KM et al (2010) Iron and zinc concentrations in grain and flour of winter wheat as affected by foliar application. J Agric Food Chem 58(23):12268–12274. https://doi.org/10.1021/jf103039k

Zhu C, Sanahuja G, Yuan D et al (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11(2):129–141. https://doi.org/10.1111/j.1467-7652.2012.00740.x

Biofortification of Vegetables

5

Milan Kumar Lal, Awadhesh Kumar, Hemant Balasaheb Kardile, Pinky Raigond, Sushil Sudhakar Changan, Nitasha Thakur, Som Dutt, Rahul Kumar Tiwari, Kumar Nishant Chourasia, Dharmendra Kumar, and Brajesh Singh

Abstract

In the past few decades, the major concern on this planet was food security. After making a successful lead in food security now, the developing nations are focusing on nutritional security, which includes food that is enriched in minerals and vitamins. Micronutrients and vitamins are essential for human growth and development. Any deficiency of these components leads to "hidden hunger." Enhancing these components can alleviate malnutrition in women and children in the developing world. Micronutrients like Fe, Zn, Se, Mg, Ca, Iodine, and vitamins like provitamin A and folate are an important component of the biofortification program. Biofortification of vegetable with vitamins and micronutrients is the present need of an hour to fight different health issues faced by the developing countries. For biofortification of vegetable and other staple crops, three major techniques are used, viz. conventional breeding, agronomic approach (use of mineral fertilizer), and genetic engineering. These approaches have enormous potential to address this vitamin and micronutrient malnutrition. Many genes are available for the target traits by which it will be possible to improve micronutrient in vegetables. These tools can be very much helpful in improving the level of micronutrients and vitamins by several-fold in staple cereals and vegetables.

Keywords

Biofortification · Vegetable · Iron · Zinc · Iodine · Selenium · Provitamin A

M. K. Lal (✉) · H. B. Kardile · P. Raigond · S. S. Changan · N. Thakur · S. Dutt · R. K. Tiwari · K. N. Chourasia · D. Kumar · B. Singh
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

A. Kumar
ICAR-National Rice Research Institute, Cuttack, Odisha, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_5

5.1 Introduction

Agricultural research in developing countries is mainly focusing on increasing cereals production and productivity. This scenario is due to as cereal is a cheaper source of food for the poor population, and along with carbohydrates, it also provides protein, lipid, micronutrients, vitamins, phenols, antioxidants, which have huge health benefits. But most importantly, the challenges may be come across by the year 2050 when the world population will exceed 9 billion, and to feed this population, huge pressure will be on agriculture (Hubert et al. 2010). The modern technologies used in agriculture and allied fields are successful in fulfilling the demand for food and feed of poor populations in developing countries. In the last few decades, research and development in developing countries focused mainly on the production of food rather on the nutritional aspects. But now the time has come where the food production along with better nutritional quality to be taken care (Bechoff et al. 2009). The production of food and feed must be increased many folds, while on the other hand, this has to be done by preserving the agroecosystem so that the balance has to be maintained. Micronutrient undernourishment is a major issue for more than half of the world's population, which is prominent in developing countries (Ortiz-Monasterio et al. 2007). The primary source of vitamins and minerals in developing countries mainly depends upon staple food. But staple foods like rice, wheat, and maize are rich in carbohydrate but deficient in vitamins and minerals. The human need to consume a relatively small amount of macro-elements, a trace amount of microelement (Fe, Cu, Zn, I, and Se) and vitamins along with a large amount of carbohydrate, protein, and lipids (Welch 2002) to maintain a healthy lifestyle.

The edible part of plants like grain or seed can be widely used to prepare foods which contain a low level of micronutrients and have low bioavailability to human. This needs to be improved, and uptake efficiency should be increased in order to get more bioavailable micronutrients and vitamins. But more than half of the world's population suffers from micronutrient undernourishment as most of the staple food which is consumed in the developing countries are low in vitamins and minerals (Ortiz-Monasterio et al. 2007). Thus, biofortification is an essential requirement for the enhancement of these vitamins and minerals may be through conventional breeding or by genetic engineering approach.

After World War II, agricultural research is diverted towards cereals production and productivity. But this has to be a shift from cereals to vegetables where there is ample scope to increase the nutrition quality and reduce the hidden hunger. Kennedy et al. (2003) reported that the out of three-person in one person suffers from hidden hunger due to lack of minerals and vitamins in their diet, which ultimately leads to serious health consequences. Agricultural crops are the primary source of micronutrients and vitamins, which are essential for human growth and development. In the developing countries, the women, infants, and children of low-income families mostly suffer from iron, zinc, and vitamin A deficiencies, which account for over three billion people (Welch 2005). These in micronutrient deficiencies result in increased morbidity, and mortality rates, slow development of the nation, and

permanent impairment of cognitive development in children and infants as all categories of people cannot afford the high quality and costly fruits and vegetables for gaining nutrition. Thus, an agricultural-based approach has been proposed as supplementary strategies for the breeding of staple food crops for higher vitamins and micronutrients. This method of increasing the vitamins and minerals by breeding approach or by genetic engineering has been termed as biofortification (Nestel et al. 2006). Thus, biofortified staple cereals and vegetables will be beneficial for the sparse population of the world and will help to increase nutritional status. This will lead to a significant advantage in health and economic benefits.

The important concern for biofortification is that after the development of variety, there should be widespread adoption by the farmer. The crop has to reach the needy poor people. Vegetables, fruits, dairy, and meat products are rich in vitamins and micronutrients, but they are expensive for poor people. They rely on few starchy staples (rice, wheat, maize, and potato); as a result, the intake of dietary diversity becomes a luxury, and poor people cannot afford it (Gómez et al. 2013). The extent of diseases due to malnutrition and mineral deficiencies is so high that the World Bank estimated the combined economic cost of mineral deficiency in developing countries and could waste as much as 5% of its gross domestic product (GDP). The deficiency of micronutrient and vitamins has a significant impact and burden on society which ultimately leads to an increase in susceptibility to infectious diseases, physical impairment, cognitive losses, blindness, and premature mortality. Comprehensively, the deficiency of provitamin A, Fe, I, Zn, and Se is reported to have a maximum percentage of disease burden, negative impact on the public (Black et al. 2008; Stein 2010).

5.2 What Is Biofortification

Biofortification can be defined as the development of micronutrient-dense staple crops (cereals and vegetables) using traditional plant breeding practices, modern biotechnology, and agronomical approached. In this process, the concentration of plant-derived nutrition and vitamins is increased in the edible organ during the growth and development of the plant. (O'Hare 2015). This is the process of breeding nutrients in the food crops and provides a low cost, sustainable, and long term delivery of adequate micronutrients. It is a technique where the edible parts like grain, straw, roots, fruits, and tubers are enriched with micronutrients and vitamins through the appropriate breeding method and biotechnological tools (Bouis 2000; Saltzman et al. 2013). Biofortified staple food may not contain a high level of essential vitamins and micronutrients as compared to industrially fortified foods, but they can help to reduce "hidden hunger" by increasing the daily adequacy of micronutrients uptake by the individual throughout the life cycle (Bouis et al. 2011). The strategies of biofortification involve agronomic approach, conventional breeding, and genetic engineering approach (Fig. 5.1). Moreover, biofortification provides a possible means of disseminating the technology and food to a malnourished population where there may have limited access to diverse kinds of diet,

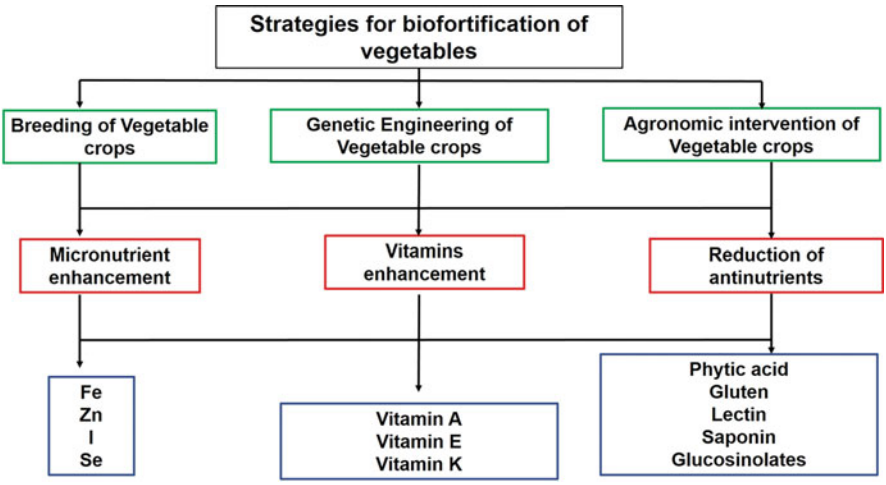

Fig. 5.1 Strategies for biofortification of vegetables with the enhancement of mineral and vitamin, reduction of anti-nutrients to increase the bioavailability of micronutrients

supplements, and food, which are commercially fortified (Saltzman et al. 2013). Biofortification is the different types of fortification and can be significantly increasing the level of vitamins and nutrients in the living product (edible part), and its accumulation takes place by normal physiological process of the plant (O'Hare 2015).

The breeding of plants for enhancement of bioavailability of micronutrients in the targeted edible food is the low-cost approach for poor countries and their people who cannot afford high-quality food. Thus mitigation of micronutrient malnutrition can be done by this approach, and this will ensure better health (Waters and Sankaran 2011). The enhancement of micronutrients and vitamins in the staple food and vegetable will lead to more consumption of micronutrients, particularly in the poor people, which ultimately leads to a reduction of malnutrition (Das et al. 2017).

Increasing phytonutrients and vitamins in a plant with a lesser life cycle which have short juvenile period to reach the flowering stage like in vegetable can be easily achieved. But this will be difficult for the tree-fruit and nuts where the juvenile period is much longer (O'Hare 2015). The principal objective of biofortification in vegetables should be the production of the nutritionally enriched vegetable crop so that the negative economic and health consequences can be overcome by managing mineral and vitamin deficiencies in humans. Among crops cultivated the fruits and vegetables have a rich source of genetic diversity for micronutrients, and hence they can be easily biofortified through conventional breeding, marker-assisted breeding, or genetic engineering (Das et al. 2017).

5.3 Importance of Micronutrients and Vitamins

Micronutrients and vitamins are an essential component in the growth and development of plants and animals. It is essential for metabolism like in redox reaction and also acts as the cofactor in various biochemical reactions inside the cell. Humans hardly synthesize vitamins, and the major vitamins are taken by plant or animal sources (Grivetti and Ogle 2000; Asensi-Fabado and Munné-Bosch 2010). Vitamins found in fruits and vegetables have strong antioxidant potential, which includes vitamin A, E, and K (lipid-soluble); vitamin B and C (water-soluble). These vitamins are synthesized and stored in the plant part, which are edible like in grain if seeds are used to prepare food. Application of breeding method and genetic engineering for biofortification with various micronutrient (Fe, Zn, I, and Se) and vitamins (vitamin A, B, C, E, and K) is relatively cost-effective and efficient method for counteracting deficiency for human and farm animals (Lyons et al. 2004; Nestel et al. 2006; Zhao and McGrath 2009). It was estimated over 60% of the world's population is deficient in Fe, 30% are deficient in Zn as well as in iodine, and 15% are Se deficient (White and Broadley 2009). Fruits and vegetables are rich in natural vitamins and minerals, which can act as an antioxidant that limits the cell damage from free radicals, which are produced during stress. There are studies related to cancer, where it was found that eating fruits and vegetables can prevent various types of cancer (Wargovich 2000; Tuama and Mohammed 2019). Thus for a healthy lifestyle, the consumption of fruits and vegetables should be done, and a balanced and complete diet should be maintained.

To meet metabolic need human requires at least 49 nutrients, out of which 20 minerals elements are essential for human health. There are seven major elements (Ca, P, K, S, Na, Cl, and Mg) and 12 trace elements (Fe, I, Cu, Zn, Mn, Co, Cr, Se, Mo, F, Sn, Si, and V). All the minerals are needed to supplemented or taken from other sources such as plant or animal products. During the early 1960s, the green revolution made the earth capable of fighting against food security (Pinstrup-Andersen and Hazell 1985). But this reduced the local production of fruits, vegetables, and legumes, which is the primary source of micronutrients in the people (Welch and Graham 2004). Fruits and vegetables are the primary source of mineral and vitamins along with dietary fiber that helps to improve the digestive function and lower the risk of type II diabetes, obesity, and related disorder.

5.3.1 Vitamins

Fruits and vegetables play an important role in human health as they contain important essential components like nutrients, vitamins, and phytochemicals, which may reduce the risk of chronic diseases. Due to improper nutrition, the present lifestyle and unhealthy diet lead to the development of serious diseases like diabetes, obesity, certain types of cancer, stroke, inflammation, and other cardiovascular diseases (Cömert et al. 2019). Vitamin is an essential component of human health,

growth, and development. As discussed earlier, vitamins cannot be produced by humans but need to be consumed from another source like plants and animals.

It was estimated that about 250 million pre-school children are under the risk category of provitamin A deficiency along with the significant proportion of pregnant women who are at risk are suffering from provitamin A deficiency. Due to this provitamin deficiency, the children are at higher risk. It causes visual impairment, blindness, and an increase in the risk of illness from diseases like diarrhea and measles. (WHO 2016). It was estimated that in developing countries, the death of 5–10 million children takes place every year due to this above issue (Anderson 2019). Provitamin A is derived from a plant from the carotenoid pathway. It is a derivative of carotenoid, which includes retinol and its ester, retinal, and retinoic acid. This vitamin plays an important role in vision, immune response, growth of the epithelial cell, bone growth, and regulation of adult genes (Tumuhimbise et al. 2013). Moreover, childhood blindness is due to vitamin A deficiency due to which around 250,000–5,00,000 children go blind, and it is more prevalent in developing countries like sub-Saharan Africa and Southeast Asia (Nestel et al. 2006).

The primary source of provitamin A or carotenoid is commonly found in animal products like egg and milk. The precursor of vitamin A or β-carotene is abundantly found in the colored fruits and leafy vegetables. Change in β-carotene or provitamin A content was studied in dried sweet potatoes when it is processed. It was reported by Bechoff et al. (2009) that *trans-β*-carotene was retained significantly in hot air cross-flow as compared to sun drying. Drying of sweet potato slices was done in an air oven for 12 h at 60 °C; it was found that about 30% reduction in total carotenoid was observed (Hagenimana et al. 1999). Lower retention of provitamin A was reported in artificial drying as compared to natural sun drying. Thus, provitamin A or trans-β-carotene decreases after processing of sweet potato (Hagenimana et al. 1999; Van Hal 2000; Kosambo 2004).

Provitamin A or carotenoid is a powerful antioxidant which prevents the free radical that damage the cell membrane, which are produced during stress. It is used to build new cells and is critical for healthy brain development and nerve function, which ultimately slows down the aging process in humans. It also promotes the growth of strong teeth and bones. The formation of rods and cones in the retina of the eye is dependent on vitamin A which helps us to improve visual (Mata et al. 2002).

5.3.2 Iron (Fe)

Iron (Fe) is an essential micronutrient for humans, animals, and plants. It is redox-active metal, which catalyzes the oxidation-reduction reaction and regulation of cell growth and differentiation. It is an integral part of many proteins and enzymes where it acts as a cofactor for physiological function. It also plays a vital role in metabolic pathways like the electron transport chain of respiration in mitochondria. Most importantly, the human body's Fe is contained in red blood cells (Gómez-Galera et al. 2010; Jomova and Valko 2011). If Fe deficiency occurs in the body, the amount of hemoglobin is also affected, and the symptoms of anemia take place along with

tiredness, weakness, and inability to concentrate (Das et al. 2017). Deficiency of Fe during childhood and puberty stage may impair physical growth, mental development, and poor learning capacity. Moreover, severe anemia increases the risk of dying during childbirth (Beard 1994). It also catalyzes the formation of provitamin A from carotene and also induces antibodies synthesis, which ultimately enhances immunity (Semba 1994). The RDA (mg Fe day^{-1}) for Fe is 10, 8, and 18 for children, adult males, and adult females (Trumbo et al. 2001).

Fe is derived from animal products like milk and meat and also from plant products. Before absorption of Fe in body, the bound Fe should be well hydrolyzed or solubilized. But the absorption of Fe largely depends upon the presence of enhancer and inhibitory substances (Baltussen et al. 2004). The compounds like ascorbic acid and cysteine enhance Fe absorption in the gut, whereas compounds like phytic acid chelate with Fe and Zn which inhibit the absorption of Fe in the gut (Kumar et al. 2017).

5.3.3 Iodine

Iodine is a crucial microelement which plays an essential role in the metabolism of human. Its deficiency can lead to a severe disorder of thyroid and the inadequate synthesis of hormone which are released from thyroid. The RDA of iodine is 40–200 μg day^{-1} (Dai et al. 2004). It has multiple roles in human growth and development, which involve enhancement of protein, regulating the energy transfer, and maintaining the central nervous system (Gerber et al. 1999). It controls the production of thyroid hormone known as thyroxine (T4) and triiodothyronine, which is the major hormone responsible for maintaining the above function. The reports from WHO exposed that about 1.6 billion people are suffering from iodine deficiency throughout the world (WHO 2016).

The important primary source of iodine supplementation followed nowadays is seafood and iodized salt. The consumption of seafood is very much helpful to obtain iodine supplementation, but it does not meet the need of iodine-deficient area and the vegetarian population. Moreover, the supplementation of food products with iodine may be helpful in controlling the deficiency of iodine, but it may be difficult to control the losses during transport, storage, and food cooking (Winger et al. 2008). The 80% of the requirement of iodine in the human body comes from the edible vegetable, which is grown under the natural condition, and about 99% bioavailable iodine can be in this food (Welch and Graham 2004).

5.3.4 Zinc

Zinc (Zn) is an important trace microelement for microorganisms, plant as well as animal (Broadley et al. 2007; Prasad 2008). It is the cofactor for various enzymes and proteins. It is involved in different physiological functions, such as the functioning of the immune system, protein synthesis, DNA synthesis, wound healing, and

cell division (Bao et al. 2010). A large number of enzymes and proteins are dependent upon Zn to maintain their structural stability and transcription factor. It also helps to improve and maintain the immune system, thus prevent infection and diseases (Prasad 2008; Bao et al. 2010). Moreover, Zn is required to activate over 300 enzymes in the human system. In humans and animals, it plays an important role in the functioning of reproductive health, sensory function, digestive system, and neurobehavioral development (Levenson and Morris 2011). The RDA (mg Zn day^{-1}) for children is 5, for adult males is 11, and 8 for adult females (Trumbo et al. 2001).

It also participates in the synthesis and degradation of carbohydrates in plants. Along with carbohydrates, it also involves the synthesis of lipid, protein, and nucleic acid (Brown et al. 1993). It is the only element that is involved in all the six classes of enzymes, namely oxidoreductase, transferase, hydrolases, lysases, isomerases, and ligases (Barak and Helmke 1993). It occurs in the plant as a free ion and may be complexed with low molecular weight compounds. The deficiency of Zn in plants leads to inhibition of photosynthesis, decreases the production of auxin hormone (as the cofactor for the synthesis of auxin), and decreased rate of respiration. Moreover, its deficiency in the plant may also lead to an increase in the disruption of the plasma membrane as many antioxidant enzymes may be inhibited. This leads to the enhancement of reactive oxygen species in the cell, thus damaging the cellular compartment and organelles (Brown et al. 1993). Fe can have a negative effect on Zn absorption in the human gut if given together in a supplement. Most importantly, it was observed that protein meals enhance Zn absorption. Other compounds such as amino acid and low molecular weight ions (EDTA and organic acid like citrate) are shown to have a positive effect on Zn absorption and have been used for Zn supplement (Zhao et al. 2012). The bioavailability of Zn in cereals, legumes, vegetables, nuts, and wholegrain can be inhibited by phytic acid. Phytic acid inhibits the release of Zn from the food by chelating it and thus making it unavailable for absorption in the human gut (Kumar et al. 2017).

5.3.5 Selenium

Selenium (Se) is a trace element which is required by human and animal in very minute quantity, but it is essential for human growth and development. However, in higher concentrations, it has a toxic effect. The protein which contains Se is known as selenoprotein, which has a structural and enzymatic role. It also acts as antioxidant element to prevent from the damage of the free radical formation. The other property of Se also includes the catalysis of active thyroid hormone. It also prevents immune response disease and helps to maintain the immune system, which further acts as a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS (Rayman 2000). The deficiency of Se in the blood can lead to various health implications like cancer and cardiovascular diseases (Brown and Arthur 2001). It was reported that its deficiency might lead to adverse mood states, oxidative stress, and inflammation. The higher intake of Se is associated with the

reduced risk of cancer (Rayman 2012). Maintaining Se intake in the diet may prevent the risk of lung, prostate, colorectal, and bladder cancers. The deficiency also leads to muscular dystrophy and muscle weakness.

The uptake of Se can take place in the plant as selenite, selenite, or organoselenium compounds. These compounds mainly include selenocysteine and selenomethionine, but they cannot take up as colloidal elemental Se of metal selenides (White et al. 2004). Se transported inside the plant cell through high-affinity sulfate transporter (HAST) in root cell (Terry et al. 2000; Li et al. 2008). Moreover, it is also transported through phosphate transporter (Broadley et al. 2006). The uptake of Se takes place in the roots where selenite is delivered to xylem and transported the shoot, and here, assimilation takes place where selenite is converted to organoselenium compounds (White and Broadley 2009).

5.4 Physiology of Biosynthesis of Vitamins and Micronutrients

Fortification of a food product is necessary for many countries, where there is a fortification with vitamin A in butter, margarine, and sugar, iodine fortification in salt, vitamins A fortification in mild, and folate fortification in cereals and legumes (Kumar et al. 2019). However, there were various drawbacks of fortification/supplementation. So the technology of biofortification came into action where enhancement of vitamin and mineral can be done in the edible part of staple food with the help of breeding or genetic engineering. So to understand the process of accumulation of vitamin and mineral in staple food, most importantly, the physiology of crop is to be explored. The basic pillars of the biofortification program are to understand the physiological process and bioavailability of nutrients and vitamins. So, the knowledge of the physiology of nutrient uptake, phytoavailability of micronutrients in the rhizosphere, and vitamin synthesis are essential (Bowen and Rovira 1991).

5.4.1 Vitamins

Vitamins are primarily synthesized in the plant. Out of which folates and provitamin A are mainly targeted for the biofortification program. Folates are the group of water-soluble vitamin B, which is also known as Vitamin B9, which consists of pteridine ring and a para-aminobenzoate moiety (*p*-ABA) and is linked with γ-linked tail with more L-glutamates (Strobbe and Van Der Straeten 2017). Folate biosynthesis takes place in the cytosol where the pterin branch is formed, and it yields 6-hydroxymethyl-dihydro pterin (HMDHP). Folates are present in mitochondria, plastids cytosol, and vacuoles (Chen et al. 1997).

The synthesis of carotenoid takes place de novo in the plant, and it is mainly involved in the synthesis of photosynthetic pigment and precursor for other molecules such as signaling molecules. It is synthesized in plastid and is an essential component of a healthy diet, antioxidant, and also a precursor of provitamin A (Giuliano 2017). The precursor of provitamin A is a C40 isoprenoid unit, which is a

carotenoid. It is synthesized in plastid from the 2-C-methyl-d-erythritol 4-phosphate pathway. Geranylgeranyl pyrophosphate is formed after condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate in the presence of isopentenyl diphosphate isomerase. Geranylgeranyl pyrophosphate is the precursor of other isoprenoid molecules, like gibberellins, quinones, provitamin A, and the isoprenoid moieties of chlorophylls and tocopherols which is the major constituents of chloroplast (Giuliano et al. 2000). The enzymes responsible for the synthesis of β-carotene are phytoene synthase (Psy), phytoene desaturase (Pds), ζ-carotene desaturase (Zds), and lycopene cyclase (Lcy) (Sandmann 2001).

5.4.2 Minerals

The uptake of micronutrients like Fe, Zn, I, and Se are generally taken place from the rhizosphere. These micronutrients are then transported from root to edible part of the plant. The uptake, transport, and accumulation of micronutrients are highly regulated and tightly controlled mechanism (Welch 1995).

5.4.3 Iron

The absorption of Fe takes place in the rhizosphere. The proton is released from plants in the rhizosphere, which lowers the pH of soil solution and thus increases the solubility of Fe^{3+} (Santi and Schmidt 2008). There are two strategies for the uptake of Fe in plant, viz. the strategy I and strategy II (Santi and Schmidt 2008). Strategy I takes place in all the plants except plant from Graminaceae family. The Fe uptake in this strategy takes place by reduction of ferric iron, and this is then bound to chelates (citrate and nicotianamine) and subsequent uptake of liberated ferrous iron. This step is mediated by an iron-regulated enzyme known as ferric oxidoreductase (FRO). The strategy II of uptake of Fe takes place in microorganism and grasses. In this strategy, there is a release of protein known as phytosiderophores in the rhizosphere. This protein takes up the ferric iron-loaded phytosiderophores-metal complex inside the plant through membrane-bound transporter (Robinson et al. 1999).

5.4.4 Zinc

The uptake of Zn primarily takes place by Zn transporter of the ZIP family, which is highly regulated in root and other tissues. The transcription factor which is responsible for Zn uptake in the plant is two bZIP families, and it is upregulated under Zn deficiency (Grotz et al. 1998). It is generally absorbed from soil as Zn^{2+} or $Zn(OH)_2$. Phytosiderophores have the same mechanism of uptake for Zn. Phytosiderophores like mutagenic acid, avenic acid, and nicotinamine are responsible for the uptake of Zn from the soil. Under deficiency condition, Zn chelates with phytosiderophores, and its uptake rate was found to be increased (Ueno et al. 2007). The mode of

transportation of Zn between the cells can be symplastic or apoplastic. The factor which is responsible for absorption and desorption of Zn includes chemical form of Zn, total concentration of Zn in soil, pH of soil, organic matter content of soil, temperature of soil, carbonate and phosphate content of soil, microorganism content of soil, and other relative biological activities of plant (White and Broadley 2005).

5.4.5 Iodine

The soil contains a very low amount of iodine, and this amount is insufficient for humans and animals in comparison to their nutritional needs (Halka et al. 2019). It is present in a very trace amount which is fixed with organic matter, clay, and oxides of Fe and Al. The WHO recommended the daily dose of 5 g/day iodized salt (WHO 2013); however, the uptake of iodine is less. Iodine is taken by the plant in the form of iodate and iodite. The most prevalent form of iodine in soil solution is iodite (Fuge and Johnson 1986). The experiments on Chinese cabbage revealed that iodine uptake by this Chinese cabbage was more effective when iodine was supplied as iodate as compared to iodite in low concentration (Weng et al. 2008a). The iodine concentration in root was greater in as compared to leaf. The spray of iodine solution in cabbage and spinach increases the level of iodine in both roots and leaves (Weng et al. 2013). It was reported that the degree of phytotoxicity of iodine exists in soil solution, and iodide is more phytotoxic than iodate; this may be due to plants absorb a more reduced form of iodine (Weng et al. 2013).

5.4.6 Selenium

The micronutrient Se is not that essential for plant growth and development, but it is much more essential micronutrient for humans and animals (Fordyce 2013). In the plant, it enhances the antioxidant capacity, which helps to alleviate the heavy metal stress. The uptake of Se in the plant does not take in its colloidal elemental or selenide form, but in plant roots, the uptake takes place in the form of selenite, selenite and organoselenium compounds (White and Broadley 2009). The Se content in normal soil is around 0.01 and 2.0 mg Se kg^{-1}, whereas seleniferous soil contains Se concentration up to 1200 mg Se Kg^{-1} (White and Broadley 2009). It is mobile in the soil solution, and sometimes it strongly gets fixed with Fe and Al in soil (Broadley et al. 2006). It was reported by Hawkesford and Zhao 2007 that high-affinity sulfate transporter is involved in the transport of selenite across the plasma membrane of root cell, and phosphate transporter is involved in the transportation of selenite. Selenite is converted to organoselenium in root tissue and further transported all over in plant via xylem and redistributed in the plant in a similar manner as sulfur is distributed (Li et al. 2008).

5.4.7 Silicon

Recently silicon is getting wide attention due to numerous studies demonstrating beneficial role of the element for plant as well as human health (Deshmukh et al. 2017; Ratcliffe et al. 2017). Recently, International Plant Nutrition Institute (IPNI, http://www.ipni.net/) has announced silicon as beneficial element for the plant health. Similarly, numerous silicon based tonics and health supplements have been released worldwide (Scholey et al. 2018). In this regard, efforts are being made towards the enhancement of silicon uptake more particularly in vegetable crops (D'Imperio et al. 2016; Montesano et al. 2016). In a study performed by Montesano et al. (2016) performed Si biofortified in green bean pods by growing plants with the supplementation of Si-enriched nutrient solution. They have also showed effect of boiling and steaming cooking methods on Si content in the cooked green beans. Similarly, De Souza et al. (2019) study has shown that the silicon spray on leaves can promote biofortification and also help to increase the biomass and ascorbate content in Chard and Kale. However, most of the vegetables belong to Solanaceae and Brassicaceae family cannot uptake silicon from soil and such species are well known poor silicon accumulators (Deshmukh et al. 2015; Deshmukh and Bélanger 2016; Sonah et al. 2017). Efforts can be made through transgenic or genome editing approaches to make such species genetically capable to uptake and accumulate significant amount of silicon (Vats et al. 2019; Mushtaq et al. 2020).

5.5 Agronomic Biofortification of Vegetables

The agronomic biofortification of vegetables is one of the simplest and easy methods of biofortification. However, this strategy requires a long period and adequate funds, and this technique is useful in the countries where the genetic engineering method of biofortification is not well accepted. In this approach, generally, fertilizer is used either in the form of spray on leaves or the application of fertilizer in soil (Weng et al. 2008b). The biofortification of Fe and Zn was reported to be successful where the foliar application was used to enhance these nutrients in plant tissue and edible part (Saltzman et al. 2013). The agronomic approach for biofortification also includes management practices during the crop growing season. The package and practices like tillage, water management, and nutrient interaction are involved in enhancing micronutrient. Foliar application is the better option for agronomic biofortification, which requires less amount of Fe and Zn fertilizer as compared to soil application (Prasad et al. 2014).

5.6 Breeding of Vegetable for Biofortification of Vegetables

The current knowledge of all the processes for enhancement of micronutrients and vitamins in the edible part of the plant is very limited. More basic research is needed to accumulate the micronutrients, making micronutrients in bioavailable form and

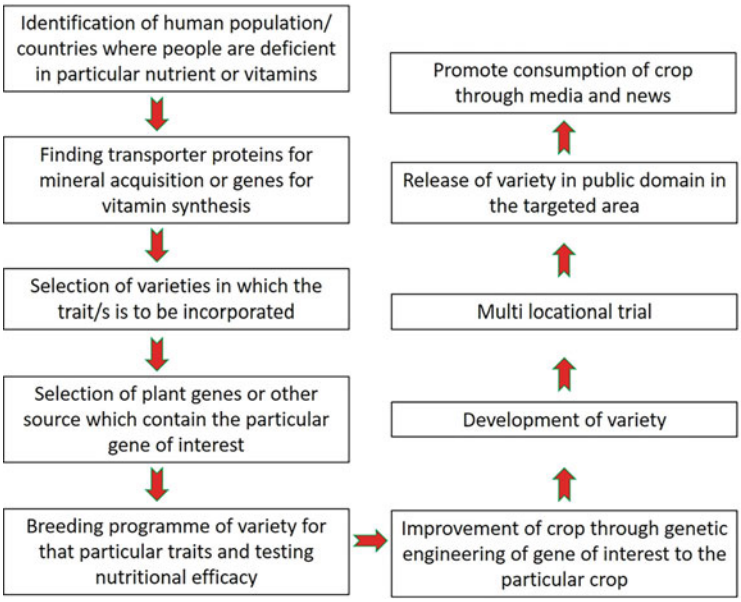

Fig. 5.2 Flow chart for biofortification of vegetable using a breeding program or genetic engineering as a tool

ultimate increasing vitamin in seed, grain, tuber, flesh, and other edible parts of staple cereals and vegetables. Using classical or modern breeding techniques and also with the help of genetic engineering, this can be possible to biofortify large amounts of crops and can be disseminated throughout the world (Welch and Graham 2004). The effort is for one time in the breeding of a biofortified crop. Once the plant is developed and is adopted by the farmer, then the seed can be reproduced, multiplied, and shared among all the farmer groups of the targeted area (Fig. 5.2). This will ultimately help to maintain high nutrient traits, overtime in that particular crop (Graham et al. 2001). The conventional breeding is based on natural variation and maybe a good alternative to genetic engineering experiment. It was found that the folate content in vegetables such as tomato and potato was found to be increased twofold in newly developed breeding lines (Hanson and Gregory 2011).

Due to the increase in the demand for the biofortified product, the production has to be increased using the genetic basis of plant breeding. The criteria have to be set for the breeding of vegetables for micronutrients and vitamins, which will meet the demand of farmers as well as the targeted people (Fig. 5.3). Firstly, vegetable production and productivity should be maintained so that it will be widely accepted by the farmer, and they must get the revenue as invested by them. The yield of biofortified vegetables must be more or at par with the previous version of that vegetable. Secondly, the micronutrient concentration in the vegetable should be achieved significantly so that it will have an impact on human health. Thirdly, the particular trait for the biofortification of vegetables should be stable between

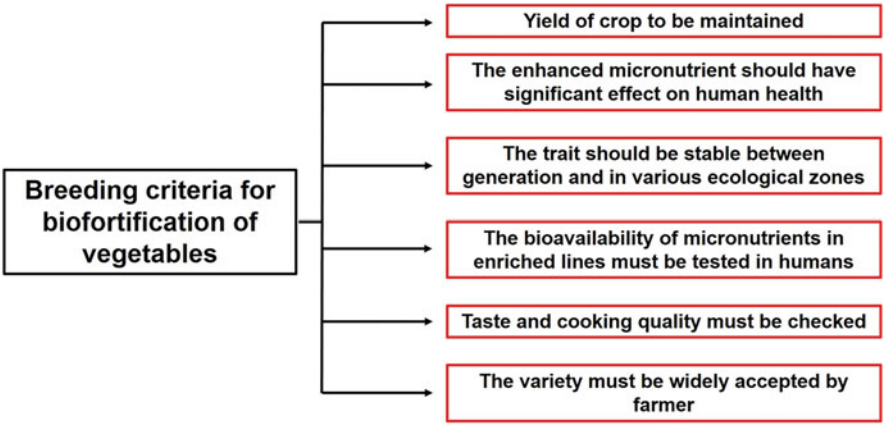

Fig. 5.3 Breeding criteria for biofortification of vegetables

generations. This is applicable to the various ecological and climatic zone where the vegetable is suitable for its growth. Fourthly, the micronutrient bioavailability should be maintained while cooking, and its level should not alter after cooking. Fifthly, the taste of biofortified products should be acceptable to the consumer. And the final criteria should be the acceptance by the farmer. More publicity and importance of crops should be disseminated to the farmer so that they will know the importance of growing of biofortified vegetables (Welch and Graham 2004).

The aim of CGIAR and HarvestPlus is to determine the genetic variability and heritability of the mineral trait and their stability across the different climatic and ecological zone, soil conditions, and a number of the gene responsible for its uptake and feasibility of breeding increased concentration of several minerals. The conventional breeding for more nutrition relies on the inheritance of genes responsible for the particular trait and favorable quantitative trait loci (QTL) from sexually compatible parental lines. Thus, biofortification by breeding has its own constraints of finding a natural variation of desired traits and the collection of vegetable germplasm, which is a time-consuming process Shimelis and Laing 2012. The recent advances in plant breeding techniques such as genome-wide association studies (GWAS) and marker-assisted breeding (MAB) is the powerful and important tools for biofortification. These newly advanced techniques helped breeder to identify the QTLs responsible for the increase in β-carotene and α-tocopherol up to 3.22 and 5.76 fold respectively (Azmach et al. 2013; Lipka et al. 2013).

A horticultural crop like cassava was targeted for enhancement of provitamin A. The variation in β-carotene concentration on cassava roots was screened from CIAT core collection (5500 genotypes). It was reported that after breeding for β-carotene content in cassava its content ranged from 0.1 ± 2.4 mg /100 g (Chávez et al. 2000). It has been suggested that the biofortification breeding programme for Se and iodine should be done simultaneously and the primary target for both the nutrient is thyroid and its metabolism (Lyons et al. 2005). Along with the breeding

strategies, some research intervenes the agronomic practices to get a better result. Weng et al. (2008b) applied iodine fertilizer along with diatomaceous earth to radish (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L.), and Chinese cabbage (*Brassica chinensis* L.) and found that iodine concentration was high in the leaves of these vegetables. In another experiment, Zhu et al. 2003 reported that iodate ion had a less detrimental effect on biomass production of spinach as compared to iodide ion (Zhu et al. 2003). Landini et al. (2011) study the uptake of iodine in tomato and its concentration in its fruit using radioactive iodine. They concluded that the iodine concentration was higher when plants were supplied with iodine hydroponically. Greenhouse experiment on soil-grown spinach revealed that the addition of iodate and iodide in the soil does not lead to an increase in biomass of spinach (*Spinacia oleracea* L.), but there was an increase in iodine concentration in leaf tissues. However, iodate is accumulated in the leaf tissues as compared to iodide (Dai et al. 2006). To understand the genetic variability of micronutrient content in potato germplasm breeding program was carried out in eight clones of potatoes. After analysis for Cu, Fe, Mn, and Zn content in potato clones, it was found that the difference between the clones was significant for micronutrient (Haynes et al. 2012). These variations in potato germplasm for micronutrients are large and can be used to improve the quality of the potato through a breeding program.

The identification of the genetic variability of the crop should be screened in which there is more accumulation of targeted micronutrients. The screening process should be emphasized on the rate and proportion of accumulation of micronutrients in the edible part (Calderini and Ortiz-Monasterio 2003). This is a one-time process, and once the high-yielding, high vitamins, and nutrient lines are developed, this has to be tested in multilocation for confirming its stability to grow in region-specific (Fig. 5.2). Conventional plant breeding is a cost-effective method, which is a widely accepted method for making plant biofortified and stable. The sustainable and cost-effective solution may be provided by plant breeding, and this will help to deliver micronutrients and vitamins to the targeted population. However, the uptake and accumulation of micronutrients in crops such as vegetables are regulated by polygene and having minor effects. Therefore, the conventional breeding of biofortification approaches has met with only marginal success (Naqvi et al. 2009). But in the absence of adequate genetic variability and variation among traits gene effect, genetic engineering will be more viable for the enhancement of micronutrients at the desired level. Despite various techniques in conventional breeding such as heterosis, transgressive segregants, mutational breeding, quantitative genetics, marker-assisted breeding, QTL mapping, etc. to explore the genetic variability for vitamins and micronutrients, it takes more time and labor as compared to genetic engineering. With the powerful tools such as "omics" technologies, gene editing tools like transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9 help to increase the opportunity for new biofortification strategy in very less period of time.

There are some other examples of successful biofortification programs where the products are disseminated to the public. The Cowpea varieties Pant Lobia-1 and Pant Lobia-2 were released by G.B. Pant University of Agriculture and Technology,

Pantnagar, India which was biofortified with high Fe and Zn respectively. Pant Lobia-1 and Pant Lobia-2 were released by Uttarakhand Government, India in 2008 and 2010, respectively.

5.7 Biotechnological Tools for Biofortification of Vegetables

Biotechnology is a powerful tool for the biofortification tool, which is being used worldwide to combat the seriousness of mineral and vitamin deficiency. The recent advancement in the tools and techniques of genetic engineering enables to incorporate the trait which cannot be possible through conventional breeding (Chaudhary et al. 2019; Rana et al. 2019). Not only one trait, but multiple traits or pathway can be targeted using this technology. The classic example is of increasing the bioavailability of micronutrients, enhancing β-carotene, ascorbate, and folate in a single plant (multivitamin corn) (Carvalho and Vasconcelos 2013).

The goal for biofortification of vegetable by genetic engineering includes several points which need to be considered before designing the crop for enhancing particular component (Fig. 5.4). The micronutrient which is fixed in soil should be made available for the plant before absorption. Various transporter systems present in plant cells involved in the uptake of mineral from soil (Ram et al. 2019; Vishwakarma et al. 2019). The uptake efficiency of these mineral uptakes should be increased using a genetic tool (Zhu et al. 2013; Pinto and Ferreira 2015). The second goal is the redistribution of micronutrients within the plant system. The source and sink relationship will help to maintain the nutrient in the plant system. The accumulation of micronutrients like Zn can be enhanced in the shoot by foliar application, but accumulation in fruit, seeds, and tubers is limited by Zn transport in the phloem (White and Broadley 2011). Thirdly, using the genetic tool, the pathway is

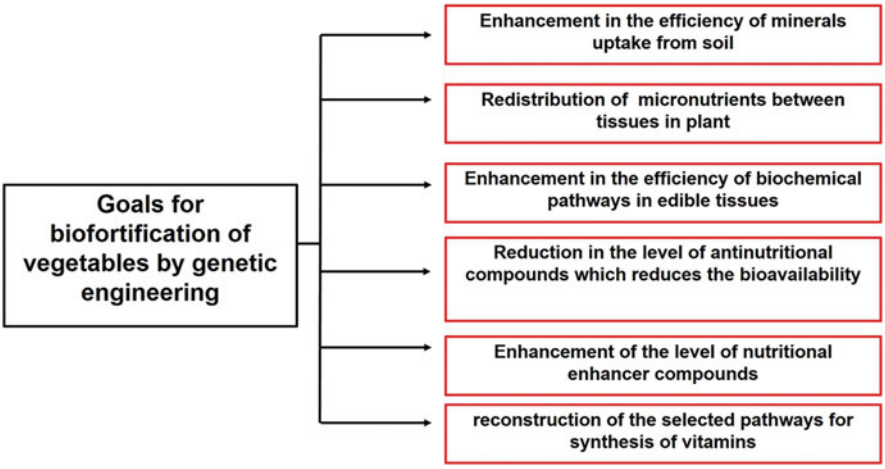

Fig. 5.4 Goals for biofortification of vegetable by a genetic engineering approach

responsible for enhancing the efficiency of the biochemical pathway in edible tissues. Fourthly, the biotechnological tool can be helpful in reducing the anti-nutritional compound, which ultimately affects the bioavailability of micronutrients. Fifthly, the level of nutritional enhancer can be increased by the overexpression of gene, which is responsible for nutrient bioavailability (Rawat et al. 2013). Lastly, the modification in the pathway leads to the production of higher vitamin in the edible part of the plant and consumption of which can reduce the incidence of malnutrition.

The studies on the bioaccessibility and bioavailability of β-carotene from plant matrixes revealed that the transgenic crops such as cassava, sweet potato, melon, sorghum, and potato perform better in terms of bioaccessibility and bioavailability as compared to non-transgenic plant (Failla et al. 2009, 2012; Thakkar et al. 2009; Fleshman et al. 2011; Lipkie et al. 2013). The major factor that affects the bioaccessibility and bioavailability is processing and cooking. The pathway of bacterial origin was incorporated in potato where there is a synthesis of β-carotene (provitamin A) from geranylgeranyl diphosphate in the MEP pathway (Diretto et al. 2007). They used a tuber-specific or constitutive promoter to express three gene encoding phytoene synthase (CrtB), phytoene desaturase (CrtI), and lycopene beta-cyclase (CrtY) from *Erwinia* in potato. It was reported that the carotenoid content and β-carotene content was enhanced 20 and 3600-fold respectively in transgenic potatoes (Diretto et al. 2007). The other technique of making a transgenic plant is by blocking the rate-limiting enzyme in the pathway. Römer et al. (2002) developed transgenic potato, which was biofortified with zeaxanthin and β-carotene by silencing the ZEP gene. The transgenic line was shown to increase zeaxanthin content up to 130-fold in potato tuber. Tomato is rich in lycopene and β-carotene, 5–15% depending upon the varieties and genotype. The transgenic approach was made by overexpression of phytoene synthase and phytoene desaturase using *35S::tp::crtI* promoter. The leaves of *crtI* tomatoes, β-cyclic carotenoids were enriched (Giuliano et al. 2000). Metabolic engineering efforts that overexpressed two folate synthesis genes in combination have increased folate levels by up to 25-fold in tomato fruit and 100-fold in rice grains (Hanson and Gregory 2011).

The overexpression of gene FEA1 from *Chlamydomonas reinhardtii* in cassava and sweet potato leads to successful enhancement of Fe accumulation in edible tuber tissue (Chávez et al. 2007). Cation transporter families such as ZIP (ZRT, IRT-related protein) and CDF (Cation diffusion facilitator) play an important role in the Zn uptake and translocation in the plant. IRT2 protein of the ZIP family was identified in Arabidopsis thaliana root cell, which significantly contributes to Zn uptake (Korshunova et al. 1999). The studies were carried out to enhance Fe content using a transgenic approach. Lactoferrin is a Fe-chelating glycoprotein from human milk and is a family of transferrin family, and ferritin is the protein that can store 4500 Fe in the bioavailable form (Kanyshkova et al. 2001). The transgenic plant was developed using the rice glutelin-1 promoter to increase the Fe content and was found that Fe content was increased significantly as compared to control (Nandi et al. 2002). The overexpression of the gene encoding Fe (III) reductases that enhance Fe uptake gene in the non-graminaceous plant is one of the strategies to biofortify the plant (Connolly et al. 2003).

Anti-nutrients such as phytic acid and tannins inhibit the absorption of Fe, Zn, and Ca in human and animal gut, thus reducing the bioavailability of micronutrients (Welch and Graham 2004). There is intra-specific variation in the phytate content in the edible portion of cereal grain and is independent of Fe and Zn concentration. However, Fe and Zn are inhibited by phytic acid content, and the bioavailability of Fe and Zn depends on cooking and processing (Kumar et al. 2017). By reducing phytic acid in cereals and vegetables, the bioavailability of Fe and Zn can be increased. It was reported that by knocking down enzymes of the IP6 pathway, the mineral bioavailability was enhanced. Moreover, the overexpression of phytase and phytate-degrading enzyme in the edible portion can also decrease the level of phytic acid, which ultimately enhance micronutrient bioavailability (Goto and Yoshihara 2001).

Iodine is an essential component of the hormones produced from the thyroid gland in humans, and it plays many vital roles with respect to the growth and development process (Velasco et al. 2018). It was reported in tomato that the expression of a gene such as HMT (encode for methyltransferase), SAMT (encode for salicylic acid carboxyl methyltransferase), and S3H (encode for salicylic acid 3-hydroxylase) could enhance iodine concentration in tomato fruit. In potato, the content of Se was increased under tropical climate by application of Se in small doses. The concentration of Ca, along with Se, was reported to be increased (de Oliveira et al. 2019).

5.8 Future Thrust

The current researches are now focusing on maintaining a healthy life by consumption of nutraceutical supplements as well as healthier diet from fruits and vegetable. So, there is scope to enhance micronutrients and vitamins in vegetables on a large scale through biofortification program. This will help developing countries to overcome the issue of malnutrition or "the hidden hunger." However, many breeding programs are focused on the improvement of production and productivity, tolerance to abiotic stress, resistance to biotic stress. But enhancing the quality of vegetables will help those developing countries to save revenue, which may be spent against the disease, which is caused by micronutrient and vitamin deficiency. To achieve biofortification of vegetable it requires the collaboration of plant breeder, plant physiologist, biochemist, molecular biologist and other nutrition scientists. However, the genetically modified crop may require regulatory approval from various committees before it is released. The recent advanced technology in the field of genetics and genome editing (TALENS, CRISPR/Cas9, etc.) will help this biofortification program to move at a greater pace. More particularly, use of CRISPR/Cas9 based genome editing is being widely used in crop plants including many vegetables (Vats et al. 2019; Mushtaq et al. 2020). The recent advancement in the genome editing provides numerous approaches to get desired genetic modification bypassing the regulatory issues associated with transgenic technologies.

5.9 Conclusion

The major area of research for developing countries after food security is nutritional security. Because the major population of the developing world is suffering from "hidden hunger" and combating this problem, the agricultural scientist is capable of changing the physiology of crops by biofortification of vegetables and cereals. There is much scope for plant breeders, molecular scientists, and genetic engineers to improve micronutrient density and vitamin content of staple food crops and vegetables for developing countries. Moreover, after the development of variety, which is rich in micronutrients and vitamins, it should be adopted by the farmer on a large scale without hindering its production and productivity. There is enough genetic diversity of vegetables available, and it has to be screened for a particular trait. For enhancing micronutrient in the plant, there should be a clear understanding of the mechanism of ion uptake from soil, redistribution within tissues, and homeostasis in the plant. Working on enhancing micronutrient and vitamin with the help of conventional breeding or by genetic engineering both requires particular traits that need to be incorporated. The recent advances in genetics made it possible to enhance micronutrient by reducing anti-nutrients such as reduction of phytic acid or tannins. Genome editing tools like ZFN, TALENS, CRISPR-Cas9, etc. have the potential to edit plant genes or knockdown undesirable traits and can be exploited for the biofortification of vegetables.

References

Anderson MD (2019) 6 comparing the effectiveness of structures for addressing hunger and food insecurity. In: Civil society and social movements in food system governance. Routledge, New York, p 124

Asensi-Fabado MA, Munné-Bosch S (2010) Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends Plant Sci 15:582–592

Azmach G, Gedil M, Menkir A, Spillane C (2013) Marker-trait association analysis of functional gene markers for provitamin A levels across diverse tropical yellow maize inbred lines. BMC Plant Biol 13:227

Baltussen R, Knai C, Sharan M (2004) Iron fortification and iron supplementation are cost-effective interventions to reduce iron deficiency in four subregions of the world. J Nutr 134:2678–2684

Bao B, Prasad AS, Beck FWJ et al (2010) Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am J Clin Nutr 91:1634–1641

Barak P, Helmke PA (1993) The chemistry of zinc. In: Zinc in soils and plants. Springer, Dordrecht, pp 1–13

Beard JL (1994) Iron deficiency: assessment during pregnancy and its importance in pregnant adolescents. Am J Clin Nutr 59:502S–510S. https://doi.org/10.1093/ajcn/59.2.502S

Bechoff A, Dufour D, Dhuique-Mayer C et al (2009) Effect of hot air, solar and sun drying treatments on provitamin A retention in orange-fleshed sweetpotato. J Food Eng 92:164–171. https://doi.org/10.1016/j.jfoodeng.2008.10.034

Black RRE, Allen LHL, Bhutta ZZA et al (2008) Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371:243–260

Bouis HE (2000) Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. Nutrition 16:701–704

Bouis HE, Hotz C, McClafferty B et al (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32:S31. https://doi.org/10.1177/15648265110321s105
Bowen GD, Rovira AD (1991) The rhizosphere: the hidden half of the hidden half. In: Plant roots: the hidden half. Marcel Dekker, New York
Broadley MR, White PJ, Bryson RJ et al (2006) Biofortification of UK food crops with selenium. Proc Nutr Soc 65:169–181
Broadley MR, White PJ, Hammond JP et al (2007) Zinc in plants. New Phytol 173:677–702
Brown K, Arthur J (2001) Selenium, selenoproteins and human health: a review. Public Health Nutr 4:593–599. https://doi.org/10.1079/PHN2001143
Brown PH, Cakmak I, Zhang Q (1993) Form and function of zinc plants. In: Zinc in soils and plants. Springer, Dordrecht, pp 93–106
Calderini DF, Ortiz-Monasterio I (2003) Are synthetic hexaploids a means of increasing grain element concentrations in wheat? Euphytica 134:169–178
Carvalho SMP, Vasconcelos MW (2013) Producing more with less: strategies and novel technologies for plant-based food biofortification. Food Res Int 54:961–971
Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari A, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H (2019) Advances in omics approaches for abiotic stress tolerance in tomato. Biology 8(4):90
Chávez AL, Bedoya JM, Sánchez T et al (2000) Iron, carotene, and ascorbic acid in cassava roots and leaves. Food Nutr Bull 21:410–413
Chávez AL, Sánchez T, Ceballos H et al (2007) Retention of carotenoids in cassava roots submitted to different processing methods. J Sci Food Agric 87:388–393. https://doi.org/10.1002/jsfa.2704
Chen L, Chan SY, Cossins EA (1997) Distribution of folate derivatives and enzymes for synthesis of 10-formyltetrahydrofolate in cytosolic and mitochondrial fractions of pea leaves. Plant Physiol 115:299–309
Cömert ED, Mogol BA, Gökmen V (2019) Relationship between color and antioxidant capacity of fruits and vegetables. Curr Res Food Sci 2:1. https://doi.org/10.1016/J.CRFS.2019.11.001
Connolly EL, Campbell NH, Grotz N et al (2003) Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. Plant Physiol 133:1102–1110
Dai JL, Zhu YG, Zhang M, Huang YZ (2004) Selecting iodine-enriched vegetables and the residual effect of iodate application to soil. Biol Trace Elem Res 101:265–276. https://doi.org/10.1385/BTER:101:3:265
Dai JL, Zhu YG, Huang YZ et al (2006) Availability of iodide and iodate to spinach (Spinacia oleracea L.) in relation to total iodine in soil solution. Plant Soil 289:301–308. https://doi.org/10.1007/s11104-006-9139-7
Das A, Laha S, Mandal S et al (2017) Preharvest biofortification of horticultural crops. Elsevier Inc., Amsterdam
de Oliveira VC, Faquin V, Andrade FR et al (2019) Physiological and physicochemical responses of potato to selenium biofortification in tropical soil. Potato Res 62:315–331. https://doi.org/10.1007/s11540-019-9413-8
De Souza JZ, De Mello PR, Silva SLO, Farias TP, Neto JG, Souza Junior JP (2019) Silicon leaf fertilization promotes biofortification and increases dry matter, ascorbate content, and decreases post-harvest leaf water loss of chard and kale. Commun Soil Sci Plant Anal 50(2):164–172
Deshmukh R, Bélanger RR (2016) Molecular evolution of aquaporins and silicon influx in plants. Funct Ecol 30(8):1277–1285
Deshmukh RK, Vivancos J, Ramakrishnan G, Guérin V, Carpentier G, Sonah H, Labbé C, Isenring P, Belzile FJ, Bélanger RR (2015) A precise spacing between the NPA domains of aquaporins is essential for silicon permeability in plants. Plant J 83(3):489–500
Deshmukh RK, Ma JF, Bélanger RR (2017) Role of silicon in plants. Front Plant Sci 8:1858
D'Imperio M, Renna M, Cardinali A, Buttaro D, Santamaria P, Serio F (2016) Silicon biofortification of leafy vegetables and its bioaccessibility in the edible parts. J Sci Food Agric 96(3):751–756

Diretto G, Al-Babili S, Tavazza R et al (2007) Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. PLoS One 2:e350
Failla ML, Thakkar SK, Kim JY (2009) In vitro bioaccessibility of $β$-carotene in orange fleshed sweet potato (Ipomoea batatas, lam.). J Agric Food Chem 57:10922–10927
Failla ML, Chitchumroonchokchai C, Siritunga D et al (2012) Retention during processing and bioaccessibility of $β$-carotene in high $β$-carotene transgenic cassava root. J Agric Food Chem 60:3861–3866
Fleshman MK, Lester GE, Riedl KM et al (2011) Carotene and novel apocarotenoid concentrations in orange-fleshed Cucumis melo melons: determinations of $β$-carotene bioaccessibility and bioavailability. J Agric Food Chem 59:4448–4454
Fordyce FM (2013) Selenium deficiency and toxicity in the environment. In: Essentials of medical geology. Springer, Dordrecht, pp 375–416
Fuge R, Johnson CC (1986) The geochemistry of iodine—a review. Environ Geochem Health 8:31–54
Gerber H, Peter H-J, Bürgi E et al (1999) Colloidal aggregates of insoluble inclusions in human goiters. Biochimie 81:441–445
Giuliano G (2017) Provitamin A biofortification of crop plants: a gold rush with many miners. Curr Opin Biotechnol 44:169–180. https://doi.org/10.1016/J.COPBIO.2017.02.001
Giuliano G, Aquilani R, Dharmapuri S (2000) Metabolic engineering of plant carotenoids. Trends Plant Sci 5:406–409. https://doi.org/10.1016/s1360-1385(00)01749-0
Gómez MI, Barrett CB, Raney T et al (2013) Post-green revolution food systems and the triple burden of malnutrition. Food Policy 42:129–138. https://doi.org/10.1016/j.foodpol.2013.06.009
Gómez-Galera S, Rojas E, Sudhakar D et al (2010) Critical evaluation of strategies for mineral fortification of staple food crops. Transgenic Res 19:165–180. https://doi.org/10.1007/s11248-009-9311-y
Goto F, Yoshihara T (2001) Improvement of micronutrient contents by genetic engineering—development of high iron content crops. Plant Biotechnol 18:7–15
Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Adv Agron 70:77–142
Grivetti LE, Ogle BM (2000) Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. Nutr Res Rev 13:31–46. https://doi.org/10.1079/095442200108728990
Grotz N, Fox T, Connolly E et al (1998) Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. Proc Natl Acad Sci 95:7220–7224
Hagenimana V, Carey EE, Gichuki ST et al (1999) Carotenoid contents in fresh, dried and processed sweetpotato products. Ecol Food Nutr 37:455–473. https://doi.org/10.1080/03670244.1998.9991560
Halka M, Smoleń S, Czernicka M et al (2019) Iodine biofortification through expression of HMT, SAMT and S3H genes in Solanum lycopersicum L. Plant Physiol Biochem 144:35–48. https://doi.org/10.1016/J.PLAPHY.2019.09.028
Hanson AD, Gregory JF (2011) Folate biosynthesis, turnover, and transport in plants. Annu Rev Plant Biol 62:105–125. https://doi.org/10.1146/annurev-arplant-042110-103819
Hawkesford MJ, Zhao F-J (2007) Strategies for increasing the selenium content of wheat. J Cereal Sci 46:282–292
Haynes KG, Yencho GC, Clough ME et al (2012) Genetic variation for potato tuber micronutrient content and implications for biofortification of potatoes to reduce micronutrient malnutrition. Am J Potato Res 89:192–198
Hubert B, Rosegrant M, van Boekel MAJS et al (2010) The future of food: scenarios for 2050. Crop Sci 50:33–50. https://doi.org/10.2135/cropsci2009.09.0530
Jomova K, Valko M (2011) Importance of iron chelation in free radical-induced oxidative stress and human disease. Curr Pharm Des 17:3460–3473. https://doi.org/10.2174/138161211798072463

Kanyshkova TG, Buneva VN, Nevinsky GA (2001) Lactoferrin and its biological functions. Biochemist 66:1–7

Kennedy G, Nantel G, Shetty P (2003) The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. Food Nutr Agric 32:8–16

Korshunova YO, Eide D, Clark WG et al (1999) The IRT1 protein from Arabidopsis thaliana is a metal transporter with a broad substrate range. Plant Mol Biol 40:37–44

Kosambo L (2004) Effect of storage and processing on all trans-$β$ carotene content in fresh Sweet potato (Ipomoea batatas Lam) roots and its products. CIP Funded Res Proj Annu Rep (July 2003–June 2004) Kenya Ind Res Dev Institute, Nairobi, 11p

Kumar A, Lal MK, Kar SS et al (2017) Bioavailability of iron and zinc as affected by phytic acid content in rice grain. J Food Biochem 41:e12413. https://doi.org/10.1111/jfbc.12413

Kumar S, Palve A, Joshi C et al (2019) Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches. Heliyon 5:e01914

Landini M, Gonzali S, Perata P (2011) Iodine biofortification in tomato. J Plant Nutr Soil Sci 174:480–486

Levenson CW, Morris D (2011) Zinc and neurogenesis: making new neurons from development to adulthood. Adv Nutr 2:96–100

Li H-F, McGrath SP, Zhao F-J (2008) Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytol 178:92–102

Lipka AE, Gore MA, Magallanes-Lundback M et al (2013) Genome-wide association study and pathway-level analysis of tocochromanol levels in maize grain. G3: Genes Genomes Genetics 3:1287–1299

Lipkie TE, De Moura FF, Zhao Z-Y et al (2013) Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. J Agric Food Chem 61:5764–5771

Lyons GH, Stangoulis JCR, Graham RD (2004) Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the *HarvestPlus* program. Nutr Rev 62:247–252. https://doi.org/10.1111/j.1753-4887.2004.tb00047.x

Lyons G, Ortiz-Monasterio I, Stangoulis J, Graham R (2005) Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? Plant Soil 269:369–380

Mata NL, Radu RA, Clemmons RS, Travis GH (2002) Isomerization and oxidation of vitamin A in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. Neuron 36:69–80. https://doi.org/10.1016/S0896-6273(02)00912-1

Montesano FF, D'Imperio M, Parente A, Cardinali A, Renna M, Serio F (2016) Green bean biofortification for Si through soilless cultivation: plant response and Si bioaccessibility in pods. Sci Rep 6(1):1–9

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Nandi S, Suzuki YA, Huang J et al (2002) Expression of human lactoferrin in transgenic rice grains for the application in infant formula. Plant Sci 163:713–722

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 106:7762–7767

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006) Biofortification of staple food crops. J Nutr 136:1064–1067. https://doi.org/10.1093/jn/136.4.1064

O'Hare TJ (2015) Biofortification of vegetables for the developed world. Acta Hortic 1106:1–8. https://doi.org/10.17660/ActaHortic.2015.1106.1

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E et al (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J Cereal Sci 46:293–307. https://doi.org/10.1016/j.jcs.2007.06.005

Pinstrup-Andersen P, Hazell PBR (1985) The impact of the green revolution and prospects for the future. Food Rev Int 1:1–25

Pinto E, Ferreira IM (2015) Cation transporters/channels in plants: tools for nutrient biofortification. J Plant Physiol 179:64–82
Prasad AS (2008) Zinc in human health: effect of zinc on immune cells. Mol Med 14:353–357
Prasad R, Shivay YS, Kumar D (2014) Agronomic biofortification of cereal grains with iron and zinc. Adv Agron 125:55–91. https://doi.org/10.1016/B978-0-12-800137-0.00002-9
Ram H, Kaur A, Gandass N, Singh S, Deshmukh R, Sonah H, Sharma TR (2019) Molecular characterization and expression dynamics of MTP genes under various spatio-temporal stages and metal stress conditions in rice. PLoS One 14(5):e0217360
Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). CRC Crit Rev Food Sci Nutr:1–17. https://doi.org/10.1080/10408398.2019.1685454
Ratcliffe S, Jugdaohsingh R, Vivancos J, Marron A, Deshmukh R, Ma JF, Mitani-Ueno N, Robertson J, Wills J, Boekschoten MV (2017) Identification of a mammalian silicon transporter. Am J Phys Cell Phys 312(5):C550–C561
Rawat N, Neelam K, Tiwari VK, Dhaliwal HS (2013) Biofortification of cereals to overcome hidden hunger. Plant Breed 132:437–445
Rayman MP (2000) The importance of selenium to human health. Lancet 356:233–241. https://doi.org/10.1016/S0140-6736(00)02490-9
Rayman MP (2012) Selenium and human health. Lancet 379:1256–1268. https://doi.org/10.1016/S0140-6736(11)61452-9
Robinson NJ, Procter CM, Connolly EL, Lou GM (1999) A ferric-chelate reductase for iron uptake from soils. Nature 397:694
Römer S, Lübeck J, Kauder F et al (2002) Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. Metab Eng 4:263–272
Saltzman A, Birol E, Bouis HE et al (2013) Biofortification: progress toward a more nourishing future. Glob Food Sec 2:9–17
Sandmann G (2001) Carotenoid biosynthesis and biotechnological application. Arch Biochem Biophys 385:4–12
Santi S, Schmidt W (2008) Laser microdissection-assisted analysis of the functional fate of iron deficiency-induced root hairs in cucumber. J Exp Bot 59:697–704
Scholey D, Belton D, Burton E, Perry C (2018) Bioavailability of a novel form of silicon supplement. Sci Rep 8(1):1–8
Semba RD (1994) Vitamin A, immunity, and infection. Clin Infect Dis 19:489–499
Shimelis H, Laing M et al (2012) Timelines in conventional crop improvement: pre-breeding and breeding procedures. Aust J Crop Sci 6:1542
Sonah H, Deshmukh RK, Labbé C, Bélanger RR (2017) Analysis of aquaporins in Brassicaceae species reveals high-level of conservation and dynamic role against biotic and abiotic stress in canola. Sci Rep 7(1):2771
Stein AJ (2010) Global impacts of human mineral malnutrition. Plant Soil 335:133–154
Strobbe S, Van Der Straeten D (2017) Folate biofortification in food crops. Curr Opin Biotechnol 44:202–211
Terry N, Zayed AM, De Souza MP, Tarun AS (2000) Selenium in higher plants. Annu Rev Plant Biol 51:401–432
Thakkar SK, Huo T, Maziya-Dixon B, Failla ML (2009) Impact of style of processing on retention and bioaccessibility of $β$-carotene in cassava (Manihot esculanta, Crantz). J Agric Food Chem 57:1344–1348
Trumbo P, Yates AA, Schlicker S, Poos M (2001) Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Acad Nutr Diet 101:294
Tuama AA, Mohammed AA (2019) Phytochemical screening and in vitro antibacterial and anticancer activities of the aqueous extract of Cucumis sativus. Saudi J Biol Sci 26:600–604

Tumuhimbise GA, Namutebi A, Turyashemererwa F, Muyonga J (2013) Provitamin A crops: acceptability, bioavailability, efficacy and effectiveness. Food Nutr Sci 4:430
Ueno D, Rombolà AD, Iwashita T et al (2007) Identification of two novel phytosiderophores secreted by perennial grasses. New Phytol 174:304–310. https://doi.org/10.1111/j.1469-8137.2007.02056.x
Van Hal M (2000) Quality of sweetpotato flour during processing and storage. Food Rev Int 16:1–37
Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8 (11):1386
Velasco I, Bath SC, Rayman MP (2018) Iodine as essential nutrient during the first 1000 days of life. Nutrients 10:290
Vishwakarma K, Mishra M, Patil G, Mulkey S, Ramawat N, Pratap Singh V, Deshmukh R, Kumar Tripathi D, Nguyen HT, Sharma S (2019) Avenues of the membrane transport system in adaptation of plants to abiotic stresses. Crit Rev Biotechnol 39(7):861–883
Wargovich MJ (2000) Anticancer properties of fruits and vegetables. HortScience 35:573–575
Waters BM, Sankaran RP (2011) Moving micronutrients from the soil to the seeds: genes and physiological processes from a biofortification perspective. Plant Sci 180:562–574. https://doi.org/10.1016/J.PLANTSCI.2010.12.003
Welch RM (1995) Micronutrient nutrition of plants. CRC Crit Rev Plant Sci 14:49–82. https://doi.org/10.1080/07352689509701922
Welch RM (2002) Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J Nutr 132:495S–499S. https://doi.org/10.1093/jn/132.3.495s
Welch RM (2005) Biotechnology, biofortification, and global health. Food Nutr Bull 26:S304–S306
Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364
Weng H-X, Hong C-L, Yan A-L et al (2008a) Mechanism of iodine uptake by cabbage: effects of iodine species and where it is stored. Biol Trace Elem Res 125:59–71. https://doi.org/10.1007/s12011-008-8155-2
Weng HX, Weng JK, Yan AL et al (2008b) Increment of iodine content in vegetable plants by applying iodized fertilizer and the residual characteristics of iodine in soil. Biol Trace Elem Res 123:218–228. https://doi.org/10.1007/s12011-008-8094-y
Weng HX, Hong CL, Xia TH et al (2013) Iodine biofortification of vegetable plants—an innovative method for iodine supplementation. Chin Sci Bull 58:2066–2072. https://doi.org/10.1007/s11434-013-5709-2
White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593
White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84
White PJ, Broadley MR (2011) Physiological limits to zinc biofortification of edible crops. Front Plant Sci 2:80
White PJ, Bowen HC, Parmaguru P et al (2004) Interactions between selenium and sulphur nutrition in Arabidopsis thaliana. J Exp Bot 55:1927–1937
WHO (2016) Micronutrient deficiencies. WHO, p 3–5
WHO IGN (2013) Salt reduction and iodine fortification strategies in public health. Report of a joint technical meeting convened by World Health Organization (WHO) and The George Institute for Global Health in collaboration with the International Council for the Control. World Health Organization
Winger RJ, König J, House DA (2008) Technological issues associated with iodine fortification of foods. Trends Food Sci Technol 19:94–101

Zhao FJ, McGrath SP (2009) Biofortification and phytoremediation. Curr Opin Plant Biol 12:373–380

Zhao LI, Yuan L, Wang Z et al (2012) Phytoremediation of zinc-contaminated soil and zinc-biofortification for human nutrition. In: Phytoremediation and biofortification. Springer, Berlin, pp 33–57

Zhu Y-G, Huang Y-Z, Hu Y, Liu Y-X (2003) Iodine uptake by spinach (Spinacia oleracea L.) plants grown in solution culture: effects of iodine species and solution concentrations. Environ Int 29:33–37

Zhu C, Sanahuja G, Yuan D et al (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11:129–141

Biofortification in Fruits

6

Anjum Ayoub, Muntazir Mushtaq, Zahoor A. Mir, and Aejaz Ahmad Dar

Abstract

The increasing population, lack of proper nutrition and hunger are the most significant challenges faced by many countries over the globe. The dificiency of vitamins and minerals is of major public health concern. Vitamin deficiency, especially vitamin A deficiency (VAD), is dominant in many developing nations and is the leading cause of death in these nations. VAD leads to >600,000 deaths among women and children across the globe every year specifically the kids <5 years of age group are majorly affected. Malnutrition due to micronutrients such as selenium, iodine, iron and zinc affects 15%, 30%, 60% and 30% of the population. Various health issues and physical disorders among individuals are seen due to the insufficient availability of these micronutrients and minerals. Customary farming practices can enhance the content of nutrition in plant foods up to a certain level, but in order to address the negative impacts of the vitamins and minerals and to achieve the long-term goals, biofortification is used. Biofortification is the process of enhancing the nutrient content in food crops using agronomic, conventional, and transgenic breeding methods. Biofortification is undertaken in many fruit crops like banana, cassava, beans, tomato, orange sweet potato (OSP), cowpea, pumpkin, and so forth. Also, few traditional and transgenic varieties have been released, while many varieties are in the development pipeline. The consequences of adequacy and efficacy studies, even recent accomplishments in delivery, give proof that biofortification is a promising procedure for battling hidden hunger. This article provides a general outline of biofortification strategies, and Strengths, Weaknesses, Opportunities

A. Ayoub (✉)
Division of Food Science and Technology, SKUAST, Jammu, India

M. Mushtaq · A. A. Dar
School of Biotechnology, SKUAST, Jammu, India

Z. A. Mir
National Institute for Plant Biotechnology, New Delhi, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_6

and Threats (SWOT) analysis of every strategy. In addition, it also addresses present and future challenges for biofortification efforts in agriculture crops.

Keywords

Malnutrition · Biofortification · Micronutrients · Fruits

6.1 Introduction

In the previous 40 years, agriculture reseach in developing countries has focused mainly on the higher production of cereals. However, the present day agriculture is aimed at not only producing more calories to minimize hunger but also increase the nutrients in the foods and food supplements to reduce hidden hunger. One in every three individuals on the planet experience the ill effects of hidden hunger, caused because of the lack of minerals and nutrients, which prompts adverse health effects (Fig. 6.1) (Kennedy et al. 2003). Biofortification, the way towards breeding for enhancement of the nutrients in food crops, gives a relatively practical, cost-effective, sustainable, and long-term method for delivering more micronutrients.

Biofortification in staple foods cannot produce as high level of minerals and nutrients every day as of industrially or mechanically fortified food; however, they can help by accomplishing the daily intake of micronutrient among people throughout the lifecycle (Bouis et al. 2011). It should be noted that biofortification may not be relied upon to treat micronutrient deficiencies or elimination of deficiencies in all

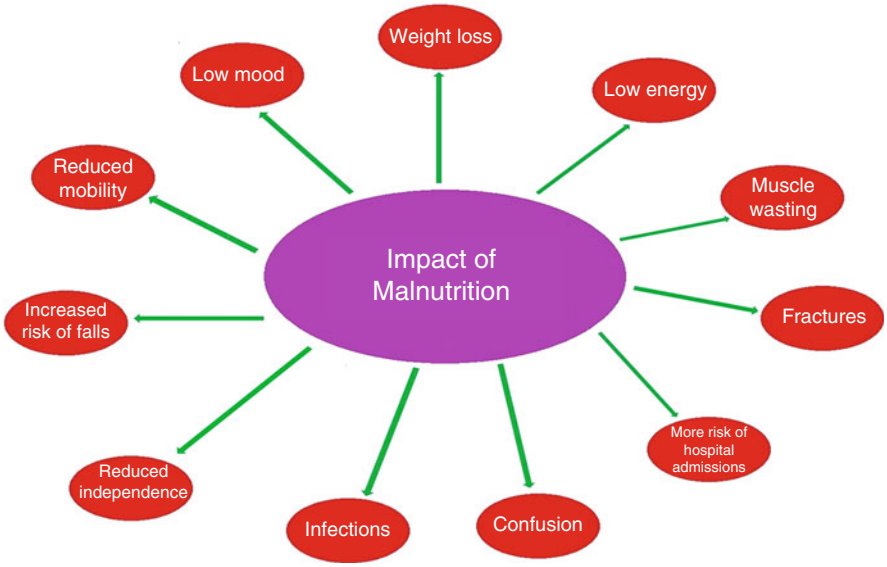

Fig. 6.1 Various impacts of malnutrition on human health

population groups. No single intervention will take care of the issue of micronutrient deficiency, but the biofortification and existing complementary methods can provide micronutrients reasonably to many vulnerable people in an cost-effective way which helps to combat malnutrition (Bouis 1999; Nestel et al. 2006; Pfeiffer and McClafferty 2007; Qaim et al. 2007; Meenakshi et al. 2010).

Biofortification is a secure and achievable method for reaching to a malnourished population having limited access to the supplements and industrially fortified foods. The biofortification system looks to enhance micronutrient in those varieties that already have favored agronomic and consumption attributes. Surpluses of these yields may make their way into retail outlets so that they can reach first to the rural zones and then to the urban zones, as compared to the interventions by complementary methods, for example, fortification and supplementation, that start in urban centers.

6.1.1 Why Biofortification

Vitamin A deficiency (VAD) is dominant in many developing nations and is the main cause of deaths in these nations. VAD leads to the >600,000 deaths among women and children across the globe every year specifically affecting the kids <5 years of age group. According to the Government of India, the statistics provided to the World Health Organization (WHO), 62% of all preschool-age kids are suffering from VAD. Similarly, iron (Fe), zinc (Zn), and selenium (Se) are important micronutrients and their lack or deficiency in food and fruit crops is a serious issues related to the public health, especially in developing countries (WHO 2009). Various techniques involved in the biofortification of food and fruit crops are shown in Fig. 6.2.

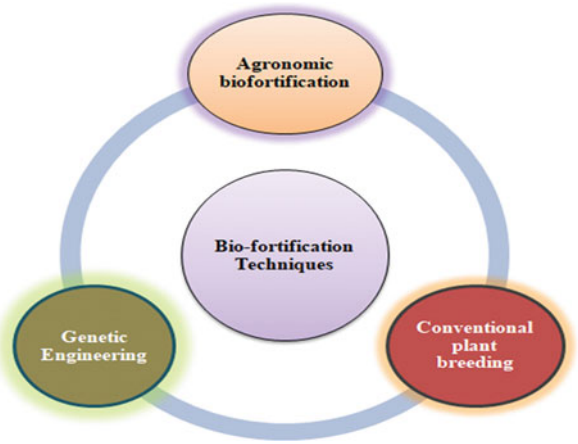

Fig. 6.2 Different techniques for biofortification of fruit crops

6.2 Agronomic Biofortification

Application of manure or fertilizers to increase the micronutrients in edible or consumable parts. The level of accomplishment in agronomic biofortification is proportional to the mobility of mineral components in the soil and also in the plant (White and Broadley 2003). Most important micronutrients used for agronomic biofortification are zinc, (foliar application of $ZnSO_4$), iodine (soil use of iodide or iodate), selenium (as selenate). The use of inorganic Se fertilizers resulted in about more than a ten fold increment in concentration of Se. The utilization of inorganic I and Zn had an impact on crop nutritional improvement at a national scale in China and Thailand. Fe ($FeSO_4$) shows a low mobility in soil because of it's conversion to Fe^{+3} form, which is inaccessible to plant roots. To avoid this, synthetic metal chelators are used for example, EDTA-Fe and EDTA-Zn chelates, which are powerful in increasing concentrations of minerals in consumable vegetable and fruit tissues (Shuman 1998). Foliar application is the rapid, effective, and simple strategy for supplementation or fortification of micronutrients (Fe, Zn, Cu, etc.) in plants. Several researchers have discovered that the mycorrhizal mediated enhancement of Fe, Se, Zn, and Cu fixations in crop plants (Cavagnaro 2008).

6.2.1 Conventional Plant Breeding

From the previous four decades, the conventional or traditional breeding mostly focused on yield related characteristics and resistance breeding. The absence of priority for nutritional aspects lead to a reduced amount of nutrients in the existing varieties. The conventional approaches for biofortification involve three main steps: discovery, development and dissemination (Fig. 6.3). Minerals such as Fe, Zn, Cu, and Mg showed decline in their mean concentration in few plant-based foods (Susana et al. 2013). Recent advancement in conventional plant breeding has mainly focused on fortification of essential antioxidants, vitamins and minerals. The potential to build the micronutrient thickness of staple foods by conventional plant breeding requires sufficient and adequate genetic variation in concentrations of these minerals among existing cultivars, making it possible to select appropriate breeding material.

In beans and peas, 6.6 fold variation in Fe and Zn levels has been observed (Gregorio et al. 2000). Such genotypic variation is commonly progressively diminished in tubers (White and Broadley 2003), and in fruit crops. For example, less than two fold variation in Fe, Zn, Ca, and Mg concentrations has been reported in strawberry (Hakala et al. 2003).

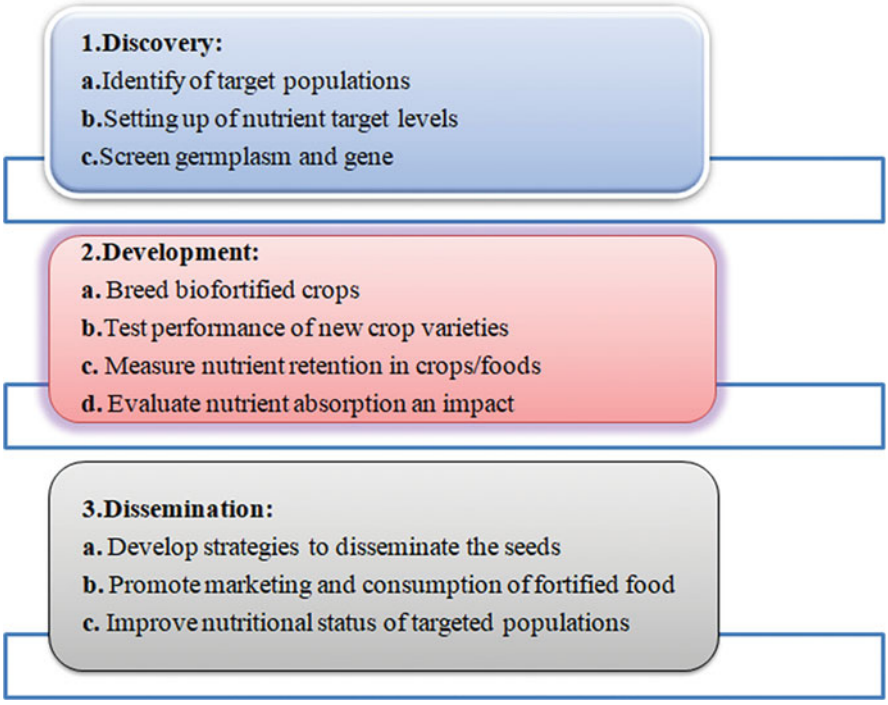

Fig. 6.3 Steps involved in biofortification by conventional plant breeding

6.3 Genetic Engineering

The absence of genetic variation among the genotypes for the character/trait or the non amenability of a crop for conventional plant breeding (because of absence of sexuality; for example, banana) will hinder biofortification process. Under such circumstance, the genetic engineering offers a valid alternative to enhance micronutrient concentration in tissues of the edible crop and their bioavailability. One of the principal concerns about this technique is the problem associated with environment i.e gene flow, which involves the transfer of foreign genes from transgenic crop to non-target species (Winkler 2011). Key features of selected transgenes for biofortification includes redistributing micronutrients between tissues, accelerating the biochemical pathways in edible tissues, or even the recreation of selected pathways. Some strategies involved in the expulsion of "anti-nutrients". One of the first biofortified crops was golden rice, which was engineered in a way to create beta-carotene or pro-vitamin A in the edible grains (Van Eenennaam et al. 2003). Since then there has been a comparable success with different crops, giving us a variety of carotenoid-enhanced foods (Zhu et al. 2007) as well as enrichment of crops with vitamin E (Van Eenennaam et al. 2003) and folate (Bekaert et al. 2008).

The different biofortified crops includes maize, orange, cauliflower, tomato, yellow potatoes, and brilliant canola (Susana et al. 2013). Subsequently the crops are engineered to enhance multiple nutrients by transfer of multigenes (Winkler 2011), for example, multivitamin corn which is engineered to deliver more elevated levels of pro-vitamin A, vitamin B9, and vitamin C (β -carotene, folate, and ascorbate). Promising lines among engineered plants have been identified, which contained 169-fold more β-carotene, 6.1-fold more ascorbate, and double the amount of folate as found in the endosperm (Cakmak 2010).

6.3.1 Biofortification Is a Strategy to Meet the Challenges of Micronutrient Malnutrition

Current system of biofortification of crops utilizes both conventional and transgenic approaches. Improvement of nutrient content through conventional plant breeding depends on the exploitation of genetic variation that exists in crop species, which can be used to introduce new alleles through cross-breeding. Conventional plant breeding has extraordinary potential, and its prosperity and success are particularly connected with the green revolution, where the rate of increased yield of crops such as rice, wheat, and maize in developing nations outpaced population growth to such an extent that the costs of rice and wheat fell by 30–40% between 1970 and 1979 (Bouis et al. 2003). Presently, at global level the institutes like Consultative Group on International Agricultural Research (CGIAR) have set out projects to consider the crop genetic variation to ascrtain the feasibility of breeding programs for enhancement of nutrients. The conventional breeding inherently has less impact on the communities however having few regulatory constraints as compared to the genetically modified varieties (Zhu et al. 2007). In certain fruit crops like bananas where the cultivated varieties are infertile, vegetatively propagated, and show limited cross-breeding. Early reports from coventional breeding of banana showed that the development of hybrid banana is hindered by the complex genetic constitution, long cropping cycles, the trisomic pattern of genetic inheritance, and low female fertility, which limits application of classical breeding methods. This demonstrates it is probably going to takemany years to develop a satisfactory banana hybrid using conventional methods of breeding. In such scenario, for crops like banana, the application of genetic engineering is a significant alternative option and an invaluable complementary solution for improvement. Through genetic engineering, well-characterized novel traits can be directly introduced into the plant genome, and their expression can be targeted to specific parts of the plant such as fruit, leaves, seeds with the help of the tissue-specific promoters (Zhu et al. 2007).

The huge amount of initial investment is required in the biofortification research for product development, to cover the costs of dissemination, efficacy tests, and subsequent evaluation of both nutritional safety and impact (Hunt 2002). From such an investment, there are high expectations of return because once this is acheived, it will provide long lasting nutritional benefits. Also, the benefits can be extended to the other communities and countries with a minimum investment in

dissemination and adaptive breeding. This is possible as long as there is an effective and adequate agriculture infrastructure (Hunt 2002; Bouis et al. 2003). Since there are no recurrent expenses, particularly for the farmers, biofortification is a procedure that has the potential for extensive use in poor communities where there is a lack of supplements or malnutrition is the main problem. The biofortification of staple foods specifically has incredible long term potential for elevating the nutrients in the deficient foods (Bouis et al. 2003). In spite of the fact that transgenic approaches can be undermined by political, social, economic, and intellectual property constraints, eradication of malnutrition and attaining food security remain the main focus of developing countries.

6.3.2 Banana Biofortification: Strategy for Alleviating the Micronutrient Malnutrition

A lot of banana cultivars among cultivation have been selected over time and have evolved deep cultural significance within communities. The consumers/farmers have their preferences to the specific varieties that are already integrated into the farming system. Under such situation, the main barrier would be accepting a new cultivar that may not be agronomically suitable or cannot be put for similar use. Similarly in Uganda attempts to introduce the new crops failed consistantly in the already established subsistence cropping system (Carswell 2003). Besides being a staple food crop, bananas, such as east african high land bananas (EAHB), are also a regional cash crop of Uganda. Therefore rather than introducing a new food crop, the nutrient quality of the edible parts of staple food crops are increased by the help of the biofortification. In a adapted cropping framework, a biofortified staple crop would guarantee the delivery of sufficient micronutrients through a normal daily diet without recurrent costs. Due to many reasons, biofortification through genetic engineering appears to be the most appropriate methodology for bananas. Firstly, the most common cultivated varieties are basically sterile, making their breeding impossible by conventional methods. Secondly, they are vegetatively propagated. Finally, with advances in banana transformation techniques and the availability of a number of gene sequences, it is possible to develop new banana varieties through transgenic approach.

6.3.3 Strategies for Improving provitamin A (PVA) in Banana

Bananas with high β-carotene have been identified and characterized (Englberger et al. 2003). The communities which mainly rely upon banana for food can preferentialy grow high β-carotene varieties. However, as discussed above, there is a constraint, particularly where certain varities are well adopted under subsistence cropping frameworks. Transgenic approaches, therefore, offer a viable alternatives to introduce the novel genes that promote uptake of iron or synthesis PVA in the cultivated varities. A unique methodology was used to develop a nutritionally

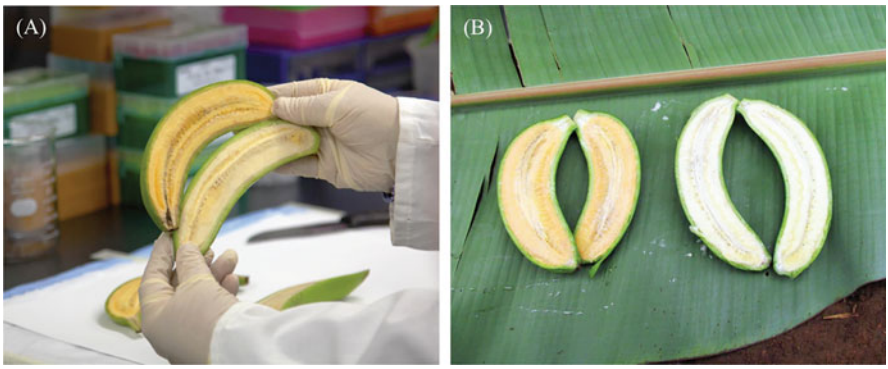

Fig. 6.4 (**a**) Pro-vitamin A (PVA)-biofortified banana in genetic background of "Cavendish" cultivar developed in Australia and (**b**) PVA-biofortified (left) banana in "Nakitembe" genetic background developed in Uganda and wild-type control (right). The figure is reproduced from Paul et al. (2018) which is available under the terms of the Creative Commons Attribution License (CC BY)

rich golden rice, a similar approach can be utilized for banana improvement. The information about genes such as Psy from daffodil (*N. pseudonarcissus*) (Ye et al. 2000) and CrtI from bacteria (*E. uredovora*) (Misawa et al. 1990) used for development of golden rice is publically available. Based on the available sequence information the Psy homologs have been characterized from different crop plants including maize and banana (Paine et al. 2005). The banana homologs of Psy have been isolated and characterized from the high β-carotene bananas (cv Asupina) at the Queensland University of Technology in Brisbane, Australia with the aim of transforming them into cooking banana for the improvement of PVA content of the fruit (Mlalazi 2010). Researchers at the Queensland University of Technology have conducted the human trial to test the efficiency of genetically modified (GM) nutritionally enhanced banana, the Super Banana, to provide a good source of beta-carotene (Waltz 2014). Subsequently, efforts are made to transfer the technology worldwide more particularly in areas affected with malnutrition and where banana is a staple food. For instance in Uganda, Banana21 project initiated to alleviate micronutrient deficiencies through the generation of banana with increased PVA and iron (Paul et al. 2018) (Fig. 6.4). Similarly, genome editing approaches look promising to achieve PVA biofortified non-transgenic banana cultivars (Vats et al. 2019; Mushtaq et al. 2020). However, nutritional improvement of any crop species through different advanced approaches needs precise understanding of genetic regulations of the desired trait (Rana et al. 2019).

6.3.4 Systems for Improving Bananas for Increased Iron Content

There are no reports of iron-rich banana varieties, unlike β-carotene. Hence increasing the iron content in banana can only rely upon the biotechnological techniques

and methods. The existing knowledge of iron uptake from the soil and storage could be exploited in order to increase the iron content of the banana. In soil, iron exists as Fe^{3+}, which is insoluble and toxic and not accessible to plants until it is chelated and reduced to the forms that can be taken up by the plants. Three primary genes involved in iron transport i.e FRO2, IRT1, and plant ferritin, whose functions have been shown in different plants, can be used in genetic transformation of banana. The soybean (*Glycine max*) ferritin (Ragland et al. 1990) *A. thaliana* (cv. Landsburg) FRO2 (Robinson et al. 1999) and IRT1 (Eide et al. 1996) genes are very well characterized.

Researchers at the Queensland University of Technology have sequenced the banana ferritin gene. These genes can be overexpressed in combination or individually, to evaluate their potential for enhancement of iron in banana.

6.3.5 Banana Transformation and Generation of Transgenic Plants

The generation and transformation of transgenic plants requires a well-established tissue culture, cell, and regeneration framework. Over the past two decades, advances in banana biotechnology have prompted the advancement of plant regeneration for a range of banana varieties. Particularly cell suspensions of embryonic cell cultures have been formed and regenerated by somatic embryogenesis from high proliferating meristems (Dhed'a et al. 1991), zygotic embryos (Marroquin et al. 1993), and immature male flowers (Côte et al. 1996; Becker et al. 2000).

Genetic transformation of banana has been reported utilizing electroporation of protoplasts (Sagi et al. 1994) and wounded co-cultivating meristems (May et al. 1995; Tripathi et al. 2008). The electroporation of protoplasts involves tedious and time-consuming procedures for the generation of protoplasts, while the transformation of meristems is constrained due to the potential of generating chimeric plants. In this way, these two methods are not routinely utilized.

Successful regeneration and transformation of banana have been achieved in various cultivars using particle bombardment (Becker et al. 2000) and *Agrobacterium*-mediated transformation (Ganapathi et al. 2001; Khanna et al. 2004). Moreover, Khanna et al. (2004) reported an improvised agrobacterium mediated method in the banana that demonstrates the higher efficiencies of transformation by the incorporation of centrifugation steps. Although both methods of transformation are established very well and are applied routinely, while as particle bombardment provides little efficiency of transformation and is restricted to the availability of particle gun and gold particles, which are expensive. Due to the stability to produce both low copy number transgene insertion into the genome and high transformation efficiencies, *Agrobacterium*-mediated transformation is well preferred.

For the establishment of embryonic banana cell suspensions, immature male flowers are the most commonly used explants to induce callus. But it takes 9 months to 1 year to induce embryogenic callus and to get cell suspensions in sufficient volumes that ascertain the potential of regeneration and to carry out an experiment of

transformation. The transformation process of banana takes at least 8–10 months from the time of transformation to in-vitro regeneration of shoots (Côte et al. 1996; Becker et al. 2000). The vegetative cycle of banana takes at least 11–16 months from planting to fruit formation. Therefore, it would take atleast 3 years before a transgenic banana fruit could be obtained. The function of the selected target promoters and genes for the transformation of banana ideally needs to be evaluated with a relatively shorter generation in a model crop before incorporating them into a banana. Also, to ensure the expression of proteins involved in the synthesis, uptake, and accumulation of iron or PVA carotenoids, tissue-specific (root or fruit) promoters are required. In order to preserve the genetic makeup and genetic integrity of transformed cultivars, the use of the native genes and promoters is a desirable strategy to ensure transgene compatibility.

As a part of the challenges in global health (GCGH) program enzymes in the carotenoid pathway, as well as a range of promoters, genes encoding ferritin have been isolated from different cultivars of banana, with the aim of transferring them into EAST AFRICAN highland bananas (EAHIB). Some of these target promoters and genes have not yet been characterized functionally. Previous studies have suggested that rice can accumulate carotenoids (Ye et al. 2000; Paine et al. 2005) and iron (Drakakaki et al. 2000; Vasconcelos et al. 2003) following expression of the transgene. To characterize the target genes, the rice offers an ideal monocot system for initial studies.

6.4 Banana Biofortification Promoters

6.4.1 Sources of Promoters Related to Banana Fruit

The being a climacteric fruit post-harvest physiology of banana is characterized by a phase of green storage followed by ethylene production, which causes the change of starch into sugars in the ripening stage (Bapat et al. 2010). Also, other changes include during the ripening stage are peel softening and fruit pulp softening due to the degradation of the cell wall, decline in polyphenols, and increase in the enzyme activity that accelerates the formation of the aroma and induce accumulation of protein (Asha et al. 2007). There is less data available about promoters of banana and their role in controlling the gene expression and ripening process in fruit. But many studies have identified transcripts related to fruit ripening (Mlalazi 2010). The sequences of the promoters controlling the expression of these fruit-related genes can be identified that would be helpful for expression of these genes in banana fruit.

A group of genes called expansins (α- and β-family) are known to play a role in fruit ripening in addition to many other processes like cell wall expansion, seed germination, and organogenesis during the development of the plant (Trivedi and Nath 2004). Expansin transcripts (MaExpa1) were characterized from banana fruit (Cavendish, cv Robusta) and are known to be regulated by ethylene (Trivedi and Nath 2004). Four expansin related genes (MaExpA2, MaExpA3, MaExpA4, and

MaExpA5) (*Musa acuminata* (AAA), cultivar Harichal) are reported to differentially expressed during development and ripening process (Asha et al. 2007).

6.5 Particle Bombardment

The particle bombardment is a well-established transformation technique used in many laboratories, and its utilization to analyze the function of various promoters has been reported in various species of plants. In bananas, for example, the transient bombardment method of particles has been used to assess the activity of the banana bunchy top virus-derived promoter (BT6.1) in embryogenic cell suspensions of banana. Further bombardment of the particle has been used assessment transient evaluation of heterologous strawberry fruit promoters (Agius et al. 2005) in cotyledons of *Pinus pinea* (Humara et al. 1999), in roots and leaves of the creeping bentgrass (Basu et al. 2003) and in tobacco protoplast (Rigau et al. 1993).

6.6 Agrobacterium-Mediated Infiltration

In spite of the fact that the particle bombardment method is commonly used in many laboratories, there is an increase in the utilization of the agrobacterium-mediated transient assays because there is no requirement of specific instruments or equipment. The usefulness of transient GUS expression by the *Agrobacterium*-mediated infiltration (agro-infiltration) methods has been demonstrated for the analysis of promoters in a range of tissues like tobacco leaves (Lee et al. 2007) and fruit of tomato (Rasori et al. 2003). Besides this, agro-infiltration has been utilized to study transient gene expression in various fruits, including peach, pear, strawberry, tomato, and orange (Wroblewski et al. 2005). However, these methods were demonstrated to be restricted to species and tissues that are naturally and physiologically suitable for *Agrobacterium* infection (Wroblewski et al. 2005). A range of physiological components influencing the effectiveness of agrobacterium-mediated transient expression assay includes the volume and density of the agrobacterium suspension, type of tissue and age, the time span post-infection and host compatibility of bacteria (Hasan et al. 2008).

In addition to the promoter activity analysis, agro-infiltration has additionally been utilized in plants to assess the gene functions, for example, the heat shock proteins in tomato (Orzaez et al. 2006) and the expression of the *A. thaliana* early flowering genes (Apetala1-AP1) in tomato (Hasan et al. 2008).

In spite of the fact that transient assay provides the rapid results, the primary assessment of the promoter activity, conclusive and quantitative analysis in stably transformed plants are ultimately required since transient analyses results do not always provide robust results (Hamilton et al. 2000).

6.7 Biofortification of Tomato

The FAO (Food and Agriculture Organization of the United Nations) and the WHO (World Health Organization) have highlighted the importance of nutrients specifically, the plant-inferred phytochemicals in the prevention of non-transmittable diseases and cancer (FAO 2015). In this context, tomato (*Solanum lycopersicum*), one of the globally important and most consumed fruits, is a significant source of compounds with recognized healthy impacts, like reduction in digestive tract tumors, risk of cardiovascular diseases, inflammatory processes, hypertension, obesity, and diabetes (Canene-Adams et al. 2005).

These properties have been related to the hydrophilic (basically phenols and ascorbic acid) and lipophilic antioxidants including carotenoids (chiefly β-carotene and lycopene) and vitamin E tocopherols (VTE) (Raiola et al. 2014). The structural analysis of Vitamin E has shown that there are four tocopherols (α, β, δ, and γ), and four tocotrienols (α, β, δ, and γ) which are lipid-soluble non-enzymatic antioxidants. In both food and human tissues, γ-tocopherol and α-tocopherol are the more rich VTE forms.

Interestingly, the highest biological activity is shown by the α-tocopherol as compared to the other VTE forms. This is because of the particular selective retention of this compound mediated by the α-tocopherol transfer protein (α-TTP) that is regarded as the main vitamin E regulator in human beings, while on the other hand, other forms of the VTE are excreted and degraded by the liver. The incorporation of α-tocopherol into lipoproteins that circulate or distribute this compound to non-hepatic tissues which is supported by hepatic α-TTP (Jiang 2014). All four different types of the tocopherols consist of a hydrophobic phytyl tail situated in the membrane core and polar chromanol head group placed at the membrane surface and methyl groups at the chromanol ring (Munné-Bosch 2007).

Tocopherols are able to inhibit the lipid peroxidation and are synthesized only by the photosynthetic organisms. They contribute to membrane stability, protect the photosystem II, fluidity, and permeability and they prevent the damage due to the oxidation by the scavenging singlet oxygen and lipid peroxyl radicals (Quadrana et al. 2013). In plants, these antioxidants play a very important role in various physiological processes such as development, plant growth, and senescence (Horvath et al. 2006). It was reported that there is very high variability in the content of the tocochromanol and composition in plant tissues (Grusak and Della Penna 1999). Particularly, photosynthetic tissues commonly show less total tocochromanols (fresh weight < 50 μg/g) and a large percentage of α-tocopherol, while seeds contain 10–20 times of the total tocochromanols (Traber and Arai 1999).

Epidemiological studies have shown that large vitamin E intake is related to the reduced risk of cardiovascular diseases, whereas intake of other dietary antioxidants (such as vitamin C and beta-carotene) are not, this revealed that vitamin E has specific function than being an antioxidant (Stampfer et al. 1993). Recommended Dietary Allowance (RDA) was given by the Institute of Medicine report on VTE (IOM USA 2000) of around 15 mg/day of α-tocopherol.

Interestingly, it has been observed that consumption of tomatoes is related to the synergistic properties. In particular, α-tocopherol and lycopene, which have been shown to inhibit and prostate carcinoma cell proliferation, low-density lipoprotein (LDL) oxidation, and HL-60 (human promyelocytic leukemia cells) cell differentiation.

As previously known, the amount of antioxidants in tomatoes may depend upon various biotic and abiotic factors which also include environmental conditions (Raffo et al. 2002), fruit maturity level (Dumas et al. 2003), genotype (Leonardi et al. 2000), and cultivation practice (Abushita et al. 2000).

6.8 Biofortification of Vitamin E Content

Because of the high value of the nutrition and the various benefits of vitamin E in human health, metabolic engineering approaches and conventional breeding programs focused on development of novel plant lines of tomato that accumulate the more substantial levels of vitamin E since long time ago (Lu et al. 2013).

Today a huge range of genetic assets are available for tomato; these include populations created through crossing S. *lycopersicum* with wild relatives and mutant collections. Biofortified tomatoes can be developed by using natural variation of in germplasm. The nutritional quality of tomato usually exhibits quantitative variation controlled by several genes involved in many primary pathways and secondary metabolism pathways and is regulated by the development, environmental, and physiological signals. Therefore the one primary focal point of several genetic studies has been the quantitative trait loci (QTL) and identification of the genetic determinants that control the accumulation of vitamin E in tomato fruit (Quadrana et al. 2013).

Dissection of the quantitative traits was achived using the population of introgression lines (ILs) derived from wild tomato species (such as *S. pimpinellifolium*, *S. habrochaites*, and *S. pennellii*) (Eshed and Zamir 1995). A set of isogenic lines were previously developed in which marker-defined chromosome segments which are of the wild species *Solanum pennellii* were replaced with intervals of the cultivated variety M82 (Eshed and Zamir 1995). The introgression lines (ILs) of *S. pennellii* were used to map several QTLs associated with valuable traits that can be introduced into new varieties to improve specific characters or traits related to the quality of tomato fruit (Stevens et al. 2006). Stevens et al. (2006) revealed a detailed metabolite profile of 76 *Solanum pennellii* introgression lines and recognized two QTLs clarifying variation in α-tocopherol fruit content situated on chromosomes 6 and 9. Subsequently, Almeida et al. 2015 studied variations of the isoforms of the tocopherol (α, β, γ, and δ) in ripe fruits of the same introgression lines.

Recently Quadrana et al. (2013) reported the detailed fine mapping of the metabolic QTL situated in the introgressed region of the *S. pennellii* IL9-2-6 (mQTL9-2-6) to a locus encoding VTE3, a gene that encodes 2-methyl-6-phytylquinol methyltransferase which is a focal enzyme for γ-tocopherol and α-tocopherol synthesis. The authors concluded that Mqtl9-2-6 is a QTL linked

with differential methylation of a SINE retrotransposon that is situated in the promoter area of the allelesVTE3 (9). Methylation of promoter can be suddenly reverted, and this can prompt distinctive epialleles affecting vitamin E content and gene expression in tomato fruits.

In spite of versatality of conventional breeding to improve the quality of tomato fruit, this method has some limitations such as it needs long breeding programs and sexual incompatibility in comparison to the genetic engineering where new genes can be introduced into local varieties directly. Metabolic engineering of plants can employ different approaches to improve the amount of many phytonutrients in fruits of tomato. One technique to increase the amount of tocopherol in tomato plants is overexpression of biosynthetic genes in transgenic plants. Seo et al. (2011) overexpressed a gene coding for HPT (homogentisate phytyltransferase), a significant enzyme in the biosynthesis of tocopherols in tomato. Also, they have overexpressed one HPT homolog (MdHPT1) that was previously identified from the apple (*Malus domestica* Borkh. cv. Fuji). In the transgenic plants, the ectopic expression of MdHPT1 resulted in a significant increase in the level of the α-tocopherol of 3.6-fold and 1.7-fold in fruits and leaves of the transgenic plants, relative to control tissues.

Another approach is the metabolic engineering which involves coordinated expression of many genes of a complex biosynthesis pathway. Chloroplast genes are organized in operons that are often processed as monocistronic mRNAs and are coexpressed as polycistronic transcripts. Therefore, it is possible in transgenic plastids to perform proficient metabolic pathways engineered with synthetic multigene operons. Lu et al. (2013) utilized transformation, in chloroplast genome in tomato to build the tochopherol metabolic pathway. They overexpressed genes encoding the three key plastid-localized enzymes (homogentisate phytyltransferase, tocopherol cyclase, and γ-tocopherol methyltransferase) using synthetic operons and single constructs in tomato and tobacco plants and gained up to tenfold increase in total accumulation of tocochromanol. In comparison to nuclear transformation, chloroplast transformation provides significant advantages like stable and high production levels of recombinant proteins, cellular compartmentalization of compounds harmful to the plant, biological containment of recombinant products and transgenes, no silencing of gene or position effects (Rigano et al. 2012).

6.9 Transgenic Approaches for Biofortification in Tomato

6.9.1 Antioxidants

There is a wide range of antioxidants present in fruits and vegetables, including carotenoids and anthocyanins like vitamin C, vitamin E, and β-carotene. There is an increase in the levels of ascorbate and glutathione, total antioxidant activity, and in soluble antioxidants of primary metabolism in transgenic fruit which accumulates trans-resveratrol (Giovinazzo et al. 2005).

6.9.2 Carotenoid Rich Tomato

The potent antioxidant called lycopene has the tremendous potential to prevent epithelial cancers and helps in improving human health. Hence there is much interest in enhancing the levels of the carotenoid content in tomato fruit with the help of genetic manipulation and thereby improving the nutritional quality. The psy-1 enzyme catalyzes the preliminary committed step of the biosynthesis of the carotenoid pathway by making phyteone from GGPP (geranylgeranyl diphosphate). Therefore, in order to enhance the carotenoid content of tomato fruit, the Psy-1 gene is constitutively expressed in tomato (Bergougnoux 2014).

6.9.3 Anthocyanin Rich Tomato

In order to enrich the anthocyanin content of a commercially cultivated tomato cultivar, Arka Vikas Ros 1and De1 transcription factors are expressed specifically in fruits using agrobacterium-mediated transformation. The normal content of anthocyanin of transgenic fruit was 0.1–1 mg fresh weight, which is 70–100 folds much than that of the control fruits (Maligeppagol et al. 2013).

6.9.4 Flavonol rich Tomato

Tomato transformation with the gene *Petunia chi-a* encoding chalcone isomerase which resulted in 78 fold increase in flavonols of the fruit peel, particularly due to accumulation of the rutin (Muir et al. 2001). This 78 fold increase in total flavonols of the fruit was achieved by a single biosynthetic enzyme through ectopic expression (Verhoeyen et al. 2002).

6.9.5 Folate Rich Tomato

Through engineering a slight increment in pteridine generation can fundamentally improve the folate content in plants and that boosts PABA supply that (de la Garza Diaz et al. 2004). When transgenic PABA-and pteridine overproduction characteristics were combained by crossing, resulting vine-ripened tomato fruit produced up to 25-fold high folate than control (Garza et al. 2007). Expressing a yeast S-adenosylmethionine decarboxylase gene (ySAMdc; Spe2) fused with a ripening- inducible E8 promoter explicitly expand levels of the polyamines spermidine and spermine in the fruit of tomato during ripening. This prompted an increment in lycopene, enhanced vine life, and improved juice quality of fruit (Mehta and Cassol 2002).

6.10 Grape Fortification

Breeding grapes offer a variety of additional health benefits because they are natural sources of polyphenols and other antioxidants, high mineral content, which includes high vitamin C and K. Antioxidants properties and phenolic compounds of various grape cultivars which are grown in china have been assessed (Xu et al. 2010). An improved variety released by the Indian agricultural institute called Pusa Navrang, which contains maximum amounts of total soluble solids, organic acids, carbohydrates, fats, proteins, minerals, and antioxidants.

6.11 Conclusion

Biofortification is a well-established cost-effective, strategy of agriculture for enhancing the nutritional status of the malnourished populations throughout the globe. Strategies of biofortification based on the breeding of crops, specific or targeted genetic manipulations, and the application of mineral fertilizers hold extraordinary potential for addressing malnutrition in humans. The production of the food crops by biofortification with enhanced nutrient contents like zinc, iron, Se, and pro-vitamin A will provide adequate levels of nutrients that are often lacking in the diets of the developing and developed world. Initiatives at international and national levels like the Harvest Plus program are acting like pillars to accomplish these targets. These efforts have made it possible to enhance both the quantity and bioavailability of essential mineral elements in the diets of humans, particularly in staple cereals like maize, wheat, cassava, beans millets, and sweet potatoes. But the crop biofortification is a challenging endeavor. To accomplish this, coordinated efforts and collaboration between nutrition scientists, plant breeders, molecular biologists, and genetic engineers are essential.

Conventional approaches to breeding are finding easy acceptance and are being utilized to improve the quality of nutrition of foods. Although a worthy and effective emphasis is being made through transgenic means, success rates of approaches based on breeding are much larger as transgenically fortified crop plants have to confront hurdles due to constraints like acceptance within consumers and various expensive, tedious regulatory approval processes. Other than these challenges, biofortified crops have a splendid future as they have the tremendous potential to eradicate malnutrition among billions of poor people throughout the globe due to a lack of micronutrients, particularly in developing countries.

References

Abushita AA, Daood HG, Biacs PA (2000) Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. J Agric Food Chem 48:2075–2081

Agius F, Amaya I, Botella MA, Valpuesta V (2005) Functional analysis of homologous and heterologous promoters in strawberry fruits using transient expression. J Exp Bot 56 (409):37–46

Almeida L, Asís R, Molineri VN, Sestari I, Lira BS, Carrari F, Peres LE, Rossi M (2015) Fruits from ripening impaired, chlorophyll degraded and jasmonate insensitive tomato mutants have altered tocopherol content and composition. Phytochemistry 111:72–83

Asha V, Sane A, Sane AP, Nath P (2007) Multiple forms of alpha-expansin genes are expressed during banana fruit ripening and development. Postharvest Biol Technol 45(2):184–192

Bapat VA, Trivedi PK, Ghosh A, Sane VA, Ganapathi TR, Nath P (2010) Ripening of fleshy fruit: molecular insight and the role of ethylene. Biotechnol Adv 28(1):94–107

Basu C, Kausch AP, Luo H, Chandlee JM (2003) Promoter analysis in transient assays using a GUS reporter gene construct in creeping bentgrass (*Agrostis palustris*). J Plant Physiol 160 (10):1233–1239

Becker DK, Dugdale B, Smith MK, Harding RM, Dale JL (2000) Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv. "Grand Nain" via microprojectile bombardment. Plant Cell Rep 19:229–234

Bekaert S, Storozhenko S, Mehrshahi P, Bennett MJ, Lambert W, Gregory JF III, Schubert K, Hugenholtz J, Van Der Straeten D, Hanson AD (2008) Folate biofortification in food plants. Trends Plants Sci 13:28–35

Bergougnoux V (2014) The history of tomato: from domestication to biopharming. Biotechnol Adv 32:170–189

Bouis HE (1999) Economics of enhanced micronutrient density in food staples. Field Crops Res 60:165–173

Bouis HE, Chassy BA, Ochanda JO (2003) Genetically modified food crops and their contribution to human nutrition and food quality. Trends Food Sci Technol 14(5–8):191–209

Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32(Supplement 1):31S–40S

Cakmak I (2010) Biofortification of cereals with zinc and iron through fertilization strategy. In: 19th World Congress of Soil Science, 1–6 August, Brisbane, Australia, Proceedings, pp 4–6

Canene-Adams K, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW Jr (2005) The tomato as a functional food. J Nutr 135:1226–1230

Carswell G (2003) Food crops as cash crops: the case of colonial Kigezi, Uganda. J Agrar Chang 3 (4):521–551

Cavagnaro TR (2008) The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. Plant Soil 304:315–325

Côte FX, Domergue R, Monmarson S, Schwendiman J, Teisson C, Escalant JV (1996) Embryogenic cell suspensions from the male flower of *Musa* AAA cv. Grand Nain. Physiol Plant 97:285–290

de la Garza Diaz R, Quinlivan EP, Klaus SM, Basset GJ, Gregory JF, Hanson AD (2004) Folate biofortification in tomatoes by engineering the pteridine branch of folate synthesis. Proc Natl Acad Sci 101(38):137205

Dhed'a D, Dumortier F, Panis B, Vuylsteke D, De Lange E (1991) Plant regeneration in cell suspension cultures of the cooking banana cv. Bluggoe (*Musa* spp. ABB group). Fruits 46:125–135

Drakakaki G, Christou P, Stoger E (2000) Constitutive expression of soybean ferritin cDNA in transgenic wheat and rice results in increased iron levels in vegetative tissues but not in seeds. Transgenic Res 9(6):445–452

Dumas Y, Dadomo M, di Lucca G, Grolier P (2003) Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Sci Food Agric 83:369–382

Eide D, Broderius M, Fett J, Guerinot ML (1996) A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc Natl Acad Sci U S A 28(11):5624–5628

Englberger L, Schierle J, Marks GC, Fitzgerald MH (2003) Micronesian banana, taro, and other foods: newly recognized sources of provitamin A and other carotenoids. J Food Compos Anal 16(1):3–19

Eshed Y, Zamir D (1995) An introgression line population of Lycopersicon pennellii in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

FAO (2015) Increasing fruit and vegetable consumption becomes a global priority. FAO, Rome. Available online: http://www.fao.org/english/newsroom/focus/2003/fruitveg1.htm. Accessed on 20 Oct 2015

Ganapathi TR, Higgs NS, Balint-Kurti PJ, Arntzen CJ, May GD, Van Eck JM (2001) *Agrobacterium*-mediated transformation of embryogenic cell suspensions of the banana cultivar Rasthali (AAB). Plant Cell Rep 20(2):157–162

Garza RID, Gregory JF III, Hanson AD (2007) Folate biofortification of tomato fruit. Natl Acad Sci 107(10):4218–4222

Giovinazzo G, D'Amico L, Paradiso A, Bollini R, Sparvoli F, DeGara L (2005) Antioxidant metabolite profiles in tomato fruit constitutively expressing the grapevine stilbene synthase gene. Plant Biotechnol J 3(1):5769

Gregorio GB, Senadhira D, Htut H, Graham RD (2000) Breeding for trace mineral density in rice. Food Nutr Bull 21:382–386

Grusak MA, Della Penna D (1999) Improving the nutrient composition of plants to enhance human nutrition and health. Annu Rev Plant Physiol Plant Mol Biol 50:133–161

Hakala M, Lapveteläinen A, Houpalahti R, Kallio H, Tahvonen R (2003) Effects of varieties and cultivation conditions on the composition of strawberries. J Food Compos Anal 16:67–80

Hamilton DA, Schwarz YH, Rueda J, Mascarenhas JP (2000) Comparison of transient and stable expression by a pollen-specific promoter: the transformation results do not always agree. Sex Plant Reprod 12(5):292–295

Hasan M, Khan AJ, Khan S, Shah AH, Khan AR, Mirza B (2008) Transformation of tomato (*Lycopersicon Esculentum* Mill.) with *Arabidopsis* early flowering gene Apetalai (AP1) through *Agrobacterium* infiltration of ripened fruits. Pak J Botany 40(1):161–173

Horvath G, Wessjohann L, Bigirimana J, Jansen M, Guisez Y, Caubergs R, Horemans N (2006) Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. Phytochemistry 67:1185–1195

Humara JM, Lopez M, Ordas RJ (1999) Transient expression of the *uidA* gene in *Pinus pinea* cotyledons: a study of heterologous promoter sequences. Plant Cell Tissue Org Cult 56 (2):69–78

Hunt JM (2002) Reversing productivity losses from iron deficiency: the economic case. J Nutr 132 (4):794–801

Institute of Medicine (IOE) (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. National Academy Press, Washington, DC

Jiang Q (2014) Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med 72:76–90

Kennedy G, Nantel G, Shetty P (2003) The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. Food Nutr Agric 32:8–16

Khanna H, Becker D, Kleidon J, Dale J (2004) Centrifugation assisted *Agrobacterium tumefaciens*-mediated transformation (CAAT) of embryogenic cell suspensions of banana (*Musa* spp. Cavendish AAA and Ladyfinger AAB). Mol Breed 14:239–252

Lee SC, Kim DS, Kim NH, Hwang BK (2007) Functional analysis of the promoter of the pepper pathogen-induced gene, CAPIP2, during bacterial infection and abiotic stresses. Plant Sci 172 (2):236–245

Leonardi C, Ambrosino P, Esposito F, Fogliano V (2000) Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. J Agric Food Chem 48:4723–4727

Li J, Chen M, Liu X-W, Zhang H-C, Shen FF, Wang GP (2007) Transient expression of an active human interferon-beta in lettuce. Sci Hortic 112(3):258–265

Lu Y, Rijzaani H, Karcher D, Ruf S, Bock R (2013) Efficient metabolic pathway engineering in transgenic tobacco and tomato plastids with synthetic multigene operons. Proc Natl Acad Sci U S A 4:E623–E632

Maligeppagol M, Chandra GS, Prakash M, Navale PM, Deepa H, Rajeev PR, Asokan R, Babu KP, Babu CCB, Rao VK, Kumar KNK (2013) Anthocyanin enrichment of tomato (Solanum lycopersicum L.) fruit by metabolic engineering. Curr Sci 105:72–80

Marroquin CG, Paduscheck CC, Escalant JV, Teisson C (1993) Somatic embryogenesis and plant regeneration through cell suspensions in *Musa acuminata*. In Vitro Cell Dev Biol Plant 29P (1):43–46

May GD, Afza R, Mason HS, Wiecko A, Novak FJ, Arntzen CJ (1995) Generation of transgenic banana (*Musa acuminata*) plants via *Agrobacterium*-mediated transformation. Bio/Technology 13:486–492

Meenakshi JV, Johnson N, Manyong V, DeGroote H, Javelosa J, Yanggen D, Naher F et al (2010) How cost-effective is biofortification in combating micronutrient malnutrition? An ex ante assessment. World Dev 38(1):64–75

Mehta R, Cassol T (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nat Biotechnol 20:613–618

Misawa N, Nakagawa M, Kobayashi K, Yamano S, Izawa Y, Nakamura K, Harashima K (1990) Elucidation of the *Erwinia uredovora* carotenoid biosynthetic pathway by functional analysis of gene products expressed in *Escherichia coli*. J Bacteriol 172(12):6704–6712

Mlalazi B (2010) Defining the role of phytoene synthase in carotenoid accumulation of high provitamin a bananas. PhD thesis. Queensland University of Technology, Brisbane

Muir SR, Collins GJ, Robinson S, Hughes S, Arnaud B, Ric De Vos CH, van Tunen AJ, Verhoeyen ME (2001) Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. Nat Biotechnol 19:470–474

Munné-Bosch S (2007) α-Tocopherol: a multifaceted molecule in plants. Vitam Horm 76:375–392

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006) Biofortification of staple food crops. J Nutr 136:1064–1067

Orzaez D, Mirabel S, Wieland WH, Granell A (2006) Agroinjection of tomato fruits. A tool for rapid functional analysis of transgenes directly in fruit. J Exp Bot 140(1):3–11

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of Golden rice through increased pro-vitamin A content. Nat Biotechnol 23(4):482–487

Paul J-Y, Harding R, Tushemereirwe W, Dale J (2018) Banana21: from gene discovery to deregulated golden bananas. Front Plant Sci 9:558

Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47: S88–S105

Qaim M, Stein AJ, Meenakshi JV (2007) Economics of biofortification. Agric Econ 37:119–133

Quadrana L, Almeida J, Otaiza SN, Duffy T, Corrêa da Silva JV, de Godoy F, Asís R, Bermúdez L, Fernie AR, Carrari F et al (2013) Transcriptional regulation of tocopherol biosynthesis in tomato. Plant Mol Biol 81:309–325

Raffo A, Leonardi C, Fogliano V, Ambrosino P, Salucci M, Gennaro L, Bugianesi R, Giuffrida F, Quaglia G (2002) Nutritional value of cherry tomatoes (Lycopersicon esculentum Cv. Naomi F1) harvested at different ripening stages. J Agric Food Chem 50:6550–6556

Ragland M, Briat J, Gagnon J, Laulhere J, Massenet O, Theil E (1990) Evidence for conservation of ferritin sequences among plants and animals and for a transit peptide in soybean. J Biol Chem 265:18339–18344

Raiola A, Rigano MM, Calafiore R, Frusciante L, Barone A (2014) Enhancing the health promoting effects of tomato fruit for biofortified food. Mediat Inflamm 2014:139873

Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). Crit Rev Food Sci Nutr:1–17

Rasori A, Bertolasi B, Furini A, Bonghi C, Tonutti P, Ramina A (2003) Functional analysis of peach ACC-oxidase promoters in transgenic tomato and in ripening peach fruit. Plant Sci 165 (3):523–530

Rigano MM, Scotti N, Cardi T (2012) Unsolved problems in plastid transformation. Bioengineered 3:329–333

Rigau J, Capellades M, Montoliu L, Torres MA, Romera C, Martinezizquierdo JA, Tagu D, Puigdomenech P (1993) Analysis of a maize alpha-tubulin gene promoter by transient expression and in transgenic tobacco plants. Plant J 4(6):1043–1050

Robinson NJ, Procter CM, Connolly EL, Guerinot ML (1999) A ferric-chelate reductase for iron uptake from soils. Nature 397(6721):694–697

Sagi L, Remy S, Panis B, Swennen R, Volckaert G (1994) Transient gene expression in electroporated banana (*Musa* spp., cv. 'Bluggoe', ABB group) protoplasts isolated from regenerable embryogenic cell suspensions. Plant Cell Rep 13:262–266

Seo YS, Kim SJ, Harn CH, Kim WT (2011) Ectopic expression of apple fruit homogentisate phytyltransferase gene (MdHPT1) increases tocopherol in transgenic tomato (Solanum lycopersicum Cv. Micro-Tom) leaves and fruits. Phytochemistry 72:321–329

Shuman LM (1998) Micronutrient fertilizers. J Crop Prod 1:165–195

Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett W (1993) Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 328:1444–1449

Stevens R, Buret M, Garchery C, Carretero Y, Causse M (2006) Technique for rapid small-scale analysis of vitamin C levels in fruit and application to a tomato mutant collection. J Agric Food Chem 54:6159–6165

Susana MP, Carvalho M, Vasconcelos W (2013) Producing more with less: strategies and novel technologies for plant-based food biofortification. Food Res Int 54:961–971

Traber MG, Arai H (1999) Molecular mechanisms of vitamin E transport. Annu Rev Nutr 19:343–355

Tripathi L, Tripathi JN, Tushemereirwe WK (2008) Rapid and efficient production of transgenic East African Highland Banana (*Musa* spp.) using intercalary meristematic tissues. Afr J Biotechnol 7(10):1438–1445

Trivedi KP, Nath P (2004) MaExp1, an ethylene-induced expansin from ripening banana fruit. Plant Sci 167(6):1351–1358

Van Eenennaam AL, Lincoln K, Durrett TP, Valentin HE, Shewmake RCK, Thorne GM, Jiang J, Baszis SR, Levering CK, Aasen ED (2003) Engineering vitamin E content: from Arabidopsis mutant to soy oil. Plant Cell 15:3007–3019

Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, Oliveira M, Goto F, Datta SK (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci 164(3):371–378

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8 (11):1386

Verhoeyen A, Bovy G, Collins S, Muir S, Robinson S, de Vos CHR, Colliver S (2002) Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway. J Exp Bot 53:377–383

Waltz E (2014) Vitamin A Super Banana in human trials. Nat Biotechnol 32:857

White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92:487–511

WHO (2009) Global prevalence of vitamin a deficiency in populations at risk 1995–2005. WHO global database on vitamin a deficiency. WHO, Geneva

Winkler JT (2011) Biofortification: improving the nutritional quality of staple crops. In: Pasternak C (ed) Access not excess. Smith-Gordon, Cambridgeshire, pp 100–112

Wroblewski T, Tomczak A, Michelmore R (2005) Optimization of *Agrobacterium*- mediated transient assays of gene expression in lettuce, tomato and *Arabidopsis*. Plant Biotechnol J 3 (2):259–273

Xu C, Zhang Y, Cao L, Lu J (2010) Phenolic compounds and antioxidant properties of different grape cultivars grown in China. Food Chem 119:1557s–1565s

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305

Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci 12(12):548–555

Transgenic Biofortified Crops: Applicability and Challenges

7

Zahoor Ahmad Mir, Prashant Yadav, Sajad Ali, Sandhya Sanand, Muntazir Mushtaq, Javaid Akhther Bhat, Anshika Tyagi, Deepali Upadhyay, Apekshita Singh, and Anita Grover

Abstract

Throughout the world, more people suffer from malnutrition than hunger, particularly in developing countries. Some nutrients like iodine, vitamin A, iron, and zinc malnutrition are significant concerns. Biofortification is the most resilient method to improve the nutrient content of the crop plants and is a durable and cost-effective method of introducing genes to overcome the nutrient deficiencies faced by the people in developing countries. Currently, agronomic, conventional, and transgenic biofortification are three common approaches to nutrient biofortification. In this chapter, the significant progress made in transgenic biofortification development, their applicability, and future challenges has been discussed. The transgenic approach has been utilized for the successful development of crops to acquire the nutrients that do not exist naturally. Recently, several reports on the development of transgenic crops to enhance levels of essential

Z. A. Mir · S. Sanand · A. Tyagi · A. Grover (✉)
ICAR-National Institute for Plant Biotechnology, New Delhi, India

P. Yadav
ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India

S. Ali
Centre of Research for Development, University of Kashmir, Srinagar, Jammu and Kashmir, India

M. Mushtaq
School of Biotechnology, SKAUST-JAMMU, Jammu, Jammu and Kashmir, India

J. A. Bhat
State Key Laboratory for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, China

D. Upadhyay
Division of Genetics, IARI, New Delhi, India

A. Singh
Amity University, Noida, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_7

micronutrient contents in crops like tomato, sweet potato, potato, beans, cassava, and other vegetable crops have been reported.

Keywords

Biofortification · FAO · GMOs · *Arabidopsis thaliana*

7.1 Introduction

The deficiency of micronutrients, also called "hidden hunger," has posed a threat to human health worldwide. Worldwide, about 161 million children below 5 years of age are stunted because of micronutrient deficiency. This happens when the daily intake of food lacks essential vitamins and minerals (De Onis and Branca 2016). Child nutrition status and growth are strongly associated with dietary diversity (Arimond and Ruel 2004). Frequent access to dietary supplements like animal-source protein, vegetables, and fruits is a major challenge for most of the developing countries. The inaccessibility is often because of limited local availability, high cost, and distribution challenges (Fanzo 2012).

Since the Green Revolution, the focus has been on breeding new varieties of a few staple crops like rice, wheat, and maize, which is often attributed to solving world hunger. According to the United Nations Food and Agriculture Organization (FAO), 792.5 million people across the world are malnourished, out of which 780 million people live in developing countries (McGuire 2015). FAO finds that malnutrition is still responsible for more than half of the child deaths in developing countries. Hisdden hunger, also known as micronutrient deficiency, affects more than 2 billion individuals worldwide, or one in three people globally (FAO 2015) and 805 million people lack enough calories to eat (McGuire 2015).

Some studies in the USA revealed that today's food contains lower levels of vitamin C, zinc, iron, protein, calcium, and other nutrients than in the past (Marles 2017). Similar assessments have been done in the UK, India, and elsewhere, confirming what is generally viewed to be a global trend. Animal-based food products are with a high content of minerals and vitamins but are a very expensive source of dietary energy. Poor people obtain their dietary energy from staple foods because of the lower cost of these food products. Biofortification of essential micronutrients in crop plants can be accomplished using three main approaches *viz*, agronomic, conventional, and transgenic involving the use of fertilization, crop breeding, and biotechnology strategies. The genetically biofortified crops have a major concern on social acceptance and its role in elevating global hunger (Fig. 7.1).

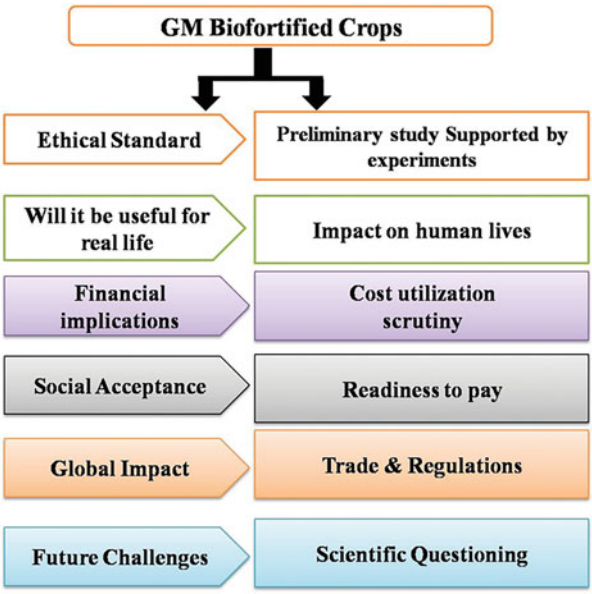

Fig. 7.1 Social impact and regulatory concerns of genetically biofortified crops

7.2 Biofortification

An important science-driven strategy that has been gaining acceptance for increasing micronutrient content in common food crops world over is to enhance the content of micronutrients in their natural form in farm produce, by an approach called as Biofortification. Biofortification is a food-based approach, which is a relatively new and attractive intervention that involves breeding food crops, using conventional or transgenic methods for enhancing their micronutrient content. Biofortification can provide a means to overcome the hidden hunger problems by providing crops with enhanced micronutrient amounts. Organizations such as the World Health Organization and the Consultative Group on International Agricultural Research (CGIAR) have included the development of nutritionally enhanced high-yielding biofortified crops as one of their main goals (Bouis 2000). Biofortification techniques can nutritionally enhance food crops with increased bioavailability to the human population that is developed and grown using modern biotechnology techniques, conventional plant breeding, and agronomic practices. The agronomic approach involves the use of fertilizers, which increases micronutrient temporary. This approach is less useful for micronutrients that cannot be absorbed directly (Lyons and Cakmak 2012). Conventional plant breeding involves crossing between well-developed parent lines with high vitamin or mineral levels for several generations in order to produce plants with the desired nutrient and agronomic traits. Transgenic plant breeding involves enhancing the micronutrient in crops where the essential nutrient does not naturally exist at the required levels. Production of

Table 7.1 Nutrient biofortification and current status of transgenic biofortified crops

Crop plants	Nutrient biofortification	Current status	Reference
Cereals			
Rice	Stacked beta-carotene	Released	Burkhardt et al. (1997)
	High folate rice	Research	Storozhenko et al. (2007)
	High lysine rice	Released	
	High leucine rice	Released	
	Iron	Released	Hurrell and Egli (2010)
	High amino acids and protein content	Research	Yang et al. (2016)
	Zinc	Research	Lee and An (2009)
Wheat	Selenium	Released	
	Iron	Released	Borg et al. (2012)
	Zinc	Released	
Legumes/pulses			
Soyabean	Vitamin E	Released	
	b-carotene	Released	Pierce et al. (2015)
	Lithium	Research	Carlos et al. (2019)
	Zinc	Research	Zou et al. (2014)
	Iron	Research	
	Oleic acid	Released	Zhang et al. (2014)
Common bean	Iron	Research	Sperotto and Ricachenevsky (2017)
Vegetable and Oilseeds			
Lettuce	Iodine	Research	Voogt et al. (2010)
	Iron	Research	Goto et al. (2000)
Potato	Folate	Research	De Lepeleire et al. (2018)
	b-carotene	Research	Ducreux et al. (2004)
	Lutein	Research	
	Provitamin A orange sweet potato	Released	Moura et al. (2016)
Mustard	Gamma-linolenic acid	Research	Hong et al. (2002)
Cauliflower	*b*-carotene	Research	Lu et al. (2006)

sufficiently and sustainably nutritious and safe foods is the main goal of biofortification (Saltzman et al. 2013). The exploitation of genes for essential nutrients, through biotechnology and conventional plant breeding programs, offers a promising route for improving the quality of food crop. This strategy has received considerable scientific attention (Bouis 2003; Bouis et al. 2003) and is the focus of a program on biofortification (Table 7.1) within the Consultative Group on International Agriculture Research (CGIAR) (www.harvestplus.org). While biofortified crops are not available in all developing countries, biofortification is expected to grow significantly in the next 5 years (Saltzman et al. 2013).

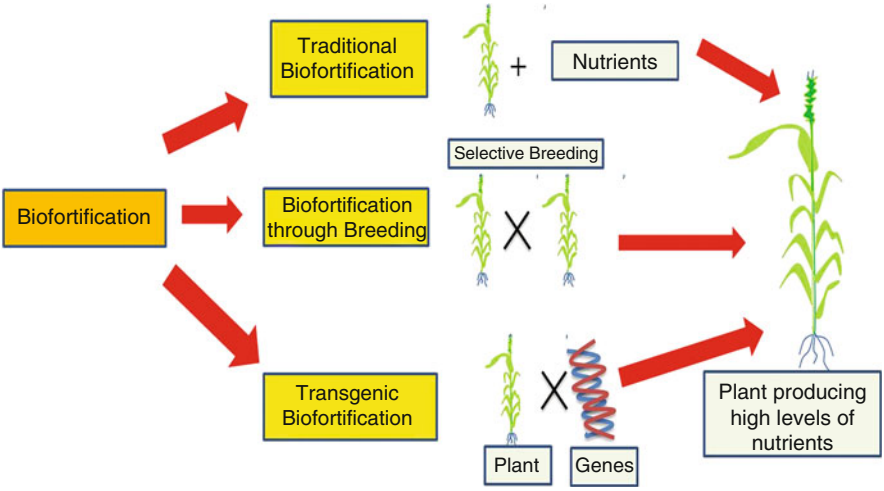

Fig. 7.2 Different types of biofortification in crop plants, traditional biofortification, biofortification through breeding, transgenic biofortification

Biofortified crops that have been released so far include vitamin A, orange sweet potato, vitamin A maize, vitamin A cassava, iron beans, iron pearl millet, zinc rice, and zinc wheat. While biofortified crops are not available in all developing countries, biofortification is expected to grow significantly in the next 5 years (Saltzman et al. 2013). The exploitation of genes for essential nutrients, through biotechnology and conventional plant breeding programs, offers a promising route for improving the quality of food crops. This strategy has received considerable scientific attention (Bouis 2003; Bouis et al. 2003) and is the focus of a program on biofortification (Table. 7.1) within the Consultative Group on International Agriculture Research (CGIAR) (Graham 2003; www.harvestplus.org). Biofortification has advanced from traditional methods to transgenic means (Fig. 7.2). This involves selecting or developing cultivars of staple crops for a high amount of specific micronutrients. Biofortification essentially improves the already grown and/or consumed and accepted crops and therefore does not require any significant change in eating behavior, food habits, educating masses, or food processing.

7.2.1 Why Biofortification?

Biofortification is a food-based approach, which is a relatively new and attractive intervention that involves breeding food crops, using conventional or transgenic methods for enhancing their micronutrient content. Biofortified crops that have been released so far include vitamin A orange sweet potato, vitamin A maize, vitamin A cassava, iron beans, iron pearl millet, zinc rice, and zinc wheat. Hidden hunger, also known as micronutrient deficiency, afflicts more than 2 billion individuals worldwide, or one in three people globally (FAO 2013) and 805 million people lack

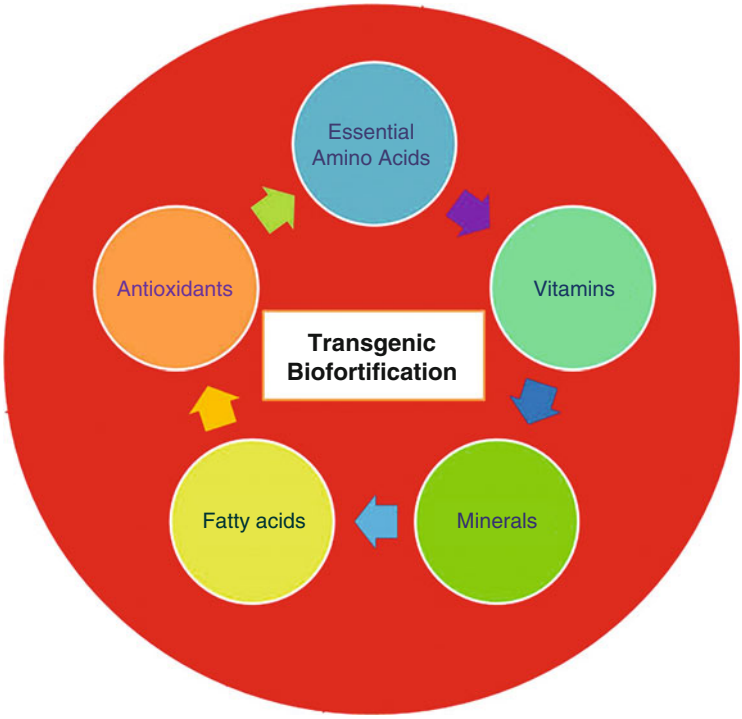

Fig. 7.3 Representation of transgenic biofortification of different essential elements, antioxidants, amino acids, minerals, vitamins, and fatty acids

enough calories to eat (McGuire 2015). An important science-driven strategy that has been gaining acceptance for increasing micronutrient content in common food crops world over is to enhance the content of micronutrients in their natural form in farm produce, by an approach called as biofortification (Fig. 7.3). This involves screening of germ plasm of staple crops for nutritional qualities and to identify QTLs for crop improvement. Biofortification is the advance version in crop breeding, whic is know a days a very conventional method of nutritional improvement in staple as well as non staple crops.

Some of the important micronutrients whose deficiency is common in Indian population include vitamin A, iron, zinc, calcium, folic acid, vitamin D, and iodine. These micronutrients need to be increased, especially in staple crops that are consumed in larger quantities by the young, old, and poor.

7.2.2 Transgenic Means of Biofortification

Biofortification of staple food crops through biotechnology is one of several strategies for improving essential micronutrients in foods for at-risk populations

(Hefferon 2015). In some cases, genetic variability for desirable target traits for biofortification is not available in the germplasm. Hence, the transgenic approach using genetically modified (GM) technology is the only practicable choice. The methodology involves the introduction of genes from novel sources for desirable target traits and has advantages of unconstrained access to the genes of interest, targeted expression in tissues of interest, rapid and direct application by introduction into popular varieties, and stacking of different genes. Several transgenic experiments in many agricultural crops targeted protein and micronutrient accumulation in target tissues.

Intended for the development of biofortified crops transgenic approach can be a valid alternative when there is an inadequate genetic variation in nutrient content among plant varieties (Brinch-Pedersen et al. 2007; Zhu et al. 2013). It depends on the huge genetic pool for the transfer and expression of desirable genes from one to other plant species, which is independent of their evolutionary and taxonomic status. Furthermore, the transgenic approaches remain the only possible alternative to fortify the crop with pacific micronutrients when it does not naturally exist in crops (Pérez-Massot et al. 2013). The techniques to recognize and characterize gene function and process these genes to engineer plant metabolism has been a key to the development of transgenic crops (Christou and Twyman 2004). Furthermore, different pathways from microorganisms like bacteria can also be introduced into crops to utilize alternative pathways for metabolic engineering (Newell-McGloughlin 2008).

A substantial amount of time, investment, and efforts are involved during the initial research and development stages of transgenically biofortified crops. However, in a distant future, it is a cost-effective and sustainable approach, contrasting to the organizational and agronomic biofortification nutrition-based programs (Hefferon 2015: White and Broadley 2005).

Genetic engineering has no taxonomic limitations, and even synthetic genes can be constructed and used to enhance micronutrient contents in transgenic crops, such as rice, wheat, soyabean, common bean and potato. Genetic engineerings holds potential to balance the micronutrient deficiency in staple crops utilized among poor people in developing countries (Hirschi 2009) (Table 7.1).

7.2.3 The Usefulness of Transgenic Biofortification

Biofortification can be a sustainable approach for achieving nutritional security along with dietary diversification, supplementation, and commercial fortification strategies. The typical advantages of biofortification are:

1. It capitalizes on the regular daily intake of a consistent and large amount of food staples across populations regardless of age, gender, and economic status.
2. It is a one-time investment to develop seeds that fortify themselves, and thus cost-effective.

3. Once in place, the biofortified crop system is highly sustainable, and hence constant monitoring is not needed.
4. More importantly, biofortification does not compromise yield per se and thus is economically sustainable to farmers.
5. Since the nutrients are provided through food in natural form, the toxicity issue does not generally arise.

7.2.4 Limitations in Transgenic Methods

The constraint of restricted genetic variation among crops in conventional breeding was overcome by transgenic crops, but the major constraint of this method is its little acceptance among masses. Biofortified crops should necessarily be adopted by farmers and the community very readily in significant enough numbers in order to improve the general nutritional health of a given community (Al-Babili and Beyer 2005).

Another limitation is that for the acceptance and commercialization of these transgenic crops, different countries have adopted various regulatory processes. Unfortunately, the present economic and political landscape is not receptive to this technology (Inaba and Macer 2004). Moreover, the regulatory procedure is highly time-consuming and expensive. Bt Brinjal was developed by an Indian seed company Mahyco, but it was not released in India. A moratorium on its release was imposed because of some opposition groups of anti-GMO activists, scientists, and farmers until further tests were conducted. However, in 2013–2014, four varieties of Bt Brinjal were given approval for commercial release in Bangladesh.

Although more efforts are required for the transgenic biofortification approach in comparison to that of breeding, still its success rate in terms of cultivar release is very low because of the time required for the whole transgenic process to understand the possible effect on other life forms. In the similar context let us take the example of golden rice, after the completion of the 8 years project the detail was first published in Science in 2000 (Ye et al. 2000), and since then due to various issues it is not ready for farmers and dissemination is not allowed.

The regulatory issues and environmental concerns can be bypassed using genome editing approaches which are being widely accepted worldwide (Vats et al. 2019). The genome editing approaches can make desired changes without introducing any transgene which helps to develop non-transgenic GMO (Vats et al. 2019; Mushtaq et al. 2020). However, not all the genome edited plants can bypass the regulations (Tsuda et al. 2019). The genome editing performed with DNA free method and which impact change in one or few nucleotide resulting in knockout of the native genes are considered as most safer approach to get the product at comitial level (Tsuda et al. 2019). However, efficient utilization of such advanced tools for the enhancement of nutritional value of crop plants needs in-depth understanding of the desired trait, genetic regulation, and omics scale information (Chaudhary et al. 2019; Rana et al. 2019).

7.3 Mineral and Vitamin Deficiencies

Some serious public health problem in developing countries is because of the deficiencies of mineral and vitamin. A large number of people throughout Asia, Africa, and Latin America are affected due to vitamin A, zinc, and iron deficiencies. For example, vitamin A deficiency in Sub-Saharan Africa, the prevalence among preschool children ranges from 40% in the west and Central Africa to about 25% in southern Africa (Chizuru et al. 2003). Nearly 40% of pregnant women and 62% of children in Africa are anemia affected, about half of which is estimated to be attributed to iron deficiency (Petry et al. 2016). The nutrient deficiency in worldwide studies suggests that 24% of Africans have insufficient zinc consumption, with young children and pregnant women at the utmost risk of deficiency (Bailey et al. 2015). Moreover, half of the children with vitamin and mineral deficiencies are suffering from multiple deficiencies (Micronutrient Initiative 2009) (Fig. 7.4).

Biofortified staple foods can increase the daily requirements of micronutrient intakes among individuals throughout the lifecycle (Bouis et al. 2011). Biofortification cannot be thought to treat micronutrient deficiencies in all population groups, but complement existing interferences to sustainably provide

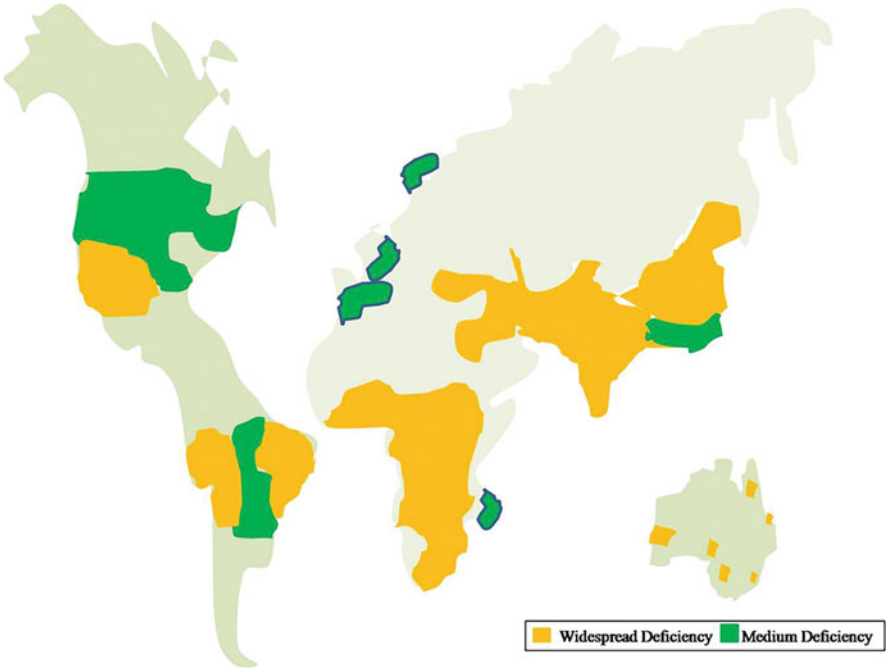

Fig. 7.4 Global map representing a deficiency of nutrients in the human population in rural and in urban areas

micronutrients to the most vulnerable people in a cost-effective way (Bouis 1999; Nestel et al. 2006; Pfeiffer and McClafferty 2007; Qaim et al. 2007).

7.4 Transgenic Biofortification of Different Cereal Crops

Among micronutrients, vitamins, minerals, essential amino acids, and essential fatty acids have been targeted by the use of various genes from different sources. To enhance the nutritional level of cereals, legumes, vegetables, oilseeds, fruits, and fodder crops by transgenic biofortification approach globally and to overcome the nutritional deficiencies in developing countries (Fig. 7.3).

7.4.1 Cereals

7.4.1.1 Rice (*Oryza sativa*)

Rice is the most important stable crop cultivated globally. Rice is consumed by every household, and it meets the demand for vitamin A deficiency causing night blindness in underprivileged children. Golden rice has revolutionized the world, especially the underdeveloped nations whose main source of vitamin A deficiency is rice. Biofortification of cereal crops was the major breakthrough in increasing the nutritional qualities and providing better health. Due to the advent of genetic engineering, researchers have identified and modified genes responsible for increased nutritional values in staple crops. The provitamin B carotene has been increased by 23-fold by targeting the carotene desaturase gene (Burkhardt et al. 1997). *Arabidopsis* GTP-cyclohydrolase I (GTPCHI) gene has been overexpressed in rice by genetic modification to increase folate content (up to 150-fold) (Storozhenko et al. 2007).

7.4.1.2 Wheat (*Triticum aestivum*)

Wheat is the second most widely grown crop after rice in the world. Wheat is the major source of amino acids, minerals, and vitamins. In recent times there is a shift from the basic research in biofortification to transgenic biofortification of wheat crops and researchers have got success in their attempt to increase the vitamin A content, nutrients like iron and protein content, especially essential amino acids lysine, methionine, cysteine, and tyrosine of wheat grains. Recently the overexpression of bacterial *PSY* and carotene desaturase genes (*CrtB, CrtI*) have increased the provitamin A content of wheat (Wang et al. 2014). Iron content has also been increased by overexpressing ferritin gene from soybean. (Xiaoyan et al. 2012) (Fig. 7.5). Wheat plants have also been targeted to improve the antioxidant activity by the transfer of regulatory genes for anthocyanin production (Doshi et al. 2006).

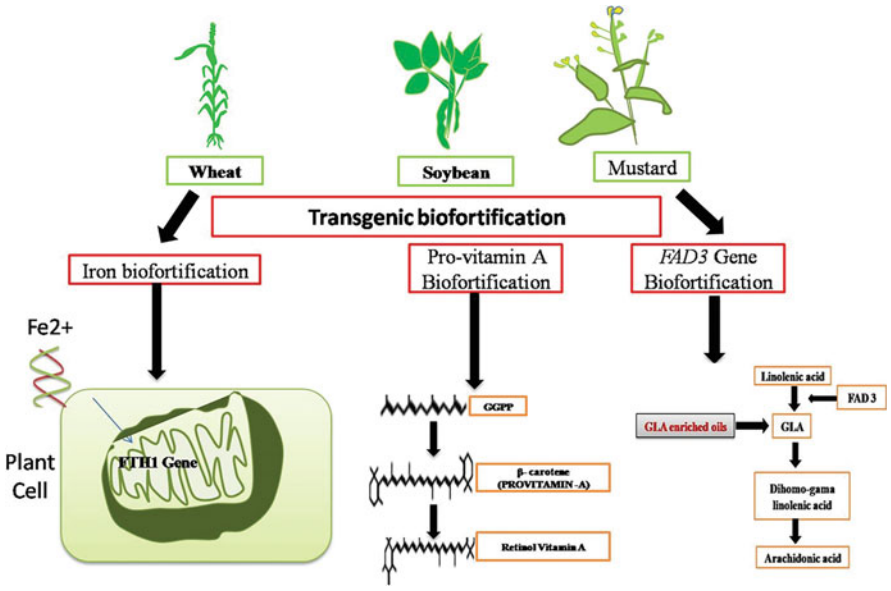

Fig. 7.5 Transgenic biofortification of some essential elements in wheat, soyabean, and mustard

7.4.2 Legumes and Pulses

7.4.2.1 Soybean (*Glycine max*)

Soybean is the major source of proteins in developing countries. Soybean is also the major source of vegetable oil globally, and it meets the demand of the world. Soybean has been exploited to enhance its nutrient content mainly provitamin A (beta-carotene) and seed proteins such as glycinin and conglycinin, which make up to 80% of the total protein of soybean (Fig. 7.5).

7.4.2.2 Common Beans (*Phaseolus vulgaris*)

The common bean is the staple crop of the world, and some researchers predict its domestication from the Peruvian–Ecuadorian region as the center of origin, but it is still debatable. The common bean is the important group in the legume family, and common beans are rich in several essential amino acids, e.g., threonine, valine, lysine, isoleucine, and leucine. Methionine content in common transgenic bean has been increased by transforming methionine-rich storage albumin (Aragão et al. 1999).

7.4.3 Vegetable and Oilseed Crops

7.4.3.1 Transgenic Potato (*Solanum tuberosum*)

Potato is the most prevalent root vegetable and is the main source of fibers, vitamins, and carbohydrates. Potato also has economic importance and is grown in almost every part of the globe. Potato has been genetically improved in many ways, but as far as the nutritional improvement through transgenic biofortification is concerned, provitamin A content has been increased in potato tubers by transfer of *PSY* and incorporation of phytoene desaturase, and lycopene β-cyclase (Diretto et al. 2006).

7.4.3.2 Carrot (*Daucus carota* subsp. *sativus*)

Carrot is an important source of vitamins, and it contains a high amount of *b* carotene and minerals. There are only a few reports of biofortification through transgenic means in carrot, recently a group of scientists has developed transgenic biofortified carrot by expressing H+/Ca+ transporter gene from Arabidopsis *CAX1* (Park et al. 2008).

7.4.3.3 Lettuce (*Lactuca sativa*)

Lettuce is mostly used as a base in the salad items, and it serves as the main source of phosphorus, magnesium, and calcium. Lettuce is also rich in fibers, and recently it has been improved for iron content by transgenic biofortification means. The iron content of lettuce is low as compared to other leafy vegetables; hence the researchers have introduced the ferritin gene of soybean to increase the iron content in lettuce (Goto et al. 2000).

7.4.3.4 Mustard (*Brassica juncea*)

Mustard is the most important oilseed crop grown mostly in the Indian subcontinent. *B. juncea* has been genetically modified by various research groups in India as well as in other parts of the globe. Mustard plants have usually been improved through breeding approaches as well as by genetic engineering means. Due to the ban on transgenic crops in India, researchers have improvised it through conventional biofortification and transgenic biofortification. Recently a group of researchers has introduced the *FAD3* gene in transgenic mustard to increase the production of gamma-linolenic acid (Fig. 7.5) (Hong et al. 2002).

7.4.3.5 Cauliflower (*Brassica oleracea*)

Cauliflower is the most important vegetable crop in the Brassica family; cauliflower is usually rich in fibers, vitamins, phosphorus, and pantothenic acid. Biofortification of cauliflower has been done to increase the *b* carotene by insertion of Ty1-copia-like LTR retrotransposons (Li et al. 2001).

7.4.4 Biofortification of Some Essential Minerals by Transgenic Means

7.4.4.1 Iron

Genetic engineering has been successfully applied to enhance mineral concentrations in cereal crops, by overexpressing the soybean or rice storage protein ferritin. Iron concentrations in polished rice grains have also been increased (Oliva et al. 2014) by coexpressing *Arabidopsis* nicotianamine synthase, common bean ferritin, and *Aspergillus* phytase (Wirth et al. 2009). Overexpression of *AtIRT1*, *AtNAS1,* and bean *FERRITIN* in rice resulted in 3.8-fold higher iron and 1.8-fold higher zinc concentrations than in the wild-type control (Boonyaves et al. 2016). Rice nicotianamine synthase *OsNAS2* and soybean ferritin *SFER-H1* overexpression has achieved dietary targets for both iron and zinc nutrition in rice grains (Trijatmiko et al. 2016). Despite the success in rice, improved mineral biofortification in dicotyledonous plants are rare and are mainly restricted to the model plant *A. thaliana.* The reduction-based mechanism for the iron acquisition was used in non-grass plants (Rodríguez-Celma and Schmidt 2013), which is mediated by membrane-bound oxidoreductase *FRO2* and the ZIP-family transporter *IRT1*. Three-fold increases in storage root iron concentrations in greenhouse-grown cassava were achieved by transgenic overexpression of the algal iron assimilatory protein FEA1 (Ihemere et al. 2012),

Narayanan et al. used genetic engineering to enhance mineral micronutrient concentrations in cassava. Overexpression of the *Arabidopsis thaliana* vacuolar iron transporter VIT1 in cassava accumulated 3–7 times higher levels of iron in transgenic storage roots than non-transgenic controls. Co-expression of a mutated *A. thaliana* iron transporter (IRT1) and ferritin (FER1) resulted in the accumulated levels of iron 7–18 and zinc 3–10 times higher than those in non-transgenic controls in the field.

7.4.4.2 Zinc

Zinc deficiency is one of the major causes of malnutrition in developing countries and is within the top five leading causes of the loss of healthy life years (Bouis et al. 2011). Zinc deficiency causes an increased risk of death from diarrhea, stunting, and hindered cognitive development (Black et al. 2008). Plants can obtain zinc directly from soil, zinc plays many physiological roles in plants that are involved in plant growth and development and increased zinc concentrations in storage roots have been achieved by overexpression of the *A. thaliana* zinc transporters *AtZIP1* and *AtMTP1*, but shoot development in transgenic plants is impaired (Gaitán-Solís et al. 2015).

7.4.4.3 Zinc and Iron

Masuda et al. (2012) overexpressed the iron (II)-nicotine amine transporter *OsYSL2* in rice endosperm, which resulted in the accumulation of the iron storage protein ferritin as well as enhanced iron translocation. While yield was similar to conventional rice, but transgenic rice produced more iron and zinc than conventional rice.

The author concluded that more than one gene introduction is essential for iron biofortification. Milling removes the nutrient-rich

outer layers of the embryo, which results in the low iron and zinc in rice. To overcome this, Paul et al. (2014) generated a transgenic high-yielding indica rice cultivar, which expressed the ferritin gene from soybeans. These plants overexpressed the level of ferritin, which resulted in a higher level of iron and zinc content when milled. In the same way, Tan et al. (2015) used the iron transporter gene *MxIRT1*, but here it was from apple trees to generate transgenic rice. This also increased the iron and zinc content to threefold in rice plants expressing *MxIRT1*. This was also an effective way to biofortified rice and zinc in rice plants. In the same vein rice, nicotianamine synthase (*OsNAS2*) and soybean ferritin (*SferH-1*) genes were overexpressed by Trijatmiko et al. (2016) which showed endosperm Fe and Zn enrichment, while other genes like nicotine amine and 20-deoxymugenic acid (DMA) were overexpressed to develop the transgenic rice plants to accumulate iron and zinc in rice endosperm (Banakar et al. 2017). Due to this way

transgenic rice plants developed this way accumulated fourfold more iron (Fe) and twofold more zinc (Zn). While the almost same increase in iron and zinc was obtained when overexpression of *AtIRT1*, *AtNAS1*, and bean *FERRITIN* in rice was done by Boonyaves et al. 2017.

Masuda et al. 2013 successfully increased iron and zinc accumulation by many folds in transgenic plants. Rice transgenic plants were generated by expressing the soybean ferritin gene (SoyferH2) driven by two endosperm-specific promoters, along with the barley nicotianamine synthase gene (HvNAS1), two nicotine amine aminotransferase genes (HvNAAT-A and -B), and a mugineic acid synthase gene (IDS3) to increase mugineic acid production in rice plants. The use of ferritin and mugineic acid expressed in transgenic rice to increase accumulation in the seed, even under iron-limited conditions, was successfully achieved.

7.4.4.4 Protein

Protein and amino acid profiles in rice have been used to characterize the varieties. An old variety, "Heera" grains were found to have >10% protein. While CR Dhan 310' with >10% protein in polished rice developed by NRRI has been nationally released. Genes responsible for high grain protein content in rice have been deciphered, genetic resources like wild tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) with higher levels of micronutrient and grain protein content have been identified. In cultivated bread and durum wheat for higher grain protein and mineral content, a QTL (*Gpc-B1*) was identified in *dicoccoides* and transferred (Distelfeld et al. 2007). Reserachers, in India have identified and transferred *Gpc-B1* QTL in wheat by Punjab Agricultural University (PAU), IIWBR, and IARI, which are being tested under AICRP-Wheat.

Traditional maize possesses poor endosperm protein due to low levels of essential amino acids, such as lysine and tryptophan. With the discovery of the enhanced nutritional quality of the maize mutant *opaque2*, diverse open-pollinated varieties and hybrids in quality protein maize (QPM) genetic backgrounds have been released worldwide, and marker-assisted selection has been applied to develop locally adapted QPM germplasm29. India released its first soft endosperm-based nutritious

maize composites in 1970, the first hard-endosperm QPM composite in 1997, and the first QPM hybrid in 2001. Since then, research efforts at various ICAR institutes and SAUs have led the development of the QPM version of elite commercial hybrids for different agro-ecologies of the country. The first report of marker-aided selection (MAS) of *opaque2* led to the commercial release of "Vivek QPM-9" in India (Gupta et al. 2009).

7.4.4.5 Provitamin A

Three research groups in India, viz. IARI, IIRR, and Tamil Nadu Agricultural University (TNAU) have been involved in the development of Indian versions of golden rice from the original prototype in collaboration with the International Rice Research Institute (IRRI) supported by the Department of Biotechnology (DBT), India. Provitamin A content was low in traditional maize (<2.5 ppm) as compared to the global target. This screening of 380 maize inbred lines led to the identification of favorable alleles of *lycopene epsilon cyclase* (*lcyE*) and *β-carotene hydroxylase 1* (*crtRB1*) (Muthusamy et al. 2014). These alleles are capable of enhancing carotene to 15 ppm. In India first ever provitamin A rich version Vivek QPM9 was developed through breeding efforts by IARI.

7.5 Future Directions

Conventional plant breeding does not face the same regulatory hurdles and is widely accepted, and it is considered to be the fastest route to getting more nutritious crops into the hands of farmers and consumers. However, one of the very significant limitations of conventional plant breeding thus far is that the density of a *single* nutrient has been increased for each staple food crop—and that particular nutrient has been dictated by the variation of the nutrient density available in varieties stored in germplasm banks maintained by agricultural research centers. In crops where the target nutrient does not naturally exist at the required levels in the tens of thousands of varieties in germplasm banks, transgenic plant breeding is a promising approach to produce biofortified crops with the desired nutrient and agronomic traits—for single nutrients and for multiple nutrients as well. For example, transgenic iron and zinc rice have been developed and tested in confined field trials that can provide +30% of the EAR for iron and +50% of the EAR for zinc in the same event (Trijatmiko et al. 2016). Golden rice, which contains beta-carotene, can provide more than 50% of the EAR for vitamin A. Despite being available as a prototype since early 2000, golden rice has not been introduced in any country, in large part due to highly risk-averse regulatory approval processes (Wesseler and Zilberman 2014). High iron-zinc and high provitamin A rice can be crossed to give transgenic rice with high levels of all three nutrients. While these transgenic varieties have tremendous potential for nutritional impact, release to farmers depends on approval through very strict national biosafety and regulatory processes, which ignore scientific recommendations that transgenic crops are safe (Nicolia et al. 2014; Howell et al. 2018).

7.6 Conclusion

Biofortification has truly reached a defining moment; out of the research and data analysis it is now quite obvious that biofortification is a promising, cost-effective, agricultural approach to improve the nutritional status of malnourished populations throughout the world. Biofortification strategies using targeted genetic manipulation hold great potential for addressing mineral malnutrition in humans. Biofortified crops with increased levels of micronutrients such as iron, zinc, provitamin A, etc. can provide sufficient micronutrients that are deficient in the diets of the people living in developing countries. National and international initiatives have been launched to achieve these targets, and these efforts have delivered bio-availability of staple cereal crops like wheat, cassava, maize, sweet potatoes, beans, and millets with the increased essential mineral elements.

Instead of such enormous advantages, transgenically fortified crops have to face challenges due to acceptance constraints among consumers and different expensive and time-consuming regulatory approval processes, adopted by different countries. Besides these challenges, transgenically fortified crops hold a promising future, to remove micronutrient malnutrition among billions of poor people, especially in the developing countries.

References

Al-Babili S, Beyer P (2005) Golden Rice–five years on the road–five years to go? Trends Plant Sci 10(12):565–573

Aragão FJL, Barros LMG, De Sousa MV, Grossi de Sá MF, Almeida ERP, Gander ES, Rech EL (1999) Expression of a methionine-rich storage albumin from the Brazil nut (Bertholletia excelsa HBK, Lecythidaceae) in transgenic bean plants (Phaseolus vulgaris L., Fabaceae). Genet Mol Biol 22(3):445–449

Arimond M, Ruel MT (2004) Dietary diversity is associated with child nutritional status: evidence from 11 demographic and health surveys. J Nutr 134(10):2579–2585

Bailey DP, Broom DR, Chrismas BC, Taylor L, Flynn E, Hough J (2015) Breaking up prolonged sitting time with walking does not affect appetite or gut hormone concentrations but does induce an energy deficit and suppresses postprandial glycaemia in sedentary adults. Appl Physiol Nutr Metab 41(3):324–331

Banakar R, Alvarez Fernandez A, Díaz-Benito P, Abadia J, Capell T, Christou P (2017) Phytosiderophores determine thresholds for iron and zinc accumulation in biofortified rice endosperm while inhibiting the accumulation of cadmium. J Exp Bot 68(17):4983–4995

Black RE, Bhutta ZA LHA, Caulfield LE, De Onnis M, Ezzati M, Mathers C, Rivera J (2008) Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371:243–260

Boonyaves K, Gruissem W, Bhullar NK (2016) NOD promoter-controlled AtIRT1 expression functions synergistically with NAS and FERRITIN genes to increase iron in rice grains. Plant Mol Biol 90(3):207–215

Boonyaves K, Wu TY, Gruissem W, Bhullar NK (2017) Enhanced grain iron levels in rice expressing an iron-regulated metal transporter, nicotianamine synthase, and ferritin gene cassette. Front Plant Sci 8:130

Borg S, Brinch-Pedersen H, Tauris B, Madsen LH, Darbani B, Noeparvar S, Holm PB (2012) Wheat ferritins: improving the iron content of the wheat grain. J Cereal Sci 56(2):204–213

Bouis HE (2003) Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proc Nutr Soc 62(2):403–411
Bouis HE (1999) Economics of enhanced micronutrient density in food staples. Field Crop Res 60:165–173. https://doi.org/10.1016/S0378-4290(98)00138-5
Bouis H, Howarth E (2000) Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. Nutrition (Burbank, Los Angeles County, Calif) 16(7–8):701–704
Bouis HE, Chassy BM, Ochanda JO (2003) 2. Genetically modified food crops and their contribution to human nutrition and food quality. Trends Food Sci Technol 14(5–8):191–209
Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32(1 Suppl):S31–S40
Brinch-Pedersen H, Borg S, Tauris B, Holm PB (2007) Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. J Cereal Sci 46(3):308–326
Burkhardt PK, Beyer P, Wünn J, Klöti A, Armstrong GA, Schledz M, von Lintig J, Potrykus I (1997) Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (Narcissus pseudonarcissus) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. Plant J 11(5):1071–1078
Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari A, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H (2019) Advances in omics approaches for abiotic stress tolerance in tomato. Biology 8(4):90
Chizuru N, Ricardo U, Shiriki K, Prakash S (2003) The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. Public Health Nutr 7(1a):245–250. https://doi.org/10.1079/PHN2003592
Christou P, Twyman RM (2004) The potential of genetically enhanced plants to address food insecurity. Nutr Res Rev 17(1):23–42
De Lepeleire J, Strobbe S, Verstraete J, Blancquaert D, Ambach L, Visser RG, Stove C, Van Der Straeten D (2018) Folate biofortification of potato by tuber-specific expression of four folate biosynthesis genes. Mol Plant 11(1):175–188
De Onis M, Branca F (2016) Childhood stunting: a global perspective. Matern Child Nutr 12:12–26
Diretto G, Tavazza R, Welsch R, Pizzichini D, Mourgues F, Papacchioli V, Beyer P, Giuliano G (2006) Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. BMC Plant Biol 6(1):13
Distelfeld A, Cakmak I, Peleg Z, Ozturk L, Yazici AM, Budak H, Saranga Y, Fahima T (2007) Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. Physiol Plant 129(3):635–643
Doshi KM, Eudes F, Laroche A, Gaudet D (2006) Transient embryo-specific expression of anthocyanin in wheat. Vitro Cell Dev Biol-Plant 42(5):432–438
Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA (2004) Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. J Exp Bot 56(409):81–89
Fanzo J. The nutrition challenge in sub-Saharan Africa (No. 2012–012). United Nations Development Programme, Regional Bureau for Africa; 2012
FAO (2013) FAO statistical yearbook: world food and agriculture
FAO (2015) IFAD, and Unicef. "WFP." The state of food insecurity in the world 46
Gaitán-Solís E, Taylor NJ, Siritunga D, Stevens W, Schachtman DP (2015) Overexpression of the transporters AtZIP1 and AtMTP1 in cassava changes zinc accumulation and partitioning. Front Plant Sci 6:492
Goto F, Yoshihara T, Saiki H (2000) Iron accumulation and enhanced growth in transgenic lettuce plants expressing the iron-binding protein ferritin. Theor Appl Genet 100(5):658–664
Graham H, Peter H, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131(3):872–877

Gupta HS, Agrawal PK, Mahajan V, Bisht GS, Kumar A, Verma P, Srivastava A, Saha S, Babu R, Pant MC, Mani VP (2009) Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular marker assisted breeding. Curr Sci 96(2):230–237

Hefferon KL (2015) Nutritionally enhanced food crops; progress and perspectives. Int J Mol Sci 16 (2):3895–3914

Hirschi A (2009) Career adaptability development in adolescence: multiple predictors and effect on sense of power and life satisfaction. J Vocat Behav 74(2):145–155

Hong H, Datla N, Reed DW, Covello PS, MacKenzie SL, Qiu X (2002) High-level production of γ-linolenic acid in Brassica juncea using a Δ6 desaturase from Pythium irregulare. Plant Physiol 129(1):354–362

Howell EL, Wirz CD, Brossard D, Jamieson KH, Scheufele DA, Winneg KM, Xenos MA (2018) National Academies of Sciences, Engineering, and Medicine report on genetically engineered crops influences public discourse. Politics Life Sci 37(2):250–261

Hurrell R, Egli I (2010) Iron bioavailability and dietary reference values. Am J Clin Nutr 91 (5):1461S–1467S

Ihemere U, Narayanan N, Sayre R (2012) Iron biofortification and homeostasis in transgenic cassava roots expressing an algal iron assimilatory protein, FEA1. Front Plant Sci 3:171

Inaba M, Macer D (2004) Policy, regulation and attitudes towards agricultural biotechnology in Japan. J Int Biotechnol Law 1(2):45–53

Initiative M (2009) Investing in the future: a united call to action on vitamin and mineral deficiencies. Micronutrient Initiative, Ottawa

Lee S, An G (2009) Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. Plant Cell Environ 32(4):408–416

Li L, Paolillo DJ, Parthasarathy MV, DiMuzio EM, Garvin DF (2001) A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (Brassica oleracea var. botrytis). Plant J 26(1):59–67

Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran DM, Cosman KM, Conlin BJ, Paolillo DJ, Garvin DF, Vrebalov J, Kochian LV, Küpper H, Earle ED, Cao J, Li L (2006) The cauliflower Or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. Plant Cell 18(12):3594–3605

Lyons G, Cakmak I (2012) Agronomic biofortification of food crops with micronutrients. Fertilizing crops to improve human health: a scientific review. Food Nutr Secur 1:97–122

Marles RJ (2017) Mineral nutrient composition of vegetables, fruits and grains: the context of reports of apparent historical declines. J Food Compos Anal 56:93–103

Masuda H, Ishimaru Y, Aung MS, Kobayashi T, Kakei Y, Takahashi M, Higuchi K, Nakanishi H, Nishizawa NK (2012) Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. Sci Rep 2:543

Masuda H, Aung MS, Nishizawa NK (2013) Iron biofortification of rice using different transgenic approaches. Rice 6(1):40

McGuire S (2015) FAO, IFAD, and WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO, 2015. Adv Nutr 6(5):623–624. https://doi.org/10.3945/an.115.009936

Moura MDG, Lopes LC, Biavatti MW, Busse JW, Wang L, Kennedy SA, Bhatnaga N, de Cássia Bergamaschi C (2016) Brazilian oral herbal medication for osteoarthritis: a systematic review protocol. Syst Rev 5(1):86

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:8

Muthusamy V, Hossain F, Thirunavukkarasu N, Choudhary M, Saha S, Bhat JS, Prasanna BM, Gupta HS (2014) Development of β-carotene rich maize hybrids through marker-assisted introgression of β-carotene hydroxylase allele. PLoS One 9(12):e113583

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006) Biofortification of staple food crops. J Nutr 136(4):1064–1067

Newell-McGloughlin M (2008) Nutritionally improved agricultural crops. Plant Physiol 147 (3):939–953

Nicolia A, Manzo A, Veronesi F, Rosellini D (2014) An overview of the last 10 years of genetically engineered crop safety research. Crit Rev Biotechnol 34(1):77–88

Oliva N, Chadha-Mohanty P, Poletti S, Abrigo E, Atienza G, Torrizo L, Garcia R, Dueñas C Jr, Poncio MA, Balindong J, Manzanilla M, Montecillo F, Zaidem M, Barry G, Hervé P, Shou H, Slamet-Loedin IH (2014) Large-scale production and evaluation of marker-free indica rice IR64 expressing phytoferritin genes. Mol Breed 33(1):23–37

Paul S, Ali N, Datta SK, Datta K (2014) Development of an iron-enriched high-yieldings indica rice cultivar by introgression of a high-iron trait from transgenic iron-biofortified rice. Plant Foods Hum Nutr 69(3):203–208

Park HS, Rene ER, Choi SM, Chiu AS (2008) Strategies for sustainable development of industrial park in Ulsan, South Korea—from spontaneous evolution to systematic expansion of industrial symbiosis. J Environ Manag 87(1):1–13

Pérez-Massot E, Banakar R, Gómez-Galera S, Zorrilla-López U, Sanahuja G, Arjó G, Miralpeix B, Vamvaka E, Farré G, Rivera SM, Dashevskaya S, Berman J, Sabalza M, Yuan D, Bai C, Bassie L, Twyman RM, Capell T, Christou P, Zhu C (2013) The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. Genes Nutr 8 (1):29

Petry N, Olofin I, Hurrell R, Boy E, Wirth J, Moursi M, Donahue Angel M, Rohner F (2016) The proportion of anemia associated with iron deficiency in low, medium, and high human development index countries: a systematic analysis of national surveys. Nutrients 8(11):693

Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47 (S3):S88–S105

Pierce EC, LaFayette PR, Ortega MA, Joyce BL, Kopsell DA, Parrott WA (2015) Ketocarotenoid production in soybean seeds through metabolic engineering. PLoS One 10(9):e0138196

Qaim M, Stein AJ, Meenakshi JV (2007) Economics of biofortification. Agric Econ 37:119–133

Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). Crit Rev Food Sci Nutr:1–17. https://doi.org/10.1080/10408398.2019.1685454

Rodríguez-Celma J, Schmidt W (2013) Reduction-based iron uptake revisited: on the role of secreted iron-binding compounds. Plant Signal Behav 8(11):1473–1485

Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y, Pfeiffer WH (2013) Biofortification: progress toward a more nourishing future. Glob Food Sec 2(1):9–17

Sperotto RA, Ricachenevsky FK (2017) Common bean Fe biofortification using model species' lessons. Front Plant Sci 8:2187

Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, Lambert W, Van Der Straeten D (2007) Folate fortification of rice by metabolic engineering. Nat Biotechnol 25(11):1277

Tan S, Han R, Li P, Yang G, Li S, Zhang P, Wang WB, Zhao WZ, Yin LP (2015) Over-expression of the *MxIRT1* gene increases iron and zinc content in rice seeds. Transgenic Res 24(1):109–122

Trijatmiko KR, Dueñas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C, Balindong J, Oliva N, Sapasap MV, Borrero J, Rey J, Francisco P, Nelson A, Nakanishi H, Lombi E, Tako E, Glahn RP, Stangoulis J, Chadha-Mohanty P, Johnson AA, Tohme J, Barry G, Slamet-Loedin IH (2016) Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. Sci Rep 6:19792

Tsuda M, Watanabe KN, Ohsawa R (2019) Regulatory status of genome-edited organisms under the Japanese Cartagena Act. Front Bioeng Biotechnol 7:387

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8 (11):1386

Voogt W, Holwerda HT, Khodabaks R (2010) Biofortification of lettuce (Lactuca sativa L.) with iodine: the effect of iodine form and concentration in the nutrient solution on growth, development and iodine uptake of lettuce grown in water culture. J Sci Food Agric 90(5):906–913

Wang C, Zeng J, Li Y, Hu W, Chen L, Miao Y, Deng P, Yuan C, Ma C, Chen X, Zang M, Wang Q, Li K, Chang J, Wang Y, Yang G, He G (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

Wesseler J, Zilberman D (2014) The economic power of the golden rice opposition. Environ Dev Econ 19(6):724–742

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10(12):586–593

Wirth J, Poletti S, Aeschlimann B, Yakandawala N, Drosse B, Osorio S, Tohge T, Fernie AR, Günther D, Gruissem W, Sautter C (2009) Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. Plant Biotechnol J 7(7):631–644

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

Yang QQ, Zhang CQ, Chan ML, Zhao DS, Chen JZ, Wang Q, Li QF, Yu HX, Gu MH, Sun SS, Liu QQ (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 X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451):303–305

Zhang L, Yang XD, Zhang YY, Yang J, Qi GX, Guo DQ, Xing GJ, Yao Y, Xu WJ, Li HY, Li QY, Dong YS (2014) Changes in oleic acid content of transgenic soybeans by antisense RNA mediated posttranscriptional gene silencing. Int J Genom 2014:921950

Zhu C, Sanahuja G, Yuan D, Farré G, Arjó G, Berman J, Zorrilla-López U, Banakar R, Bai C, Pérez-Massot E, Bassie L, Capell T, Christou P (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11 (2):129–141

Zou T, Xu N, Hu G, Pang J, Xu H (2014) Biofortification of soybean sprouts with zinc and bioaccessibility of zinc in the sprouts. J Sci Food Agric 94(14):3053–3060

Biofortified Fodder Crops: An Approach to Eradicate Hidden Hunger

8

Pravin V. Jadhav, Sayali Magar, Pranita Thakur, Mangesh Moharil, Himanshu Yadav, and Rushil Mandlik

Abstract

Biofortification of crop plants is one of the most critical challenges for today's world. Considerable efforts are being made towards well optimization of the nutritional composition of the food crops. However, very limited is being done to get the desired nutritious feed and fodder for the poultry birds, dairy animals, and animals reared for the meat purpose. The inadequate and imbalanced nutrition to domestic animals affects not only their health but also reduces the quality of the derived product. A most important aspect of the nutritional quality of feed and fodder involved total calories, mineral content, and, more particularly, the bio-availability of the nutrients. The strategies being used for the biofortification of food crops and fodder crops are mostly similar. The biofortification approaches mainly include agronomical practices, breeding efforts, transgenic, and advanced genome editing methods. The present chapter addresses the nutritional aspect of feed and fodder crops, approaches for biofortification, and successful examples where significant efforts are made towards biofortification. The areas required for adequate attention are also highlighted. Advanced strategies based on recent development in genome editing are also discussed concerning their use for fodder biofortification.

Keywords

Biofortification · Nutritional quality · Breeding · Transgenic approaches · Genome editing · Fertilizers

P. V. Jadhav (✉) · S. Magar · P. Thakur · M. Moharil
Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

H. Yadav · R. Mandlik
National Agri-Food Biotechnology Institute, Mohali, Punjab, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_8

8.1 Introduction

Humans and other animals are dependent on various plant species to provide dietary minerals. More than 22 different microelements are needed for the normal function of the human organism (Graham et al. 2007b). In practice, diets are not always diverse enough, and consumed in sufficient quantities, to assure adequate intake of all minerals. The elements like iron (Fe), copper (Cu), zinc (Zn), iodine (I) and selenium (Se) are important for human and livestock health, as they manage physical and mental development. Similarly they play vital role in defence mechanism in response to the various diseases (Dimkpa and Bindraban 2016). Many enzymes, proteins, and other biological compounds that control important biological functions in humans are not entirely functional if there is a lack of microelements. Similarly, for livestock, mineral needs for optimal growth or productivity are hardly ever met by plant foods alone; supplemental minerals habitually are added to animal feeds (Grusak and Cakmak 2009). It is estimated that about half of the human population suffers from the lack of Fe and Zn and their deficiency is widely known as "hidden hunger," which causes slower growth and psychomotor development in children, weaker immunity, decreased muscle mass, hair loss, infertility, and in acute cases even death (Stein 2010). Over 30% of the human population suffers from I deficiency and 15% from the lack of Se. The lack of Cu is most common in developed and developing countries (Rawat et al. 2013). However, microelements are usually present in insufficient amounts in the soil, in fodder crops, animals, and ultimately in the food of end-users i.e. humans. Changes in dietary habits of humans and a diet based on processed cereals, which have low concentrations of minerals in general, together with the lack of fish, fruit, and foodstuff of animal origin, greatly contribute to the problems associated with the lack of microelements (Gómez-Galera et al. 2010). Anti-nutrition factors, such as phytic acid, fibers, and tannins, further diminish the bioavailability of food microelements by preventing their absorption in the intestines (Pfeiffer and McClafferty 2007). The lack of these microelements in food or microelements malnutrition can be conquered by a nutrition variety, supplementing micronutrients in people's usual dietary intakes, by enriching the food with specific minerals (fortification) and by increasing the concentration of minerals in edible crops (biofortification) (Stein 2010).

To provide higher quantities of plant-based dietary minerals, researchers have been working to enhance the mineral density of plant foods (Rana et al. 2019). This is not proving to be an easy task, as minerals must be acquired from the rhizosphere and are partitioned to edible tissues via a complex, integrated series of short- and long-distance transport events (Grusak 2002a). While some gains have been realized through conventional breeding, especially for micronutrients, continued efforts to understand the molecular mechanisms and regulation of plant mineral nutrition are essential if the desire is to make significant improvements in the food supply. Genomic studies are beginning to provide us with some of the necessary knowledge and tools to effect these changes. This chapter emphasizes the importance of plants in the dietary food chain, why research on plant mineral is needed, and how the

existing genomic technologies can be applied to initiate improvements in plant mineral content.

8.2 Plants as Sources of Dietary Minerals

8.2.1 Mineral Elements Required by Humans

It is important to understand the status of the food and mineral requirement of human and livestock so that we can enhance the level of required mineral content in the food. There are at least 22 mineral elements that are essential for human health, required to be supplied from outside as an appropriate diet (Graham et al. 2007a; White and Broadley 2005). However, a large fraction of the world's population is mineral deficient. According to estimation, over 60% of the world's population is suffering from iron (Fe) deficiency, over 30% from zinc (Zn), 30% from Iodine (I), and 15% from selenium. Additionally, people from developed and developing countries are calcium (Ca), magnesium (Mg), and copper (Cu) deficient (Thacher et al. 2006). This is due to the intake of a plant-based diet with low mineral concentration and a lack of animal food products in the diet (Graham et al. 2007a; White and Broadley 2009).

Plant foods can provide all of these elements, especially those that have been determined as essential for growth and reproductive development of plants themselves viz., N, S, P, K, Ca, Mg, Cl, Fe, Zn, Mn, Cu, B, Mo, and Ni (Marschner 1995). Plants acquired these elements from soils through various transport mechanisms and found in all plant foods. Similarly, elements not identified as plant essential, but which are essential for humans like Na, Cr, I, Se, Si, and V can enter the plant through various non-selective transport mechanisms when these minerals are available in the soil (Kochian 1991) and make their way into the food supply for humans and livestock. However, levels of these minerals may vary across tissues within a single plant, across genotypes of a species, or across species. Thus, although plants have the potential to deliver many dietary minerals, that delivery is not always optimal in any given food source.

Presently, humankind is facing a more serious challenge of mineral malnutrition, which needs to be managed through fortification of food with increased minerals, dietary diversification, and mineral supplementation (Consensus 2004). Biofortification of crops should be promoted with the application of mineral fertilizers along with breeding varieties with an aim to increase the plant's ability to acquire more mineral not only for mineral enhancement but also for the yield improvement in infertile soil (Graham et al. 2001, 2007a; Pfeiffer and McClafferty 2007). The daily intake dose for different minerals and vitamins is well studied and recommendations have been made for better health (Table 8.1). However, very less work has been done to define such daily required dose for different domesticated animals.

Table 8.1 Recommended daily intake of various food supplements for humans

Vitamins	*Recommended daily intake*	*Overdosage (mg or μg/day)*
Biotin (B-complex)	30 μg	No information found
Folate (B-complex)	400 μg	Doses larger than 400 μg may cause anemia and may mask symptoms of a vitamin B_{12} deficiency
Vitamin A	600 μg	Extremely high doses (>9000 mg) can cause dry, scaly skin, fatigue, nausea, loss of appetite, bone and joint pains, and headaches
Vitamin B_1 (thiamine)	1.4 mg	No toxic effects resulting from high doses have been observed
Vitamin B_2 (riboflavin)	1.6 mg	Doses higher than 200 mg may cause urine color alteration
Vitamin B_3 (niacin)	18 mg	Doses larger than 150 mg may cause problems ranging from facial flushing to liver disease
Vitamin B_5 (pantothenic acid)	6 mg	The dose should not exceed 1200 mg; this may cause nausea and heartburn
Vitamin B_6 (pyridoxine)	2 mg	Doses larger than 100 mg may cause numbness and tingling in hands and feet
Vitamin B_{12} (cobalamin)	6 μg	Doses larger than 3000 μg may cause eye conditions
Vitamin C (ascorbic acid)	75 mg	No impacts of overdose have been proven so far
Vitamin D (cholecalciferol)	5 μg	Large doses (>50 μg) obtained from food can cause eating problems and ultimately disorientation, coma, and death
Vitamin E (tocopherol)	10 mg	Doses larger than 1000 mg cause blood clotting, which results in an increased likelihood of hemorrhage in some individuals
Vitamin K	80 μg	Large doses of one form of vitamin K (menadione or K_3) may result in liver damage or anemia
Minerals	*Recommended daily intake*	*Overdosage*
Boron	<20 mg	No information found
Calcium	1000 mg	Doses larger than 1500 mg may cause stomach problems for sensitive individuals
Chlorine	3400 mg	No information found
Chromium	120 μg	Doses larger than 200 μg are toxic and may cause concentration problems and fainting
Copper	2 mg	As little as 10 mg of copper can have a toxic effect
Fluorine	3.5 mg	No information found
Iodine	150 μg	No information found
Iron	15 mg	Doses larger than 20 mg may cause stomach upset, constipation, and blackened stools
Magnesium	350 mg	Doses larger than 400 mg may cause stomach problems and diarrhea

(continued)

Table 8.1 (continued)

Manganese	5 mg	Excess manganese may hinder iron adsorption
Molybdenum	75 μg	Doses larger than 200 μg may cause kidney problems and copper deficiencies
Nickel	<1 mg	Products containing nickel may cause skin rash in case of allergies
Phosphorus	1000 mg	Contradiction: The FDA states that doses larger than 250 mg may cause stomach problems for sensitive individuals
Potassium	3500 mg	Large doses may cause stomach upsets, intestinal problems, or heart rhythm disorder
Selenium	35 μg	Doses larger than 200 μg can be toxic
Sodium	2400 mg	No information found
Vanadium	<1.8 mg	No information found
Zinc	15 mg	Doses larger than 25 mg may cause anemia and copper deficiency

Source: https://www.lenntech.com/recommended-daily-intake.htm

8.2.2 Bioavailability of Minerals

Although the total quantity of a mineral present in a food does not reflect its available amount in the human body and livestock via absorption because only a certain amount is bioavailable (Jafari and McClements 2017). Bioavailability is a term used to describe the digestion, absorption, and subsequent utilization of dietary compounds (Linder 1991). Bioavailability of a mineral takes place through three main steps, including (1) nutrient absorption by improving its accessibility in the intestinal lumen, (2) maintenance and/or absorption/uptake in the body, and (3) utilization (consumption) by the body. It depends on digestion, release from the food matrix, the absorption rate of the target ingredient by intestinal cells, and its transport amount to body cells. Several organic molecules can also influence mineral bioavailability. These include tannins and various polyphenolics as inhibitors, or ascorbic acid and S-amino acids as promoters (Welch and Graham 2004). Efforts have been undertaken to manipulate these compounds in plants, especially the reduction of tannins; the potential also exists to manipulate inorganic constituents directly.

8.2.3 Mineral Nutrition for Livestock

Globally, the forage grasslands represent 26% of the total land area and 70% of the agriculture area, used for livestock feeding purposes (Fao and Isric 2010). Forage crops are usually members of grasses (*Poaceae*) and herbaceous legumes (*Fabaceae*), but some members like mulga (*Acacia aneura*) and lead tree (*Leucaena leucocephala*) belonging to tree legumes are also grown in desert and grasslands (Muir et al. 2011). Consumption of these crops by livestock can be done either directly or after partial drying and pre-digestion. Their nutritional status and

digestibility (D-value) are defined by the concentration (and ratios) of carbohydrates, proteins, and lipids (Capstaff and Miller 2018; Osbourn 1980). They are the main source of minerals required in cattle nutrition (Suttle 2010). There are various determinants that determine the elemental profile of forage crops, including soil type and plant species. Soil is the major source of both micro- and macroelements for plants. Being an abundance of these elements in soil, a very less amount is available to plants (Marijanušić et al. 2017). Elements required for the optimal growth of plants are considered to be essential elements without which a plant cannot complete its life cycle. There are several macro- and microelements listed as essential elements. Examples of macro- and microelements include phosphorous (P), potassium (K), and calcium (Ca) which come under macroelement category, while copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and molybdenum (Mo) are part of microelements category. Both absence and excess of these elements show retardation in growth and disrupt the normal functioning of a living organism. Elements like Na, Cr, I, Se, Si, and V, which are non-essential for plants but essential for animals and humans enter in plants through various non-selective transport mechanisms (Kochian 1991), thus makes their way into humans and livestock food supply (Kabata-Pendias 1992).

The daily requirements for specific minerals vary considerably from one animal species to another, but the basis for establishing mineral needs is also varying in livestock than in humans. Although mineral intakes to prevent deficiency have been studied in the past, current efforts are more focused on the economics of production. Mineral intake levels often are set to maximize growth (e.g., swine, beef cattle, broiler chickens) or to optimize productivity (e.g., dairy cattle, laying hens) (Coleman and Moore 2003). Thus, with recommended high mineral intakes for certain livestock, it is common for commercial feed to contain supplemental minerals (Pond et al. 1995, 2004). Efforts to enhance mineral concentrations in plant foods would reduce the need for some of these additions, and thereby lower costs to the livestock producer. Interestingly, these mineral needs are not static but are rather a moving target. The production potential and nutritional requirements of animals are also under constant manipulation through conventional breeding and biotechnology (Bonneau and Lavard 1999). The type of plant material fed to livestock differs, in part, from that consumed by humans. Although cereals and grain legumes are fed widely to many animals, forages are a significant part of some diets, especially for ruminant animals (Pond et al. 2004; Reddy et al. 2003). Both monocots (e.g., *Lolium* spp., *Pennisetum* spp.) and dicots (e.g., *Medicago sativa*, *Trifolium* spp.) serve as forage and thus plant scientists should not overlook these species as targets for mineral improvement. In the case of forages, strategies to increase mineral concentration would be worthwhile, but efforts to ensure an appropriate balance of minerals would be equally important.

8.2.4 Strategies for Mineral Improvement

8.2.4.1 Agronomic Biofortification

Agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvement of the solubilization and mobilization of mineral elements in the soil. When crops are grown where mineral elements become immediately unavailable in the soil, the targeted application of soluble inorganic fertilizers to roots or to leaves is practiced. Therefore, the use of inorganic fertilizers must be included in any future strategy for food security. If the widespread use of inorganic fertilizers is facilitated, it might be possible to incorporate mineral elements essential for human nutrition before their distribution, as is practiced for Se in Finland and Zn in Turkey.

8.2.4.2 Inorganic Fertilizers

Soils often contain large amounts of Fe, but little of this is phytoavailable. The application of inorganic Fe fertilizers to such soils is usually ineffective as it rapidly becomes unavailable to plant roots through adsorption, precipitation, and oxidation reactions. For this reason, Fe-chelates are often used as soil Fe fertilizers (Rengel et al. 1999). Similarly, the availability of Fe in the rhizosphere can be increased by soil acidification with elemental S (Shuman 1998). Foliar applications of Zn fertilizers can increase leaf, tuber, and fruit Zn concentrations (Broadley et al. 2007). In some soils, the residual effects of a single application of Zn fertilizer can be appreciated over several years.

The phytoavailability of Cu in many agricultural soils is low, and Cu applied to the soil often becomes rapidly unavailable to plants, and concentrations in forage crops can be increased by Cu fertilization (Gupta 1979; Tamoutsidis et al. 2002). However, the combination of crop variety and Cu fertilization must be managed appropriately to ensure that Cu fertilization is adequate but not excessive, as too much Cu can be toxic to both plants and livestock (Puig et al. 2007). Magnesium is generally supplied to crops as its sulfate (Epsom salts or kieserite), carbonate, or, most commonly, oxide. In addition, the use of magnesium ammonium phosphate (struvite) has recently received attention, as it has potential as a sustainable P source for agriculture (Parsons and Smith 2008). Magnesium fertilizers are frequently applied to the soil surface or, when less soluble, incorporated into the subsoil. Magnesium sulfate provides readily available Mg^{2+}, whereas MgO behaves as a slow-release fertilizer (Draycott and Allison 1998). The foliar application of Mg fertilizers increases Mg concentrations in plant tissues and exhibited a strong positive association between Mg^{2+} in the soil solution and Mg concentrations in produce (Oury et al. 2006).

Tissue Se concentrations in plants can be increased by soil or foliar applications of Se fertilizers, and this has been shown to have beneficial effects on animal health and deliver Se to the human diet (Hawkesford and Zhao 2007; Rayman 2008). The I is present in solution as iodide, although iodate can also be present under strongly oxidizing conditions (Fuge and Johnson 1986). Fertilization with soluble iodide and/or iodate salts has been practiced in agriculture, and the iodination of irrigation

water has successfully increased the delivery of I to humans/livestock through edible crops (Lyons et al. 2004). It has been suggested that dietary I requirements are quite low, I fertilizers might be added to large areas of agricultural production from aeroplanes (Graham et al. 2007a). From the foregoing discussion, it is clear that the mineral elements can be increased in the mineral-deficient edible crops with the application of inorganic fertilizers.

8.2.4.3 Acquisition of Mineral Elements from Unfertilized Soils

Levels of Fe, Zn, and Cu in most soils would be sufficient to support mineral-dense crops if these elements were phytoavailable (Rengel 2001; White and Broadley 2009). Hence, there is considerable interest in developing management systems that exploit soil and fertilizer sources of mineral elements more effectively and in breeding mineral-efficient crops that produce high yields and accumulate minerals from previously infertile soils. The acquisition of mineral elements with restricted mobility in the soil, such as P, K, Fe, Zn, and Cu, can be improved by investing more biomass in the root system, by producing a greater number and more even spread of roots, by developing a more extensive root system, with longer, thinner roots with more root hairs, and by proliferating lateral roots in mineral-rich patches (White and Hammond 2008). Similarly, the efflux of organic acids, which displace cations from their binding sites in the soil, and the secretion of enzymes capable of degrading organic compounds, such as phytate, that chelate cations can also improve the acquisition of Fe, Zn, and Cu (Lynch 2007; Morgan et al. 2005). There is considerable intraspecific genetic variation in root architecture and root exudation that might improve the acquisition of all these elements from unfertilized soils (Lynch 2007; White and Hammond 2008).

Rotations and intercropping of plants that are better able to access and mobilize mineral elements with low solubility and/or movement in the soil solution can be utilized to increase their tissue concentrations and crop yield (Graham et al. 2007a; Inal et al. 2007; Jolley et al. 2004; Rengel et al. 1999). The diversification of rotations to include species with greater concentrations of essential mineral elements for human nutrition in their edible tissue also has the potential to increase the delivery of these elements to the human diet independently (Graham et al. 2007a). Moreover, soil microorganisms can also be exploited to increase the volume of soil explored by crop plants and the phytoavailability of mineral elements (Kirkby and Johnston 2008). Many crops are associated with mycorrhizal fungi, which have the potential to increase the volume of soil exploited for the acquisition of immobile mineral elements and release organic acids, siderophores, and enzymes capable of degrading organic compounds (Smith and Read 2010). Relationships with N2-fixing bacteria, whether symbiotic or associative, are especially important in N-limited environments (Hardarson and Broughton 2003). Thus, the deployment of legumes in N-limited environments is essential, but is often, although not always (Houlton et al. 2008), compromised by their high demand for P and other mineral elements for growth (White and Hammond 2008). It reveals that exudates from plant roots and mycorrhizal fungi can provide carbon for other soil microbes that affect the phytoavailability of mineral elements. Hence, inoculants of growth-promoting

bacteria can increase the acquisition of Fe, Zn, and Cu by plant roots, tissue mineral concentrations, plant growth, and yield (Barea et al. 2005; Whiting et al. 2001).

8.2.5 Forage Nutritional Content

8.2.5.1 Digestibility

The digestibility (D-value) of forage crops is determined by the concentration and composition of carbohydrates, proteins, and lipids along with minerals and vitamins, which are required for the production of energy by animals i.e. metabolisable energy (ME measured in MJ/Kg dry matter) (Osbourn 1980). The need for digestibility calculation is required when the crops grown are specific to ruminants and non-ruminant animals. In forage crops, carbohydrates hold for 50–80% of dry matter (DM); and if it drops too low, then the addition of supplement grains is required. Different types of primary carbohydrates like cellulose, hemicelluloses, starch, and fructans are degraded into simple sugars via cleavage of glycosidic bonds, either through animals itself or via microbial enzymatic actions prior to animal feeding. These simple sugars further produce monosaccharides which are metabolized to produce energy. Also, the digestibility gets altered due to different ratio of carbohydrates, especially when there are constraints in microbial digestion due to complex cell wall structure and limiting cell wall penetration (Bergman 1990; Weimer 1996).

8.2.5.2 Protein

Animals fulfill their need for nitrogen (N) predominantly from forage crops. Ribulose 1,5 bisphosphate carboxylase/oxygenase (RuBisCO) is the major form of protein available to the animal, though its amount varies from species to species (Wallace et al. 1997). Evidence of RuBisCO variation in different plant species leading to different available protein content can be seen during the comparison between grasses with herbaceous legumes, red clover (*Trifolium pratense*), white clover (*Trifolium repens*),and Lucerne (*Medicago sativa*) which are grown for their high protein content (Ruckle et al. 2017). There are several molecules which severely affect the protein digestibility. Lignin, tannins, and micronutrients like proanthocyanidins represent such groups. Tannins and proanthocyanidins inhibit protein degradation through binding and proven to be advantageous in preventing bloat. However, a high level of tannins will cause the passing of unabsorbed protein through the digestive tract and thus loss in nutritional value (Piluzza et al. 2014).

8.2.5.3 Lipids

Dietary lipids are crucial for the better quality of animal products; forage diets having low PUFA levels produce leaner meat in comparison to cereal diets (Van Elswyk and McNeill 2014). In forage crops, lipids are mostly present in the form of polyunsaturated fatty acids (PUFAs) in the range of 10–30 g kg^{-1} (Hatfield et al. 2007). α-Linolenic acid is the most abundant lipid constituting 62% of total lipid content (Clapham et al. 2005). It is evident through numerous studies that the use of

fresh forage enhances the milk quality because of low PUFA content and high trans-fatty acid (Chilliard et al. 2007; Elgersma et al. 2006).

8.2.5.4 Trace Elements

Minerals and trace elements are important elements controlling livestock health which minimize usages of antibiotics during production of animal derived products. Hence, balance of trace elements like Zinc and selenium are essentially required to maintain immune system and overall health of the animal. Imbalance of levels of such trace elements may possess adverse or toxic effects. For instance, an adequate amount of Zn is important for the immune system, but when added in greater quantity, it results in wasteful excretion. Also, selenium can be beneficial when given in the right amount, but prove to be toxic in greater quantity (Zhu et al. 2009) reviewed in Brugger and Windisch (2015). Contrastingly, avoiding the accumulation of toxic minerals can also be important for forage crops. Some elements accumulated in plants can make them unpalatable for livestock, but the ability of forage crops to grow fast and quickly recover from cutting makes them ideal crops for phytoremediation [e.g., Napier grass (Ishii et al. 2015)].

8.2.5.5 Biomass Production

Biomass production is the most desirable property of any forage crop, as they are cut or directly gazed by animals. The nutritional status of any forage crop directly depends on the rate of biomass production. Due to intensive production and faster growth, there is a decrement in the nutritional quality of forage crops. However, this is completely dependent on species grown, as some cultivars show better recovery after defoliation. The ability of shoot meristem to respond after cutting or grazing is essential in biomass production. In some of the forage cultivars, increased root exudation has been directly linked to the aboveground grazing and cutting (Paterson and Sim 1999). Exudates from the roots act as stimulants for rhizospheric microbes, which in turn mobilizes the soil nutrients to assist the aboveground growth. In addition to this, an optimal supply of nutrients and water is also required for forage biomass production (Dawson et al. 2004).

8.2.6 Genetic Biofortification Strategies for Improving Forage Crops

8.2.6.1 Cultivar Breeding

Plant nutritional science is at an exciting juncture because of the wealth of sequence information available for mineral-related genes in various models and agronomic species, and because of the breadth of technologies available to study the expression and function of relevant genes and gene products (Grusak 2002b). However, in comparison to other crops, there are few improvements in the area of forage crops due to their recent cultivation. Plant breeding can be considered as the starting point of the food chain all over the world, as shown in the simple food chain model in Fig. 8.1. Traditional breeding, as well as “green” biotechnology or green chemistry,

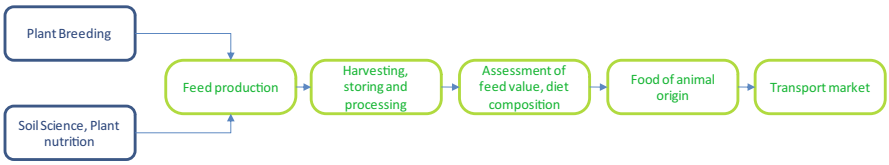

Fig. 8.1 Key elements of the production of food of animal origin in the human food chain

may result in changing of composition and nutritive value of feed plants. Recently, there comes the advancement in the field of forage crop breeding due to present agricultural trends and the global economic importance of forages. Various new cultivars have been developed by breeding many closely related wild lines with an aim to increase nutritional values, biomass production, crop durability, resistance against biotic stress (fungal and nematodes), and digestibility of DM (Boller and Greene 2010).

The various crops which have achieved the greatest improvements through breeding involve *Medicago* spp., *Lolilum*, *Trifolium* spp., and *Festuca*. Individual plants possessing high genotypic and phenotypic heterogeneity have been observed along with polyploidy, which is augmented by in-breeding across many kinds of grass and linkage of few agronomic traits to distinct genes. Several studies focus on this problem, particularly in legumes and grasses (Blackmore et al. 2016; Collins et al. 2012; de Araüjo et al. 2002; Luo et al. 2016; Piano et al. 2007; Vogel and Pedersen 1993). In spite of these problems, there has been a major success in developing breeding lines for forage crops, especially in *Lolium* and *Medicago*. Figure 8.2 represents a historic, present and future timeline of *Lolium* cultivation. *Festulolium*, another success forage crop, represents such one crop, which is developed by exploiting two closely related species of *Lolium* and *Festuca* (Humphreys et al. 2003). When backcrossed, a novel hybrid with more stable protein content in comparison to the parental line has been generated (Humphreys et al. 2014).

8.2.6.2 Strategies for Mineral Improvement

Increasing the microelement concentrations in plants through the application of mineral fertilizers can be complemented by growing crops with increased ability to acquire and accumulate these elements in their edible parts. Significant within-species genetic variations in the concentration of Fe, Zn, Cu, and Se were found in edible tissues of crop plants, but variations in the concentration of I in plant tissue are much fewer compared to the above-mentioned microelements. However, variations that are hereditary in the concentration of I in the leaves of the same species of ryegrass and white clover are found, indicating that they are under the influence of genetic control (Alderman and Jones 1967). The concentrations of Fe and Zn in grain cereal vary (1.5–4-fold) among genotypes depending on how many of them are genetically different (White and Broadley 2009). The strong correlation between Fe and Zn concentrations in corn, wheat, sorghum, and pearl millet was determined. This fact greatly increases the possibility of simultaneous cultivation for increased concentration of Zn and Fe in the aforementioned cultures. Wild wheat varieties

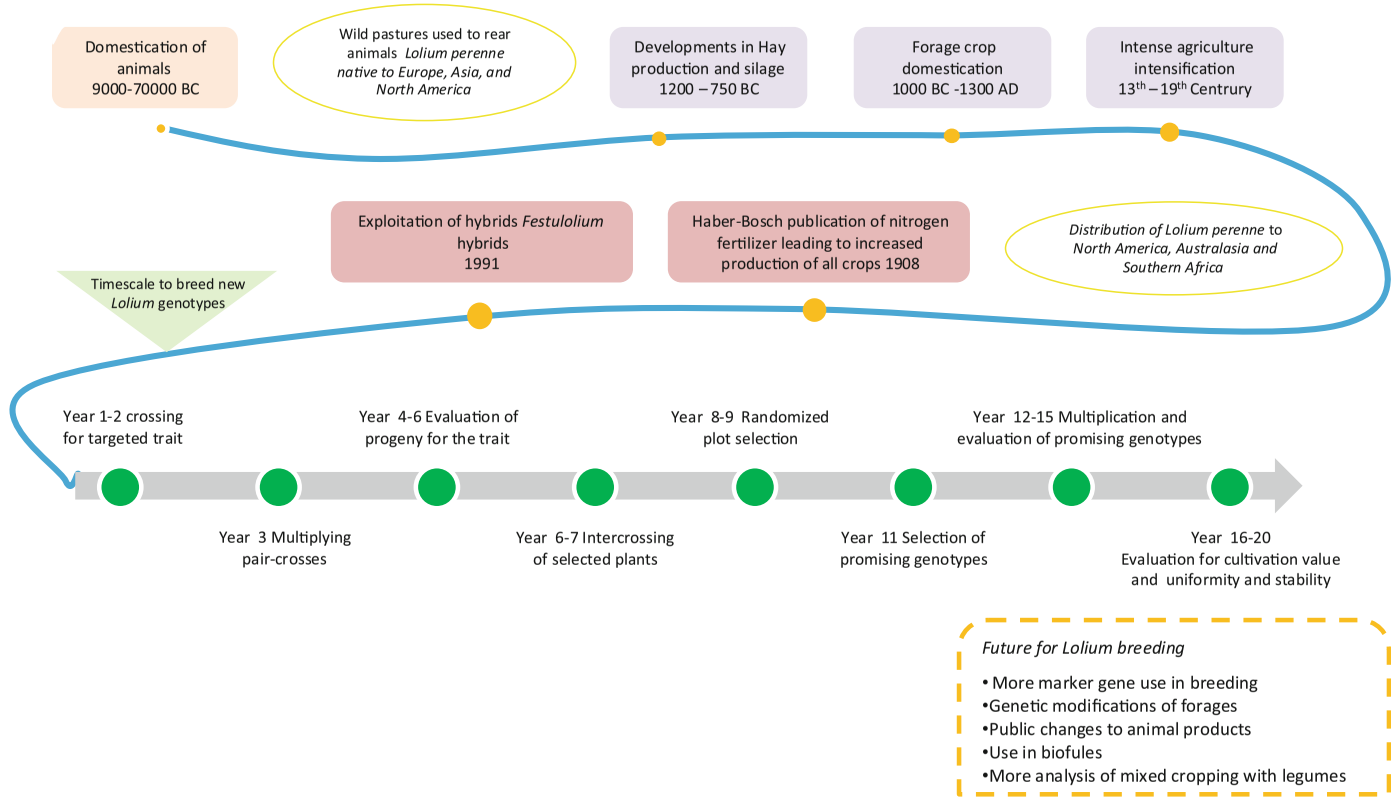

Fig. 8.2 Cultivation timeline of perennial ray grass including current and future breeding strategies (Brunis 2016; Capstaff and Miller 2018)

have a significantly higher concentration of Se compared to cultivated varieties, which can be used in breeding programs (White and Broadley 2009). Large amounts of microelements can be removed with the process of polishing and milling of cereal seeds or potential food. The amount of this loss is genotype-dependent (grain morphology, grain size, embryo size, number, and thickness of the tissue layers) because the highest concentrations of microelements are often in the husk and aleuronic layers (Bechoff and Dhuique-Mayer 2016). This fact can be largely used in the biofortification approach, with regard to the number of aleurone cell layers, which is cultivar dependent. However, according to Yang et al. (2003), there is little information available on the within-species variations in seed concentration of Se and I in the legumes. The concentration of Zn is often much higher in vegetable leaves than in grain, seeds, fruit, or tubers. Large genetic variations in shoots of Fe, Zn, and S concentration have also been found in spinach, beet, and onion (Grusak and Cakmak 2009).

Strategies for mineral improvement, therefore, must take into account the role of several short- and long-distance transport processes, as well as the availability of a given mineral in different tissues. The availability and transportation of mineral from soil to plant include series of sequential compartments with distinct capabilities of transporting each mineral from one to another compartment. The total flux of a mineral from the soil to a terminal tissue in the plant will be determined both by the rate limitations of transport steps along the pathway and by the size of the mineral pool in each successive compartment. The partitioning of some minerals to seeds is quite high compared to other tissues which reveal that efforts to double the Fe or Cu concentration in pea seeds would require improved transport into the plant to increase the vegetative pool of these minerals and may require elevated rates of transport from leaves to seeds. For most minerals, further research is needed to identify and characterize the full inventory of molecular- and tissue-level components that contribute to whole-plant mineral dynamics. Draft genomes have been increased for such crops and cultivars (Byrne et al. 2015; VanBuren et al. 2015) along with the evidence of using Brachypodium as a model research plant (Rancour et al. 2012). Utilizing this knowledge, candidate genes can be identified for nutritional enhancement purposes.

8.2.6.3 Identification of Candidate Genes for Nutritional Enhancement

Potential candidate genes can be identified through different genetic approaches, including quantitative trait loci (QTL) analysis or marker-assisted selection (MAS) using completed genomes and induced mutagenesis (Bansal et al. 2019; Chaudhary et al. 2019a, b; de Ronne et al. 2019; Kumawat et al. 2019). Several studies have been conducted in relation to biomass and growth traits. QTL has been used for various traits in *M. sativa* like vigor and lodging resistance, plant height, regrowth after harvest, flowering, stem height, and biochemical marker of ROS resistance genes for drought tolerance co-related to DM (Herrmann et al. 2010; Maghsoodi et al. 2017; McCord et al. 2014; Robins et al. 2007). Another ROS associated gene, member of the iron superoxide dismutase family, can increase the DM in both legume *M. sativa* (McKersie et al. 2000) and grass *Lolium* cultivars (Warnke et al.

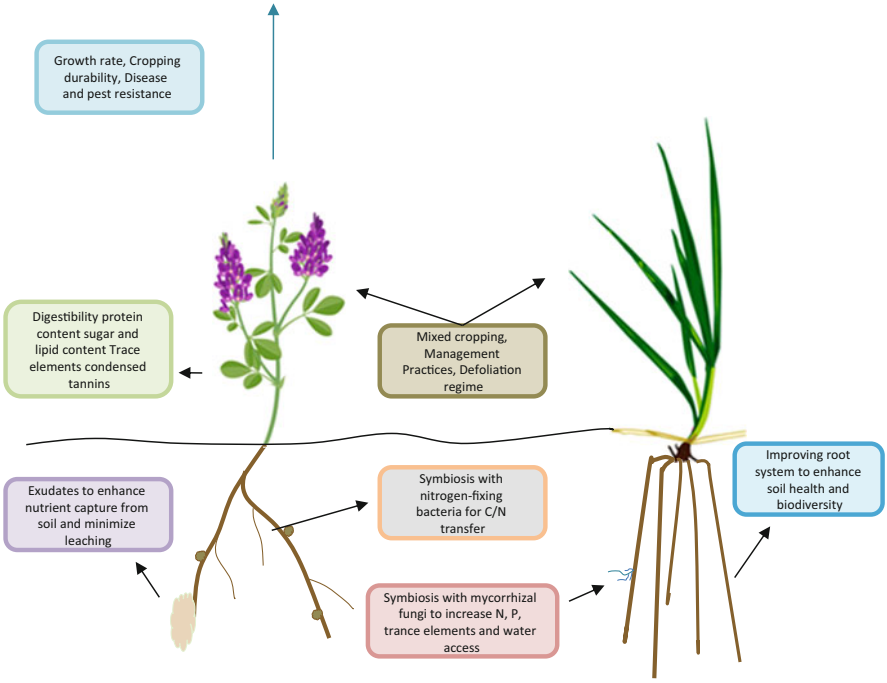

Fig. 8.3 Targets for impotent in forage crops

2002). Differential expression of genes between wild-type and a dwarf mutant of *Lolium* led to the identification of three dwarfism associated genes that were utilized for forward screening (Li et al. 2017a). To aid breeding, various molecular markers are used to infer phenotypic traits and track inheritance. Investigation of chloroplast SSR in *Lolium* represents such an example (Diekmann et al. 2012). Software like BioMercator is also used for iterative mapping to perform meta-QTL in *Lolium*, exploiting the old data to find new candidate genes (Shinozuka et al. 2012). Instead of enhancing optimal nutrition content, rapid biomass production is the most attractive trait, especially for those forage crops, which are subject to extensive cutting during the growing season. Because of this, candidate genes associated with photosynthesis and nitrogen use efficiency (NUE) become most desirable. Apart from these, there is a huge opportunity for improving traits of forage crops for biotic and abiotic stress also. Some of the traits that can be considered for all forage crops are depicted in Fig. 8.3.

Improvement in such traits could be achieved through transformation and molecular marker-assisted breeding programs. Proficient transformation has been developed for temperate cultivars of *Festuca* (Wang and Ge 2005; Zhang et al. 2006) and *Lolium* (Badenhorst et al. 2016; Bajaj et al. 2006); and recently for some of the tropical grasses, *Pennisetum* (Gondo et al. 2017) and *Brachiaria* (Cabral et al. 2015). Various approaches have been used for identification of candidate genes biomass

and different growth related traits. However, genomic approach permit us to explore information not only related to the target plant but also helps to understand rhizosphere microbiome associated with the target plant under study which will be advantageous for improving nutritional quality of crop.

Microorganisms also play a very important role in maintaining crop health and its nutritional value. Epiphytic bacteria and nitrogen-fixing bacteria perform such functions. Due to production of different specific binding molecules and/or siderophores, bacteria living in association with plants helps in digestion and absorption of forage eaten by livestock as they improve the uptake of trace elements in the soil, the rhizosphere microbiome is important as they are responsible for nutrient cycling and uptake, particularly in low input systems like those grown in the tropics.

As far as digestibility per se, availability of energy (ME) in livestock is directly linked to the digestion of macromolecules and their uptake (Nocek and Russell 1988; McCarthy Jr et al. 1989). Water-soluble carbohydrates (WSC) help in improving the digestibility (D-Value). Fructans which is water soluble carbohydrate and are the major storage plysaccherides present in *Lolium* (Miller et al. 2001; Chalmers et al. 2005). Distinct sequence variants of fructan: Fructan 6G-fructosyltransferase can be used to increase fructan levels in lolium at warmer climates, thus aiding in the development of new forage crops for the hot climate with high sugar content (Rasmussen et al. 2014). Also, the availability of N to the root is directly related to the amount of WSCs, highlighting importance of C:N balance in the growth of vegetative tissue (Louahlia et al. 2008; Roche et al. 2017). With recent advancements, several genes, including promoters, have been identified and manipulated to generate new cultivar. Transgenic lines of *Lolium perenne* have been generated through identification of photosynthesis promoters RBCS and chlorophyll a/b binding (CAB) (Panter et al. 2017). The obtained transgenic line is better in terms of yield, fiber, and digestibility and the fructans content in both pseudostems and leaf blades providing a platform for future studies to identify a promoter having importance in nutritional importance (Wang and Brummer 2012; Badenhorst et al. 2018). Other genes are also being targeted to increase biomass production. Delaying in the leaf senescence in *Lolium multiforum* is attained by introducing the 50 flanking regions of Zea mays cysteine protease gene SEE1 in Lolium. The resulting transgenic shows delayed leaf lifespan by approximately 8–16 days (Li et al. 2004). Along with fast growth, the ability of meristematic tissues responding after cutting is another desirable trait. Both these traits for fast growth rate and faster recovery are easy to select in various breeding trials and are of great interest to researchers in both *Lolium* and *Medicago* (Wilman et al. 1977; Vance et al. 1979). Besides different techniques used to enhance the quality of forage crops, mixed crop schemes are also used due to its advantages as observed in growing legumes and grasses together. Breeding in forage crops follows the monoculture selection regime; however, there is a vast scope in using mixed species to generate new cultivars. The advantages and disadvantages of using mixed forage crops are listed in Table 8.2.

Table 8.2 The advantages and disadvantages of growing forage crops in mixed systems

	Mixed cropping	
S. no	Advantages	Disadvantages
1	Soil nutrient availability: each species may have different strategies to mobilize nutrients	Growth rates and optimal harvest date can differ
2	Pathogen and pest susceptibility is different	Specialist equipment may be needed
3	Legume can supply N	Competition for resources
4	Stem support in the canopy	One species may host pathogens
5	Root depth for water access and improved soil structure	Monitoring more than one species at a time to keep up with needs

8.2.6.4 Integrating Genomic Technologies for Mineral Improvement

There is significant genetic diversity for almost any mineral of interest within existing germplasm collections and/or other unique genetic populations. This diversity offers tremendous opportunities to utilize various genomic resources and technologies, in an effort to manipulate mineral levels in plants. Fortunately, following the successes and advances that came out of the sequencing of the *Arabidopsis* genome (Arabidopsis Genome Initiative, 2000), most major crop species (e.g., the legumes *Medicago truncatula* and *Lotus japonicus*) have had genome projects in operation for several years.

Genome projects are contributing significantly towards generation of molecular markers for mapping, construction of genetic maps, comparative maps of crop of intrest and its related species. Transcriptomic studies helps for global gene expression studies and providing mutants for functional studies [i.e. T-DNA lines, or lines identified through high throughput screening of targeting induced lesions in genome (TILLING)] (McCallum et al. 2000; Scholte et al. 2002). Figure 8.4 presents a generalized flowchart that includes many of these tools and resources, and in the remainder of this chapter, we will discuss how they can impact cultivar development.

8.2.6.5 Transgenic Approach in Biofortification

The transgenic approach combines agronomic and genetic biofortification, i.e., the aim is to improve the phytoavailability of microelements in the soil, their uptake from the rhizosphere, the transfer to younger parts of the plants and their accumulation in edible tissues (Davies 2007). In addition, in the transgenic biofortification approach, the aim can also be to reduce the concentration of ant nutrient substances and to increase the promoter substances, which increase the absorption of microelements in the intestine (White and Broadley 2009). GM forage crops may be more acceptable to the public as if fed to animals; their entry into the human food chain is indirect. Also, fortification of fodder crops for enhanced nutrients can be done to reduce the expenditure of additional supplements given in animal diet. Several fortified fodder crops have been documented, including *Medicago sativa* and *Sorghum bicolor*. *Sorghum bicolor* is an appealing crop to be fortified using biotechnological approaches as it possesses the qualities of highly drought

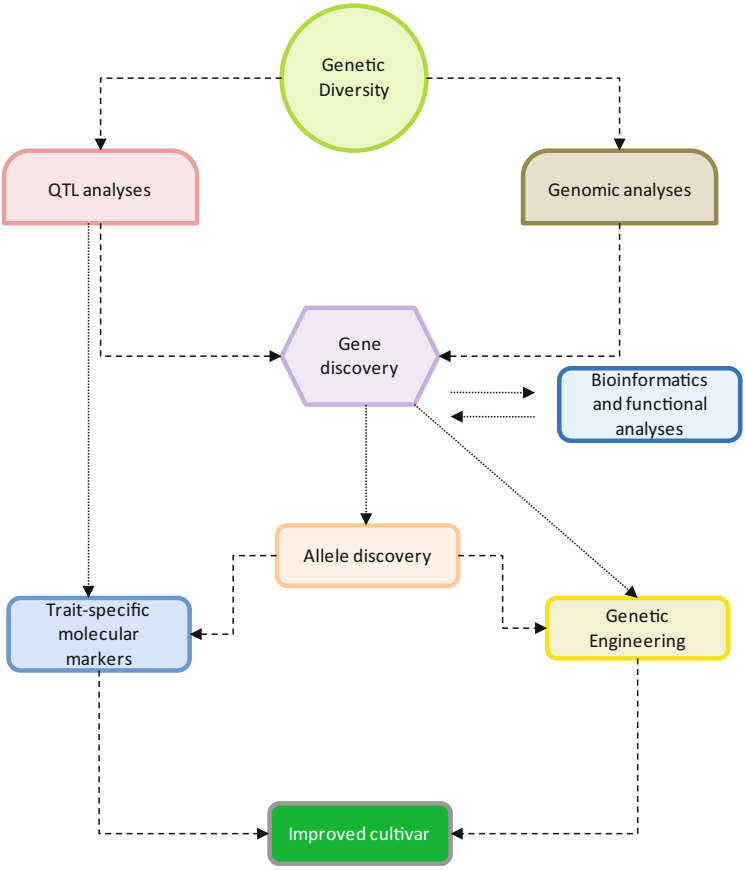

Fig. 8.4 Flowchart deciphering contribution of various nutritional genomics tools and resources that can contribute towards cultivar development

resistance, high biomass production, and C4 photosynthesis. Vegetative parts of crop plants contain a low percentage of fats and TAGs (triacylglycerols) as compared to seeds (OECD 2008; Vanhercke et al. 2019). The use of a multigene strategy for combinatorial overexpression of *Zea mays* WRINKLED (WRI1) *Umbelopsis ramanniana* DGAT2 (acyl CoA: diacylglycerol acyltransferase) and *Sesamum indicum* oleosin-L which plays a role in positively regulating the fatty acid synthesis, catalyzing the final TAG assembly step and accumulation of fatty acid, respectively, shows the feasibility of increasing the oil content in leaf tissue to 3 and 8.4% on a dry weight basis depending on leaf and developmental stages. The strategy used to upregulate the TAGs level in *Zea mays* can be used to get similar improvements in sorghum which is one of the most widely used fodder crop after maize (Fig. 8.5). Subsequently, when the expression of WRI1 is driven by different promotors (constitutive and mesophyll cell-specific promotor), it revealed changes in the

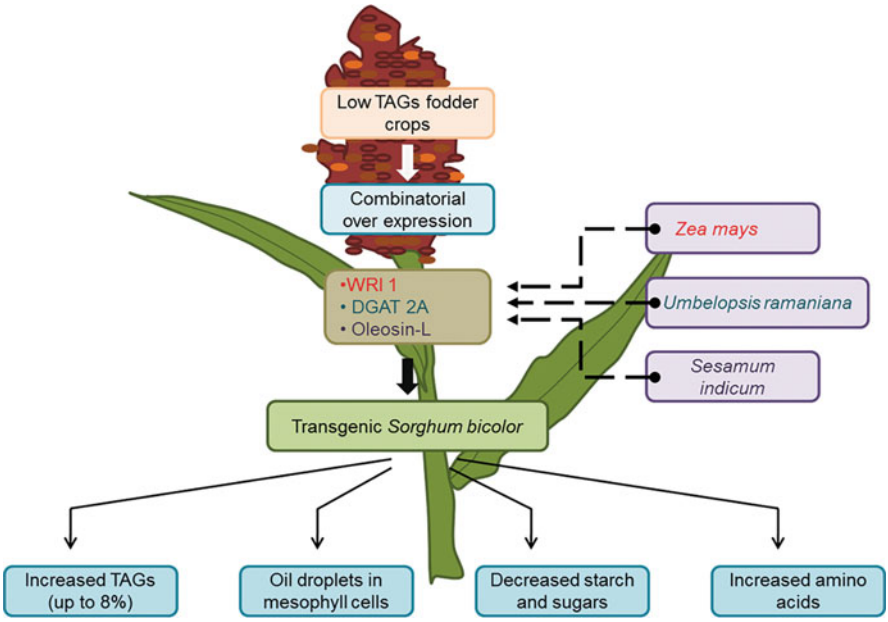

Fig. 8.5 Strategy adopted to upregulate oil expression in *Sorghum bicolor* through transgenic approach. Transgenic plants are developed by overexpressing three genes WRI 1, DGAT 2A, and Oleosin-L (shown in brown box), derived from different eukaryotic organisms (shown in purple boxes). Resulting transgenic lines show enhancement of TAGs up to 8% in leaves as oil droplets in mesophyll cells

lipidome profile as well as levels of starch and soluble sugar (Vanhercke et al. 2019). The pictorial representation of the multigene construct is shown in Fig. 8.6.

CRISPR/Cas9 technology can be used to generate crops with enhanced nutrition value (Mushtaq et al. 2019; Vats et al. 2019). Nowadays, it is also used in forage crop enhancement. Several gene editing lines of forage crops conferring stress tolerance have been generated, aiding both biomass increment and nutritional quality. In *Medicago sativa*, a mutation has been successfully done in Squmaosa Promoter Binding Protein (SPL 9) gene using CRISPER/Cas9. Gao et al. (2018), revealed advancement using gene editing approach for the improvement of fodder crops is limited. Many genes have been identified, which can be used to enhance forage crops. But due to limited transformation technology, development of new forage crop is lagging behind. Genome editing is a promising technology and will be more accepted in the future for forage crop development (Lee et al. 2012; Li et al. 2017b; Zheng et al. 2017).

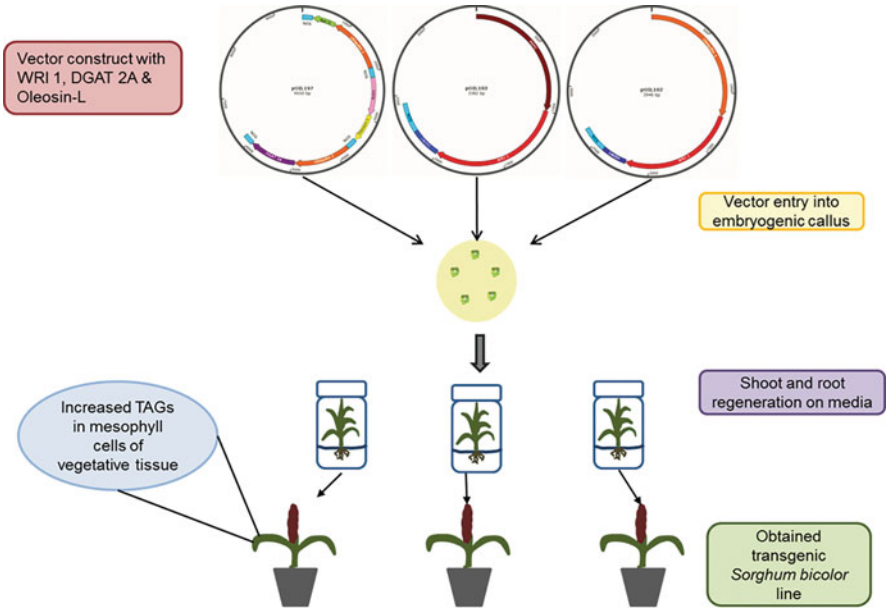

Fig. 8.6 Pictorial representation of multigene construct for combinatorial overexpression of *Sorghum bicolor* and regeneration of transgenic plants from embryogenic callus

8.3 Conclusion

Advancement in genomics and bioinformatics has revolutionized all biology; due to expanding databases and the addition of more species and cultivars, it is very helpful for breeders to improve forage crop performance. Nutritional quality and yield of forage crops can be increased by using GWAS for traits like NUpE or specific trace elements and high vegetative tissue concentration. SNPs identification in the genes of model plants also aids in the genome targeting in forage crops (Bonhomme et al. 2014; Slavov et al. 2014; Thorogood et al. 2017). The function of genes in forage can be studied using TILLING lines (Carelli et al. 2013; Dalmais et al. 2013; Manzanares et al. 2016). Limitations in transferring genes in forage crops are the biggest bottleneck due to which less work in the development of forage crops has been accomplished. In conclusion, by utilizing the information gathered from grain crop and model plants like Arabidopsis, we can improve the nutritional quality and yield of forage crops.

References

Alderman G, Jones D (1967) The iodine content of pastures. J Sci Food Agric 18:197–199

Badenhorst P, Smith K, Spangenberg G (2016) Development of a molecular breeding strategy for the integration of transgenic traits in outcrossing perennial grasses. Agronomy 6:56

Badenhorst P, Panter S, Palanisamy R, Georges S, Smith K, Mouradov A, Mason J, Spangenberg G (2018) Molecular breeding of transgenic perennial ryegrass (Lolium perenne L.) with altered fructan biosynthesis through the expression of fructosyltransferases. Mol Breed 38:21

Bajaj S, Ran Y, Phillips J, Kularajathevan G, Pal S, Cohen D, Elborough K, Puthigae S (2006) A high throughput agrobacterium tumefaciens-mediated transformation method for functional genomics of perennial ryegrass (Lolium perenne L.). Plant Cell Rep 25:651–659

Bansal R, Rana N, Kumawat S, Kumar N, Preeti P, Bhatt V, Waqar A, Altaf N, Katara JL, Samantaray S et al (2019) Advances in induced mutagenesis and mutation mapping approaches in rice. Oryza 56(Special issue):8

Barea J-M, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Bechoff A, Dhuique-Mayer C (2016) Factors influencing micronutrient bioavailability in biofortified crops. Ann N Y Acad Sci 1390:74–87

Bergman E (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev 70:567–590

Blackmore T, Thorogood D, Skøt L, McMahon R, Powell W, Hegarty M (2016) Germplasm dynamics: the role of ecotypic diversity in shaping the patterns of genetic variation in Lolium perenne. Sci Rep 6:22603

Boller B, Greene SL (2010) Genetic resources. In: Fodder crops and amenity grasses. Springer, New York, pp 13–37

Bonhomme M, André O, Badis Y, Ronfort J, Burgarella C, Chantret N, Prosperi JM, Briskine R, Mudge J, Debellé F (2014) High-density genome-wide association mapping implicates an F-box encoding gene in Medicago truncatula resistance to Aphanomyces euteiches. New Phytol 201:1328–1342

Bonneau M, Lavard B (1999) Biotechnology in animal nutrition, physiology and health. Livest Prod Sci 59:223–242

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702

Brugger D, Windisch WM (2015) Environmental responsibilities of livestock feeding using trace mineral supplements. Anim Nutr 1:113–118

Bruins M (2016) Breeding targets for ryegrass in Europe 3. Available at: http://european-seed.com/breeding-targets-ryegrass-europe/. Accessed 04 Dec 2017

Byrne SL, Nagy I, Pfeifer M, Armstead I, Swain S, Studer B, Mayer K, Campbell JD, Czaban A, Hentrup S (2015) A synteny-based draft genome sequence of the forage grass Lolium perenne. Plant J 84:816–826

Cabral GB, Carneiro VT, Rossi ML, da Silva JP, Martinelli AP, Dusi DM (2015) Plant regeneration from embryogenic callus and cell suspensions of Brachiaria brizantha. In Vitro Cell Dev Biol Plant 51:369–377

Capstaff NM, Miller AJ (2018) Improving the yield and nutritional quality of forage crops. Front Plant Sci 9:535

Carelli M, Calderini O, Panara F, Porceddu A, Losini I, Piffanelli P, Arcioni S, Scotti C (2013) Reverse genetics in Medicago truncatula using a TILLING mutant collection. In: Legume genomics. Springer, New York, pp 101–118

Chalmers J, Lidgett A, Cummings N, Cao Y, Forster J, Spangenberg G (2005) Molecular genetics of fructan metabolism in perennial ryegrass. Plant Biotechnol J 3:459–474

Chaudhary J, Alisha A, Bhatt V, Chandanshive S, Kumar N, Mir Z, Kumar A, Yadav SK, Shivaraj S, Sonah H (2019a) Mutation breeding in tomato: advances, applicability and challenges. Plan Theory 8:128

Chaudhary J, Deshmukh R, Sonah H (2019b) Mutagenesis approaches and their role in crop improvement. Plan Theory 8:467

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M (2007) Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur J Lipid Sci Technol 109:828–855

Clapham WM, Foster JG, Neel JP, Fedders JM (2005) Fatty acid composition of traditional and novel forages. J Agric Food Chem 53:10068–10073
Coleman SW, Moore JE (2003) Feed quality and animal performance. Field Crop Res 84:17–29
Collins RP, Helgadóttir Á, Frankow-Lindberg BE, Skøt L, Jones C, Skøt KP (2012) Temporal changes in population genetic diversity and structure in red and white clover grown in three contrasting environments in northern Europe. Ann Bot 110:1341–1350
Consensus C (2004) Copenhagen consensus: the results. Cambridge University Press, Cambridge
Dalmais M, Antelme S, Ho-Yue-Kuang S, Wang Y, Darracq O, d'Yvoire MB, Cezard L, Légée F, Blondet E, Oria N (2013) A TILLING platform for functional genomics in Brachypodium distachyon. PLoS One 8:e65503
Davies KM (2007) Genetic modification of plant metabolism for human health benefits. Mutat Res 622:122–137
Dawson L, Thornton B, Pratt S, Paterson E (2004) Morphological and topological responses of roots to defoliation and nitrogen supply in Lolium perenne and Festuca ovina. New Phytol 161:811–818
de Araüjo M, Coulman B, Rakow G (2002) Genetic variation, heritability and progeny testing in meadow bromegrass. Plant Breed 121:417–424
de Ronne M, Labbé C, Lebreton A, Sonah H, Deshmukh R, Jean M, Belzile F, O'Donoughue L, Bélanger R (2019) Integrated QTL mapping, gene expression and nucleotide variation analyses to investigate complex quantitative traits: a case study with the soybean–Phytophthora sojae interaction. Plant Biotechnol J 3. https://doi.org/10.1111/pbi.13301
Diekmann K, Hodkinson TR, Barth S (2012) New chloroplast microsatellite markers suitable for assessing genetic diversity of Lolium perenne and other related grass species. Ann Bot 110:1327–1339
Dimkpa C, Bindraban P (2016) Micronutrients fortification for efficient agronomic production. Agron Sustain Dev 36:1–26
Draycott P, Allison M (1998) Magnesium fertilisers in soil and plants: comparisons and usage. Proceedings-Fertiliser Society, United Kingdom
Elgersma A, Tamminga S, Ellen G (2006) Modifying milk composition through forage. Anim Feed Sci Technol 131:207–225
Fao I, Isric I (2010) JRC. 2009. Harmonized world soil database (version 1.1). Food and Agriculture Organization, Rome, Italy and International Institute for Applied Systems Analysis, Laxenburg, Austria
Fuge R, Johnson CC (1986) The geochemistry of iodine—a review. Environ Geochem Health 8:31–54
Gao R, Feyissa BA, Croft M, Hannoufa A (2018) Gene editing by CRISPR/Cas9 in the obligatory outcrossing Medicago sativa. Planta 247:1043–1050
Gómez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T, Christou P (2010) Critical evaluation of strategies for mineral fortification of staple food crops. Transgenic Res 19:165–180
Gondo T, Umami N, Muguerza M, Akashi R (2017) Plant regeneration from embryogenic callus derived from shoot apices and production of transgenic plants by particle inflow gun in dwarf napiergrass (Pennisetum purpureum Schumach.). Plant Biotechnol 34:143
Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Adv Agron 70:77
Graham RD, Welch RM, Saunders DA, Ortiz-Monasterio I, Bouis HE, Bonierbale M, De Haan S, Burgos G, Thiele G, Liria R (2007a) Nutritious subsistence food systems. Adv Agron 92:1–74
Graham RL, Nelson R, Sheehan J, Perlack R, Wright LL (2007b) Current and potential US corn Stover supplies. Agron J 99:1–11
Grusak MA (2002a) Enhancing mineral content in plant food products. J Am Coll Nutr 21:178S–183S

Grusak MA (2002b) Phytochemicals in plants: genomics-assisted plant improvement for nutritional and health benefits. Curr Opin Biotechnol 13:508–511
Grusak MA, Cakmak I (2009) 12 methods to improve the crop-delivery of minerals to humans and livestock. In: Plant nutritional genomics. Wiley, Williston, p 265
Gupta U (1979) Copper in the environment. Part 1. Wiley, New York
Hardarson GG, Broughton WJ (2003) Maximising the use of biological nitrogen fixation in agriculture, vol 99. Springer, Dordrecht
Hatfield RD, Jung H-JG, Broderick G, Jenkins TC (2007) Nutritional chemistry of forages. Forages 2:467–485
Hawkesford MJ, Zhao F-J (2007) Strategies for increasing the selenium content of wheat. J Cereal Sci 46:282–292
Herrmann D, Barre P, Santoni S, Julier B (2010) Association of a CONSTANS-LIKE gene to flowering and height in autotetraploid alfalfa. Theor Appl Genet 121:865–876
Houlton BZ, Wang Y-P, Vitousek PM, Field CB (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327
Humphreys MW, Canter P, Thomas HM (2003) Advances in introgression technologies for precision breeding within the Lolium-Festuca complex. Ann Appl Biol 143:1–10
Humphreys MW, O'Donovan SA, Farrell MS, Gay AP, Kingston-Smith AH (2014) The potential of novel Festulolium (2n= 4x= 28) hybrids as productive, nutrient-use-efficient fodder for ruminants. Food Energy Secur 3:98–110
Inal A, Gunes A, Zhang F, Cakmak I (2007) Peanut/maize intercropping induced changes in rhizosphere and nutrient concentrations in shoots. Plant Physiol Biochem 45:350–356
Ishii Y, Hamano K, Kang D-J, Idota S, Nishiwaki A (2015) Cadmium phytoremediation potential of napiergrass cultivated in Kyushu, Japan. Appl Environ Soil Sci 2015:1
Jafari SM, McClements DJ (2017) Nanotechnology approaches for increasing nutrient bioavailability. In: Advances in food and nutrition research. Elsevier, Amsterdam, pp 1–30
Jolley V, Hansen N, Shiffler A (2004) Nutritional and management related interactions with iron-deficiency stress response mechanisms. Soil Sci Plant Nutr 50:973–981
Kabata-Pendias A (1992) Trace metals in soils in Poland—occurrence and behaviour. Soil Sci 140:53–70
Kirkby EA, Johnston AEJ (2008) Soil and fertilizer phosphorus in relation to crop nutrition. In: The ecophysiology of plant-phosphorus interactions. Springer, Dordrecht, pp 177–223
Kochian LV (1991) Mechanisms of micronutrient uptake and translocation in plants. In: Micronutrients in agriculture. Soil Science Society of America, Madison, pp 229–296
Kumawat S, Rana N, Bansal R, Vishwakarma G, Mehetre ST, Das BK, Kumar M, Kumar Yadav S, Sonah H, Sharma TR (2019) Expanding avenue of fast neutron mediated mutagenesis for crop improvement. Plan Theory 8:164
Lee K-W, Cha J-Y, Kim K-H, Kim Y-G, Lee B-H, Lee S-H (2012) Overexpression of alfalfa mitochondrial HSP23 in prokaryotic and eukaryotic model systems confers enhanced tolerance to salinity and arsenic stress. Biotechnol Lett 34:167–174
Li Q, Robson PR, Bettany AJ, Donnison IS, Thomas H, Scott IM (2004) Modification of senescence in ryegrass transformed with IPT under the control of a monocot senescence-enhanced promoter. Plant Cell Rep 22:816–821
Li W, Katin-Grazzini L, Gu X, Wang X, El-Tanbouly R, Yer H, Thammina C, Inguagiato J, Guillard K, McAvoy RJ (2017a) Transcriptome analysis reveals differential gene expression and a possible role of gibberellins in a shade-tolerant mutant of perennial ryegrass. Front Plant Sci 8:868
Li Z, Long R, Zhang T, Wang Z, Zhang F, Yang Q, Kang J, Sun Y (2017b) Molecular cloning and functional analysis of the drought tolerance gene MsHSP70 from alfalfa (Medicago sativa L.). J Plant Res 130:387–396
Linder MC (1991) Nutritional biochemistry and metabolism: with clinical applications. Elsevier, New York

Louahlia S, Laine P, MacDuff J, Ourry A, Humphreys M, Boucaud J (2008) Interactions between reserve mobilization and regulation of nitrate uptake during regrowth of Lolium perenne L.: putative roles of amino acids and carbohydrates. Botany 86:1101–1110

Luo K, Jahufer M, Wu F, Di H, Zhang D, Meng X, Zhang J, Wang Y (2016) Genotypic variation in a breeding population of yellow sweet clover (Melilotus officinalis). Front Plant Sci 7:972

Lynch JP (2007) Roots of the second green revolution. Aust J Bot 55:493–512

Lyons GH, Stangoulis JC, Graham RD (2004) Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the HarvestPlus program. Nutr Rev 62:247–252

Maghsoodi M, Razmjoo J, Gheysari M (2017) Application of biochemical markers for the assessment of drought tolerance in alfalfa (Medicago sativa L.) cultivars. Grassl Sci 63:3–14

Manzanares C, Yates S, Ruckle M, Nay M, Studer B (2016) TILLING in forage grasses for gene discovery and breeding improvement. New Biotechnol 33:594–603

Marijanušić K, Manojlović M, Bogdanović D, Čabilovski R, Lombnaes P (2017) Mineral composition of forage crops in respect to dairy cow nutrition. Bulgarian J Agr Sci 23(2):204–212

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London

McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeting induced local lesions in genomes (TILLING) for plant functional genomics. Plant Physiol 123:439–442

McCarthy R Jr, Klusmeyer T, Vicini J, Clark J, Nelson D (1989) Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J Dairy Sci 72:2002–2016

McCord P, Gordon V, Saha G, Hellinga J, Vandemark G, Larsen R, Smith M, Miller D (2014) Detection of QTL for forage yield, lodging resistance and spring vigor traits in alfalfa (Medicago sativa L.). Euphytica 200:269–279

McKersie BD, Murnaghan J, Jones KS, Bowley SR (2000) Iron-superoxide dismutase expression in transgenic alfalfa increases winter survival without a detectable increase in photosynthetic oxidative stress tolerance. Plant Physiol 122:1427–1438

Miller L, Moorby JM, Davies DR, Humphreys MO, Scollan ND, MacRae JC, Theodorou MK (2001) Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.): milk production from late-lactation dairy cows. Grass Forage Sci 56:383–394

Morgan J, Bending G, White P (2005) Biological costs and benefits to plant–microbe interactions in the rhizosphere. J Exp Bot 56:1729–1739

Muir J, Pitman W, Foster J (2011) Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? Grass Forage Sci 66:301–315

Mushtaq M, Mukhtar S, Sakina A, Dar AA, Bhat R, Deshmukh R, Molla K, Kundoo AA, Dar MS (2020) Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiol Biochem 147:242

Nocek J, Russell J (1988) Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J Dairy Sci 71:2070–2107

OECD (2008) OECD-FAO agricultural outlook 2008–2017: OECD agricultural outlook 2008–2017 (Organisation for Economic Co-operation and Development)

Osbourn D (1980) The feeding value of grass and grass products. In: Grass, its production and utilization. Blackwell Scientific Publications, Oxford, pp 70–124

Oury F-X, Leenhardt F, Remesy C, Chanliaud E, Duperrier B, Balfourier F, Charmet G (2006) Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. Eur J Agron 25:177–185

Panter S, Mouradov A, Badenhorst P, Martelotto L, Griffith M, Smith K, Spangenberg G (2017) Re-programming photosynthetic cells of perennial ryegrass (Lolium perenne L) for fructan biosynthesis through transgenic expression of fructan biosynthetic genes under the control of photosynthetic promoters. Agronomy 7:36

Parsons SA, Smith JA (2008) Phosphorus removal and recovery from municipal wastewaters. Elements 4:109–112

Paterson E, Sim A (1999) Rhizodeposition and C-partitioning of Lolium perenne in axenic culture affected by nitrogen supply and defoliation. Plant Soil 216:155–164
Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47:S-88–S-105
Piano E, Annicchiarico P, Romani M, Pecetti L (2007) Effect of the number of parents and their combining ability on the performance of synthetic varieties in tall fescue. Aust J Agric Res 58:1100–1105
Piluzza G, Sulas L, Bullitta S (2014) Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. Grass Forage Sci 69:32–48
Pond CM, Mattacks CA, Gilmour I, Johnston M, Pillinger C, Prestrud P (1995) Chemical and carbon isotopic composition of fatty acids in adipose tissue as indicators of dietary history in wild arctic foxes (A lopex lagopus) on Svalbard. J Zool 236:611–623
Pond WG, Church DB, Pond KR, Schoknecht PA (2004) Basic animal nutrition and feeding. Wiley, Hoboken
Puig S, Andrés-Colás N, García-Molina A, Penarrubia L (2007) Copper and iron homeostasis in Arabidopsis: responses to metal deficiencies, interactions and biotechnological applications. Plant Cell Environ 30:271–290
Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). Crit Rev Food Sci Nutr:1–17. https://doi.org/10.1080/10408398.2019.1685454
Rancour D, Marita J, Hatfield RD (2012) Cell wall composition throughout development for the model grass Brachypodium distachyon. Front Plant Sci 3:266
Rasmussen S, Parsons AJ, Xue H, Liu Q, Jones CS, Ryan GD, Newman JA (2014) Transcript profiling of fructan biosynthetic pathway genes reveals association of a specific fructosyltransferase isoform with the high sugar trait in Lolium perenne. J Plant Physiol 171:475–485
Rawat N, Neelam K, Tiwari VK, Dhaliwal HS (2013) Biofortification of cereals to overcome hidden hunger. Plant Breed 132:437–445
Rayman MP (2008) Food-chain selenium and human health: emphasis on intake. Br J Nutr 100:254–268
Reddy B, Reddy PS, Bidinger F, Blümmel M (2003) Crop management factors influencing yield and quality of crop residues. Field Crop Res 84:57–77
Rengel Z (2001) Genotypic differences in micronutrient use efficiency in crops. Commun Soil Sci Plant Anal 32:1163–1186
Rengel Z, Batten G, Crowley Dd (1999) Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crop Res 60:27–40
Robins JG, Bauchan GR, Brummer EC (2007) Genetic mapping forage yield, plant height, and regrowth at multiple harvests in tetraploid alfalfa (Medicago sativa L.). Crop Sci 47:11–18
Roche J, Turnbull MH, Guo Q, Novák O, Späth J, Gieseg SP, Jameson PE, Love J (2017) Coordinated nitrogen and carbon remobilization for nitrate assimilation in leaf, sheath and root and associated cytokinin signals during early regrowth of Lolium perenne. Ann Bot 119:1353–1364
Ruckle ME, Meier MA, Frey L, Eicke S, Kölliker R, Zeeman SC, Studer B (2017) Diurnal leaf starch content: an orphan trait in forage legumes. Agronomy 7:16
Scholte M, d'Erfurth I, Rippa S, Mondy S, Cosson V, Durand P, Breda C, Trinh H, Rodriguez-Llorente I, Kondorosi E (2002) T-DNA tagging in the model legume Medicago truncatula allows efficient gene discovery. Mol Breed 10:203–215
Shinozuka H, Cogan NO, Spangenberg GC, Forster JW (2012) Quantitative trait locus (QTL) meta-analysis and comparative genomics for candidate gene prediction in perennial ryegrass (Lolium perenne L.). BMC Genet *13*:101
Shuman LM (1998) Micronutrient fertilizers. J Crop Prod 1:165–195

Slavov GT, Nipper R, Robson P, Farrar K, Allison GG, Bosch M, Clifton-Brown JC, Donnison IS, Jensen E (2014) Genome-wide association studies and prediction of 17 traits related to phenology, biomass and cell wall composition in the energy grass Miscanthus sinensis. New Phytol 201:1227–1239

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic Press, Amsterdam

Stein AJ (2010) Global impacts of human mineral malnutrition. Plant Soil 335:133–154

Suttle NF (2010) Mineral nutrition of livestock, vol 13. CABI Publishing, Wallingford, pp 347–383

Tamoutsidis E, Papadopoulos I, Tokatlidis I, Zotis S, Mavropoulos T (2002) Wet sewage sludge application effect on soil properties and element content of leaf and root vegetables. J Plant Nutr 25:1941–1955

Thacher TD, Fischer PR, Strand MA, Pettifor JM (2006) Nutritional rickets around the world: causes and future directions. Ann Trop Paediatr 26:1–16

Thorogood D, Yates S, Manzanares C, Skot L, Hegarty M, Blackmore T, Barth S, Studer B (2017) A novel multivariate approach to phenotyping and association mapping of multi-locus gametophytic self-incompatibility reveals S, Z, and other loci in a perennial ryegrass (Poaceae) population. Front Plant Sci 8:1331

Van Elswyk ME, McNeill SH (2014) Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: the US experience. Meat Sci 96:535–540

VanBuren R, Bryant D, Edger PP, Tang H, Burgess D, Challabathula D, Spittle K, Hall R, Gu J, Lyons E (2015) Single-molecule sequencing of the desiccation-tolerant grass Oropetium thomaeum. Nature 527:508

Vance CP, Heichel GH, Barnes DK, Bryan JW, Johnson LE (1979) Nitrogen fixation, nodule development, and vegetative regrowth of alfalfa (Medicago sativa L.) following harvest. Plant Physiol 64:1–8

Vanhercke T, Belide S, Taylor MC, El Tahchy A, Okada S, Rolland V, Liu Q, Mitchell M, Shrestha P, Venables I (2019) Up-regulation of lipid biosynthesis increases the oil content in leaves of Sorghum bicolor. Plant Biotechnol J 17:220–232

Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R (2019) Genome editing in plants: exploration of technological advancements and challenges. Cell 8:1386

Vogel K, Pedersen J (1993) Plant breeding reviews. Wiley, Newark

Wallace R, Onodera R, Cotta M (1997) Metabolism of nitrogen-containing compounds. In: The rumen microbial ecosystem. Springer, Dordrecht, pp 283–328

Wang Z-Y, Brummer EC (2012) Is genetic engineering ever going to take off in forage, turf and bioenergy crop breeding? Ann Bot 110:1317–1325

Wang Z-Y, Ge Y (2005) Agrobacterium-mediated high efficiency transformation of tall fescue (Festuca arundinacea). J Plant Physiol 162:103–113

Warnke S, Barker R, Brilman L, Young W, Cook R (2002) Inheritance of superoxide dismutase (Sod-1) in a perennial× annual ryegrass cross and its allelic distribution among cultivars. Theor Appl Genet 105:1146–1150

Weimer PJ (1996) Why don't ruminal bacteria digest cellulose faster? J Dairy Sci 79:1496–1502

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84. https://doi.org/10.1111/j.1469-8137.2008.02738.x

White PJ, Hammond JP (2008) Phosphorus nutrition of terrestrial plants. In: The ecophysiology of plant-phosphorus interactions. Springer, Dordrecht, pp 51–81

Whiting SN, de Souza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by Thlaspi caerulescens. Environ Sci Technol 35:3144–3150

Wilman D, Droushiotis D, Mzamane MN, Shim J (1977) The effect of interval between harvests and nitrogen application on initiation, emergence and longevity of leaves, longevity of tillers and dimensions and weights of leaves and 'stems' in Lolium. J Agric Sci 89:65–79

Yang F, Chen L, Hu Q, Pan G (2003) Effect of the application of selenium on selenium content of soybean and its products. Biol Trace Elem Res 93:249–256

Zhang W-J, Dong J-L, Liang B-G, Jin Y-S, Wang T (2006) Highly efficient embryogenesis and plant regeneration of tall fescue (Festuca arundinacea Schreb.) from mature seed-derived calli. In Vitro Cell Dev Biol Plant 42:114–118

Zheng G, Fan C, Di S, Wang X, Xiang C, Pang Y (2017) Over-expression of Arabidopsis EDT1 gene confers drought tolerance in alfalfa (Medicago sativa L.). Front Plant Sci 8:2125

Zhu Y-G, Pilon-Smits EA, Zhao F-J, Williams PN, Meharg AA (2009) Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. Trends Plant Sci 14:436–442

Global Scenario of Vitamin Deficiency and Human Health

9

Roni Chaudhary, Siddhant Chaturvedi, Renu Sharma, and Siddharth Tiwari

Abstract

Vitamins are either fat-soluble (vitamin A, D, E, and K) or water-soluble (vitamin B and C) and required in small amounts for maintaining the healthy growth and normal function of the human body. Vitamin deficiency is a major public health issue worldwide. More than two billion people have found vitamin deficient and are mostly belong to developing countries. Vitamin A deficiency is of severe health concern under the age of 5 years children in several South-East Asian and African countries. Similarly, vitamin D and vitamin B_{12} deficiencies are the other major growing health concern. The global community must prioritize their action to eradicate hunger as well as vitamin deficiency in low- and middle-income countries. Various vitamin supplementation, food fortification, biofortification, and health policy programs are needed to improve the scenario of vitamin deficiency. Nutrition policy should transit from a simple reductionist method to multifaceted solutions and also work in the synergy of the scientific community to improve the health quality of large population. This chapter is mainly focused on vitamin types and their roles in human health, the global scenario of vitamin deficiency, and a significant source of vitamins to eradicate their deficiency.

R. Chaudhary · R. Sharma · S. Tiwari (✉)
National Agri-Food Biotechnology Institute (NABI), Department of Biotechnology, Ministry of Science and Technology (Government of India), Mohali, Punjab, India
e-mail: siddharth@nabi.res.in

S. Chaturvedi
National Agri-Food Biotechnology Institute (NABI), Department of Biotechnology, Ministry of Science and Technology (Government of India), Mohali, Punjab, India

Department of Biotechnology, Panjab University, Chandigarh, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_9

Keywords

Biofortification · Global vitamin deficiency · Vitamins · Fat-soluble vitamins · Water-soluble vitamins · Vitamin sources

9.1 Introduction

Vitamins are required in adequate amounts for maintaining body functions and good health. They are micronutrients and play a vital role in several enzymatic reactions as precursor and cofactor (MacFabe et al. 2007; Obrenovich et al. 2015). Vitamins are classified into two types, fat-soluble (Vitamin A, D, E, and K) and water-soluble (Vitamin B and C). Vitamin A is a vital nutrient that enters in the human body either in the form of precursors they consume or directly from the dietary sources (Bates 1995). Vitamin A is essential for several functions including, a good visual system, maintaining epithelial cell integrity, growth and development, red blood cell production, and immunity (Sommer and West 1996; Imdad et al. 2017). The major populations of South African and Asian regions usually eat monotonous food, so vitamin deficiencies are prevalent in these areas. A study conducted by the South African National Health and Nutrition Examination Survey (SANHANES) in 2013 shows a significant population of children faced vitamin deficiency (Faber and Wenhold 2018). It was reported that every third woman with rein Africa had a vitamin A or vitamin B_{12} deficiency (Faber and Wenhold 2018). The impact on vitamin A deficiency (VAD) has studied in 19 countries that include India, Africa, Latin America, and Australia. It is found that the VAD is associated with high mortality due to diarrhea, measles, meningitis, and respiratory diseases. Vitamin A sufficiency or supplementation reduces the risk of diarrhea and measles but has no significant effect on the respiratory disease (Imdad et al. 2017). Different province of South Africa is affected by VAD as it is noted 18.5% in Northern Cape, 21.0% in Western Cape, 38.4% in KwaZulu-Natal, and 43.5% in Limpopo (Faber and Wenhold 2018). Case studies related to vitamin B_{12} deficiency shows the incidence of an autoimmune disease called pernicious anemia as 0.13% in Northern Europe, 80% in the Gambia, 13.33% in India, and 69% in Middle East countries (Stabler and Allen 2004). A population-based cross-sectional survey for vitamin C deficiency shows different values in two different parts of India. The 73.9% prevalence of vitamin C deficiency is reported in North and 45.7% in South part of India (Ravindran 2011). Vitamin D is a fat-soluble vitamin which helps to maintain health by regulating calcium and phosphorus level in the body (Holick 2005; Aguiar et al. 2019). The main source of vitamin D is sunlight exposure, but the risk of vitamin D deficiency (VDD) is increased by excessive use of sunscreen, air pollution, full-body clothing, and living in the high latitude (Bates 1995; Munns et al. 2016). VDD can lead to osteomalacia, muscle weakness, bone pain, and the consequent risk of falls. In children, severe VDD causes hypocalcemia that leads to seizures, tetany, heart failure, and rickets with osteomalacia leg bowing (Maiya et al. 2008; Uday et al. 2018). The International Osteoporosis Foundation and DSM nutritional products surveys had concluded that the VDD had significantly higher in North America than

Europe (Wahl et al. 2012; Hilger et al. 2014; Spiro and Buttriss 2014). Shallow amount of vitamin E in the human body results in neurodegenerative disorder that is known as Ataxia with vitamin E deficiency (AVED). AVED is a rare disorder that resulted from a mutation in the gene coding for α-TTP, required for vitamin E retention (Khadangi and Azzi 2019). Hence, this chapter is broadly summarized the global scenario of different vitamin deficiencies and their impact on human health.

9.2 Vitamins and Their Impact on Human Health

Vitamins and minerals are considered micronutrients because they required in low or traceable amounts for normal human growth and metabolism. Minerals, vitamins, fatty acids, and necessary amino acids are unavoidable as they play a crucial role for maintaining human health (MacFabe et al. 2007; Obrenovich et al. 2015; Castro et al. 2016; Saghazadeh et al. 2017; El-Ansary et al. 2018). Deficiency of vitamins and minerals through poor food habits is one of the main contributing factors to numerous health problems. Anemia, hypothyroidism, scurvy and rickets occur due to lack of iron, iodine, vitamin C, and vitamin D, respectively (Kocovska et al. 2012; Cannell 2017; Kocovska et al. 2017; Adams et al. 2018). According to the Food and Agriculture Organization (FAO 2017), the undernourishment level had decreased from 20% to nearly 10% of the total world population in 2016. However, there is a need for continuous efforts in order to reduce undernourishment in people all over the world. The successful fall in the level of malnourishment can be attained by increasing the yield and nutrition quality of staple food crops. Indeed, in the last 25 years, the production per hectare of wheat, rice, and potato has risen by 30% (FAO 2017). However, these crops often deficient in adequate amounts of micronutrients, thereby augmenting the prevalence of micronutrients deficiency that is known as “hidden hunger”. Different classes of vitamins, their major sources, role in the human body, and consequences of improper levels are summarized in Table 9.1.

9.3 Vitamins and Global Deficiency

Vitamins are essential for human health, but their deficiency is prevalent in the whole world. Anthropometric and micronutrient deficiency are the indicators of human health and wellness. South African and Asian countries are facing poverty and dependency on one food that leads to a deficiency of several vitamins. Sociocultural limitations, economic constraints, insufficient dietary intake, and poor absorption are leading to a reduction in the bioavailability of the vitamin in the body. These conditions are considered as the potential cause of the prevalence of different vitamin deficiencies in several developing countries. The vitamin deficiency is aggravated by the absence of new legislation, lack of education, poor sanitation, enforcement of existing food laws, and week monitoring and surveillance system. Several recent studies reported higher morbidity and mortality among pregnant

Table 9.1 Classes of vitamins and their possible role and consequences

Vitamins	Type of vitamins	Source	Role in the human body	Probable consequences of improper level	Case studies related to vitamin deficiency	References
Vitamin A (retinol)	Fat-soluble	*Gracilaria chilensis, Codium fragile, Moringa oleifera,* orange, vegetables (such as carrots, pumpkins, sweet potato), dark green vegetables (such as spinach, collards, broccoli, turnip greens, Swiss chard), orange flesh fruits (such as apricot, cantaloupe, mango, nectarine, orange, papaya, peach	Improvement of gut microbiota; increased plasma retinol, CD38, and RORA mRNA	Measles, meningitis, impairment of central nervous system development; increased serum 5-hydroxytryptamine (5-HT) levels, autism spectrum disorder, diarrhea	16 studies in India; eight in Africa; seven in Latin America, two in Australia; sub-Saharan African region (48%) VAD, South Asian (43%) VAD, In 2013 94,500 deaths recorded in sub-Saharan Africa from diarrhea and measles	Blomhoff et al. (1991), D'Ambrosio et al. (2011), Khillan (2014), Faber and Wenhold (2018)
Vitamin B complex						
Vitamin B_1 (thiamine)	Water-soluble	Legumes	The functioning of nervous system and metabolism of carbohydrates	Central nervous system (CNS) diseases; Wernicke-Korsakoff syndrome, beriberi; language deficiency; ASD	Vitamin B_{12} deficiency: Northern Europe (0.13%), Gambia (80%), Middle East (69%), and India (13.33%)	Stabler and Allen (2004), Obrenovich et al. (2015)

Vitamin B_2 (riboflavin)	Water-soluble	Green vegetables	The precursor of coenzyme flavin adenine mononucleotide and flavin adenine dinucleotide, mitochondria bioenergetic process	The degenerative nervous system, endocrine dysfunction anemia, and also skin disorders, hyperemia (excessive blood), cheilosis (cracked and swollen lips)		Rasmussen et al. (2001), Jiang et al. (2005)
Vitamin B_3 (niacin or nicotinic acid)	Water-soluble	Almonds, cape gooseberry, avocado	Bioenergetics and redox reactions of metabolism	Pellagra		Savvidou (2014)
Vitamin B_5 (pantothenic acid)	Water-soluble	Peas, beans, nuts, broccoli, mushroom, potatoes, sweet potatoes	The precursor of coenzyme A, lipid and carbohydrate metabolism	Upper respiratory infections, insomnia, fatigue		Jiang et al. (2005), Allen (2008)
Vitamin B_6 (pyridoxine)	Water-soluble	Grapes, beans, banana, spinach, cauliflower, cabbage, avocados, prunes	Neurotransmitter synthesis, gene expression; transamination, decarboxylation reaction; brain development	Anemia associated with depression; impaired immune function; may be associated with convulsive seizures		McCormick (2006), Saposnik et al. (2009), Ebbing et al. (2010)
Vitamin B_7 (biotin)	Water-soluble	Raw egg yolk, liver, peanuts, certain vegetables	Deamination of amino acids and decarboxylation reaction	Lethargy, tingling of extremities, hallucination, hair loss, ataxia		Savvidou (2014)
Vitamin B_9 (folate)	Water-soluble	Green leafy vegetables, whole grain, citrus fruits, and beans, etc.	Synthesis of nucleic acids, the functioning of the nervous system, regulates stress management	Affective disorders (depression, anger)		He and Shui (2014), Bailey et al. (2015)

(continued)

Table 9.1 (continued)

Vitamins	Type of vitamins	Source	Role in the human body	Probable consequences of improper level	Case studies related to vitamin deficiency	References
Vitamin B_{12} (riboflavin)	Water-soluble	Meat, poultry, fish, eggs, milk and milk products and dried purple laver	Methionine transmethylation/ transculturation metabolism	Anemia, cognitive impairment, affective disorders (depression, anger)		Strand et al. (2007), Allen (2008), Honzik et al. (2010), Hendren et al. (2016)
Vitamin C (ascorbic acid)	Water-soluble	Tomatoes, broccoli, citrus fruits, strawberries, turnip, leafy greens such as spinach and mustard greens	Antioxidant, and participates in several enzymatic reactions	Scurvy, impaired immune function	Two different parts of India show 73.9% prevalence of vitamin C deficiency in North India and 45.7% in South India	Li and Schellhorn (2007), Rautiainen et al. (2009), Ravindran (2011)
Vitamin D	Fat-soluble	Fishes, egg yolks, shiitake mushrooms, liver, fortified milk, irradiated foods, salmon, tuna, sardines, cod liver oil	Brain development and function, mood regulation, neuronal differentiation, axonal connectivity, dopamine ontogeny, immunological modulation and transcriptional control of several genes	Pathogenesis of certain psychiatric disorders like, depression and ASD	Vitamin D north-south gradient in Europe with Scandinavian countries which show higher vitamin d content than southern Europe, case studies show higher vitamin d value in North America than Europe	MacFarlane et al. (2004), McGillivray et al. (2007), Wahl et al. (2012), Tassone et al. (2013), Hilger et al. (2014), Albahrani and Greaves (2016)
Vitamin E (tocopherol)	Water-soluble	Avocado, nuts (such as almonds, cashew nuts, filberts, macadamia nut, peanuts, pistachio, walnuts), lentils, chickpeas, green leafy vegetables	Antioxidant, protect against peroxidative damage in plasma and red blood cells. Protect tissue lipids from free radical scavenging	Arthritis, cataracts, neurological disease, immunological disorders	Vitamin E deficiency in America 11%, middle east 27%, Africa 27%, Europe 8%, Asia pacific 16%	Dror and Lindsay (2011), Valtuena et al. (2013), Masri et al. (2015), Shamim et al. (2013), Yahia et al. (2019), Peter et al. (2013)

Vitamin K: Phylloquinone or K1	Fat-soluble	Spinach, kale, cabbage, kiwi, avocado, and grapes	Blood clotting, a cofactor in protein carboxylation	Diabetes, renal calculi, osteoporosis	Vitamin K insufficiency in Uganda predicted by the lower level of protein induced in vitamin K absence or prothrombin (PIVKA-II) as 33.3% mothers and 66% newborns	Choi et al. (2011), Ferland (2012), Manna and Kalita (2016)
Menaquinone or K2	Fat-soluble	Mostly fermented food and dairy products	Osteocalcin and matrix GLa protein synthesis, calcium transport	Cognitive impairment		

women and children. Worldwide, one-third of the total deaths among children caused by vitamin deficiency. The highest mortality and child malnutrition are reported in South Asia, where 178 million of the children below 5 years of age found stunted growth while the global estimate of wasting is ~55 million children. Other studies confirmed that approximately 48% of children under the age of 5 years in India were stunted, followed by 37% in Pakistan and 43% in Bangladesh (Bhutta et al. 2008; Black 2008). The prevalence of vitamin A, B_{12}, D, and E deficiency in various countries like America, Africa, Europe, India, and the province of South Africa is presented in Fig. 9.1 and Table 9.2. Therefore, rigorous efforts are needed to control vitamin deficiency in several lower- and middle-income countries to minimize the negative health effect in human life (Akhtar et al. 2013).

9.4 Fat-Soluble Vitamins

Fat-soluble vitamins (A, D, E, and K) are absorbed in the body along with fats in the diet. They dissolved in organic solvents (fats or oil) during the process of extraction and purification and stored in fatty tissues of the body.

9.4.1 Vitamin A

Vitamin A term is used for the retinol and its related compounds that exhibit the biological activity. The major forms of vitamin A are retinol, retinoic acid, and retinal (Albahrani and Greaves 2016; Kaur et al. 2016). It is stored in the liver in the form of retinyl palmitate (Ross and Ternus 1993). Vitamin A includes all naturally occurring nutritionally active form (retinoic acids, retinol) and plant-derived carotenoids. The major source of vitamin A is either a retinyl ester that is derived from an animal source, e.g., meat, egg, liver, milk, or plant-derived pigmented carotenoids (Blomhoff et al. 1991; Ross and Ternus 1993; Faber et al. 2015). β-carotene and other carotenoids converted to vitamin A in the body. β-carotene is a molecule that carries the highest activity to convert into vitamin A. Theoretically one molecule of β-carotene is converted into two molecules of retinol. However, due to physiological efficiency, the maximum conversion of β-carotene to retinol is found to be nearly 50%. Additionally, absorption efficiency depends on the source of food and food matrix. A study in India showed that absorption of β-carotene is higher from green vegetables than carrots and papayas (Schweiggert et al. 2014). Normally, 70–90% of vitamin A is absorbed by the gut by the presence of intestinal juice and bile salts (D'Ambrosio et al. 2011). Extracellular transportation of vitamin A mainly occurs through retinol-binding protein (RBP) and thyroxine-binding protein transthyretin (TTR). RBP-TTR complex is required for protecting vitamin A against oxidation, esterification, and also helps in vitamin A solubility (Blomhoff et al. 1991; Khillan 2014).

Deficiency of vitamin A is well known to cause night blindness. Vitamin A also plays an essential role in cell division, cell differentiation, apoptosis, and immune response. It also regulates the metabolism of macronutrients like carbohydrate, lipid,

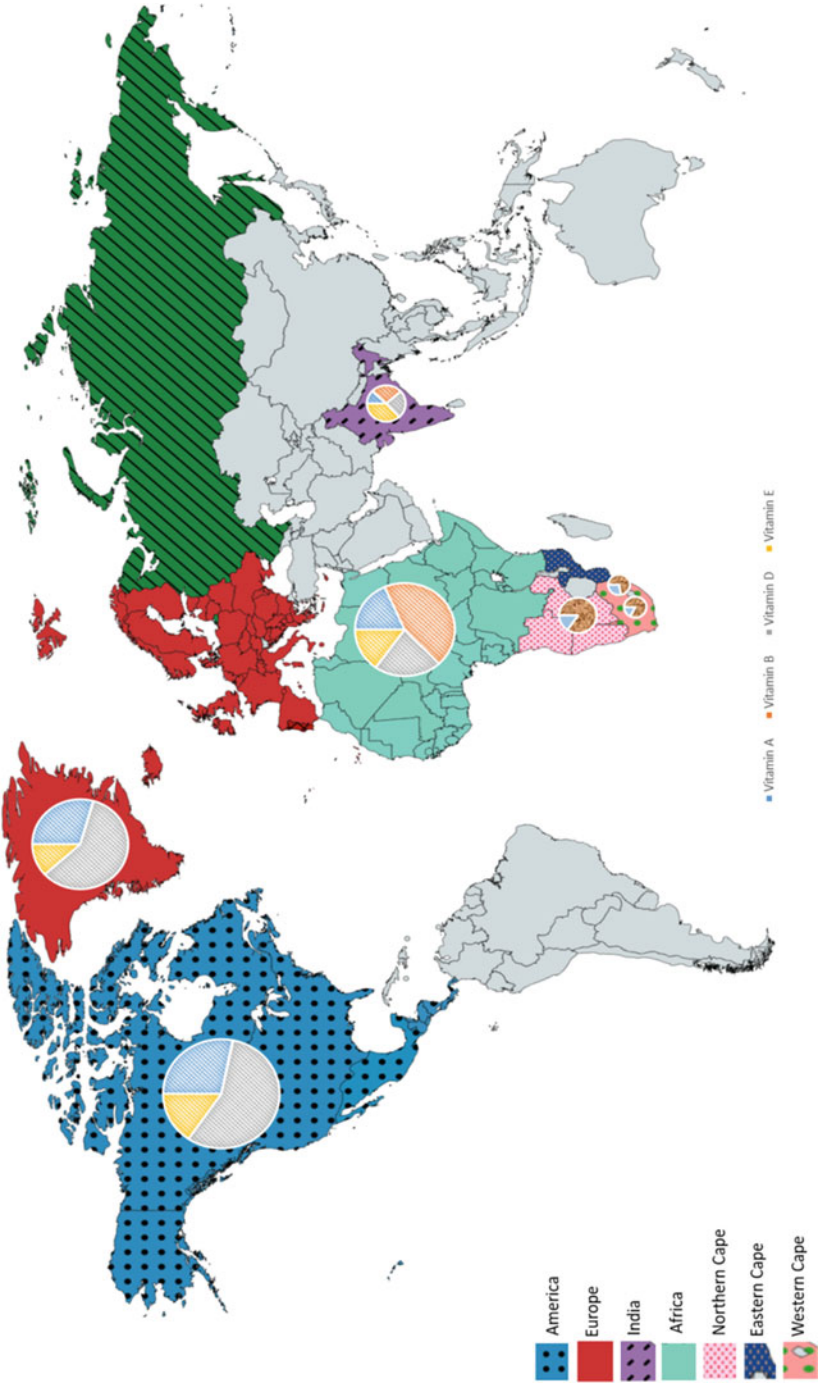

Fig. 9.1 Global scenario of vitamin deficiency

Table 9.2 Status of different vitamin deficiency in major countries

	Vitamin A	Vitamin B_{12}	Vitamin D	Vitamin E	References
America	5–20% (Approx. 20%)	0.151%	40%	11%	Stabler and Allen (2004), Forrest and Stuhldreher (2010), Dror and Lindsay (2011), Valtuena et al. (2013), Durazo-Arvizu et al. (2014), Cashman et al. (2015)
Europe	5–20% (Approx. 20%)	0.13%	40%	8%	Stabler and Allen (2004), Durazo-Arvizu et al. (2014), Faber and Wenhold (2018), Cashman et al. (2015), Dror and Lindsay (2011), Valtuena et al. (2013)
Africa	33%	80%	39%	27%	Stabler and Allen (2004), Durazo-Arvizu et al. (2014), Cashman et al. (2015), Dror and Lindsay (2011), Valtuena et al. (2013)
India	32.6%	70%	66.2%	98% (pregnant mother)	Stabler and Allen (2004), Durazo-Arvizu et al. (2014), Cashman et al. (2015), Dror and Lindsay (2011), Valtuena et al. (2013)
Province of South Africa	Vitamin A deficiency (VAD)	Anemia (vitamin B_{12}) deficiency			
Northern cape	18.5%		–		Wenhold and Faber (2008), Faber and Wenhold (2018)
Western cape	21.0%		28.6%		Wenhold and Faber (2008), Faber and Wenhold (2018)
Kwazulu-natal	38.4%		10.4%		Wenhold and Faber (2008), Faber and Wenhold (2018)
Limpopo	43.5%		34.2%		Wenhold and Faber (2008), Faber and Wenhold (2018)

and protein (Chen and Chen 2014). VAD causes dryness and keratinization in epithelial cells of the skin, respiratory, gastrointestinal, and urogenital tracts. These are the initial preventative symptoms against infection (Blomhoff et al. 1991). The major cause of VAD is the regular monotrophic diet, which provides low intake and bioavailability of vitamin A (Underwood et al. 2000). A study conducted by the

South African National Health and Nutrition Examination Survey (SANHANES) in 2013 mentioned that mostly children and women in Africa suffered from VAD (Faber and Wenhold 2018). The report also showed that nearly 43.6% of children under the age of 5 faces the VAD. Other report published by the South African Vitamin A Consultative Group (SAVACG) in 1994 showed the lowest prevalence of VAD in Northern Cape (18.5%) and Western Cape (21.0%), while it is highest in KwaZulu-Natal (38.0%) and Limpopo (43.5%) in children of age group 6 months to 6 years (Faber and Wenhold 2018). The population living in rural areas and with poorly educated mothers were most affected by VAD (Faber et al. 2007). The survey conducted by the UN standing committee on nutrition in 2013 showed that the Sub-Saharan African (48%) and South Asian (43%) regions had the highest VAD. Ninety-four thousand five hundred children deaths recorded in 2013 due to diarrhea and measles that had shown a link with VAD.

9.4.2 Vitamin D

Vitamin D is a group of sterols that are directly or indirectly associated with the number of pathologies. The most abundant form of vitamin D is 25-hydroxyvitamin D {25(OH)D}, while the active form of vitamin D is 1,25-dihydroxyvitamin D3 (1,25-$(OH)_2$ D3). Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are the two main forms of vitamin D. Vitamin D2 is derived from the plant after irradiation of UV lights, while vitamin D3 is derived from the animal source. The greatest natural source of vitamin D3 is sunlight from which endogenous synthesis occurs (Albahrani and Greaves 2016). According to WHO, the minimum Recommended Dietary Allowance (RDA) for vitamin D is 5 μg/day (World Health Organization 2004). The deficiency of vitamin D is known for rickets disease in children and osteomalacia in adults (Holick 2006). The VDD is linked with soft bone and bone fracture (Cranney et al. 2007). Various studies showed that it is related to immune response, cardiovascular disease, and stroke (Beard et al. 2011). Severe VDD is found in the patient of HIV and breast cancer; those are associated with body immune response (Welsh 2004; Beard et al. 2011). VDD is a major concern in many countries. Previously it was thought that VDD occurs in areas where the sunny climate is not found throughout the year. Several recent studies have shown that VDD is also common in a sunny area. The study was conducted on 834 Saudi people between the age group of 20–74 years, where 87% of persons showed VDD, mostly older and obese people (Ardawi et al. 2012).

Vitamin D containing foods are oily fishes, egg yolks, shitake mushrooms, liver, and organ meats. 90% of vitamin D is synthesized dermally under the sunlight (Pillai et al. 2011). 7-dehydrocholesterol (cholesterol-like substance) is converted into pre-vitamin D by UV-B, which further isomerizes to vitamin D3 and D2. Vitamin D3 and D2 both are an inactive form while the biological active form of vitamin D is 1,25(OH)2D3, which is also known as calcitriol (Albahrani and Greaves 2016). Various studies showed that South-East Asia and Pacific regions were more susceptible to VDD (Torti 2012). In Middle East Asia and Africa, VDD found low in children than the adult (McGillivray et al. 2007). It may be due to children live more

outside than adults. In North America, VDD is more common than in the European region (Tassone et al. 2013). VDD also increases with increasing the distance with the equator (Hilger et al. 2014). In Asia, most of the countries are suffering from VDD. In India, several studies were showed that the low serum level of 25(OH)D had detected across the population. In North India, 96% of infants, 91% of schoolgirls, 78% healthy hospital staff, and 84% of pregnant women were found hypovitaminosis D (Pillai et al. 2011; Goswami et al. 2008). More than 45% of women and 40.5% of men were VDD in Bangladesh and Sri Lanka. Prevalence of hypovitaminosis {D (25(OH)D < 75 nmol/L} in postmenopausal women was found 47% in Thailand, 49% in Malaysia, 90% in Japan, and 92% in South Korea (Setiati 2008; Kuwabara et al. 2010). Thailand showed high serum levels of 25(OH)D, possibly related to its geographical location close to the equator (Hilger et al. 2014). In China, VDD prevalent across the country. In North China, 89% of pubescent girls and 48% of old men reported with VDD (Strand et al. 2009). In Europe, 25–30% of adults had shown VDD, which increases to more than 75% in the old persons (MacFarlane et al. 2004). VDD in postmenopausal women in Mexico, Chile, and Brazil was found 67%, 50%, and 42%, respectively (Morales 2010). In North America, VDD in children, adults, and older people had 2%, 13%, and 11%, respectively (Hilger et al. 2014).

9.4.3 Vitamin E

Vitamin E is another fat-soluble vitamin that acts as an antioxidant metabolite (Traber and Stevens 2011). Tocopherols and tocotrienols are the two major groups of naturally available vitamin E. Each group has four isomers (α, β, γ, and δ) based on methyl group position on the chromanol ring (Albahrani and Greaves 2016). Most of the sources of vitamin E are rich in γ-tocopherol while in the bloodstream, the α-tocopherol level is dominant. The major sources of vitamin E are from leafy vegetables, whole grains, vegetable oils, and nuts. All of the eight isoforms are found in foods, but their content varies. In general, vitamin E deficiency is rare in developed countries.

Vitamin E mainly acts as an antioxidant. It is essential for the normal shape of erythrocyte and also involved in the slowing of the aging process. Furthermore, it also plays a protective role against arthritis, cataracts, neurological disease, and immunological disorders (Packer 1991; Clarke et al. 2008). Vitamin E is involved in various biological activities associated with cancer like anti-inflammation, anti-oxidation, and anti-proliferation. Further Vitamin E also overturns the synthesis of interleukins (IL-1, 6, 8) and Tumour Necrosis Factor (TNF) in the case of human breast cancer, it shows an inhibitory effect on cancer cells and in apoptosis (Kline et al. 2003; Pierpaoli et al. 2010). Vitamin E is absorbed in the small intestine. RDA for vitamin E in adult persons is 15 mg/day, while for pregnant and lactating women, it is reported 19 mg/day. The normal range of vitamin E in the serum of healthy adults should be in between 5 μg/mL (11 μmol/L) and 15 μg/mL (33 μmol/L) (McBurney et al. 2015). Vitamin E deficiency causes several neurodegenerative diseases, skeletal myopathies, weak immunity, ataxia, and hemolytic anemia

(Mansoor and Ahmad 2016). It is uncommon but reported in South Asian and sub-Saharan children and adults who are carrying acute protein deficiency, fat malabsorption syndrome, liver disease, and acute respiratory tract infection (Kilmarx 2009; Dror and Lindsay 2011). The study on Bedouin children (0.5–5.5 years) showed 89.2% vitamin E deficiency as the serum α-tocopherol cutoff was considered <17.2 μmol/L (Khatib and Elmadfa 2009). In Vietnam, the schoolgirls of age group 7–9 years of 20% rural and 27.1% urban areas had faced vitamin E deficiency. Nearly 43% of pregnant Bangladeshi women were found vitamin E deficient (Shamim et al. 2014).

9.4.4 Vitamin K

Vitamin K is a fat-soluble vitamin that considered an essential cofactor in humans for the production of protein, which is involved in the coagulation homeostasis and calcium homeostasis. Vitamin K term derived from the Germanic word "Koagulation" means able to clot blood or prevent hemorrhage (Schwalfenberg et al. 2017). Naturally, vitamin K occurs in K1 and K2 forms. The major source of vitamin K1 is green vegetables, cabbage, spinach, kiwi, avocado, grapes, and plant chlorophylls, while vitamin K2 main source is fermented food, meat, and dairy products (Dismore et al. 2003; Tarento et al. 2019). Vitamin K essential role is reported in the synthesis of sphingolipid and protein Gas6 in the peripheral and central nervous system (Ferland 2012). Vitamin K has an essential role in the pathogenesis of Alzheimer's disease as it improves the cognitive function by regulating the sulfotransferase activity, growth factors, and tyrosine kinase receptor activity in the brain (Presse et al. 2008; Ferland 2012; Presse 2013). Intake of vitamin K also suppresses growth and invasion of human hepatocellular carcinoma via protein kinase A activation, which results in moderate suppression of tumor recurrence (Yoshida et al. 2008). Supplements of vitamin K reduce diabetes risk by 51%. Its deficiency was reported to reduce insulin release from the pancreas, as shown in model organism rat (Choi et al. 2011; Manna and Kalita 2016). Vitamin K inhibits vascular calcification by matrix GLa protein that is involved in the prevention of calcium precipitation and soft tissue calcification (El Asmar et al. 2014). Deficiency of vitamin K1 and K2 causes vascular calcification, osteoporosis, coronary heart disease, arthritis, and renal calculi (Geleijnse et al. 2004; El Asmar et al. 2014). The suggested RDA for vitamin K is 65 μg/day for men and 55 μg/day for women (Marles et al. 2017).

9.5 Water-Soluble Vitamins

Water-soluble vitamins are essential for growth, development, and regular cellular functions. These compounds are structurally and functionally unrelated. All water-soluble vitamins are essential for the human as they cannot synthesize inside the body. Their deficiency can lead to a variety of medical abnormalities like anemia, neurological disorders. Ascorbic acid (vitamin C), thiamin (vitamin B_1), riboflavin

(vitamin B_2), niacin (vitamin B_3), pyridoxine/pyridoxal/pyridoxamine (vitamin B_6), folacin/folic acid/folate (vitamin B_9), cobalamin (vitamin B_{12}), biotin (vitamin B_7 or H), and pantothenic acid (vitamin B_5) are considered as a water-soluble vitamin as they dissolve in water.

9.5.1 Vitamin B Complex

Vitamin B occurs in the multiform and is easily available, except vitamins B_9 (folate) and B_{12} (riboflavin). Vitamins B_9 and B_{12} are crucial during infancy and early childhood (Rasmussen et al. 2001). Deficiency of vitamin B_9 and B_{12} are responsible for megaloblastic anemia, stunted growth, and increased infection (Strand et al. 2009). In Nepal, the predominant food is vegetarian, and the study showed that among 1125 rural pregnant women, 33% were anemic in which 10% were due to vitamin B deficiency. In urban areas, out of 80% anemic Nepali pregnant women, 48% were reported with vitamin B complex deficient (Jiang et al. 2005).

9.5.1.1 Vitamin B_1 (Thiamin)

Thiamine or vitamin B_1 acts in the body as a coenzyme that provides support in the transfer of the active aldehyde in the carbohydrate metabolism. An active form of thiamine (vitamin B_1) is thiamine pyrophosphate (TPP). It plays an important role in several critical metabolic reactions in the body to reduce cellular oxidative stress. The deficiency of vitamin B_1 at the cellular level can lead to abnormal energy metabolism. Pregnant women are prone to thiamine deficiency. It is due to the increase in the metabolic rate in their body. Vitamin B_1 deficiency is high in both developing and developed countries. Poor dietary intake is the main cause of vitamin B_1 deficiency in developing countries. Chronic alcoholism, along with people with diseases like AIDS, cancer, and inflammatory bowel, has shown thiamine deficiency in developed countries. In adults, the heart, blood vessels, gastrointestinal tract, and nervous system are severely affected due to the deficiency of thiamine.

9.5.1.2 Vitamin B_2 (Riboflavin)

Riboflavin or vitamin B_2 acts as a coenzyme in the form of riboflavin-5-phosphate and flavin adenosine dinucleotide. Riboflavin has a significant role in many oxido-reduction reactions in the respiration chain and also in amino acid, carbohydrate, and lipid metabolism. The reported rich source of riboflavin is liver, green leafy vegetables, bread, cereals, and milk. Deficiency of riboflavin can lead to various medical abnormalities, degenerative nervous system, anemia, endocrine dysfunction, skin disorders, hyperemia (excessive blood), and cheilosis (cracked and swollen lips).

9.5.1.3 Vitamin B_3 (Niacin)

Vitamin B_3 or niacin present in nicotinic acid and nicotinamide forms. It is the precursor of the coenzyme nicotinamide adenine dinucleotide and nicotinamide

adenine dinucleotide phosphate. High doses of vitamin B_3 can be used in the treatment of hypercholesterolemia due to its lipid-lowering effect. Two types of the food source are available for niacin. One is animal-derived that includes beef, fish, birds in the highly bioavailable form, and the second is plant-based food like grains, nuts, and legumes that serves less bioavailability of vitamin B_3. The deficiency of niacin leads to pellagra. This disease is related to pigmented rashes, discoloration, and sunburn-like appearance of the skin.

9.5.1.4 Vitamin B_6 (Pyridoxine/Pyridoxal/Pyridoxamine)

Vitamin B_6 referred to as pyridoxine, pyridoxal, and pyridoxamine, while pyridoxal phosphate and pyridoxamine phosphate are its coenzyme forms. Vitamin B6-dependent enzymes are played an important role in carbohydrate, lipid, and protein metabolisms (McCormick 2006). Vitamin B_6 is absorbed in jejunum by passive diffusion in the body (Bailey et al. 2015). Vitamin B_6 status is measure by plasma pyridoxal-5-phosphate (PLP). PLP tends to present in reduced levels in alcoholic and obese people.

Vitamin B_6 deficiency is usually associated with the concentration of vitamin B_{12} (cobalamin) and vitamin B_9 (folic acid). Its supplementation with vitamin B_9 and vitamin B_{12} could help to decrease cardiovascular disease-related risk by reducing the level of homocysteine (Saposnik et al. 2009; Ebbing et al. 2010). Vitamin B_6 deficiency is associated with microcytic anemia, glossitis, electroencephalographic abnormalities, depression, confusion, dermatitis, and weakened immune function. In infants, vitamin B_6 deficiency can cause convulsive seizures, irritability, and abnormal hearing.

9.5.1.5 Vitamin B_9 (Folacin/Folic Acid/Folate)

Vitamin B_9 or folacin/folate/folic acid is required for the synthesis of thymidine, purine, methionine, and catabolism of histidine. Methylated vitamin B_{12} is needed for the activity of vitamin B_9 (Bailey et al. 2015).

Folate deficiency is rare but usually linked with other nutrient deficiencies. Folate deficiency is strongly associated with malabsorptive disorders, poor diet, and alcoholism. Folate deficiency causes soreness of throat, ulcers on tongue, alteration in pigments of hair, skin, and fingernails, and high concentrations of homocysteine in the blood. Several epidemiological studies have shown that there is an inverse relationship between folate consumption, status, and risk of esophageal, cervical, lung, ovarian, bladder, and pancreatic cancers (He and Shui 2014; Bailey et al. 2015).

9.5.1.6 Vitamin B_{12} (Cobalamin)

Vitamin B_{12} or cobalamin is present only in animal-derived foods such as meat, eggs, fish, milk. Vegetarian people often suffer from vitamin B_{12} deficiency (Allen 2008). Different forms of vitamin B_{12} contains cobalt and also known as cobalamin. The coenzyme forms of cobalamin are methyl-cobalamin and adenosylcobalamin. The two forms of vitamin B_{12} play a vital role in the metabolism of amino acids, and propionate and formation of methionine by methylate homocysteine. Vitamin B_{12}

requires red blood cell formation, DNA synthesis, and neurological functions. Vitamin B_{12} is present in a protein-bound arrangement in the food and released by the activity of gastric protease and hydrochloric acid (HCl) in the stomach.

The deficiency of vitamin B_{12} leads to growth retardation, megaloblastic anemia, and neurological disorders. Vitamin B_{12} deficiency is prevalent in developing countries, but developed countries are also facing the problem. The serum vitamin B_{12} level was found low in 56% of patients those who suffered from megaloblastic anemia in Pakistan (Modood et al. 1995; Mannan et al. 1995).

9.5.2 Vitamin C (Ascorbic Acid/Ascorbate)

Vitamin C or ascorbate/ascorbic acid (AA) exists in the form of dehydroascorbic acid (DHAA) (Li and Schellhorn 2007). The major source of vitamin C in the human body is derived from fruits and vegetables. The highest level of vitamin C is found in tomatoes, broccoli, citrus fruits, strawberries, turnip, spinach, and mustard greens. Vitamin C level decreases with cooking at a higher temperature. It acts as a cofactor for the critical metabolic reactions, which include the formation of collagen, carnitine, catecholamine, peptide amidation, and the metabolism of tyrosine. The human body does not synthesize vitamin C due to the absence of enzyme L-gluconolactone oxidase (Rautiainen et al. 2009). Intestinal absorption of vitamin C takes place in the human body. The tissue sample analysis of 29 patients having abdominal aortic aneurysms found a low level of ascorbic acid. The fortification of food items and the high supply of citrus fruits and green vegetables in the diet lead to an increase the vitamin C concentration in the human body. It was reported that vitamin C intake from commonly available vegetables/fruits along with nitrate had a durable and highly protective effect against cancer (Risch et al. 1985).

9.6 Conclusion

The global scenario of vitamin deficiency showed that various South-East and African countries are severely affected by various vitamin deficiencies. However, developed countries are mostly affected by vitamins D and E deficiency. Vitamin deficiency is prevalent in pregnant women, infants, and children below the 5-years age. Various research programs and surveys have suggested that vitamin deficiency can be managed by supplementation programs, staple crops biofortification, and nutrition policy. However, vitamin toxicity governed by the high dose of supplementation should also be considered that can adversely affect human health. The funding bodies and policy-makers should work together with the scientific community to ultimately eliminate vitamin deficiency worldwide and improve the healthy life of the large population.

Acknowledgements The authors express their gratitude to the Biotechnology Industry Research Assistance Council (BIRAC) for a banana biofortification project grant and to the Department of Biotechnology (DBT), Government of India for the grant (BT/PR25789/GET/119/97/2017) under the scheme of Genome Engineering Technology and Their Applications. The authors would like to acknowledge National Agri-Food Biotechnology Institute (NABI) for research facility, and the DBT-eLibrary Consortium (Del-CON) for providing access to online journals. RC is thankful to Regional Center for Biotechnology (RCB) and SC is thankful to Panjab University, Chandigarh for Ph.D. registration.

References

Adams J, Audhya T, Geis E, Gehn E, Fimbres V, Pollard E, Quig D (2018) Comprehensive nutritional and dietary intervention for autism spectrum disorder—a randomized, controlled 12-month trial. Nutrients 10(3):369. https://doi.org/10.3390/nu10030369

Aguiar M, Andronis L, Pallan M, Högler W, Frew E (2019) The economic case for prevention of population vitamin D deficiency: a modelling study using data from England and Wales. Eur J Clin Nutr:1–9. https://doi.org/10.1038/s41430-019-0486-x

Akhtar S, Ahmed A, Randhawa MA, Atukorala S, Arlappa N, Ismail T, Ali Z (2013) Prevalence of vitamin a deficiency in South Asia: causes, outcomes, and possible remedies. J Health Popul Nutr 31(4):413

Albahrani AA, Greaves RF (2016) Fat-soluble vitamins: clinical indications and current challenges for chromatographic measurement. Clin Biochem Rev 37(1):27–47

Allen LH (2008) Causes of vitamin B12 and folate deficiency. Food Nutr Bull 29(Suppl. 1):S20–S34. https://doi.org/10.1177/15648265080292S105

Ardawi MSM, Sibiany AM, Bakhsh TM, Qari MH, Maimani AA (2012) High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. Osteoporos Int 23(2):675–686

Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF III, Mills JL, Pfeiffer CM, Fazili Z, Zhang M, Ueland PM, Molloy AM (2015) Biomarkers of nutrition for development—folate review. J Nutr 145(7):1636S–1680S

Bates CJ (1995) Vitamin A. Lancet 345(8941):31–35

Beard JA, Bearden A, Striker R (2011) Vitamin D and the anti-viral state. J Clin Virol 50:194–200

Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, Haider BA, Kirkwood B, Morris SS, Sachdev HPS, Shekar M (2008) What works? Interventions for maternal and child undernutrition and survival. Lancet 371:417–440

Black MM (2008) Effects of vitamin B12 and folate deficiency on brain development in children. Food Nutr Bull 29:S126–S131. https://doi.org/10.1177/15648265080292S117

Blomhoff RUNE, Green MH, Green JB, Berg TROND, Norum KR (1991) Vitamin A metabolism: new perspectives on absorption, transport, and storage. Physiol Rev 71(4):951–990

Cannell JJ (2017) Vitamin D and autism, what's new? Rev Endocr Metab Disord 18(2):183–193. https://doi.org/10.1007/s11154-017-9409-0

Cashman KD, Dowling KG, Skrabakova Z, Kiely M, Lamberg-Allardt C, Durazo-Arvizu RA, Sempos CT, Koskinen S, Lundqvist A, Sundvall J, Linneberg A (2015) Standardizing serum 25-hydroxyvitamin D data from four Nordic population samples using the vitamin D standardization program protocols: shedding new light on vitamin D status in Nordic individuals. Scand J Clin Lab Invest 75(7):549–561

Castro K, Klein LDS, Baronio D, Gottfried C, Riesgo R, Perry IS (2016) Folic acid and autism: what do we know? Nutr Neurosci 19(7):310–317. https://doi.org/10.1179/1476830514y.0000000142

Chen W, Chen G (2014) The roles of vitamin A in the regulation of carbohydrate, lipid, and protein metabolism. J Clin Med 3:453–479

Choi HJ, Yu J, Choi H, An JH, Kim SW, Park KS, Jang HC, Kim SY, Shin CS (2011) Vitamin K2 supplementation improves insulin sensitivity via osteocalcin metabolism: a placebo-controlled trial. Diabetes Care 34(9):e147

Clarke MW, Burnett JR, Croft KD (2008) Vitamin E in human health and disease. Crit Rev Clin Lab Sci 45(5):417–450

Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, Atkinson S, Ward L, Moher D, Hanley D, Fang M (2007) Effectiveness and safety of vitamin D in relation to bone health. Evid Rep Technol Assess 158:235–225

D'Ambrosio DN, Clugston RD, Blaner WS (2011) Vitamin A metabolism: an update. Nutrients 3 (1):63–103

Dismore ML, Haytowitz DB, Gebhardt SE, Peterson JW, Booth SL (2003) Vitamin K content of nuts and fruits in the US diet. J Am Diet Assoc 103(12):1650–1652

Dror DK, Lindsay HA (2011) Vitamin E deficiency in developing countries. Food Nutr Bull 32 (2):124–143

Durazo-Arvizu RA, Camacho P, Bovet P, Forrester T, Lambert EV, Plange-Rhule J, Hoofnagle AN, Aloia J, Tayo B, Dugas LR, Cooper RS (2014) 25-Hydroxyvitamin D in African-origin populations at varying latitudes challenges the construct of a physiologic norm. Am J Clin Nutr 100(3):908–914

Ebbing M, Bønaa KH, Arnesen E, Ueland PM, Nordrehaug JE, Rasmussen K, Njølstad I, Nilsen DW, Refsum H, Tverdal A, Vollset SE (2010) Combined analyses and extended follow-up of two randomized controlled homocysteine-lowering B-vitamin trials. J Intern Med 268 (4):367–382

El Asmar MS, Naoum JJ, Arbid EJ (2014) Vitamin K dependent proteins and the role of vitamin K2 in the modulation of vascular calcification: a review. Oman Med J 29(3):172

El-Ansary A, Cannell JJ, Bjørklund G, Bhat RS, Al Dbass AM, Alfawaz HA, Chirumbolo S, Al-Ayadhi L (2018) In the search for reliable biomarkers for the early diagnosis of autism spectrum disorder: the role of vitamin D. Metab Brain Dis 33(3):917–931

Faber M, Wenhold F (2007) Nutrition in contemporary South Africa. Water SA 33(3)

Faber M, Van Jaarsveld PJ, Kunneke E, Kruger HS, Schoeman SE, Van Stuijvenberg ME (2015) Vitamin A and anthropometric status of South African preschool children from four areas with known distinct eating patterns. Nutrition 31(1):64–71

Faber M, Wenhold F (2018) Nutrition in contemporary South Africa. Water SA 33(3):393. https://doi.org/10.4314/wsa.v33i3.180609

FAO (2017) Vitamin and mineral requirements in human nutrition, 2nd edn. FAO, Geneva. Annex 2. 2004

Ferland G (2012) Vitamin K, an emerging nutrient in brain function. Biofactors 38(2):151–157. https://doi.org/10.1002/biof.1004

Forrest KYZ, Stuhldreher WL (2010) Prevalence and correlates of vitamin D deficiency in US adults. Nutr Res 31(1):48–54. https://doi.org/10.1016/j.nutres..12.001

Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, Van Der Meer IM, Hofman A, Witteman JC (2004) Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. J Nutr 134(11):3100–3105

Goswami R, Kochupillai N, Gupta N, Goswami D, Singh N, Dudha A (2008) Presence of 25 (OH) D deficiency in a rural North Indian village despite abundant sunshine. J Assoc Physicians India 56:755–757

He H, Shui B (2014) Folate intake and risk of bladder cancer: a meta-analysis of epidemiological studies. Int J Food Sci Nutr 65:286–292

Hendren RL, James SJ, Widjaja F, Lawton B, Rosenblatt A, Bent S (2016) Randomized, placebo-controlled trial of methyl B12 for children with autism. J Child Adolesc Psychopharmacol 26 (9):774–783

Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, Pierroz DD, Weber P, Hoffmann K (2014) A systematic review of vitamin D status in populations worldwide. Br J Nutr 111(1):23–45

Holick MF (2005) The vitamin D epidemic and its health consequences. J Nutr 135(11):2739S–2748S. https://doi.org/10.1093/jn/135.11.2739s

Holick MF (2006) Resurrection of vitamin D deficiency and rickets. J Clin Invest 116:2062–2072

Honzik T, Adamovicova M, Smolka V, Magner M, Hruba E, Zeman J (2010) Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B12 deficiency–what have we learned? Eur J Paediatr Neurol 14(6):488–495

Imdad A, Mayo-Wilson E, Herzer K, Bhutta ZA (2017) Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. Cochrane Database Syst Rev (3):CD008524

Jiang T, Christian P, Khatry SK, Wu L, West KP Jr (2005) Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. J Nutr 135(5):1106–1112

Kaur N, Shivani, Pandey A, Tiwari S (2016) Provitamin A enrichment for tackling malnutrition. In: Mohandas S, Ravishankar KV (eds) Banana: genomics and transgenic approaches for genetic improvement. Springer-Verlag Ltd, Singapore, pp 277–299

Khadangi F, Azzi A (2019) Vitamin E-the next 100 years. IUBMB Life 71(4):411–415. https://doi.org/10.1002/iub.1990

Khatib IMD, Elmadfa I (2009) Poor nutritional health of Bedouin preschool children in Jordan: the irony of urbanization. Ann Nutr Metab 54:301–309

Khillan JS (2014) Vitamin A/retinol and maintenance of pluripotency of stem cells. Nutrients 6:1209–1222

Kilmarx PH (2009) Global epidemiology of HIV. Curr Opin HIV AIDS 4:240–246

Kline K, Lawson KA, Yu W, Sanders BG (2003) Vitamin E and breast cancer prevention: current status and future potential. J Mammary Gland Biol Neoplasia 8:91–102

Kocovska E, Fernell E, Billstedt E, Minnis H, Gillberg C (2012) Vitamin D and autism: clinical review. Res Dev Disabil 33(5):1541–1550

Kocovska E, Gaughran F, Krivoy A, Meier UC (2017) Vitamin-D deficiency as a potential environmental risk factor in multiple sclerosis, schizophrenia, and autism. Front Psych 8:47. https://doi.org/10.3389/fpsyt.2017.00047

Kuwabara A, Himeno M, Tsugawa N, Kamao M, Fujii M, Kawai N, Fukuda M, Ogawa Y, Kido S, Okano T, Tanaka K (2010) Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly. Asia Pac J Clin Nutr 19(1):49

Li Y, Schellhorn HE (2007) New developments and novel therapeutic perspectives for vitamin C. J Nutr 137:2171–2184

MacFabe DF, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, Boon F, Taylor AR, Kavaliers M, Ossenkopp KP (2007) Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behav Brain Res 176(1):149–169

MacFarlane GD, Sackrison JL Jr, Body JJ, Ersfeld DL, Fenske JS, Miller AB (2004) Hypovitaminosis D in a normal, apparently healthy urban European population. J Steroid Biochem Mol Biol 89:621–622

Maiya S, Sullivan I, Allgrove J, Yates R, Malone M, Brain C, Archer N, Mok Q, Daubeney P, Tulloh R, Burch M (2008) Hypocalcaemia and vitamin D deficiency: an important, but preventable, cause of life-threatening infant heart failure. Heart 94(5):581–584

Manna P, Kalita J (2016) Beneficial role of vitamin K supplementation on insulin sensitivity, glucose metabolism, and the reduced risk of type 2 diabetes: a review. Nutrition 32 (7–8):732–739. https://doi.org/10.1016/j.nut.2016.01.011

Mannan M, Anwar M, Saleem M, Wiqar A, Ahmad M (1995) A study of serum vitamin B12 and folate levels in patients of megaloblastic anaemia in Northern Pakistan. J Pak Med Assoc 45:187–188

Mansoor S, Ahmad A (2016) Progressive ataxia due to alphatocopherol deficiency in Pakistan. Iran J Neurol 15(2):103–105

Marles RJ, Roe AL, Oketch-Rabah HA (2017) US pharmacopeial convention safety evaluation of menaquinone-7, a form of vitamin K. Nutr Rev 75(7):553–578
Masri OA, Chalhoub JM, Sharara AI (2015) Role of vitamins in gastrointestinal diseases. World J Gastroenterol 21(17):5191
McBurney MI, Elaine AY, Ciappio ED, Bird JK, Eggersdorfer M, Mehta S (2015) Suboptimal serum α-tocopherol concentrations observed among younger adults and those depending exclusively upon food sources, NHANES 2003-20061-3. PloS One 10(8):e0135510
McCormick D (2006) Vitamin B6. In: Bowman B, Russell R (eds) Present knowledge in nutrition, 9th edn. International Life Sciences Institute, Washington, DC
McGillivray G, Skull SA, Davie G, Kofoed SE, Frydenberg A, Rice J, Cooke R, Carapetis JR (2007) High prevalence of asymptomatic vitamin D and iron deficiency in East African immigrant children and adolescents living in a temperate climate. Arch Dis Child 92 (12):1088–1093
Modood-ul-Mannan, Anwar M, Saleem M, Wiquar A, Ahmad M (1995) A study of serum vitamin B12 and folate levels in patients of megaloblastic anaemia in northern Pakistan. J Pak Med Assoc 45:187–188
Morales J (2010) Vitamin D status in Latin America. International Osteoporosis Foundation, Washington, DC, pp 1–4
Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Mäkitie O, Ramos-Abad L (2016) Global consensus recommendations on prevention and management of nutritional rickets. Horm Res Paediatr 85(2):83–106
Obrenovich ME, Shola D, Schroedel K, Agrahari A, Lonsdale D (2015) The role of trace elements, thiamin (e) in autism and autistic spectrum disorder. Front Biosci 7:263–277
Packer L (1991) Protective role of vitamin E in biological systems. Am J Clin Nutr 53 (Suppl):1050S–1055S
Peter S, Moser U, Pilz S, Eggersdorfer M, Weber P (2013) The challenge of setting appropriate intake recommendations for vitamin E: considerations on status and functionality to define nutrient requirements. Int J Vitam Nutr Res 83(2):129–136. https://doi.org/10.1024/0300-9831/a000153
Pierpaoli E, Viola V, Pilolli F, Piroddi M, Galli F, Provinciali M (2010) γ-and δ-tocotrienols exert a more potent anticancer effect than α-tocopheryl succinate on breast cancer cell lines irrespective of HER-2/neu expression. Life Sci 86:668–675
Pillai DK, Iqbal SF, Benton AS, Lerner J, Wiles A, Foerster M, Ozedirne T, Holbrook HP, Payne PW, Gordish-Dressman H, Teach SJ (2011) Associations between genetic variants in vitamin D metabolism and asthma characteristics in young African Americans: a pilot study. J Investig Med 59(6):938–946
Presse N (2013) Vitamin K status and cognitive function in healthy older adults. Neurobiol Aging 34(12):2777–2783. https://doi.org/10.1016/j.neurobiolaging.2013.05.031
Presse N, Shatenstein B, Kergoat MJ, Ferland G (2008) Low vitamin K intakes in community-dwelling elders at an early stage of Alzheimer's disease. J Am Diet Assoc 108(12):2095–2099
Rasmussen SA, Fernhoff PM, Scanlon KS (2001) Vitamin B12 deficiency in children and adolescents. J Pediatr 138(1):10–17
Rautiainen S, Lindblad BE, Morgenstern R, Wolk A (2009) Vitamin C supplements and the risk of age-related cataract: a population-based prospective cohort study in women. Am J Clin Nutr 91 (2):487–493
Ravindran RD (2011) Prevalence and risk factors for vitamin C deficiency in north and South India: a two Centre population based study in people aged 60 years and over. PLoS One 6(12):e28588. https://doi.org/10.1371/journal.pone.0028588
Risch HA, Jain M, Choi NW, Fodor JG, Pfeiffer CJ, Howe GR, Harrison LW, Craib KJ, Miller AB (1985) Dietary factors and the incidence of cancer of the stomach. Am J Epidemiol 122 (6):947–959
Ross AC, Ternus ME (1993) Vitamin a as a hormone: recent advances in understanding the actions of retinol, retinoic acid, and beta carotene. J Am Diet Assoc 93(1285–90):1291–1292

Saghazadeh A, Mahmoudi M, Ashkezari AD, Rezaie NO, Rezaei N (2017) Systematic review and meta-analysis shows a specific micronutrient profile in people with down syndrome: lower blood calcium, selenium and zinc, higher red blood cell copper and zinc, and higher salivary calcium and sodium. PloS One 12(4):e0175437. https://doi.org/10.1371/journal.pone.0175437

Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E, HOPE 2 Investigators (2009) Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. Stroke 40(4):1365–1372

Savvidou S (2014) Pellagra: a non-eradicated old disease. Clin Pract 4:637

Schweiggert RM, Kopec RE, Villalobos-Gutierrez MG, Högel J, Quesada S, Esquivel P, Schwartz SJ, Carle R (2014) Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. Br J Nutr 111:490–498. https://doi.org/10.1017/S0007114513002596

Schwalfenberg GK (2017) Vitamins K1 and K2: the emerging group of vitamins required for human health. J Nutr Metab:1–7

Setiati S (2008) Vitamin D status among Indonesian elderly women living in institutionalized care units. Acta Med Indones 40:78–83

Shamim AA, Kabir A, Merrill RD, Ali H, Rashid M, Schulze K, Labrique A, West KP, Christian P (2013) Plasma zinc, vitamin B 12 and α-tocopherol are positively and plasma γ-tocopherol is negatively associated with Hb concentration in early pregnancy in north-west Bangladesh. Public Health Nutr 16(8):1354–1361

Shamim AA, Schulze K, Merrill RD, Kabir A, Christian P, Shaikh S, Wu L, Ali H, Labrique AB, Mehra S, Klemm RD (2014) First-trimester plasma tocopherols are associated with risk of miscarriage in rural Bangladesh. Am J Clin Nutr 101(2):294–301

Sommer A, West KP (1996) Vitamin A deficiency: health, survival and vision. Am J Epidemiol 147 (12):1175–1176

Spiro A, Buttriss JL (2014) Vitamin D: an overview of vitamin D status and intake in Europe. Nutr Bull 39:322–350. https://doi.org/10.1111/nbu.12108

Stabler SP, Allen RH (2004) Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 24:299–326

Strand TA, Taneja S, Bhandari N, Refsum H, Ueland PM, Gjessing HK, Bahl R, Schneede J, Bhan MK, Sommerfelt H (2007) Folate, but not vitamin B-12 status, predicts respiratory morbidity in north Indian children. Am J Clin Nutr 86(1):139–144

Strand MA, Perry J, Zhao J, Fischer PR, Yang J, Li S (2009) Severe vitamin D-deficiency and the health of North China children. Matern Child Health J 13(1):144–150

Tarento TD, McClure DD, Talbot AM, Regtop HL, Biffin JR, Valtchev P, Dehghani F, Kavanagh JM (2019) A potential biotechnological process for the sustainable production of vitamin K1. Crit Rev Biotechnol 69:1–19

Tassone F, Gianotti L, Baffoni C, Visconti G, Pellegrino M, Cassibba S, Croce CG, Magro G, Cesario F, Attanasio R, Borretta G (2013) Vitamin D status in primary hyperparathyroidism: a Southern European perspective. Clin Endocrinol 79:784–790

Torti J (2012) Floods in Southeast Asia: a health priority. J Glob Health 2(2):020304

Traber MG, Stevens JF (2011) Vitamins C and E: beneficial effects from a mechanistic perspective. Free Radic Biol Med 51:1000–1013

Uday S, Fratzl-Zelman N, Roschger P, Klaushofer K, Chikermane A, Saraff V, Tulchinsky T, Thacher TD, Marton T, Högler W (2018) Cardiac, bone and growth plate manifestations in hypocalcemic infants: revealing the hidden body of the vitamin D deficiency iceberg. BMC Pediatr 18(1):183

Underwood BA (2000) Dietary approaches to the control of vitamin A deficiency: an introduction and overview. Food Nutr Bull 21(2):117–123

Valtuena J, Gonzalez-Gross M, Huybrechts I, Breidenassel C, Ferrari M, Mouratidou T, Gottrand F, Dallongeville J, Azzini E, Sioen I, Gomez-martinez S (2013) Factors associated with vitamin D deficiency in European adolescents: the HELENA study. J Nutr Sci Vitaminol 59(3):161–171

Wahl DA, Cooper C, Ebeling PR, Eggersdorfer M, Hilger J, Hoffmann K, Josse R, Kanis JA, Mithal A, Pierroz DD, Stenmark J (2012) A global representation of vitamin D status in healthy populations. Arch Osteoporos 7(1–2):155–172

Welsh J (2004) Vitamin D and breast cancer: insights from animal models. Am J Clin Nutr 80 (Suppl):1721S–1724S

Wenhold F, Faber M (2008) Nutritional status of South Africans: links to agriculture and water. Water research commission, Pretoria

World Health Organization (2004) Vitamin and mineral requirements in human nutrition. World Health Organization, Geneva, pp 108–129

Yahia EM, García-Solís P, Celis MEM (2019) Contribution of fruits and vegetables to human nutrition and health. In: In Postharvest physiology and biochemistry of fruits and vegetables. Woodhead Publishing, pp 19–45

Yoshida M, Jacques PF, Meigs JB, Saltzman E, Shea MK, Gundberg C, Dawson-Hughes B, Dallal G, Booth SL (2008) Effect of vitamin K supplementation on insulin resistance in older men and women. Diabetes Care 31:2092–2096. https://doi.org/10.2337/dc08-1204

Plant Polyphenols and Gut Bacteria: Role in Obesity-Induced Metabolic Endotoxaemia and Inflammation

10

Ruchika Maurya, Mahendra Bishnoi, and Kanthi Kiran Kondepudi

Abstract

Plant polyphenols (PPs) play an important role in human nutrition due to their antioxidant capacity and the ability to reduce reactive oxygen species (ROS). PPs have been shown to alleviate diseases like type-2 diabetes, obesity, and cardiovascular diseases. Obesity is a disorder that arises due to a sedentary lifestyle besides genetic factors. Its prevalence is rapidly increasing over the past three decades all over the world, including India. Many preclinical studies suggested that plant phenolics could alleviate diet-induced obesity. The usage of PPs is gaining importance, and it is the subject of intensive research due to the ability of gut microbes in metabolizing these compounds. Among PPs, phenolic acids account for one-third of the total intake and flavonoids for the remaining two-thirds of the total intake. In this chapter, the impact of plant-derived PPs is emphasized based on its role in the prevention of obesity and associated low-grade inflammation through their gut modulatory microbial effects is highlighted.

Keywords

Cardiovascular disease · Obesity · Diabetes · High fat diet · Polyphenols · Catechin

R. Maurya · M. Bishnoi · K. K. Kondepudi (✉)
Healthy Gut Research Group, Food and Nutrition Biotechnology Division, National Agri-Food Biotechnology Institute, Mohali, Punjab, India
e-mail: kiran@nabi.res.in

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_10

10.1 Introduction

Obesity is becoming an alarming health complication in the world, which needs to be eradicated. Obesity is defined as an excess accumulation of fat that impairs health. The prevalence of overweight and obesity has steadily increased in the past three decades in the USA and worldwide and, therefore, an ultimatum for the health of society. America and Europe have the highest prevalence of obesity and overweight. It has been noted that more than 135 million individuals are affected by obesity in India (Ahirwar and Mondal 2019). According to WHO, BMI more than 30 kg per square meter with protracting positive energy balance is associated with chronic metabolic diseases like type-2 diabetes, heart diseases, hypertension, and several types of cancers. The inflammation that is associated with obesity causes insulin resistance and, ultimately, to type-2-diabetes. It can be prevented by negative energy balance through increased energy expenditure (Ferguson 2009). As anti-obesity therapeutics have certain kinds of side effects, phytonutrients and foods that are rich in bioactive compounds like polyphenols (PPs) and omega-3 fatty acids are gaining tremendous interest because of their ability to reduce inflammation (Tresserra-rimbau et al. 2018). PPs are plant secondary metabolites, which are predominantly present in fruits, green tea, cereals, seeds, cocoa, coffee, and certain vegetables (Ozdal et al. 2016). PPs are produced by metabolic pathways such as shikimate/phenyl propanoic of polyketide pathway in plants, which is mainly responsible for protection, antimicrobial, and antioxidant activities (Ashley et al. 2019). These micronutrients have antioxidant properties and are found to increase energy expenditure by enhancing the metabolic rate of the body. It has been proven through studies that PPs could prevent diabetes, cardiovascular diseases, etc. (Priya and Rathinavel 2017).

They have the lipid-lowering capacity, antioxidant properties (Fig. 10.1) that are helpful in alleviating metabolic syndrome (Alissa and Ferns 2012). At thermal

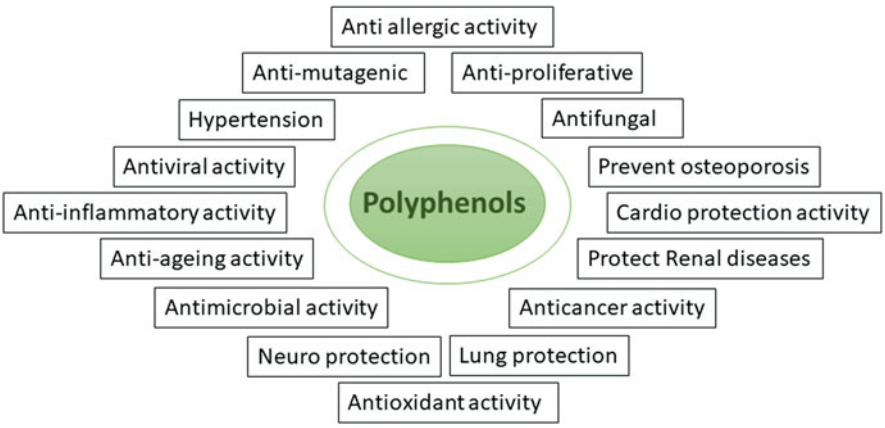

Fig. 10.1 Functional properties of plant polyphenols

processing conditions, many bioactive get inactivated, thereby reproducing the nutritional value due to the modification of PPs and poor bioavailability (Cardona et al. 2013). Hence, appropriate measures need to be taken in order to enhance bioavailability of PPs while processing.

10.2 Classification

PPs represent a wide variety of over 8000 compounds, and among them 4000 flavonoids are identified. Plant-derived food contains diversified PPs having a wide range of complex structures. Their chemical structure comprises one or more aromatic rings attached to one or more hydroxyl groups (Brglez Mojzer et al. 2016). These are also found as conjugates with sugars or organic acids or as polymers (flavonoids) instead of unconjugated aglycones (Sripad et al. 1982). PPs are classified into flavonoids and non-flavonoids or subdivided into many subclasses depending on their phenolic units within their molecular structure, substituent group, or linkage-type between phenol units. PPs are classified from basic monomer phenolic acids to a composite structure like ellagitannins and lignin (Fig. 10.2). PPs belong to a well-known group of phenol, which is characterized by two phenyl rings and one or more hydroxyl substituents (Singla et al. 2019).

In plants, PPs are widely distributed as glycosides, whereas the elementary structure is aglycones. Among the non-flavonoid group, the phenolic acids comprise hydroxybenzoic (vanillic, protocatechuic acid, gallic and syringic acid) and hydroxycinnamic acids (p-coumaric, caffeic, sinapic, ferulic, sinapic, and chlorogenic acid). These two classes are derived from a non-phenolic molecule of benzoic and cinnamic acid, respectively, which is synthesized from shikimate pathway having L-phenylalanine and L-tyrosine as the precursor molecule (Vuolo

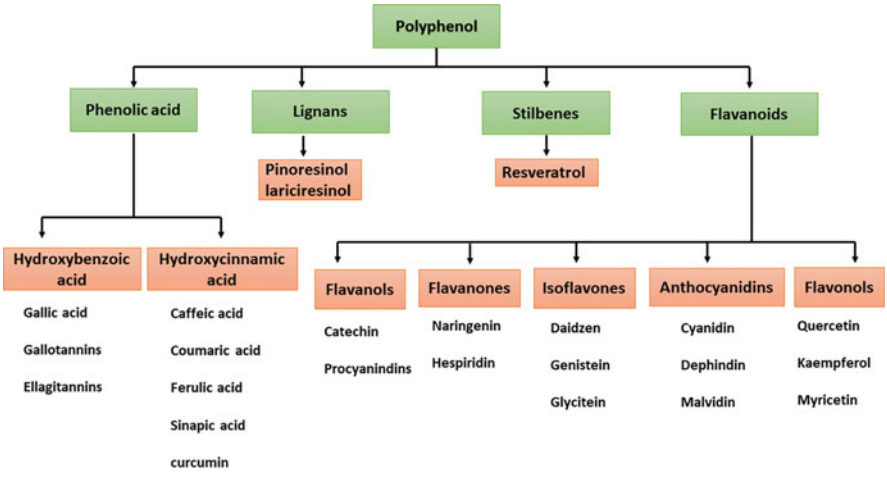

Fig. 10.2 Classification of polyphenols (Singla et al. 2019)

et al. 2019). The existence of hydroxybenzoic acids in the human diet is rare, whereas hydroxycinnamic acids are present in a substantial amount in the diet such as fruit, vegetables, etc. (Cheynier 2005). The average consumption of hydroxycinnamic acid, a major phenolic compound in coffee in an average person, is 211 mg/day (Vuolo et al. 2019). The polyphenols are further classified as lignans like pinoresinol and lariciresinol. Lignans are non-flavonoid diphenolic compound which is formed by two phenylpropanoid units (C6-C3-C3-C6). Although it is present at low concentrations in cereals, fruits, nuts, vegetables, etc., they are most abundant in flaxseed (Cooper and Nicola 2014). Stilbenes have C6-C2-C6 skeleton and represent non-flavonoids in grape and wine. One such example is trans-resveratrol, which is the most abundant polyphenol found in red wine and grapes (Pandey and Rizvi 2009).

Phenolic acids exist in the plant as free, soluble conjugate, and insoluble bound forms. Flavonoids constitute an enormous group of phenolic compounds. They are characterized by low molecular weight 15 carbon skeletons. The origin of flavonoid is from malonate/acetate pathway and stored in a plant as glycosides (bound to sugar), which is stable than free form (Vuolo et al. 2019). The feature which is shared by almost all flavonoids is the existence of diphenyl propane (C6-C3-C6), which is a closed pyran. The distinction among flavonoid arises because of the variation in the central pyran ring due to hydroxylation and oxidation pattern (Abbas et al. 2016). The conjugated chromophore present in flavonoids is accountable for red and yellow color development. An example is anthocyanidins, such as cyanidin, which demonstrate magenta and red color (Khoo 2017). Flavonoid subgroups are ubiquitous throughout the plant kingdom and comprise Flavones, Flavonols, Flavanones, and Flavanonols. Flavanols contain monomeric forms (catechin and epicatechin) as well as polymeric form (tannic acid), which are found in food as aglycones only. The monomeric catechin has the trans configuration, while epicatechin has cis configuration, whereas polymeric form tannin has high water solubility along with the high molecular weight. Tannin is also subdivided into hydrolyzable tannin and condensed tannin. Condensed tannins are polymer of catechin and epicatechin, whereas hydrolyzable tannin is the ester of gallic and ellagic acids (Tsimogiannis and Oreopoulou 2019).

10.3 Biological Functions of Polyphenols

Phenolic acids have been linked to lower the risk of chronic diseases, including cancer, cardiovascular diseases, etc. It has been reported that many diseases are interconnected with oxidative stress due to excess reactive oxygen or other nitrogen species. Dietary PPs eradicate the oxidative stress by neutralizing the free radicals through the donation of electron and as direct radical scavengers of the lipid peroxidation chain reactions (Caro-Gómez et al. 2019; Murtaza et al. 2014). Flavonoids and phenolic acids are predominantly involved in controlling weight gain and obesity in which catechins, quercetin, anthocyanins, and procyanidins have shown to play a profound role. Chiefly flavanones do not take part in weight loss or

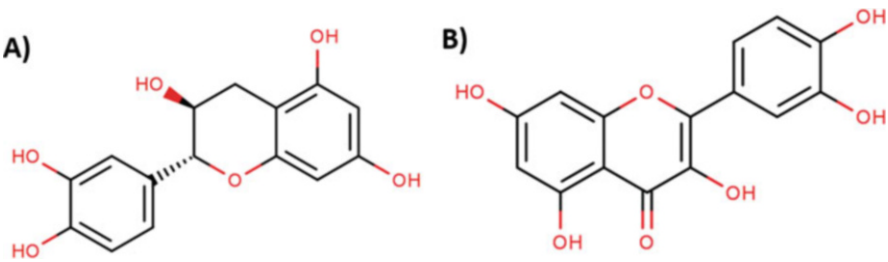

Fig. 10.3 Structure of (**a**) Catechin and (**b**) Quercetin showing double bonds of the benzene rings and oxo functional group

obesity (Lin et al. 2019). Some polyphenols like curcumin, flavonols, and ellagic acids are involved in reducing obesity. Catechins are predominantly found in green tea, which is more economical for weight loss in the Asian population (Farhat et al. 2017). Nevertheless, the chief hurdle with PPs is their bioavailability as they reach the target cell in very little concentration because it is in glycosylated form instead of aglycones and some glucosides, which are mainly absorbed in the small intestine and rest in colon (Alara et al. 2017). The efficiency of PPs absorbed in colon is 15–20%. Being hydrophilic and phenolic nature, it can be extracted by solvents like methanol, ethanol, acetonitrile, and acetone. It has been found that methanol is a more efficient solvent for the extraction of lower molecular weight PPs and aqueous acetone for a higher one. Ambient temperature for conventional extraction is 20–50 °C (Brglez Mojzer et al. 2016). Quercetin is the main flavonol that is abundant in fruits, vegetables as well as in beverages, while catechins are the main flavanols present in tea. Proanthocyanidins are flavanols which are polymeric and are present as complex mixtures of polymers with an average degree of polymerization between 4 and 11 (Scalbert and Williamson 2000). Out of the dietary phenols, quercetin and Catechin have higher antioxidant activity due to the presence of the double bonds of the benzene ring along with the Oxo functional group (Fig. 10.3) (Lin et al. 2016).

10.3.1 Alleviation of Oxidative Stress

Metabolism of molecular oxygen endogenously produces reactive oxygen species (ROS). The production of ROS inside the cells includes hydrogen peroxide (H_2O_2), singlet oxygen, free radicals such as the hydroxyl radical (OH^-), and superoxide free radical ($O_2^{\cdot -}$). Hydroxyl radical is the key player in the oxidative damage that is highly unstable and reacts non-specifically with most biological molecules. These radicals are virulent to the cells as they liberate iron molecule from Fe-containing proteins, i.e., ferroproteins. Majorly reactive electrophiles such as ROS can damage the cells by oxidation, which leads to lipid peroxidation and oxidation of DNA or proteins, which leads to mutation and then cancer possible (Luna-Vital et al. 2017). ROS production is normal up to some extent to maintain cell homeostasis and signal transduction, but overproduction of ROS causes irreversible damage to the cells

leading to cell death by the apoptotic and necrotic mechanism. The upregulated ROS leads to the initiation and progression of the inflammatory response (Chelombitko 2018). Inflammation leads to the activation of nuclear factor kappa B (NFκB) and tumor necrosis factor alpha (TNF-α) production. In obesity and diabetic condition, there is an overproduction of ROS and hence oxidative stress that leads to production of TNF-α and interleukin-6 (IL-6) and certain inflammatory cytokines like vascular cell adhesion molecule (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and NFκB leading to neuron degeneracy (Khan et al. 2019).

Phenolics act as antioxidants because of their hydrophobic benzenoid rings and the hydrogen-bonding potential of the phenolic OH groups (Ruth et al. 2017). Phenolic compounds and flavonoids act as free radical scavengers and interact with the overproduction pathway and terminate the chain reaction because of their lower redox potential that chelate metal ions release during ROS formation. PPs react with non-polar compounds present in the hydrophobic inner layer in the plasma membrane resulting in changes that may affect the oxidation rate of lipid/proteins. Interaction of PP with nitric oxide synthases (NOS) governs the production of Nitric Oxide. Some PPs like quercetin, silibinin, etc. inhibit the free radical formation by interacting with Xanthine oxidase (XO), which generates ROS (Cardona et al. 2013). Obesity is prevented by dietary polyphenols by lowering the food intake, decreasing lipogenesis, promoting lipolysis and fatty acid β-oxidation, inhibiting the adipocyte differentiation, and attenuating the inflammatory responses and oxidative stress. Green tea catechins (GTC), especially epigallocatechin gallate (EGCG), lowers food intake by overproduction and synthesis of cholecystokinin with hunger suppressing effects (González-abuín et al. 2018). GTC, resveratrol, and curcumin decrease fat accumulation in adipocytes by activating AMPK and downregulate the lipogenic gene expression (Guo et al. 2017). The pre-adipocytes undergo apoptosis and cell cycle arrest upon intervention with flavonoids (Khalilpourfarshbafi et al. 2019). They also help in arresting the G0/G1 and G2/M cell cycle and apoptosis of adipocytes, which are shown in the increase of DNA fragmentation and caspase-3 activation. PPs suppress the inflammatory responses by inhibiting the TNF and Matrix Metalloproteins (MMPs). Further PPs also help in decreasing oxidative stress and increasing antioxidant capacity in adipose tissue (Wang et al. 2014). Dietary PPs also bind to electrophile responsive elements on the promoter sites and enhances the expression of genes of the endogenous antioxidant enzyme (Shi et al. 2019). They make epigenetic changes by affecting DNA methylation and several histone modifications, thereby regulating cell cycle progression, gene expression (Fang et al. 2007). Treatment of polyphenols like genistein, quercetin, and resveratrol in primary human adipocytes and 3T3-L1 murine adipocyte shows higher inhibition of adipogenesis than an individual molecule.

10.3.2 Modulating Gene Expression Through miRNA

The microRNAs (miRNAs) are short non-coding RNAs having a length of 20–24 nucleotides that control gene expression programs. They play a regulatory role in the

biological process along with the modulation of cellular gene expression at the transcriptional and post-translational level repressing protein-coding genes translation (Heneghan et al. 2010). There are 8273 mature miRNA sequences till to date exist. They are transcribed from DNA by RNA polymerase II in the nucleus and form prior-miRNAs, which are cleaved by RNase III Drosha in the nucleus. Then it gives rise to the precursor of the mature miRNAs (pre-miRNAs) with 70–100 nucleotides (Bladé et al. 2013). The pre-miRNAs formed are then imported into the cytoplasm by exportin-5, which are further cleaved by RNase III Dicer to generate miRNAs. It then associates with the Argonaute family of proteins and incorporated in the RNA-induced silencing complex (RISC) that binds to the 3'UTR of the target mRNA. miRNA regulates more than 60% of all human genes. Over 2000, various types of miRNA have been described in humans. They regulate different types of cellular processes such as proliferation, growth factors, and apoptosis and are also associated with inflammation, impaired adipogenesis, insulin signaling, apoptosis, and oxidative stress (Brglez Mojzer et al. 2016). Inflammation associated with cardiovascular diseases (CVD), type-2 diabetes (T2D), obesity, etc. are caused by the dysregulation of the miRNAs, which may provide early biomarkers for the clinical diagnosis (Milenkovic et al. 2013). The miRNA regulates the production and secretion of insulin and also influence the resistance by the target tissue (Heneghan et al. 2010). Blood glucose homeostasis occurs through a beta-cell function regulated by pancreatic islet-specific miR-375. Moreover, miRNA-124a and let-7b are abundantly expressed in islet cells of the pancreas, which regulate blood glucose by repressing myotrophin mRNA, which encodes a protein that regulates cell membrane fusion (Fig. 10.4). Some novel biomarkers reported that are associated with obesity are miR-142-3p, miR-140-5p, miR-15a, miR-520c-3p, and miR-423-5p (Ortega et al. 2013).

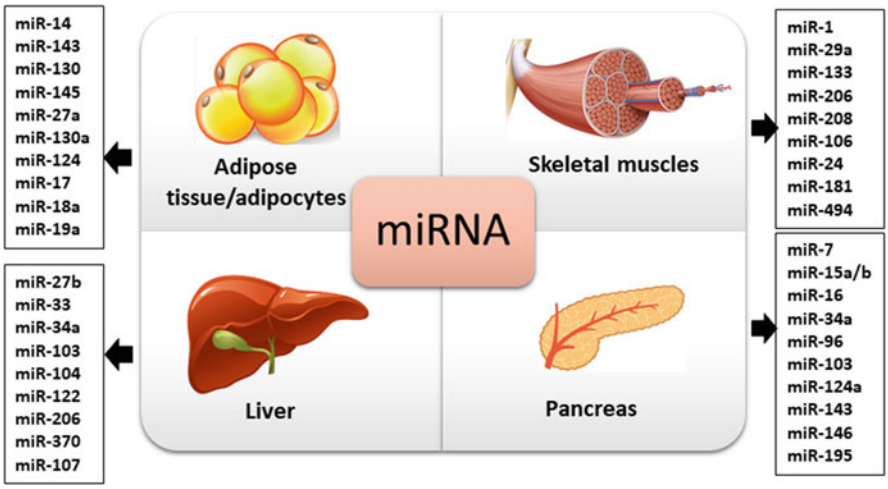

Fig. 10.4 Overview of miRNA associated with obesity in different tissues (Iacomino and Siani 2017)

Adipose tissue is a storage site of triglycerides and is the key machinery for maintaining energy homeostasis. miR-14 has a role in suppressing fat metabolism by targeting p38 and MAPK. miR-143 and miR-130 are widely studied and has linkage to adipogenesis. miR-143 and miR-145 are closely located and co-transcribed. miR-143 is a positive regulator of differentiation of human adipocyte, which acts via ERK5 signaling (Fig. 10.4) (Tresserra-rimbau et al. 2018). Adipocyte differentiation is suppressed by miR-27a and miR-130a. A panel of 40 miRNAs is predominantly expressed in the islets that have been recently identified in the case of diabetes in relation to pancreatic development. Overexpression of miR-375 is observed during pancreatic islet development. Several types of miRNA are referred to as myomiR family and are detectable in muscle tissue and act as modulators of muscle myogenesis. The myomiR include miR-1, miR-133a, miR-133b, miR-206, miR-208b, etc. (Zhang et al. 2019).

10.3.3 Polyphenol Related Signature of miRNA in Obesity

It has been reported that miRNAs are influenced by PPs by binding directly with PPs and regulating the controlling molecule bind to them. Epigallocatechin 3-gallate (EGCG) works by binding with HIF-α, which is a transcriptional activator of miR-210 and interferes with the hydroxylation of a proline residue in the oxygen-dependent degradation domain, thereby increases the expression. Quercetin increased the expression of miR-146a (Table 10.1), which is known for its posttranscriptional modulating properties of various genes like MIRLET7E and MIR17 (Corrêa and Rogero 2019).

Table 10.1 Regulation of miRNAs by phenolic compounds

Phenolics	Expression of miRNA		Target action	Reference
	Upregulate	Downregulate		
Resveratrol	miR-33a miR-122 miR-328	miR-17 miR-520 h miR-21	Hepatic cells	Kang (2019)
Epigallocatechin 3-gallate (EGCG)	miR-33a miR-122 miR-34a miR-145 miR-200 miR-210	miR-210 miR-449c miR-17 miR-21	Lung tumor	Pandima Devi et al. (2017)
Curcumin	miR-21 miR-29 miR-22 miR-200 miR-205	miR-19	Pancreatic cancer	Corrêa and Rogero (2019)
Quercetin	miR-146a	miR-27a	Breast cancer cells	Dostal and Modriansky (2019)

Table 10.2 Active ingredients of plant extract and their health effects

Plant extracts	Active ingredients	In-vitro/in-vivo effects	Reference
White tea	Epigallocatechin gallate (EGCG)	Inhibit adipogenesis and stimulate lipolysis in 3T3-L1 cells	Ferguson (2009)
Coffee polyphenols	Mono or di caffeoylquinic acids	Decrease body weight	Habauzit and Morand (2012)
Green tea polyphenols	Catechin. Epicatechin, epigallocatechin gallate, EGCG	Body weight regulation in HFD induced obese rats	Guo et al. (2017)
Apple polyphenols	Hydroxycinnamates, flavanols (catechin and epicatechin)	Cure Alzheimer's disease (suppress overexpression of presenilin-1)	Francini and Sebastiani (2013)
Citrus polyphenols	Flavanones	Neuroprotective actions	Rosaria et al. (2018)
Camu camu extract	Quercetin, cyanidin-3-glucoside, ellagic acid, and ellagitannins	Anti-hypertension Antimicrobial	Fujita et al. (2015)
Acacia polyphenol	Flavan-3-ol, catechin, procyanidin monomer	Anti-hypertension	Ikarashi et al. (2018)

PPs can interact with cell signaling pathways and modulate the activity of transcription factors and, thereby, gene expression. It modulates the development of adipose tissues, inflammation markers (Khalilpourfarshbafi et al. 2019). Over 100 miRNAs are involved in the regulation of different cell processes, such as apoptosis and inflammation, are shown to modulate by polyphenols, and it appears to become the strategy for regulating metabolic-related diseases. PPs may bind to Dicer and RISC components, which are involved in miRNA biogenesis (Guo et al. 2017). Among PPs, epigallocatechin 3-gallate (EGCG) improve homeostasis of glucose, lipid metabolism, and endothelial function and also reduce blood pressure and oxidative stress. Modification of genes occurs when flavonoids interact with signaling cascades or with epigenetic factors (Tanabe et al. 2017). An in-vitro treatment using acacia extract rich in anthocyanidins suggested a reduction in leptin and increased adiponectin levels in the murine adipocyte (Table 10.2). However, this extract is also shown to reduce oxidative stress and inhibit the NF-kB pathway along with the expression of TNF-α, IL-6, IL-8, and monocyte chemotactic peptide-1 (MCP-1), which demonstrates the role and importance of PPs in reducing inflammation in the adipose tissue. Furthermore, it is suggested that EGCG, quercetin, resveratrol, curcumin are also shown to modulate miRNA under in-vitro studies (Table 10.3) (Roopchand et al. 2015). PPs also showed the effect on the hypothalamic satiety centers and modulate insulin signaling in the brain suggesting that PPs might cross the blood-brain barrier (Chiva-Blanch and Badimon 2017).

Table 10.3 Effects (in-vivo and in-vitro) of dietary polyphenols on lipid adipogenesis

Dietary polyphenol	In-vitro/in-vivo effects	Reference
Apigenin	Suppressed lipid accumulation and adipogenesis	Pereira et al. (2009)
Capsaicin	Stimulated lipolytic activity	Chiva-Blanch and Badimon (2017)
Catechins	Adipocyte differentiation	Farhat et al. (2017)
Curcumin	Inhibit adipocyte differentiation	Wang et al. (2014)
Epigallocatechin gallate (EGCG)	Inhibit proliferation and suppress adipose phenotype expression	Chandrasekara and Shahidi (2011)
Genistein	Inhibit lipid accumulation	Williamson and Manach (2005)
Myricetin	Inhibit adipogenesis	Alissa and Ferns (2012)
Resveratrol	Induce apoptosis in adipocyte	Meydani and Hasan (2010)
Quercetin	Lower visceral adiposity and weight gain	Williamson and Manach (2005)

10.3.4 Modulating Gut Microbiota in Obesity

The human intestinal tract has more than 100 trillion microbes, which are mostly dominated by five phyla that densely populated the large intestine (Muscogiuri et al. 2019). Gut microbiota colonization throughout the gastrointestinal tract is not uniform as they have limited occurrence in the stomach and small intestine followed by a dense and diverse population in the colon due to lower digestive secretion and slow peristalsis (Sivamaruthi et al. 2019). Gut microbiome protects the intestinal mucosa in a close symbiotic relationship, maintains energy homeostasis and body weight. Among different phyla, 90% of microbial species belong to *Firmicutes* and *Bacteroidetes* followed by *Actinobacteria* (*Bifidobacterium* spp.), *Proteobacteria* (*Escherichia*, *Helicobacter*) and *Akkermansia* spp. *Akkermansia* is a Gram-negative, anaerobe, and mucin degrading bacterium that colonizes the mucus layer of the intestine and represents 1–3% of total gut microbiota (Lin et al. 2019). These microorganisms in the gut extract calories from indigestible dietary components and produce short-chain fatty acids (SCFA), amino acids, and vitamins (Tresserra-rimbau et al. 2018). The fecal concentration of SCFA, such as propionate, butyrate, and acetate fatty acids, is linked to the abundance of Bacteroidetes in the gut (Muscogiuri et al. 2019). Gut microbiota gets altered specifically to the dietary intervention, and in metabolic conditions, there is a dysbiosis where an imbalance between beneficial and pathogenic bacteria as a result of the loss of foundation species or enhanced pathogens is vastly reported.

It has been found that in the microbiota of obese people, the proportion of Bacteroidetes decreases, and that of *Firmicutes* (*Eubacterium dolichum*) is higher (Muscogiuri et al. 2019). Diet rich in protein are associated with *Bacteroides* enterotype, whereas a high carbohydrate diet is linked to *Prevotella* enterotype. The predisposition of obesity in relation to gut microbiota is also associated with genetic factors and epigenetic signatures (Cuevas-Sierra et al. 2019). High-fat diet-induced

(HFD) obesity is linked to lower *Bifidobacterium* count in caecal content (Sarma et al. 2017). Reduction of butyrate-producing bacteria due to low bacterial richness has been shown to cause endotoxemia, which is characterized by intense hinderance in gastrointestinal mobility in the small and large intestine and chronic low-grade inflammation (Muscogiuri et al. 2019).

In a recent study, the alteration of the fecal microbiota profile is observed after feeding the mice with a high-fat diet for 12 weeks (Caesar et al. 2015). The colonic epithelium, along with the colonizing bacteria, represents an initial site of interactions between diet and the host immune system, and this can impact on the composition of the gut microbiota. This directly affects the gut-immune homeostasis and permeability of the intestine (Bladé et al. 2013). The potential source of pro-inflammatory molecules that can affect whole-body metabolism is the intestine and its microbiota (Khalilpourfarshbafi et al. 2019). This evidence supports the fact that the gut microbiota plays a distinct role in energy homeostasis through energy balance modulation, glucose metabolism, and the chronic inflammatory state related to obesity. The lipopolysaccharide (LPS), which is derived from the gut microbiota is a potent inducer of inflammation and metabolic diseases (Lin et al. 2019).

The increasing population of beneficial bacteria such as *Akkermansia* can protect against obesity-linked metabolic syndrome, and it contributes to beneficial metabolic effects of gastric bypass surgery and the antidiabetic metformin (Anhê et al. 2015). Directly there is a lot of interest regarding how gut bacteria are controlling various clinical symptoms. So among them, PPs being a dietary component also reported stimulating gut bacteria, which are the beneficial cure.

10.3.5 Polyphenols in Gut Microbiota and Its Bioavailability

PPs have the capacity to modulate the gut microbiota in a beneficial way (Awika et al. 2018). It reshapes the composition of the microbiota, thereby increase the beneficial short-chain fatty acid (SCFA) production and downregulates the pro-inflammatory markers. It has been reported that *Bifidobacterium* increased in the presence of polyphenol-rich nuts and berries along with *Firmicutes*, *Proteobacteria*, *Akkermansia*. PPs are being metabolized by *Firmicutes* as the main bacterial metabolism (Wu et al. 2018). Certain bacterial repression was observed in *Clostridium perfringens*, *Clostridium difficile*, and *Bacteroides* spp. without significantly affecting commensals like *Bifidobacterium* spp. and as *Lactobacillus* spp. in case of phenolic rich diet administration (Yuan et al. 2018).

Flavonols are first metabolized into their aglycones and free phenolic acids by colonic microbiota enzyme machinery. Quercetin metabolites are 2-(3,4-dihydroxyphenyl)-acetic acid and protocatechuic acid, whereas gallic acid is the primary metabolite of myricetin. Flavanones are metabolized to sulfonated hesperidin as a final metabolite, whereas catechin metabolizes to propionic acid (Marín et al. 2015). Therefore, consumption of polyphenol-rich food is important in order to eradicate infectious diseases along with obesity and its comorbodities. Several other studies are also reported in the context of PPs and gut bacterial modulation that is given in the table below (Table 10.4).

Table 10.4 The effect on gut bacteria in relation to phenolic compounds

Phenolic compound/food	Gut bacteria		Reference
	Growth enhancement	Growth inhibition	
(+)(−) Catechin	*Lactobacillus spp.* *Enterococcus spp.* *Bifidobacterium spp.* *Clostridium coccoides-Eubacterium rectale* group *Escherichia coli*	*C. histolyticum* group	Dueñas et al. (2015)
Blueberry extract	*Lactobacilli* *Bifidobacteria*	ND	Dueñas et al. (2015)
Pomegranate extract	*Bifidobacterium spp.* *Lactobacillus-Enterococcus* group	ND	Tresserra-rimbau et al. (2018)
Grape seed extract	*Lactobacillus reuteri* *Lactobacillus acidophilus* *Enterococcus spp.*	*C. histolyticum* group	Williamson and Manach (2005)
Red wine extract	*Lactobacillus spp.* *Bifidobacterium spp.* *Bacteroides spp.* *Ruminococcus spp.*	*C. histolyticum* group *Blautia coccoides* *Anaeroglobus spp.* *Victivallis spp.*	Anhê et al. (2015)
Tea polyphenol	*Lactobacilli*	*Bacteroidaceae* *C. perfringens*	Dueñas et al. (2015)
Green tea extract	ND	*C. perfringens* *Bifidobacterium spp.* *Lactobacillus spp.*	Yuan et al. (2018)
Proanthocyanidins	*Bacteroides fragilis group* *Bacteroides-Prevotella-Porphyromonas group* *Enterobacteriaceae*	*C. leptum group*	Fracassetti et al. (2013)
Resveratrol	*Lactobacilli* *Bifidobacterium*	*Enterobacteria*	Dueñas et al. (2015)
Epicatechin	*Eubacterium rectale* *Clostridium coccoides*	*C. perfringens* *Clostridium difficile* *Bacteroides spp.*	Cardona et al. (2013)
3-O-methylgallic acid	ND	*C. perfringens* *Clostridium difficile* *Bacteroides spp.*	Williamson and Manach (2005)

(continued)

Table 10.4 (continued)

Phenolic compound/food	Gut bacteria		Reference
	Growth enhancement	Growth inhibition	
Gallic acid	*Total bacteria* *Atopobium spp.*	*C. perfringens* *Clostridium difficile* *Bacteroides spp.*	Popa et al. (2018)
Caffeic acid	*Bifidobacterium spp.*	*C. perfringens* *Clostridium difficile* *Bacteroides spp.*	Lin et al. (2019)
Anthocyanin	*Lactobacillus-Enterococcus spp.* *Bifidobacterium spp.*	*Clostridium difficile*	Luna-Vital et al. (2017)
Cocoa fraction	*Bifidobacterium spp.* *Lactobacillus spp.*	ND	Wu et al. (2018)
Soy germ extract	*Enterobacteriaceae* *Coliforms* *Lactobacillus spp.* *Staphylococcus spp.* *Clostridium spp.*	ND	Scalbert and Williamson (2000)
Black tea extract	*Klebsiella spp.* *Enterococci* *Akkermansia spp.*	*Bifidobacteria* *Blautia coccoides* *Anaeroglobus spp.* *Victivallis spp.*	Awika et al. (2018)
Lowbush wild blueberries	*Thermomonospora spp.* *Corynebacteria spp.* *Slackia spp.*	*Lactobacillus spp.* *Enterococcus spp.*	Dueñas et al. (2015)
Apple juice	*Lactobacilli* *Bifidobacteria*	ND	Faria et al. (2014)

In spite of many reports on PPs modulating gut bacteria, it is largely remained unknown as how polyphenols exterts its effects on the specific gut bacterium. Hence, it is important to understand the phenolic compound effect efficiently on individual intestinal bacterium, which is implicated in the dietary polyphenol's metabolism. One of the main drawbacks of previously reported studies is that most phenolic compounds have been analyzed without taking the consideration of bioavailability and their chemistry in the colon. The best model for studying the effect of PPs on gut microbiota is to perform interventional studies on humans directly and to understand specific microbial responses upon PPs administration using a systems biology approach (Fig. 10.5).

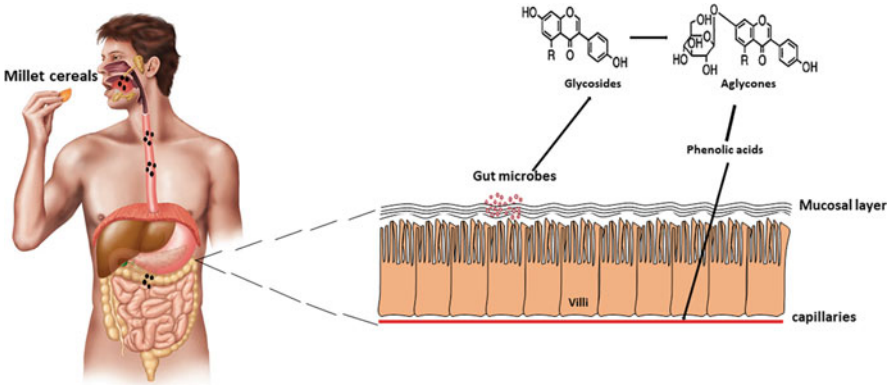

Fig. 10.5 Bioavailability of polyphenol

10.4 Conclusions and Future Perspective

Polyphenols play an important role in human nutrition. Daily intake of PPs can help in overcoming oxidative stress due to ROS generation and can prevent inflammation due to lifestyle associated and other diseases. The interaction between dietary components, especially phenolic compounds and gut microbiota, is important due to their relevance to bioavailability and in improving human health. Bioavailability varies among PPs depending on their structural feature, and the PPs that are most well absorbed in humans are isoflavones and gallic acid followed by catechins, flavanones, and quercetin glucosides with different kinetics. Finally, the metabolism of PPs by microbiota need to be understood because gut microbiota plays a special role in the biotransformation of PPs and hence their biological activities at the functional level. Mechanistic studies using systems biology approach are critical to confirm the pathways and mode of action of different types of PPs that could contribute to their evidence-based applications to improve human health.

References

Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, Ishtiaq A, Hussain S, Suleria HAR (2016) Natural polyphenols: An overview. Int J Food Prop 20(8):1689–1699

Ahirwar R, Mondal PR (2019) Prevalence of obesity in India: A systematic review. Diabetes Metab Syndr Clin Res Rev 13(1):318–321. https://doi.org/10.1016/J.DSX.2018.08.032

Alara OR, Abdurahman NH, Olalere OA (2017) Journal of King Saud University – Science Ethanolic extraction of flavonoids, phenolics and antioxidants from Vernonia amygdalina leaf using two-level factorial design. J King Saud Univ Sci:1–10. https://doi.org/10.1016/j.jksus.2017.08.001

Alissa EM, Ferns GA (2012) Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. J Nutr Metab 2012:569486

Anhê FF, Roy D, Pilon G, Dudonné S, Matamoros S, Varin TV, Garofalo C, Moine Q, Desjardins Y, Levy E, Marette A (2015) A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. Gut 64(6):872–883

Ashley D, Marasini D, Brownmiller C, Lee JA, Carbonero F, Lee SO (2019) Impact of grain sorghum polyphenols on microbiota of normal weight and overweight/obese subjects during in vitro fecal fermentation. Nutrients 11(2):217

Awika JM et al (2018) Complementary effects of cereal and pulse polyphenols and dietary fiber on chronic inflammation and gut health. Food Funct 9(3):1389–1409

Bladé C, Baselga-Escudero L, Salvadó MJ, Arola-Arnal A (2013) miRNAs, polyphenols, and chronic disease. Mol Nut Food Res 57(1):58–70. https://doi.org/10.1002/mnfr.201200454

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., Bren, U. (2016) Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effectsMolecules Basel, Switzerland, 21(7). https://doi.org/10.3390/molecules21070901

Caesar R, Tremaroli V, Kovatcheva-datchary P, Patrice D (2015) Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Article Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling:658–668. https://doi.org/10.1016/j.cmet.2015.07.026

Cardona F, Andrés-lacueva C, Tulipani S, Tinahones FJ, Queipo-ortuño MI (2013) Benefits of polyphenols on gut microbiota and implications in human health. J Nutr Biochem 24 (8):1415–1422

Caro-Gómez E, Sierra JA, Escobar JS, Álvarez-Quintero R, Naranjo M, Medina S, Ramírez-Pineda JR (2019) Green coffee extract improves cardiometabolic parameters and modulates gut microbiota in high-fat-diet-fed ApoE -/- mice. Nutrients 11(3):497

Chandrasekara A, Shahidi F (2011) Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. J Funct Foods 3(3):144–158

Chelombitko MA (2018) Role of reactive oxygen species in inflammation: a minireview. Mosc Univ Biol Sci Bull 73(4):199–202

Cheynier V (2005) Polyphenols in foods are more complex than often thought. Am J Clin Nutr 81 (1):223S–229S

Chiva-Blanch G, Badimon L (2017) Effects of polyphenol intake on metabolic syndrome: current evidences from human trials. Oxid Med Cell Longev 2017:5812401

Cooper R, Nicola G (2014) Phenolic compounds. Natural products chemistry. Elsevier, Amsterdam

Corrêa TA, Rogero MM (2019) Polyphenols regulating microRNAs and inflammation biomarkers in obesity. Nutrition 59:150–157

Cuevas-Sierra A, Ramos-Lopez O, Riezu-Boj JI, Milagro FI, Martinez JA (2019) Diet, gut microbiota, and obesity: Links with host genetics and epigenetics and potential applications. Adv Nutr 10(9):S17–S30. https://doi.org/10.1093/advances/nmy078

Dostal Z, Modriansky M (2019) The effect of quercetin on microRNA expression: a critical review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 163(2):95–106

Dueñas M, Muñoz-González I, Cueva C, Jiménez-Girón A, Sánchez-Patán F, Santos-Buelga C, Bartolomé B (2015) A survey of modulation of gut microbiota by dietary polyphenols. Figure 1. BioMed Res Int 2015:850902

Fang M, Chen D, Yang CS (2007) Dietary polyphenols may affect DNA methylation. J Nutr 137 (1):223S–228S

Farhat G, Drummond S, Al-Dujaili EAS (2017) Polyphenols and their role in obesity management: a systematic review of randomized clinical trials. Phytother Res 31(7):1005–1018

Faria A, Fernandes I, Norberto S, Mateus N, Calhau C (2014) Interplay between anthocyanins and gut microbiota. J Agric Food Chem 62(29):6898–6902

Ferguson LR (2009) Nutrigenomics approaches to functional foods. J Am Diet Assoc 109 (3):452–458

Fracassetti D, Costa C, Moulay L, Tomás-barberán FA (2013) Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (Myrciaria dubia). Food Chem 139(1–4):578–588

Francini A, Sebastiani L (2013) Phenolic compounds in apple (Malus x domestica Borkh.): compounds characterization and stability during postharvest and after processing. Antioxidants (Basel) 2(3):181–193

Fujita A, Sarkar D, Wu S, Kennelly E, Shetty K, Genovese MI (2015) Evaluation of phenolic-linked bioactives of camu-camu (Myrciaria dubia Mc. Vaugh) for antihyperglycemia, antihypertension, antimicrobial properties and cellular rejuvenation. Food Res Int 77:194–203

González-abuín N, Martínez-micaelo N, Blay M, Green BD, Ardévol A (2018) Grape-seed procyanidins modulate cellular membrane potential and nutrient-induced GLP-1 secretion in STC-1 cells, (49). https://doi.org/10.1152/ajpcell.00355.2013

Guo X, Cheng M, Zhang X, Cao J, Wu Z, Weng P (2017) Green tea polyphenols reduce obesity in high-fat diet-induced mice by modulating intestinal microbiota composition. Int J Food Sci Technol 52(8):1723–1730

Habauzit V, Morand C (2012) Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: an update for clinicians. Ther Adv Chronic Dis 3(2):87–106

Heneghan HM, Miller N, Kerin MJ (2010) Role of microRNAs in obesity and the metabolic syndrome. Obes Rev 11(5):354–361. https://doi.org/10.1111/j.1467-789X.2009.00659.x

Iacomino G, Siani A (2017) Role of microRNAs in obesity and obesity-related diseases. Genes Nutr 12:23

Ikarashi N, Toda T, Hatakeyama Y, Kusunoki Y, Kon R, Mizukami N, Kaneko M, Ogawa S, Sugiyama K (2018) Anti-hypertensive effects of acacia polyphenol in spontaneously hypertensive rats. Int J Mol Sci 19(3):700

Kang H (2019) MicroRNA-mediated health-promoting effects of phytochemicals. Int J Mol Sci 20 (10):2535

Khalilpourfarshbafi M, Gholami K, Murugan DD, Abdul Sattar MZ, Abdullah NA (2019) Differential effects of dietary flavonoids on adipogenesis. Eur J Nutr 58(1):5–25

Khan H, Sureda A, Belwal T, Çetinkaya S, Süntar İ, Tejada S, Devkota HP, Ullah H, Aschner M (2019) Polyphenols in the treatment of autoimmune diseases. Autoimmun Rev 18(7):647–657

Khoo HE (2017) Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res 61(1). https://doi.org/10.1080/16546628.2017.1361779

Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, Kong M, Li L, Zhang Q, Liu Y, Chen H, Qin W, Wu H, Chen S (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21(10):1374

Lin S, Wang Z, Lam KL, Zeng S, Tan BK, Hu J (2019) Role of intestinal microecology in the regulation of energy metabolism by dietary polyphenols and their metabolites. Food Nutr Res 63:1–12

Luna-Vital D, Weiss M, Gonzalez de Mejia E (2017) Anthocyanins from purple corn ameliorated tumor necrosis factor-α-induced inflammation and insulin resistance in 3T3-L1 adipocytes via activation of insulin signaling and enhanced GLUT4 translocation. Mol Nutr Food Res 61 (12):1–13

Marín L, Miguélez EM, Villar CJ, Lombó F (2015) Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int 2015:905215

Meydani M, Hasan ST (2010) Dietary polyphenols and obesity. Nutrients 2(7):737–751

Milenkovic D, Jude B, Morand C (2013) miRNA as molecular target of polyphenols underlying their biological effects. Free Radic Biol Med 64:40–51. https://doi.org/10.1016/j.freeradbiomed.2013.05.046

Murtaza N, Baboota RK, Jagtap S, Singh DP, Khare P, Sarma SM, Podili K, Subramanian A, Chandra TS, Bhutani KK, Boparai RK, Bishnoi M, Kondepudi KK (2014) Finger millet bran supplementation alleviates obesity-induced oxidative stress, inflammation and gut microbial derangements in high-fat diet-fed mice. Br J Nutr 112(9):1447–1458

Muscogiuri G, Cantone E, Cassarano S, Tuccinardi D, Barrea L, Savastano S, Colao A (2019) Gut microbiota: a new path to treat obesity. Int J Obes Suppl 9(1):10–19

Ortega FJ, Mercader JM, Catalán V, Moreno-Navarrete JM, Pueyo N, Sabater M, Gómez-Ambrosi J, Anglada R, Fernández-Formoso JA, Ricart W, Frühbeck G, Fernández-Real JM (2013) Targeting the circulating Microrna signature of Obesity. Clin Chem 59(5):781–792

Ozdal T, Sela DA, Xiao J, Boyacioglu D, Chen F, Capanoglu E (2016) The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. Nutrients 8(2):1–36. https://doi.org/10.3390/nu8020078

Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med Cell Longev 2(5):270–278

Pandima Devi K, Rajavel T, Daglia M, Nabavi SF, Bishayee A, Nabavi SM (2017) Targeting miRNAs by polyphenols: novel therapeutic strategy for cancer. Semin Cancer Biol 46:146–157

Pereira DM, Valentão P, Pereira JA, Andrade PB (2009) Phenolics: from chemistry to biology. Molecules 14(6):2202–2211

Popa DE, Drăgoi CM, Popa DE, Manuela C (2018) We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists TOP 1%. IntechOpen, London

Priya G, Rathinavel T (2017) Evaluation of antioxidant activity in relation with their phenolic and flavanoid content of eclipta alba l leaf collected from five different geographic regions of tamil nadu evaluation of antioxidant activity in relation with their phenolic and flavanoid, (December)

Roopchand DE, Carmody RN, Kuhn P, Moskal K, Turnbaugh PJ, Raskin I, Brunswick N (2015) Dietary polyphenols promote growth of the gut bacterium Akkermansia muciniphila and attenuate high fat diet-induced metabolic syndrome. Diabetes 64(8):2847–2858

Rosaria M, Muscatello A, Zoccali RA, Bruno A (2018) Citrus fruit polyphenols and flavonoids: applications to psychiatric disorders. In: Polyphenols: mechanisms of action in human health and disease, 2nd edn. Elsevier, Amsterdam

Ruth O, Hamid N, Kholijah S, Mudalip A (2017) Phytochemical and pharmacological properties of vernonia amygdalina: A review, 2 September, 80–96

Sarma SM, Khare P, Jagtap S, Singh DP, Baboota RK, Podili K, Boparai RK, Kaur J, Bhutani KK, Bishnoi M, Kondepudi KK (2017) Kodo millet whole grain and bran supplementation prevents high-fat diet induced derangements in a lipid profile, inflammatory status and gut bacteria in mice. Food Funct 8(3):1174–1183

Scalbert A, Williamson G (2000) Chocolate: modern science investigates an ancient medicine dietary intake and bioavailability of polyphenols 1. J Nutr 130(8):2073–2085

Shi Q, Bishayee A, Bhatia D (2019) Genetic and epigenetic targets of natural dietary compounds as anticancer agents. In: Epigenetics of cancer prevention. Elsevier, Amsterdam

Singla RK, Dubey AK, Garg A, Sharma RK, Fiorino M, Ameen SM, Haddad MA, Al-Hiary M (2019) Natural polyphenols: chemical classification, definition of classes, subcategories, and structures. J AOAC Int 102(5):1397–1400

Sivamaruthi BS, Kesika P, Suganthy N, Chaiyasut C (2019) A review on role of microbiome in obesity and antiobesity properties of probiotic supplements. BioMed Res Int. https://doi.org/10.1155/2019/3291367

Sripad G, Prakash V, Rao MSN (1982) Extractability of polyphenols of sunflower seed in various solvents. 4(2):145–152

Tanabe H, Pervin M, Goto S, Isemura M, Nakamura Y (2017) Beneficial effects of plant polyphenols on obesity. Obes Control Ther 4(3):1–16

Tresserra-rimbau A, Lamuela-raventos RM, Moreno JJ (2018) Polyphenols, food and pharma. Current knowledge and directions for future. Biochem Pharmacol 156(6):186–195

Tsimogiannis D, Oreopoulou V (2019) Classification of phenolic compounds in plants. Polyphenols in Plants:263–284. https://doi.org/10.1016/b978-0-12-813768-0.00026-8

Vuolo MM, Lima VS, Maróstica Junior MR (2019) Phenolic compounds: structure, classification, and antioxidant power. Bioactive compounds. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814774-0.00002-5

Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun I, Shen CL (2014) Novel insights of dietary polyphenols and obesity. J Nutr Biochem 25(1):1–18

Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans.II. Review of 93 intervention studies. Am J Clin Nutr 81:243S–255S

Wu T, Grootaert C, Pitart J, Vidovic NK, Kamiloglu S, Possemiers S, Van Camp J (2018) Aronia (Aronia melanocarpa) polyphenols modulate the microbial community in a simulator of the human intestinal microbial ecosystem (SHIME) and decrease secretion of proinflammatory markers in a Caco-2/endothelial cell coculture model. Mol Nutr Food Res 62(22):1–10

Yuan X, Long Y, Ji Z, Gao J, Fu T, Yan M, Zhang L, Su H, Zhang W, Wen X, Pu Z, Chen H, Wang Y, Gu X, Yan B, Kaliannan K, Shao Z (2018) Green tea liquid consumption alters the human intestinal and oral microbiome. Mol Nutr Food Res 62(12):1–15

Zhang B, Yang Y, Xiang L, Zhao Z, Ye R (2019) Adipose-derived exosomes: a novel adipokine in obesity-associated diabetes. J Cell Physiol 234(10):16692–16702

Designer Microbes for Nutraceutical Application

11

Hena Dhar, Anchal Chaudhary, and Varnika Rana

Abstract

Nutraceuticals are natural bioactive compounds, generally found in food, that possess medicinal and health-promoting properties other than the sole nutritional role. Popularity of these structurally and functionally diverse molecules, due to their beneficial effects on human health, has raised the global nutraceutical demand which could not be accomplished by their natural and chemical sources. With the progressive development in techniques for synthetic biology, system biology, genetic manipulations, and genome sequencing, microbes can be engineered to be designed as sustainable, economical, and eco-friendly source of value-added nutraceuticals. Moreover, through system metabolic engineering along with the traditional techniques, both native and non-native producers can be programmed by optimizing indigenous regulatory and metabolic pathway or introducing heterologous metabolic pathway genes to produce commercial level of desired metabolites. In addition to overexpression of rate-limiting pathway genes, competing pathways can also be blocked to increase the precursor molecules and direct the carbon flux towards desired pathway. The major steps for development of a high-performance engineered cell factory include the choice of a safe and robust host organism, the selection of best pathway enzymes from different sources to be overexpressed, and the availability of efficient tools for genetic modification. Several genetically tractable microorganisms like *Escherichia coli*, *Corynebacterium glutamicum*, *Saccharomyces cerevisiae,* and *Yarrowia lipolytica* have been engineered to produce nutraceuticals like polyunsaturated fatty acids, carotenoids, polyphenols, alkaloids, non-proteinogenic amino acid, and poly amino acids. This chapter focuses on the various metabolic engineering strategies employed to design microbes for the production of some valuable nutraceuticals.

H. Dhar (✉) · A. Chaudhary · V. Rana
National Agri-Food Biotechnology Institute, Mohali, Punjab, India
e-mail: hena@nabi.res.in

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_11

Keywords

Nutraceuticals · Metabolic engineering · PUFA · Phytochemicals · Designer microbes

11.1 Introduction

In modern era, nutraceuticals are new and promising alternative for pharmaceuticals with claimed long-term physiological benefits like physical and mental health improvement, and prevention of chronic diseases including cardiovascular diseases, diabetes, cancer, arthritis, osteoporosis and gastrointestinal diseases, etc. The idea of nutraceuticals was came into picture when diet was rated high for good health than exercise or hereditary factors by the consumers in a survey held at U.K., Germany, and France (Pandey et al. 2010). "Nutraceutical" term is a fusion of "nutrition" and "pharmaceutical" introduced by Stephen DeFelici in 1989 for the natural bioactive compounds having therapeutic effects other than nutritional value (Defelice 1995; Kalra 2003; Daliu et al. 2018). He defined nutraceuticals as "any substance that is a food or a part of food and provides medical or health benefits, including the prevention and treatment of disease." Later, health ministry of Canada defined nutraceutical as "a purified product from food with some physiological benefit that are generally sold in the form of medicine (pills or powder) and are not associated with food" (Wildman 2001). Commercially valuable nutraceuticals include antioxidants, carotenoids, dietary fibers, polyunsaturated fatty acids, polysaccharides, prebiotics, probiotics and vitamins, etc. that are generally obtained from plants, animals, and microbes. The growing popularity of diet for maintaining good health has led to increase in demand of nutraceuticals thereby expanding the global nutraceutical market from $198.7 billion in 2016 to $230.9 billion in 2018 and is estimated to reach $336.1 billion by 2023 (BCC Research Staff 2018). Considering these beneficial effects on human health, the production of these beneficial compounds needs to be improved to meet their ever-growing demands. Most of these molecules of interest are produced by the organisms that are not suitable for industrial-scale production. Moreover, mostly the traditional natural sources of these nutraceuticals are limited by the low yield, availability, quantity and cost of raw material, product stability concerns associated with the extraction methods, and dependence on climatic conditions, which ultimately increases the cost of the nutraceuticals. Alternatively, chemical methods are also available for the synthesis of some nutraceuticals but are associated with the limitations of harsh conditions and heavy pollution. Therefore to meet the demand of these high value nutraceuticals, designer microbes need to be developed via metabolic engineering approach as microbes require cheaper carbon sources, their production is easily scalable and is not affected by climate change. This chapter explicates various metabolic engineering strategies used to develop designer microbes for nutraceutical.

11.2 Polyunsaturated Fatty Acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) are important dietary substance that play crucial role in human physical and mental health. These can be mainly categorized into two types depending on the position of first double bond from methyl end: one is omega-3 fatty acids including α-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), docosahexanoic acid (DHA, C22:6 n-3); and the other one is omega-6 fatty acids including arachidonic acid (ARA, C20:5 n-4). The precursors C_{18} PUFA, linoleic acid (LA; 18:2 n-6), and α-linolenic acid (ALA; 18:3 n-3) for long chain PUFA ($\geq C_{20}$) are essential fatty acids (FA) as humans lack enzymes to convert oleic acid to LA (Δ12 desaturase) and then to ALA (Δ15 desaturase). Among these, omega-3 long chain PUFA (ω3LC-PUFAs) particularly EPA and DHA have been demonstrated to provide several health-promoting benefits like alleviation of inflammation, hypertension, cholesterol, and hypertriglyceridemia, therefore, are recognized as the miracle food of the twenty-first century (Backes et al. 2016; Sokoła-Wysoczańska et al. 2018). These ω3LC-PUFAs are essential components of cell membrane and help in maintaining fluidity of the membrane, and thereby providing several health benefits. EPA is also known to have a functional role by acting as the precursor for various biologically active molecules like eicosanoids, whereas DHA is found as a structural component in brain and retina. Although humans have the enzymes to synthesize EPA and DHA from ALA via aerobic desaturase/elongase pathway but the conversion rate is too low that only 0.2–21% of ALA is converted to EPA (8% in adult men) and 0–9% to DHA (conversion rate of EPA to DHA <0.1% in adult men and >9% in women) (Andrew et al. 2006; Williams and Burdge 2006; Abedi and Sahari 2014), thus these ω3LC-PUFA should be taken in diet. With such nutraceutical properties, the global demand of these fatty acids is increasing day-by-day and is projected at 241 thousand metric tons with a value of $4.96 billion by 2020, which cannot be accomplished by the existing sources (Industry Experts 2014). The traditional source of ω3LC-PUFAs is mainly seafood and fish oil that are associated with some limitations like availability, sustainability, mercury contamination, and unpleasant fishy smell and taste. Other sustainable sources of EPA and DHA could be bacteria and microalgae that can produce these molecules de novo by anaerobic polyketide synthase pathway directly from malonyl-CoA with no free intermediates, unlike aerobic pathway employed by higher organisms via iterative rounds of desaturation and elongation utilizing saturated fatty acids as precursors (Fig. 11.1). These biosynthetic pathways are well studied and different types of genes have been identified from various organisms with different properties; therefore researchers have gained focus on metabolic engineering of microbes to produce commercial level yield of ω3LC-PUFAs.

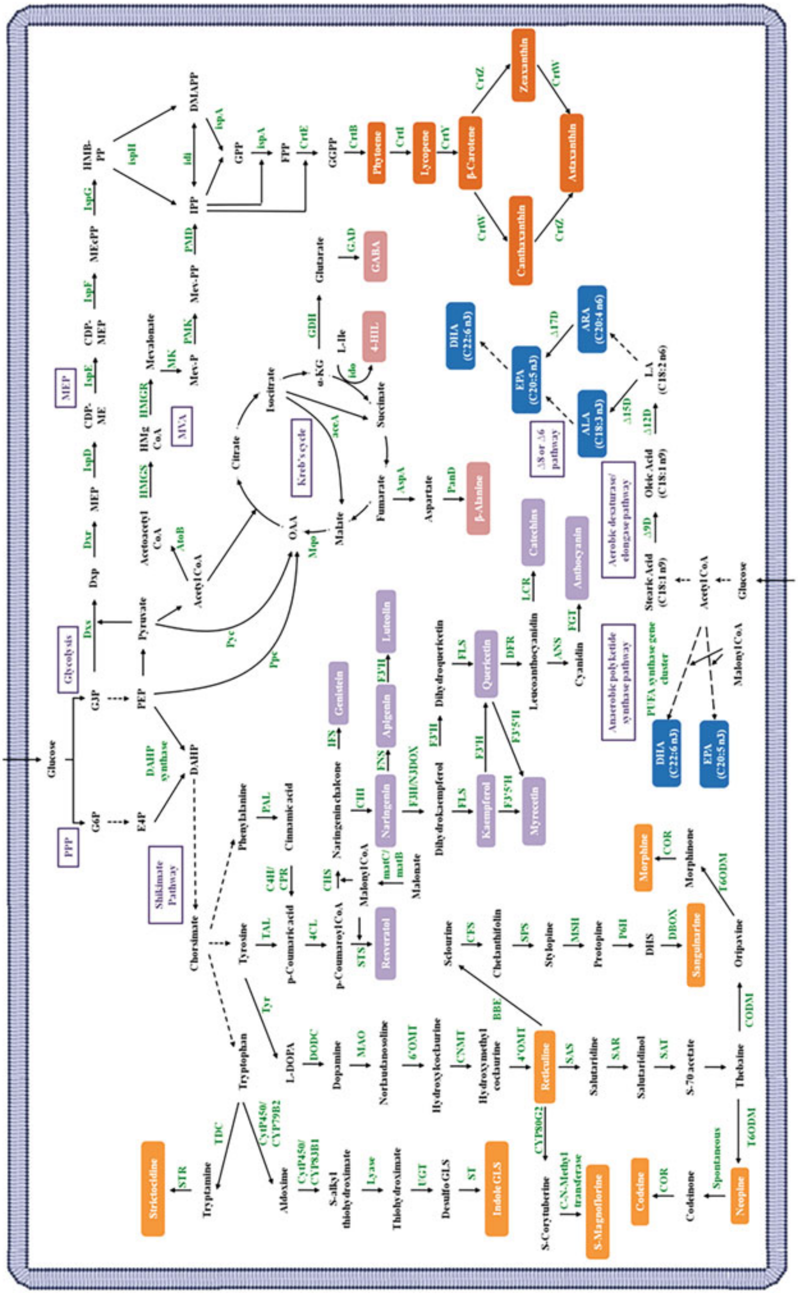
Glucose
PPP
Glycolysis
MEP
MVA
Shikimate Pathway
Kreb's cycle
G6P
G3P
E4P
PEP
Pyruvate
DAHP synthase
DAHP
Dxs
Dxp
Dxr
MEP
IspD
CDP-ME
IspE
CDP-MEP
IspF
MEcPP
IspG
HMB-PP
IspH
DMAPP
IPP
idi
IspA
GPP
FPP
CrtE
GGPP
CrtB
Phytoene
CrtI
Lycopene
CrtY
β-Carotene
CrtZ
Zeaxanthin
CrtW
Canthaxanthin
Astaxanthin
Acetyl CoA
AtoB
Acetoacetyl CoA
HMGS
HMg CoA
HMGR
Mevalonate
MK
Mev-P
PMK
Mev-PP
PMD
Pyc
Ppc
Mqo
OAA
Citrate
Isocitrate
α-KG
aceA
Succinate
Fumarate
Malate
GDH
Glutarate
GAD
GABA
L-Ile
Ido
4-HIL
AspA
Aspartate
PanD
β-Alanine
Chorismate
Phenylalanine
PAL
Cinnamic acid
C4H/CPR
Tyrosine
TAL
p-Coumaric acid
4CL
p-Coumaroyl CoA
CHS
Malonyl CoA
matC/matB
Malonate
STS
Resveratrol
Naringenin chalcone
CHI
IFS
Genistein
Naringenin
FNS
Apigenin
F3'H
Luteolin
F3H/N3DOX
Dihydrokaempferol
FLS
Kaempferol
F3'5'H
Myrecetin
F3'H
Dihydroquercetin
Quercetin
DFR
Leucoanthocyanidin
LCR
Catechins
ANS
Cyanidin
FGT
Anthocyanin
Tryptophan
TDC
Tryptamine
STR
Strictocidine
CYP450/CYP79B2
Aldoxime
CytP450/CYP83B1
S-alkyl thiohydroximate
Lyase
Thiohydroximate
UGT
Desulfo GLS
ST
Indole GLS
Tyr
L-DOPA
DODC
Dopamine
MAO
Norlaudanosoline
6'OMT
Hydroxylcoclaurine
CNMT
Hydroxymethyl coclaurine
4'OMT
Reticuline
BBE
Scolurine
CFS
Chelanthifolin
SPS
Stylopine
MSH
Protopine
P6H
DHS
DBOX
Sangolnarine
CYP80G2
S-Corytuberine
C-N-Methyl transferase
S-Magnoflorine
SAS
Salutaridine
SAR
Salutaridinol
SAT
S-70 acetate
Thebaine
CODM
Oripavine
T6ODM
Morphinone
COR
Morphine
Neopine
Spontaneous
Codeinone
Codeine
Stearic Acid (C18:1 n9)
Δ9D
Oleic Acid (C18:1 n9)
Δ12D
LA (C18:2 n6)
Δ15D
ALA (C18:3 n3)
EPA (C20:5 n3)
Δ17D
ARA (C20:4 n6)
DHA (C22:6 n3)
Δ8 or Δ6 pathway
Aerobic desaturase/ elongase pathway
Anaerobic polyketide synthase pathway
PUFA synthase gene cluster
Malonyl CoA
DHA (C22:6 n3)
EPA (C20:5 n3)

Fig. 11.1 Pathway engineering targets for microbial-based production of various nutraceuticals. Arrows with solid line represent reaction in the presence of indicated enzyme (green) and the arrows with dashed line represent multistep reaction. Enzymes and their substrate shown are—*G3P* glyceraldehyde 3-phosphate, *PEP* phosphoenolpyruvic acid, *HMg CoA* 3-hydroxymethyl-3-glutaryl coenzyme A, *MEV-P* mevalonate-5-Phosphate, *MEV-PP* mevalonate pyrophosphate, *DXP* 1-Deoxy-D-xylulose 5-phosphate, *MEP* 2-C-methylerythritol 4-phosphate, *CDP-ME* 4-diphosphocytidyl-2-C-methylerythritol, *CDP-MEP* 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate, *MEcPP* 2-C-methyl-D-erythritol 2,4-cyclodiphosphate, *HMB-PP* (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate, *IPP* isopentenyl pyrophosphate, DMAPP: Dimethylallyl pyrophosphate, *GPP* geranyl pyrophosphate, *FPP* farnesyl pyrophosphate, *GGPP* geranylgeranyl pyrophosphate, *OAA* oxaloacetic acid, *α-KG* α-ketoglutarate, *GABA* gamma-Aminobutyric Acid, *4-HIL* 4 Hydroxyisoleucine, *G6P* glucose-6-phosphate, *E4P* erythrose 4-phosphate, DAHP: 3-deoxy-d-arabino-heptulosonate-7-phosphate, DOPA: 3,4-dihydroxyphenylalanine, *DHS* dihydrosanguinarine, *GLS* glucosinolate, *LA* linoleic acid, ALA: α-linolenic acid, ARA: arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexanoic acid, *Dxs* DXP synthase, *Dxr* DXP reductoisomerase, *IspD* 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase, *IspE* 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, *IspF* 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, IspG: HMB-PP synthase, IspH: HMB-PP reductase, *AtoB* acetoacetyl-CoA thiolase, *HMGS* HMG-CoA synthase, *HMGR* HMG-CoA reductase, *MK* mevalonate-5-kinase, *PMK* phosphomevalonate kinase, *PMD* mevalonate-5-pyrophosphate decarboxylase, IPP isomerase (Idi), *IspA* isoprenyl diphosphate synthases crtE: geranylgeranyl pyrophosphate synthase, *CrtB* phytoene synthase, *CrtI* phytoene desaturase, *CrtY* lycopene cyclase, *CrtZ* β-carotene 3-hydroxylase, *CrtW* β-carotene ketolase, *Ppc* phosphoenolpyruvate carboxylase, *Pyc* pyruvate carboxylase, *GDH* glutamate dehydrogenase, *GAD* glutamate decarboxylase, *ido* isoleucine dioxygenase, *Mqo* malate:quinone oxidoreductase, *aceA isocitrate lyase AspA* L-aspartase, *PanD* L-aspartate-α-decarboxylase, *TYR* tyrosine reductase, *DODC* dopamine decarboxylase, *MAO* monoamine oxidase, *6'OMT* norcoclaurine 6-Omethyltransferase, *CNMT* S-coclaurine-N-methyltransferase, *4'OMT* 3-hydroxy-N-methylcoclaurine 4'O-methyltransferase, *BBE* berberine bridge enzyme, *CFS* cheilanthifoline synthase, *SPS* stylopine synthase, *MSH* methyl stylopine hydroxylase, *P6H* protopine 6 hydroxylase, *DBOX* dihydrobenzophenanthridine oxidase, *SAS* salutaridine synthase, *SAR* salutaridine reductase, *SAT* salutaridinol acetyl transfersae, *CODM* codeine o-demethylase, *T6ODM* Thebaine 6-0-demethylase, *COR* codeinone reductase, *CYP80G2* S-corytuberine synthase, *UGT* glucosyl transferase, *ST* sulfotransferase, *TDC* tryptophan decarboxylase, *STR* strictosidine synthase, *TAL* tyrosine ammonia lyase, *PAL* phenylalanine ammonia lyase, *4CL* coumaryl CoA ligase, *STS* stilbene synthase, *C4H* cinnamate-4-hydroxylase, *CPR* cytochrome P450 reductase, *CHS* chalcone synthase, *IFS* isoflavone synthase, *CHI* chalcone isomerase, *FNS* flavone synthase, *F3'H* flavonoid 3′ hydroxylase, *F3H* flavanone 3 hydroxylase, *N3DOX* N3 dioxygenase, *FLS* flavonol synthase, *F3'5'H* flavonoid 3′ 5′ hydroxylase, *DFR* dihydroflavonol reductase, *LCR* leucoanthocyanin reductase, *ANS* anthocyanidin synthase, *FGT* flavonoid synthase, *Δ9D* delta 9 desaturase, *Δ12D* delta 12 desaturase, *Δ15D* delta 15 desaturase, *Δ17D* delta 17 desaturase

11.2.1 EPA and DHA

Microbes have been engineered for EPA and DHA biosynthesis using both aerobic desaturase/elongase pathway and anaerobic polyketide synthase (PKS) pathway. The advancement in metabolic engineering techniques has made possible the modification of PUFA biosynthetic pathway in native organisms as well as introduction of the pathway in unnatural host organisms. Several groups have engineered bacteria with PUFA synthase genes of bacterial and microalgal origin for de novo biosynthesis of ω3LC-PUFAs. The first PUFA synthase gene cluster responsible for EPA biosynthesis was discovered in *Shewanella pneumatophori* SCRC-2738 and cloned in *E. coli* for recombinant bacterial EPA production (Yazawa 1996). Heterologous expression of PUFA synthase gene clusters from *S. pneumatophori* SCRC-2738, *Moritella marina* MP-1, or *Schizochytrium* sp. has been reported in *Escherichia coli*, *Pseudomonas putida,* and *Synechococcus* sp. Amiri-Jami et al. have identified and cloned PUFA synthase gene cluster from a marine bacterium *Shewanella baltica* MAC1, first into *E. coli* then into probiotic strains of *E. coli* and *Lactococcus lactis*, resulting in production of both EPA and DHA (Amiri-Jami and Griffiths 2010; Amiri-Jami et al. 2014). The gene cluster from DHA-producing marine bacterium *Colwellia psychrerythraea* 34H has also been cloned in *E. coli* that resulted in production of DHA (Wan et al. 2016). Peng et al. (2016) have demonstrated the role of phosphopantetheinyl transferase gene *pfaE* irrespective of its source in DHA production by expressing gene cluster of *C. psychrerythraea* 34H in *E. coli*. In a study of coexpression of DHA-producing gene cluster from *M. marina* MP-1 and EPA-producing gene cluster from *S. pneumatophori* SCRC-2738, PfaB suggested to be the key enzyme in determining the type of final product (Orikasa et al. 2009). Hayashi et al. (2016) described the important role of the structure as well as number of acyl carrier protein domains in PUFA yield. In these studies the yield of ω3LC-PUFAs was quite low to be used in commercial production.

Scientists have also cloned genes of aerobic biosynthetic pathway in yeast like *Saccharomyces cerevisiae* for EPA production with very less yield (Tavares et al. 2011). This requires the sequential modification of native fatty acids like oleic acid by expressing up to seven genes from conventional (Δ6 pathway) as well as alternate (Δ8 pathway) pathway. Xue et al. (2013) reported fairly high yield of EPA (56.6% of the total fatty acids and 15% of DCW) in *Yarrowia lipolytica* by increasing the copy number of overexpressed genes including twenty desaturase genes, eight elongase genes, and two cholinephosphotransferase genes of alternate (Δ8 pathway) pathway. Further, elimination of β-oxidation leads the carbon flux towards EPA biosynthesis pathway in *Y. lipolytica* and this engineered strain Z5567 was capable of producing 50% EPA of the total fatty acids and 25% of DCW under nitrogen limiting conditions (Zhu and Jackson 2015; Xie et al. 2016). This EPA produced using this technology has been commercialized as New Harvest™ EPA oil and Verlasso® sustainably farmed salmon. The biosynthesis of DHA in alternative host *Y. lipolytica* by modifying the desaturase/elongase pathway is generally associated with less production of <6% of total fatty acid (Damude et al. 2009). Although, marine microalgae are a good source of DHA with 30–40% of total fatty acid but the

genetic manipulation in the microalga is complicated. Therefore, research was mainly focused on strain selection and optimization of fermentation conditions to obtain increase in biomass as well as DHA yield. Metabolic engineering of native DHA producer *Schizochytrium* spp. has been performed by employing techniques like *Agrobacterium*-mediated transformation, electroporation, and particle bombardment (Yan et al. 2013; Ren et al. 2015; Zhang et al. 2018c; Li et al. 2018b). Introduction of ω-3 desaturase gene in *Schizochytrium* sp. increased ω-3/ω-6 ratio to 2.58 from 2.1 by converting 3% of docosapentaenoic acid (DPA, ω-6 fatty acid) to DHA (Ren et al. 2015). To avoid oxidation of PUFA produced by *Schizochytrium* sp. PKU#Mn4, antioxidative gene superoxide dismutase was overexpressed in the native strain with 1.37-fold increase in PUFA content (Zhang et al. 2018c). Another strategy is overexpression of malonyl-CoA: ACP transacylase (MAT) in *Schizochytrium* that increased the PUFA content by 24.5% by diverting the carbon flux towards PUFA synthesis. The engineered strain produced 47.39 g/L DHA and 1.65 g/L EPA using glucose fed-batch fermentation (Li et al. 2018b). Recently, Wang et al. (2019) engineered *Schizochytrium* sp. S31 for increased lipid content via enhancing NADPH supply by overexpressing a gene encoding malic enzyme from *Crypthecodinium cohnii* along with overexpression of codon-optimized *ELO3* gene encoding for elongase enzyme from *Mortierella alpina* for converting C_{16} into C_{18} fatty acids thereby blocking the inhibitory effect on acetyl-CoA carboxylase. This strategy enhanced the DHA production to 3.54 g/L (Wang et al. 2019).

11.3 Carotenoids

Carotenoids, belonging to terpenoids, are natural yellow- to red-colored pigments produced by plants, algae, fungi, and several bacteria. These are ubiquitously found liposoluble pigments with several physiological benefits including photosynthesis, photoprotection, coloration, cell signaling, provitamin A nutrients, and protection of cells by quenching of free radicals and singlet oxygen. According to Carotenoids Database, there are 1182 known natural carotenoids from 700 source organisms (Yabuzaki 2017). In addition to nutraceutical value due to their antioxidant properties, carotenoids are also used in food processing and cosmetics industries as food colorants, food and cosmetics additives, and feed supplements. As nutraceuticals, carotenoids have anti-cancer, anti-infectious, and anti-inflammatory properties and thus are reported to be effective against certain cancer, cardiovascular diseases, atherosclerosis, degenerative pathogenesis (e.g., Alzheimer's and Parkinson's), and diabetes. The global market for carotenoids is expected to grow from $1.5 billion in 2017 to $2.0 billion by 2022 (McWilliams 2018). Currently, carotenoids are either obtained from their natural sources or some of them are synthesized chemically from petrochemical precursors, for instance eco-efficient commercial production of enantiopure astaxanthin from α-isophorone and vinylbutinol (Jackson et al. 2008).

Carotenoids can be categorized based on their chemical composition as carotenes that are purely hydrocarbons having no oxygen such as lycopene and β-carotene, and

xanthophylls such as lutein, zeaxanthin, and astaxanthin (Das et al. 2007). Generally, these chemicals are classified as C30, C40, C45, and C50 carotenoids among which tetraterpene carotenoids C40 are the most abundant that include lycopene, β-carotene, zeaxanthin, astaxanthin, lutein, and β-cryptoxanthin. The four stages of carotenoids synthesis are precursor supply, skeleton desaturation, terminal cyclization, and product tailoring. All carotenoids are biosynthesized from isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) that can be synthesized via two distinct pathways. One is 2-*C*-methyl-D-erythritol 4-phospahte (MEP) pathway also known as the 1-deoxy-D-xylulose-5-phosphate (DXP) pathway mostly present in majority of bacteria and plant plastids, and the other one is mevalonic acid (MVA) pathway found in some bacteria, archaea, fungi, plant cytoplasm, and other eukaryotes. To one DMAPP molecule three IPPs are condensed head-to-tail in a series of prenyltransferase reactions and at first generate Geranyl pyrophosphate (GPP, C10), then Farnesyl pyrophosphate (FPP, C15) and finally geranylgeranyl pyrophosphate (GGPP, C20) by isoprenyl diphosphate synthases. Head to head condensation of two GGPPs in the presence of phytoene synthase leads to the production of colorless phytoene (C40) which is converted into red-colored lycopene after four-step desaturation by phytoene desaturase and ζ-carotene desaturase, and two isomerizations by 15-*cis*-ζ-carotene isomerase and carotenoid isomerase. Whereas, in bacteria, these four-step desaturations are carried out by carotene desaturase (CrtI) only. Lycopene gets converted into α-, β-, γ-, or δ-carotene by the addition of β- or ε-rings at its chain ends in the presence of different lycopene cyclases. These carotenes can further form a variety of xanthophylls by hydroxylases and ketolases.

Although, plants are the major source of carotenoids and also several microbes have been identified to produce various carotenoids, but owing to their slow growth rate and increased demand of these molecules, construction of designer microbes for their production is required due to advantage of fast growth rate, cost effectiveness, and eco-friendly production. Moreover, microbes can produce the two basic building blocks, IPP and DMAPP, naturally. Metabolic engineering of microbes also allow the heterologous production of new carotenoids by assembling the genes from different organisms. Metabolic engineering of non-carotenogenic genetically tractable microorganisms including *Escherichia coli*, *Saccharomyces cerevisiae,* and *Yarrowia lipolytica* for the production of these valuable carotenoids has been extensively performed.

11.3.1 Lycopene

Lycopene is a red-colored pigment which provides color to several fruits and vegetables such as tomato, watermelon, and papaya. As the common precursors for carotenoid production are IPP and DMAPP, therefore several approaches have been used to increase the content of these molecules to achieve desirable yield of the carotenoids in *E. coli*. *E. coli* has MEP pathway for the production of these precursor molecules, in which DXP synthase (Dxs) and IPP isomerase (Idi) are the major rate-

limiting native enzymes. Therefore, first approach employed engineering of the native MEP pathway in *E. coli* to increase IPP and DMAPP supply and expression of the enzymes required for condensation of these molecules into lycopene (Kajiwara et al. 1997; Kim and Keasling 2001; Kang et al. 2005; Jin and Stephanopoulos 2007). Overexpression of the two rate-limiting enzymes, Dxs and Idi, leads to increase in the supply of precursors, thereby increased the content of FPP (Alper et al. 2005). Lycopene can then be produced from FPP by introducing genes for geranylgeranyl pyrophosphate synthase (CrtE), phytoene synthase (CrtB), and phytoene desaturase (CrtI) of the carotenoid production pathway. Through metabolic engineering of MEP pathway in *E. coli*, 260 mg/L of lycopene production was achieved after 60 h in a high cell density fermentation which was very less compared to 0.93 g/L lycopene produced by carotenogenic fungus *Blakeslea trispora* after 5 days (Kim et al. 2009; Tereshina et al. 2010). In another approach, heterogenous MVA pathway either from yeast *S. cerevisiae* or from bacteria has been introduced in *E. coli* for overproduction of carotenoids. The yeast MVA pathway has HMGR as the rate-limiting enzyme, replacing of which with the bacterial enzyme along with cofactor optimization improved the carotenoid production (Pitera et al. 2007; Ma et al. 2011). An engineered *E. coli* strain capable of producing a high titer of 1.44 g/L lycopene was developed by introducing the mevalonate and lycopene pathway using a new targeted engineering strategy (Zhu et al. 2015).

Heterologous production of lycopene in non-oleaginous hosts like *S. cerevisiae* is hindered due to its lipophilic nature, thereby limiting its accumulation in cells due to cell storage capacity or cytotoxicity. Recently, *S. cerevisiae* has been engineered for lycopene production via cloning of *crtE* from *Taxus x media*, *crtB* from *Pantoea agglomerans,* and *crtI* from *B. trispora* along with overexpression of genes to divert carbon flux towards acetyl-CoA and eventually to mevalonate precursors, and overexpression of *POS5* to increase intracellular production of the cofactors. Further, overexpression of fatty acid desaturase *OLE1* to increase the supply of unsaturated fatty acid and knocking out the seipin gene *FLD1* for regulation of lipid-droplet size resulted in lycopene production of 2.37 g/L and 73.3 mg/g cdw in a two-stage fed-batch fermentation after optimization of the engineered strain (Ma et al. 2019). In the first stage, the strain was allowed to accumulate biomass and in the second stage ethanol was added for lycopene production. In an EPA-producing oleaginous yeast, *Y. lipolytica*, overexpression of codon-optimized *CrtE, CrtB, and CrtI* leads to production of lycopene to 2 mg/g DCW (Ye et al. 2012; Sharpe et al. 2014). The lycopene production increased to 16 mg/g DCW along with accumulation of lipid bodies when the codon-optimized *CrtE, CrtB, CrtI,* and HMG were integrated in an engineered strain (pox1–pox6 and gut2) (Matthaus et al. 2014).

11.3.2 β-Carotein

β-Carotene, a precursor of vitamin A, is an orange colored compound found in many fruits and vegetables like peach, mandarin, broccoli, carrot, and pumpkin.

β-Carotene can be synthesized in a heterologous host by introducing genes for lycopene production and *CrtY* gene for a lycopene cyclase. *E. coli* has been engineered by integrating genes for β-carotene synthesis (CrtEXYIB from *Pantoea agglomerans*), MEP pathway (Dxs and Idi), and central metabolic modules of carbon sources assimilation (ATP synthesis, pentosephosphate pathway, and TCA cycle) to increase supply of precursors, IPP and DMAPP, and cofactors, ATP and NADPH, for improved β-carotene production of 2.1 g/L with a yield of 60 mg/g DCW in a fed-batch culture (Zhao et al. 2013). The study also suggested more effect of increased NADPH supply than ATP on improving carotenoid production. In another study, β-carotene production was increased by 4.5-fold in *E. coli* after incorporating the T5 promoter for the rate-limiting genes of the MEP pathway in order to increase the isoprenoid flux (Suh 2012). Overexpression of both, the optimized MEP pathway (dxs and fni from *Bacillus subtilis*) along with GPPS2 from *Abies grandis* and the hybrid MVA pathway in *E. coli* for increased precursor (IPP, DMAPP) supply allows accumulation of 3.2 g/L β-carotene in a glycerol fed-batch fermentation (Yang and Guo 2014). The strategy of using MVA pathway from bacteria has also been used for the production of β-carotene. By expressing the bottom portion of MVA pathway of *Streptococcus pneumoniae* along with the β-carotene synthesis genes, an *E. coli* strain was developed capable of producing 102 mg/L lycopene and 503 mg/L β-carotene after MVA supplementation (Yoon et al. 2007). When complete MVA pathway from different sources, top portion from *Enterococcus faecalis* and bottom portion from *S. pneumoniae*, wase constructed, the engineered *E. coli* could produce 465 mg/L β-carotene at 2% (w/v) glycerol concentration (Yoon et al. 2009). Li et al. (2015b) incorporated genes for β-carotene synthetic pathway, MEP pathway, and central metabolic pathways using CRISPR–Cas9 meditated genome editing of *E. coli* and obtained a strain with 15 genomic modifications capable of producing 2 g/L β-carotene in fed-batch fermentation.

In addition to *E. coli*, certain yeast strains like *S. cerevisiae*, *Pichia pastoris,* and *Yarrowia lipolytica* have also metabolically engineered to produce β-carotene. Earlier, an engineered *S. cerevisiae* developed by expressing the bacterial *crtE*, *crtB*, *crtI,* and *crtY* genes in episomal expression vectors could produce only103 μg β-carotene/g DCW (Yamano et al. 1994). Verwaal et al. (2007) integrated the genes for crtE, crtI, and crtYB (a bi-functional phytoene synthase and lycopene cyclase) from red yeast *Xanthophyllomyces dendrorhous* into the *S. cerevisiae* chromosome along with isoprenoid genes encoding tHMG1 and GGPP synthase and achieve production of 5.9 mg β-carotene/g DCW. The production of 14.3 mg of β-carotene/L was obtained by co-expressing the genes of β-carotene synthesis pathway from *X. dendrorhous* with the mvaK1 gene encoding mevalonate kinase from *Staphylococcus aureus* (Lange and Steinbuchel 2011). The expression of MVA pathway genes could also be improved by inhibiting sterol biosynthesis. Combining the expression of HMG-CoA reductase gene with the addition of inhibitor ketoconazole increased β-carotene production by >200% (Yan et al. 2011). Xie et al. (2014) engineered *S. cerevisiae* to produce 7.41 mg β-carotene/g DCW by constructing a controllable β-carotene biosynthetic pathway using decentralized assembly strategy. Expression of β-carotene biosynthesis in non-carotenogenic

yeast *Pichia pastoris* led to production of 339 μg β-carotene/g DCW and 1.141 μg lycopene/g DCW by the engineered strain (Araya-Garay et al. 2011). These results suggested that *E. coli* is the better host for β-carotene production than the yeasts like *S. cerevisiae* and *P. pastoris*.

Owing to the lipophilic nature of β-carotene, an oleaginous yeast *Y. lipolytica* has also been explored as host for its production due to their ability to carry carotenoids in lipid droplets (Matthaus et al. 2014; Xu et al. 2016). The optimized biosynthetic genes for β-carotene synthesis (CrtE, CrtB, CrtI, and CrtY) were expressed in an EPA-producing *Y. lipolytica* to produce 5.7 mg β-carotene/g DCW (Sharpe et al. 2014). An engineered *Y. lipolytica* capable of producing 4 g/L β-carotene was developed by sequential multiple-copy integration of native genes encoding GGS1 (GGPP synthase) for enhanced GGPP precursor supply and genes for carRP (bi-functional phytoene synthase/lycopene cyclase) and carB (phytoene dehydrogenase) from *Mucor circinelloides* for β-carotene synthesis from GGPP along with strong promoters (Gao et al. 2017). Similarly, two car-cassettes containing genes for GGS1, CarB, and CarRP under the control of TEF1 promoter were integrated into an engineered lipid overproducer *Y. lipolytica* strain (JMY3501) harboring a copy of car-cassette under different promoters and a truncated version of HMG1 under the control of TEF1 promoter (Larroude et al. 2018). The resulting strain was capable of producing 454.36 mg/L of β-carotene with 61.1 mg/g DCW yield and reached to 6.5 g/L in fed-batch fermentation.

11.3.3 Zeaxanthin

Zeaxanthin (3,3′-dihydroxyl-β-carotene), a xanthophyll carotenoid, provides yellow color to the food items like corn, mangoes, saffron, bell peppers, apricots, peaches and orange, etc. The enzyme β-carotene 3-hydroxylase (crtZ) catalyzes the conversion of β-carotene to zeaxanthin by hydroxylation of its β-ionone rings. Therefore, the genes which are required for the synthesis of zeaxanthin in bacteria include *crtE*, *crtB*, *crtI*, *crtY,* and *crtZ* (Fig. 11.1). These five genes from *Pantoea ananatis* for zeaxanthin synthesis along with the three genes for dxs, idi, and ispA from *E. coli* for increased carbon flux through MEP pathway were overexpressed in both *E. coli* and a lycopene-tolerant mutant of *Pseudomonas putida* under the control of L-rhamnose-inducible promoter (Beuttler et al. 2011). Here, the lycopene-tolerant mutant used as lycopene was found to be toxic for *P. putida*. Among these, the engineered *E. coli* could produce 2.4 mg/g CDW, while, the engineered *P. putida* could produce 7.0 mg/g CDW (51 mg/L) under optimized conditions. Further, the production of zeaxanthin by the engineered *P. putida* was increased by 4.7-fold from 51 mg/L to 239 mg/L when supplemented with lecithin. Earlier, the coexpression of dxs or dxr and idi of MEP pathway along with the genes for zeaxanthin biosynthesis in *E. coli* led to production of 1.6 mg zeaxanthin/g DCW (Albrecht et al. 1999). In another approach, substrate channeling using fusion proteins as well as tunable intergenic regions (TIGRs) approach for coordinating expression of *crtY* and *crtZ* was employed to engineer lycopene producing *E. coli* LYCOP (Chen et al. 2013) for

the production of zeaxanthin from lycopene, among which the latter approach was found to be more effective by producing 1.84 mg zeaxanthin /g DCW (Li et al. 2015a). Later, the production of zeaxanthin was improved to 11.95 mg/g DCW by integrating *P. ananatis crtY* gene into the *E. coli* chromosome and transforming the codon-optimized *crtZ* gene from *P. ananatis* under the control of the P37 promoter (Li et al. 2015a). Further, overexpression of all the six MVA pathway genes from *S. cerevisiae* in this engineered *E. coli* strain using dynamically control TIGR mediated approach increased the zeaxanthin production to 23.16 mg/g DCW in 5.0 L fed-batch fermentation (Shen et al. 2016). Pollmann et al. (2017) used a dual engineering strategy for zeaxanthin production in a β-carotene accumulating mutant of *Xanthophyllomyces dendrorhous* which was developed by blocking synthesis of astaxanthin from β-carotene. In this strain, β-carotene synthesis was increased by overexpression of the genes for HMGR, crtE, and crtYB and then zeaxanthin was produced from β-carotene with a yield of 517 μg/g DCW in a shake flask culture by introducing codon-optimized *crtZ* genes from bacteria. Similarly, Breitenbach et al. (2019) blocked the astaxanthin synthesis in *X. dendrorhous* by knocking out both the alleles of astaxanthin synthase gene and also by using spontaneous mitotic recombination. This strain was then engineered to produce zeaxanthin by randomly incorporating codon-optimized bacterial *crtZ* gene either into the rDNA to reach copy number of 10 or integrating its eight copies directly into astaxanthin synthase alleles. Using both of these procedures along with insertion of *crtB* gene, the engineered strain could produce 5.2 mg/g DCW of zeaxanthin. Several attempts have also been made to increase the content of zeaxanthin in native producers via metabolic engineering. Sarnaika et al. (2018) increased the production of zeaxanthin in a native producer *Synechococcus elongatus* PCC 7942 by overexpressing β-carotene oxygenase gene (*CrtR*) for the conversion of β-carotene to zeaxanthin. Further, they cloned *GalP* gene for Hexose-H^+ symporter from *E. coli* for the uptake of extracellular glucose by the obligate photoautotrophic cyanobacterium. Zeaxanthin production in the resulting transformant was reached to 9.02 mg/g DCW under auxotrophic condition compared to 4.65 mg/g DCW production by the WT.

11.4 Polyphenols

Polyphenolic compounds (PCs) are natural phytochemicals possessing one or more hydroxyl groups containing aromatic rings with several health-promoting effects like antioxidant and anti-inflammatory properties. These secondary metabolites of plants are products of shikimate and phenylpropanoid pathways which are involved in their defense mechanism. They can be broadly categorized as flavonoids and non-flavonoids, among which flavonoids are the most diverse and broadly distributed group. The basic structural organization of flavonoids is C6–C3–C6 (known as diphenylpropane skeleton) containing two C6 aromatic rings A and B joined together by three carbons forming a heterocyclic ring C (Cheng et al. 2014). The flavonoids can be further categorized as flavanones, flavonols, flavones, flavanols, isoflavones, and anthocyanins based on hydroxylation pattern and

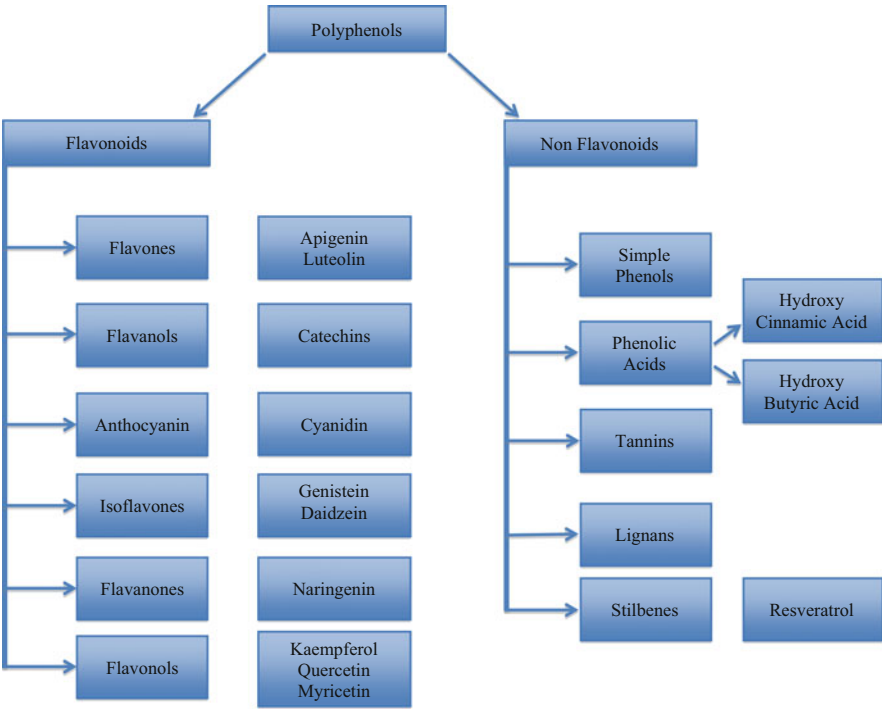

Fig. 11.2 Classification of polyphenols and their respective subcategories along with examples

differences in ring C (Fig. 11.2) (Panche et al. 2016). Different polyphenols have several different major health beneficial effects based on their structural variations and glycosylation pattern. Curcumin, resveratrol, and catechins have shown anti-obesogenic effects and also help in treatment of neurodegenerative disorders such as dementia and Alzheimer's like diseases, while, curcumin, catechins, myricetin, ginsenosides, and ginkgetin are effective in protecting against Parkinson's disease, Alzheimer's disease, and Huntington's disease. Moreover, flavonoids possess anti-cancer properties and have been shown to reduce breast, prostate, and colorectal cancer (Cory et al. 2018). All the flavonoids have major role in improving cardio-vascular health and anthocyanins particularly are associated with management and prevention of type 2 diabetes (Cory et al. 2018). Based on these diverse and useful properties, the global market of polyphenols is valued at $757 million in 2015 and expected to reach $1121 million by 2022 (Prasad 2017). As a result of increasing demand and direct connection of polyphenols to health, these natural plant products have attracted the attention of various researchers to seek novel ways for their enhanced production. Among PCs, quercetin, catechin, and kaempferol have received most research interest, majorly by the researchers of USA, China, and India (Rasouli et al. 2017, "Phenol explorer" database http://www.phenol-explorer.eu). Large scale industrial production of flavonoids from plants is

associated with limitations like requirement of specific climatic conditions for growth, long cultivation period, and low production. Therefore, researchers are focused to develop microbial cell factories for increased production of a particular flavonoid, either by incorporating codon-optimized metabolic machinery from the natural PCs producers or constructing enzyme chimeras that are suitable for bacterial expression. Metabolic engineering of *E. coli* and *S. cerevisiae* has been widely performed by introducing or reconstructing pathways for sustainable production of almost all kinds of PCs (Table 11.1).

Polyphenols are biosynthesized by combination of shikimic acid pathway that forms phenylpropanoids and acetic acid pathway for simple phenol synthesis. The ring B and chromane ring C are synthesized from phenylalanine, and ring A is synthesized on condensation of malonyl CoA with 4-coumaryl-CoA to form naringenin chalcone with an open ring C which is further closed to form naringenin by the enzyme chalcone isomerase (Tsao 2010; Cutrim and Cortez 2018).

11.4.1 Flavanones

Naringenin, belonging to flavanone subclass, is widely distributed in citrus fruits and tomatoes with several pharmacological activities like antioxidant, anti-diabetic, anti-inflammatory, antimicrobial, antiatherogenic, antidepressant, memory improving, antitumor, hypolipidemic, hypocholesterolemic, and antihypertensive (Salehi et al. 2019). Naringenin is the central precursor that can be converted to other forms of flavonoids. Biosynthesis of naringenin starts with conversion of aromatic amino acid L-tyrosine and phenylalanine to *p*-coumaric acid followed by its sequential condensations with malonyl-CoA (Lyu et al. 2017). Therefore, production of this molecule in heterologous microbial host either requires overexpression of phenylalanine ammonia lyase (PAL) for cinnamic acid production from phenylalanine and cytochrome P-450-dependent cinnamate-4-hydroxylase (C4H) to synthesize *p*-coumaric acid which has a limitation of less expression of C4H in bacteria, or overexpression of tyrosine ammonia lyase (TAL) for direct conversion of tyrosine to *p*-coumaric acid (Fig. 11.1). *p*-Coumaric acid then can be converted to naringenin by incorporating genes for 4-coumarate:CoA ligase (4CL), chalcone synthase enzyme (CHS, a type III polyketide synthase), and chalcone isomerase (CHI). Overexpression of TAL, 4CL, CHS, and CHI into engineered L-tyrosine producing *E. coli* resulted in production of 29 mg/L naringenin using glucose as carbon source, while after addition of cerulenin, a fatty acid inhibitor, increased the production to 84 mg/L (Santos et al. 2011). Koopman et al. (2012) reported production of 200 μM of naringenin using glucose as sole carbon source from *S. cerevisiae* CEN.PK engineered with overexpression of flavonoid biosynthetic genes *PAL1, C4H, CPR1* (cytochrome P450 reductase gene)*, 4CL3, CHS3,* and *CHI1* derived from *A. thaliana* and TAL1 derived from *Rhodobacter capsulatus* along with alleviation of tyrosine feedback inhibition and deleting genes for phenylpyruvate decarboxylase (Aro10, Pdc5, Pdc6). The extracellular titer of naringenin from this engineered strain reached to 400 μM in 2 L batch bioreactor. In another study, an engineered *E. coli*

Table 11.1 Metabolically engineered microbes for the production of polyphenols

Target product	Metabolic engineered host	Biosynthetic gene	Source of gene	External precursor	Titer (mg/L)	References
Flavanones						
Naringenin	*S. cerevisiae*	PAL, C4H, CPR, 4CL, CHS, CHI	*A. thaliana*	Glucose	108.9	Koopman et al. (2012)
		TAL	*Rhodobacter capsulatus*			
	E. coli	TAL	*Rhodotorula glutinis*	Glucose	100.64	Wu et al. (2014)
		4CL	*Petroselinum crispum*			
		CHS	*Petunia hybrida*			
		CHI	*Medicago sativa*			
		MatB, MatC	*Rhizobium trifolii*			
	S. cerevisiae	TAL	*Rhodosporidium toruloides*	Xylose	21.16	Zhang et al. (2017)
		4CL	*P. crispum*			
		CHS, CHI	*Petunia x hybrid*			
Flavonols						
Kaempferol	*E. coli*	FNS	*P. crispum*	Tyrosine	15.1	Miyahisa et al. (2006)
		F3H, FLS	Citrus			
	E. coli	FLS	*A. thaliana*	p-Coumaric acid	0.3	Leonard et al. (2006)
		CHI, CHS	Petunia			
		4CL	Parsley			
		F3'5'H, CPR	*Catharanthus roseus*			

(continued)

Table 11.1 (continued)

Target product	Metabolic engineered host	Biosynthetic gene	Source of gene	External precursor	Titer (mg/L)	References
	S. cerevisiae	PAL	*Populus trichocarpa*	Phenylalanine	1.3	Trantas et al. (2009)
		C4H, 4CL, IFS, CHI	*Glycine max*	Naringenin	4.6	
	S. cerevisiae	F3H	*Astragalus mongholicus*	Glucose	26.57	Rodriguez et al. (2017)
		FLS	*A. thaliana*			
	S. albus	TAL	*R. capsulatus*	–	0.212	Marın et al. (2018)
		4CL	*S. coelicolor*			
		CHS, CHI	*Glycine max*			
		N3DOX	*P. crispum*			
		FLS	*A. thaliana*			
Quercetin	*E. coli*	F3'H	*C. roseus*	p-Coumaric acid	0.05	Leonard et al. (2006)
	S. cerevisiae	F3'H	*G. max*	Phenylalanine	Traces	Trantas et al. (2009)
	S. cerevisiae	FMO	*P. hybrida*	Glucose	20.38	Rodriguez et al. (2017)
	S. albus	F3'H	*A. thaliana*	–	Traces	Marın et al. (2018)
Myricetin	*S. albus*	F3',5'H	*Petunia hybrida*	–	0.146	Marın et al. (2018)

Flavones						
Apigenin	*E. coli*	FNS	*Petroselinum crispum*	Tyrosine	13	Miyahisa et al. (2006)
		TAL	Yeast			
		4CL	*S. coelicolor*			
		CHS	Licorice			
	S. venezuelae	4CL	*S. coelicolor*	p-Coumaric acid	15.3	Park et al. (2011)
		CHS	*A. thaliana*			
		FNS	*P. crispum*			
	E. coli	4CL	*Oryza sativa*	p-Coumaric acid	30	Lee et al. (2015)
		CHS	*Populus euramericana*			
		FNS	Parsley			
	S. albus	FNS	*P. crispum*	–	0.089	Marin et al. (2017)
		F3'H	*A. thaliana*			
Flavan 3-ols						
Catechin	*E. coli*	F3H, DFR, LAR	*C. sinensis*	Glucose	0.36	Umar et al. (2012)
	E. coli	F3H	*C. sinensis*	Naringenin	374	Zhao et al. (2015)
		DFR	*Anthrium andraeanum*			
		LAR	*Desmodium uncinatum*			

(continued)

Table 11.1 (continued)

Target product	Metabolic engineered host	Biosynthetic gene	Source of gene	External precursor	Titer (mg/L)	References
Anthocyanin						
Cyanidin	*E. coli*	F3H, ANS	*Malus domestica*	Naringenin	0.006	Yan et al. (2005)
		DFR	*A. andraeanum*			
		F3GT	*P. hybrida*			
	C. glutamicum	ANS	*P. hybrida*	Catechin + glucose	40	Zha et al. (2018)
		3GT	*A. thaliana*			
Isoflavones						
Genistein	*S. cerevisiae*	CHS	*Glycyrrhiza echinata*	Galactose, *p*-coumaroyl-NAC	0.34	Katsuyama et al. (2007b)
		CHI	*Pueraria lobata*			
		IFS	*G. echinata*			
	E. coli	PAL	Yeast	Tyrosine	6 (using co-culture of *E. coli* and *S. cerevisiae*)	Katsuyama et al. (2007b)
		4-CL	*S. coelicolor*			
		Acetyl CoA carboxylase, CHS, CHI	*C. glutamicum*			
	S. cerevisiae	IFS	*G. echinata*			
Stilbenes						
Resveratrol	*E. coli*	4CL	*Nicotiana tobaccum*	p-Coumaric acid	16	Beekwilder et al. (2006)
		STS	*Vitis vinifera*			

	E. coli	4CL	*A. thaliana*	p-Coumaric acid	105	Watts et al. (2006)
		STS	*Arachis hypogaea*			
	E. coli	4CL	*A. thaliana*	p-Coumaric acid + cerulenin	2340	Lim et al. (2011)
		STS	*V. vinifera*			
	E. coli	TAL	*Saccharothrix espanaensis*	p-Coumaric acid	1.4	Choi et al. (2011)
		4CL	*S. coelicolor*			
	E. coli	TAL	*R. glutinis*	Tyrosine	35.02	Wu et al. (2013)
		4CL	*P. crispum*	Glycerol		
		STS	*V. vinifera*	Glucose		
	E. coli	PAL, C4H, 4CL	*A. thaliana*	Glucose	812	Li et al. (2016)
		Resveratrol synthase	*Vitis vinifera*			
	E. coli	TAL	*Trichosporon cutaneum*	Glucose	304.5	Wu et al. (2017)
		4CL	*P. crispum*			
		STS	*V. vinifera*			
	C. glutamicum	TAL	*F. johnsoniae*	Glucose + cerulenin	59	Braga et al. (2018)
		4CL	*Petroselinum*			
		STS	*A. hypogaea*			

Abbreviations: *PAL* phenyl ammonia lyase, *TAL* tyrosine ammonia lyase, *CPR* cytochrome p450 reductase, *4CL* coumaryl CoA ligase, *CHS* chalcone synthase, *CHI* chalcone isomerase, *N3DOX* N3 dioxygenase, *FLS1* flavonoid synthase, *F3',5'H flavonoid 3,5 hydroxylase*, *DFR* dihydroflavonol reductase, *LCR* leucocyanidin reductase, *ANS* anthocyanidine synthase, *FGT* flavonoid glycosyl transferase, *FNS* flavone synthase, *F3H-F* flavanone3 hydroxylase, *C4H* cinnamate-4-hydroxylase, *IFS* isoflavone synthase, *FMO* flavonoid monooxygenase, *STS* stilbene synthase, *LAR* leucoanthocyanidin reductase, *F3GT* flavonoid 3-*O*-glucosyltransferase

strain was developed by introducing combinatorially tuned 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase (DAHPS), chorismate mutase/prephenate dehydrogenase (CM/PDH), TAL, 4CL, CHS, CHI, malonate synthetase (matB), and malonate carrier protein (matC) using modular pathway engineering strategy. Further, the introduction of feedback resistant $tyrA_{fbr}$- $aroG_{fbr}$ cassette in this engineered *E. coli* for increased intracellular tyrosine supply led to production of 100.64 mg/L naringenin directly from D-glucose (Wu et al. 2014). Recently, a synergistic co-culture system has been developed using engineered tyrosine producing *E. coli* and naringenin-producing *S. cerevisiae* which allowed production of 21 mg/L naringenin from xylose. Here, *S. cerevisiae* harbors gene for 4CL integrated into its genome as well as in the form of plasmid. In this system, *E. coli* uses xylose as carbon source and releases acetate which is further utilized by *S. cerevisiae* (Zhang et al. 2017). In view of more favorable characteristics of sulfate conjugate of flavonoids, a naringenin 7-sulfate producing *E. coli* strain was developed by expressing gene for sulfotransferase (ST) from *Arabidopsis thaliana* along with repression of gene for 3′-phosphoadenosine-5′-phosphosulfate (PAPS) ST using CRISPRi metabolic engineering strategies. The resulting engineered *E. coli* strain produced 135.49 μM (47.7 mg/L) naringenin 7-sulfate in a 3-L fermenter after 36 h (Chu et al. 2018).

11.4.2 Flavonols

Flavonols such as quercetin, kaempferol, and myricetin are among the most popular flavonoids which are ubiquitously found in plants and have several health-promoting effects. Anti-inflammatory properties of kaempferol and quercetin are linked to their ability to inhibit tyrosine kinase, required during macrophage proliferation and that of myricetin and quercetin to inhibit lipoxygenases, involved in production of pro-inflammatory leukotrienes and hepoxilins (Comalada et al. 2006; Landolfi et al. 1984). All these three flavonols have been reported to block angiogenesis as suggested by their capability to inhibit vascular endothelial growth factor (Kim et al. 2006). Kaempferol is biosynthesized from naringenin in two steps via flavanone 3-hydroxylase (F3H) that acts on naringenin to produce dihydrokaempferol which is further catalyzed by flavonol synthase (FLS) (Fig. 11.1) (Holton et al. 1993; Lee et al. 2014; Pandey et al. 2016). Kaempferol can further be converted to quercetin and myricetin by the enzymes flavonoid 3′-hydroxylase (F3'H) and flavonoid 3′,5-′-hydroxylase (F3'5'H), respectively. Moreover, F3'H can also catalyze dihydrokaempferol to synthesize dihydroquercetin, which can be converted to quercetin by the action of FLS (Fig. 11.1). The enzyme FLS from *Ginkgo biloba* and *Citrus unshiu*, exhibiting bi-functional activity produced kaempferol in *E. coli* directly from naringenin (Lukacin et al. 2003; Xu et al. 2012). Coexpression of genes for 4CL, CHS, CHI, F3H, and FLS along with a fusion protein of P450 flavonoid 3′,5′-hydroxylase (F3'5'H) and P450 reductase produced 0.3 mg/L kaempferol and 0.05 mg/L of quercetin in *E. coli* from the substrate p-coumaric acid (Leonard et al. 2006). Upon supplementation with the flavanone naringenin in place of

phenylpropanoid acids, the engineered *E. coli* could also produce myricetin (Leonard et al. 2006). The genes encoding for full pathway enzymes for kaempferol production from phenylalanine along with the genes to increase malonyl- CoA supply, including PAL, 4CL, CHS, CHI, F3H, FLS, and acetyl CoA carboxylase, overexpressed in *E. coli* synthesized 15.1 mg/L of kaempferol (Miyahisa et al. 2006). Moreover, gene for flavone synthase I (FNS1) from *Petroselinum crispum* was also expressed in this engineered *E. coli* strain to produce flavones (13 mg/L apigenin and 9.4 mg/L chrysin). Kaempferol production of 1.3 mg/L from phenylalanine and 4.6 mg/L from naringenin was obtained from an engineered *S. cerevisiae* strain developed by employing similar strategy of cloning the genes of complete pathway (Trantas et al. 2009). A naringenin-producing *S. cerevisiae* strain was engineered by cloning F3H from *Astragalus mongholicus* and FLS from *Arabidopsis thaliana* for de novo production of kaempferol directly from glucose with a titer of 26.57 ± 2.66 mg/L (Rodriguez et al. 2017). Incorporation of cytochrome P450 flavonoid monooxygenase (FMO) from *Petunia hybrida* fused with cytochrome P450 reductase (CPR) from *Catharanthus roseus* in this kaempferol-producing strain produced 20.38 ± 2.57 mg/L quercetin from glucose (Rodriguez et al. 2017). Actinomycetes were also designed to produce these three flavonols. Two actinomycetes *Streptomyces coelicolor* and *S. albus* were engineered for kaempferol biosynthesis by expressing the six codon-optimized genes including TAL, 4CL, CHS, CHI, N3DOX (naringenin 3-dioxygenase for conversion of naringenin to dihydrokaempferol), and FLS1 under the control of promoters (Marın et al. 2018). Among these two, engineered *S. albus* could produce 0.212 μM kaempferol de novo. Addition of F3'H in the plasmid carrying the above six genes for kaempferol synthesis, resulted in an engineered *S. albus* producing 0.34 μM quercetin along with 0.155 μM kaempferol. The strain was further engineered by inserting gene for F3'5'H that allowed production of 0.146 μM myricetin and 1.984 μM quercetin along with traces of kaempferol and dihydrokaempferol.

11.4.3 Flavones

A plant specialized metabolite found ubiquitous in plant kingdom. Mainly it is present in tea, dried herbs, red wine, and citrus plants. Flavones have major biological activities like antioxidant, antitumor, and anticancerous. Major clinical implication includes anticancerous activity of Flavopiridol (CDK inhibitor), an approved drug for leukemia. Apigenin and luteolin are the major types of flavones. Naringenin is further converted to Apigenin catalyzed by FNS (Flavone synthase) and apigenin further converted to Luteolin by F3'H (Flavone 3'hydroxylase). By metabolic engineering, the production of flavones can be improved. Initial efforts by Miyahisa et al. (2006) for the introduction of the TAL (from yeast), CL (from *S. coelicolor*), CHS (from licorice plant), and flavone synthase I gene (from *Petroselinum crispum*) into *E. coli* cells has led to production of apigenin (13 mg/L) by the engineered strain using precursor tyrosine. Apigenin was synthesized from

p-coumaric acid precursor by introducing genes for 4CL from *Oryza sativa*, CHS from *Populus euramericana,* and FNS from parsley plant in *E. coli*. After optimization of expression, the yield of apigenin was increased from 13 mg/L to 30 mg/L (Lee et al. 2015). Furthermore, Park et al. (2011) clone the genes CL from *S. coelicolor*, CHS from *Arabidopsis thaliana*, and FNS from *Petroselinum crispum* in *S. venezuelae* using p-coumaric acid as a precursor. The titer obtained was 15.3 mg/L. To further improve the production, by cloning the biosynthetic genes in *E. coli* using p-coumaric acid and malonate as a precursor, the yield obtained was 110 mg/L (Leonard et al. 2008). Recently, de novo production of apigenin by heterologous expression of genes in *Streptomyces albus* is determined by introducing flavone synthase I (FSI) and flavone synthase II (FSII), both soluble and membrane bound forms from *P. crispum* without addition of supplements. Overall, the recombinant strain produced 0.089 mg/L of apigenin (Marin et al. 2017). The heterologous production of second flavone i.e. luteolin is 2 mg/L by using caffeic acid as a precursor in *S. cerevisiae* (Leonard et al. 2005) and 4 mg/L by cloning the biosynthetic genes in *E. coli* using p-coumaric acid and malonate as precursors (Leonard et al. 2008). The recent de novo production of luteolin alike apigenin by introducing additional F3'H from *Arabidopsis thaliana* in *S. albus*, the obtained titer was very low in traces without the addition of external precursors (Marin et al. 2017).

11.4.4 Flavan-3-ols

Flavanols, specifically catechin comprises 80–90% of total flavonoids. These are prominently found in green tea, cocoa, and berries. Four major catechins such as epicatechin, epicatechin 3 gallate, epigallocatechin, epigallocatechin 3 gallate (EGCG) are present in green tea (Ashihara et al. 2010). Flavanols have powerful antimicrobial activity for the prevention of infectious diseases, anti-inflammatory properties due to cytokines such as interleukins, and antioxidant properties of EGCG include scavenging of free radicals. They are scientifically proven to support a healthy heart by promoting healthy blood flow. Cocoa flavanols act on blood vessels by increasing production of nitric oxide (NO) causes dilation of blood vessel which is important for regulating blood pressure. A biosynthetic pathway of catechin starts from Phenylalanine by general polyphenol pathway. p-Coumaryl CoA and malonyl CoA are converted to chalcone by CHS (Chalcone Synthase) and further to eriodictyol catalyzed by CHI (Chalcone isomerase). Eriodictyol then converted to catechins via synthesis of intermediates dihydroflavonol and leucoanthocyanin catalyzed by enzymes F3H (Flavanone 3 hydroxylase), DFR (Dihydro flavonol reductase) and LCR (Leucoanthocyanin reductase). Metabolic engineering of catechins biosynthetic pathway by the introduction of these three important genes F3H, DFR, LCR from *Camellia sinensis* in *E. coli* BL(DE3) produced 0.36 mg/L of epicatechin gallate (Umar et al. 2012). Further, to improve the titer of catechins, constructs containing three F3H from *C. sinensis*, three DFR from *Anthurium andraeanum*, *C. sinensis*, or *Fragaria ananassa*, and two LAR from *Desmodium uncinatum* were cloned in *E. coli* and either eriodictyol or naringenin were used as

substrate. By adding more copies of genes for DFR and LAR, the titer of catechin increased to 374.6 ± 43.6 mg/L and further reached to 760.9 ± 84.3 mg/L by increasing NADPH availability (Zhao et al. 2015).

11.4.5 Anthocyanins

A class of plant specialized metabolites, a pigment which gives rich coloring to different plants such as blue (*Delphinium*), red (*Anthurium*), and purple (*Verbena*). These are found in red onions, blueberries, kidney beans, and grapes. Anthocyanins have powerful antioxidant, anti-inflammatory, antiviral, and anticancerous attributes. For example, blueberries are good for the treatment of breast cancer cells. Additionally, they are promising in the prevention of cardiovascular disorders by improving cholesterol levels. Examples of anthocyanins include cyanidin 3-*O*-glucoside (C3G) and Callistephin (red pigment in strawberry). Their extraction heavily relies on plant tissues. A suitable alternative is their sustainable production in microbes by metabolic manipulation. Biosynthesis of anthocyanins is a part of phenyl propanoid pathway. p-Coumaryl CoA and malonyl CoA are converted to Chalcone by CHS (Chalcone Synthase) and further to eriodictyol catalyzed by CHI (Chalcone isomerase). Eriodictyol is converted to catechins via the synthesis of intermediates Dihydroflavonol and leucoanthocyanin catalyzed by enzymes F3H (Flavanone 3 hydroxylase), DFR (Dihydro flavanol reductase). Further ANS (Anthocyanidin synthase) catalyzes the conversion step of Leucoanthocyanin to Cyanidin and finally converted to Anthocyanins by FGT (Flavonoid glucosyl transferase). In 2005, Yan et al. cloned the genes F3H and ANS from *Malus domestica*, DFR from *Anthurium andraeanum*, and F3GT (flavonoid 3-*O*-glucosyltransferase) from *Petunia hybrida* and expressed them in *E. coli*. The recombinant *E. coli* strain produced 6.0 μg/L of cyanidin 3-*O*-glucoside (C3G) using naringenin as a precursor. Further, in 2015, Lim et al. (2015) improved the production of cyanidin-3-O-glucoside by adding catechin as precursor. The cloning of two genes ANS from *Malus domestica* and F3GT from *Petunia hybrida* in *E. coli* improved the titer to 350 mg/L. Further, the connection of complete biosynthetic pathway was remodeled for the first time using sugar as a starter. It was accomplished by polyculturing of *E. coli*, combining 15 pathway enzymes from diverse plants and other microbes for the production of Callistephin. The titer obtained was 10 mg/L of Callistephin by combining four modules, first containing TAL, second has CL, CHS, and CHI, third containing LAR, F3H, and DFR, fourth one has 3GT, ANS (Jones et al. 2017). Safety issues in *E. coli* emphasize on the need for alternative high production host system. *Corynebacterium glutamicum* 13,032 was used to synthesize C3G by cloning genes ANS from *Petunia hybrida* and 3GT from *Arabidopsis thaliana*. Optimized expression improved the C3G production to 40 mg/L using Catechin as a precursor (Zha et al. 2018).

11.4.6 Isoflavonoid

Isoflavonoids are found in leguminosa family, predominantly in red clover, soya products, fruits, and vegetables. They have major clinical health implications including cancer prevention via anti-inflammatory and estrogen activity. Also, it is associated with the prevention of cardiovascular disorders and diabetes. Important isoflavonoids include Genistein and Daidzein. Biosynthesis of both isoflavones is a part of phenyl propanoid pathway. Biosynthesis starts with the key entry point naringenin. IFS (Isoflavone synthase) acts on naringenin for the production of Genistein. IFS in combination with CHI leads to the synthesis of Daidzein. Genistein was produced by cloning of genes for CHS and IFS from *Glycyrrhiza echinata*, and CHI from *Pueraria lobata* in *S. cerevisiae* using N-acetylcysteamine-attached p-coumarate (p-coumaroyl-NAC) and galactose as supplement. The recombinant yeast cells produced 340 μg/L of Genistein (Katsuyama et al. 2007b). Another work by the same research team, used co-incubation of engineered *E. coli* and *S. cerevisiae* for the production of genistein using tyrosine as a precursor. Co-incubation of *E. coli* cells containing a PAL gene from yeast, a 4-coumarate CoA ligase gene from *S. coelicolor*, CHS, CHI, and acetyl-CoA carboxylase genes from *Corynebacterium glutamicum*, and *S. cerevisiae* cells containing IFS gene were co-incubated resulted in the production of 6 mg/L of genistein supplemented with tyrosine.

11.4.7 Stilbenes

The stilbenes are phytoalexins, which are the antimicrobial products of plants. It includes resveratrol and piceatannol. Resveratrol commonly found in seeds of raspberries, red wine, skin of grapes, peanuts, etc. are associated with protecting brain by preventing neurodegenerative disorders. Additionally, they are associated with easing joint pain, suppressing the growth of prostate and colon cancer cells, increasing insulin sensitivity to treat diabetes. Due to the limitations such as low concentration, susceptibility to plant diseases, and product stability associated with its extraction from plant sources, the gateways for microbial production are opened. Also, chemical methods of extraction suffer from complexity of structure. A variety of approaches have been explored for the production of resveratrol. Biosynthetic pathway of resveratrol initiated from production of 3-deoxy-d-arabino-heptulosonate-7-phosphate (DAHP) by combination of PEP and E4P in the presence of enzyme DAHP synthase. The product of shikimic acid pathway, p-coumaryl CoA combined with three molecules of malonyl CoA results in the synthesis of Resveratrol catalyzed by STS (Stilbene synthase). Initial work by Beekwilder et al. in 2006, by cloning genes CL from *Nicotiana tobaccum*, STS from *Vitis vinifera* (grapevine) in *E. coli* using p-coumaric acid as a supplement resulted in the production of 16 mg/L of resveratrol. Further, Watts et al. (2006) cloned the genes for CL from *A. thaliana* and STS from *A. hypogaea* in *E. coli* and obtained a titer of 105 mg/L of resveratrol using p-coumaric acid as a precursor. Furthermore, Lim et al. in (2011), improved the titer to 1380 mg/L by expressing CL from *A. thaliana* and STS from *Vitis vinifera* using p-coumaric acid

as a precursor. Again, under the same conditions, CL from *A. thaliana* and STS from *V. vinifera* using p-coumaric acid and cerulenin as a precursor obtained the yield of 2340 mg/L. Choi et al. (2011), by cloning the genes from different sources i.e. TAL from *Saccharothrix espanaensis* and CL from *Streptomyces coelicolor* using p-coumaric acid as a precursor produced 1.4 mg/L resveratrol. The yield of 35.02 mg/L was obtained by functional expression of TAL from *R. glutinis*, CL from *P. crispum* and STS from *V. vinifera* in *E. coli* using tyrosine as a precursor (Wu et al. 2013). In addition to *E. coli*, resveratrol was also synthesized via metabolic engineering of several other hosts. Li et al. (2016) cloned the genes PAL, CL from *A. thaliana* and ACS from *S. enterica* in *S. cerevisiae* with the supplementation of glucose yielded 812 mg/L of resveratrol. Cloning and functional expression of TAL from *Trichosporon cutaneum*, CL from *P. crispum,* and STS from *V. vinifera* in *E. coli* using glucose as a precursor yielded a titer of 304.5 mg/L (Wu et al. 2017). Furthermore, *E. coli* harboring genes CL from *Lithospermum erythrorhizon*, STS from *A. hypogaea*, ACC from *C. glutamicum,* and F3H and FLS from citrus was functionally expressed with the supplementation of cinnamic acid achieved a titer of 155 mg/L (Katsuyama et al. 2007a). Recently, Braga et al. (2018) cloned the genes TAL from *F. johnsoniae*, CL from *Petroselinum,* and STS from *A. hypogaea* in *C. glutamicum* with the supplementation of glucose and cerulenin as a precursor and yielded 59 mg/L of resveratrol.

11.5 Alkaloids

Plants are a variegated source of secondary metabolites having physiological activities. Many of which exhibit anodyne properties like morphine, stimulatory effects of caffeine, anti-bacterial and anti-inflammatory properties of berberine, anticancerous properties of vinblastine and vincristine, and antimalarial activities of quinine. They also have additional protective effects related to biotic (herbivore and pathogenic microorganisms) and abiotic stress resistance (oxidative stress, temperature stress). Aromatic amino acids like phenylalanine, tyrosine, and their derivatives are the major sources of a number of pharmaceutically important alkaloids. Beyond these, basic amino acids like arginine and lysine, ornithine and nucleosides are the starting precursor to others. Alkaloids are present in vascular plants, they are omnipresent, no geographical boundation related to sourcing. Regardless of it, their production suffers many obstacles; first is related to isolation of alkaloids, second is their low levels of productivity and quality, and final is resolution of complex mixture into pure components. To combat this, microorganisms can be used as versatile biofactories to produce them in a biologically controlled environment, which ensure high levels of productivity. There are three major classes of nutraceutically relevant alkaloids namely benzylisoquinoline alkaloids (BIA), monoterpeneindole alkaloids (MIA), and glucosinolates. The major focus of metabolic engineering is on altering the genes encoding basic biosynthetic enzymes for the production of alkaloids (Table 11.2).

Table 11.2 Metabolically engineered microbes for the production of alkaloids

Target product	Metabolic engineered host	Biosynthetic gene	Source of gene	External precursor	Titer (mg/L)	References
BIA						
S-Reticulin	*E. coli*	MAO	*Micrococcus luteus*	Dopamine	55	Minami et al. (2008)
		NCS, 4'OMT, CNMT, 6'OMT	*Coptis japonica*			
	S. cerevisiae	4'OMT, CNMT, 6'OMT	*Papaver somniferum*	Norlaudanosoline	32	Hawkins and Smolke (2008)
	E. coli	MAO	*M. luteus*	Glycerol	46	Nakagawa et al. (2011)
		NCS, 4'OMT, CNMT, 6'OMT	*C. japonica*			
		TYR	*Streptomyces*			
		DODC	*Pseudomonas putida*			
	E. coli	MAO	*M. luteus*	Dopamine	54	Kim et al. (2013)
		NCS, 4'OMT, CNMT, 6'OMT	*C. japonica*			
	S. cerevisiae	NCS, 4'OMT, CNMT, 6'OMT	*P. somniferum*	Norlaudanosoline	0.659	Fossati et al. (2014)
	S. cerevisiae	NCS, 4'OMT, CNMT, 6'OMT	*P. somniferum*	Glucose	0.0806	Deloache et al. (2015)
		DODC	*P. putida*			
		CytP450	*Beta vulgaris*			
	E. coli	MtrA	*Bacillus subtilis*	Dopamine	160	Matsumura et al. (2018)
		PTPS, SPR	Rat			
Scoulerine	*S. cerevisiae*	MAO	*M. luteus*	Dopamine	7.2	Minami et al. (2008)
		NCS, 4'OMT, CNMT, 6'OMT, BBE	*C. japonica*			

Magnoflorine	*S. cerevisiae*	MAO	*M. luteus*	Dopamine	8.3	Minami et al. (2008)
		NCS, 4'OMT, CNMT, 6'OMT, CYP80G2	*C. japonica*			
Stylopine	*S. cerevisiae*	ATR1	*Arabidopsis thaliana*	Norlaudanosoline	0.676	Trenchard and Smolke (2015)
		CFS, STS	*Eschscholzia californica*			
		4'OMT, CNMT, 6'OMT, BBE	*P. somniferum*			
	S. cerevisiae	NCS, 4'OMT, CNMT, 6'OMT, P450R	*P. somniferum*	(S)-Scoulerine	0.614	Fossati et al. (2014)
		P6H	*E. californica*			
Salutaridine	*S. cerevisiae*	CPR, CYP2D6	*Homo sapiens*	Norlaudanosoline	20	Hawkins and Smolke (2008)
		4'OMT CNMT, 6'OMT	*P. somniferum*			
	S. cerevisiae	SAS, CPR	*P. somniferum*	R-Reticuline	4.911	Fossati et al. (2015)
Sanguinarine	*S. cerevisiae*	4'OMT, CNMT, 6'OMT, MSH, TNMT, BBE	*P. somniferum*	Norlaudanosoline	0.080	Trenchard and Smolke (2015)
		CFS, STS, P6H	*E. californica*			
		ATR1	*A. thaliana*			
Dihydrosanguinarine	*S. cerevisiae*	P6H	*E. californica*	Stylopine	1.9	Fossati et al. (2014)
		TNMT, MSH, P450R	*P. somniferum*			
	S. cerevisiae	P6H	*E. californica*	(S)-Scoulerine	0.257	Fossati et al. (2014)
		TNMT, MSH, P450, CFS, SPS	*P. somniferum*			
Morphine	*S. cerevisiae*	SAS, CPR, SAR, SAT, CODM, 6'ODM, CoR	*P. somniferum*	Codeine	0.143	Fossati et al. (2015)

(continued)

Table 11.2 (continued)

Target product	Metabolic engineered host	Biosynthetic gene	Source of gene	External precursor	Titer (mg/L)	References
Neopine	*S. cerevisiae*	SAS, CPR, SAR, SAT, CODM, 6'ODM, CoR	*P. somniferum*	Salutaridine	0.009	Fossati et al. (2015)
Thebaine	*S. cerevisiae*	SAS, CPR, SAR, SAT	*P. somniferum*	R-reticuline	0.311	Fossati et al. (2015)
Protopine	*S. cerevisiae*	NCS, 4'OMT CNMT, 6'OMT, MSH	*P. somniferum*	Glucose	0.252	Trenchard and Smolke (2015)
		CFS, STS, TNMT	*E. californica*			
		ATR1	*A. thaliana*			
MIA						
Strictosidine	*S. cerevisiae*	TDC, STR, GES, G8H, GOR, ISY, IO, DLGT, DLH, LAMT, SLS	*Catharanthus roseus*	Glucose	0.53	Brown et al. (2015)
		GPP	*Abies grandis*			
		FPS	*Gallus gallus*			
Indolylglucosinolates						
Glucosinolate	*S. cerevisiae*	Cyt P450, GST, Lyase, UGT	*A. thaliana*	Glucose	1.03	Mikkelsen et al. (2012)

Abbreviations: *MAO* monoamine oxidase, *NCS* norclaurine synthetase, *6'OMT* norcoclaurine 6-Omethyltransferase, *4'OMT* 3-hydroxy-N-methylcoclaurine 4'O-methyltransferase, *CNMT* (*S*-coclaurine-*N*-methyltransferase), *TYR* tyrosine reductase, *DODC* dopamine decarboxylase, *BBE* berberine bridge enzyme, *ATR* P450 reductase, *CFS* cheilanthifolinesynthase, *STS* stylopine synthase, *P6H* protopine 6 hydroxylase, *CPR* cytochrome P450 reductase, *TNMT* tetrahydroprotoberberine N-methyltransferase, *SAS* salutaridine synthase, *SAR* salutaridine reductase, *SAT* salutaridinol acetyl transfersae, *CODM* codeine o-demethylase, *T6ODM* thebaine 6-0-demethylase, *CoR* codeinonereductase, *CYP80G2-S* corytuberine synthase, *TDC* tryptophan decarboxylase, *STR* strictosidine synthase, *GES* geranoil synthase, *G8H* geraniol hydroxylase, *GOR* geraniol oxidoreductase, *ISY* iridoid synthase, *IO* iridoid oxidase, *DLGT* deoxyloganetic acid glycosyltransferase, *DLH* deoxyganic acid hydroxylase, *LAMT* loganic acid methyl transferase, *SLS* secologanin synthase, *CPR* cytochrome p450, *CYB5* cytochrome *B5 GPP* geranyl pyrophosphate, *FPS* farnesyl pyrophosphate synthetase, *UGT* glucosyltransferase, *GLS* G sulfotransferase, *MtrA* GTP cyclohydrolase, *PTPS* pyruvoyltetrahydropterin synthase, *SPR* sepiapterinreductase

11.5.1 Benzylisoquinoline Alkaloids (BIA)

One of the structurally most divergent group of nutraceutically significant alkaloids is the benzylisoquinoline alkaloids (BIAs). It includes the narcotic anodyne compounds like codeine and morphine and the antimicrobial agents sanguinarine and palmatine. These BIA products are synthesized through S-reticuline. Tyrosine is the starting precursor for the synthesis of S-reticuline. Biosynthesis of BIA starts with the conversion of tyrosine to DOPA to Dopamine by DODC (Dopamine decarboxylase) and then conversion to Norlaudanosoline via DHPPA (dihydroxyphenylacetaldehyde) by enzymes MAO (Monoamine oxidase) and NCS (Norcoclaurine synthetase). Norlaudanosoline can then be further converted to S-reticulin by enzymes 6'OMT (Norcoclaurine 6-O-methyltransferase), 4'OMT (3-hydroxy-N-methylcoclaurine 4'O-methyltransferase). S-Reticulin is a major key regulatory intermediate, a starting substrate for the synthesis of berberine, morphine, codeine, and palmatine. S-Reticulin is converted into S-coclaurine by BBE (Berberine bridge enzyme) and further conversion to sanguinarine via intermediates stylopine, protopine, and DHS (dihydrosanguinarine) by enzymes cheilanthifoline synthase (CFS), stylopine synthase (SPS), tetrahydroprotoberberine N-methyltransferase (TNMT), methyl stylopine hydroxylase (MSH), protopine 6 hydroxylase (P6H), dihydrosanguinarine oxidase (DBOX). S-Reticulin can also be further converted to morphine and codeine via thebaine by enzymes salutaridine synthase (SAS), salutaridine reductase (SAR), salutaridinol acetyl transferase (SAT), codeine-o-demethylase (CODM), thebaine 6-o-demethylase (T6ODM), and codeinone reductase (COR). S-Reticulin can also be converted to magnoflorine by S-corytuberine synthase (CYP80G2). Microbial production of S-reticulin by metabolic engineering is one of the strategy other than plant genetic transformations. Microbial production is generally highly efficient because of the shorter generation time in comparison to plant tissue culture. In addition, microbial production does not require additional complex growth supplements. Therefore it has attracted worldwide interest of researchers to enhance the product efficiency and yield. Initial efforts by Minami et al. (2008), for the development of a combined system to produce desired benzylisoquinoline alkaloids, *S*-reticuline was synthesized from dopamine by cloning the genes for MAO from *Micrococcus luteus*, NCS, OMT, CNMT from *Coptis japonica* in *Escherichia coli*. The production titer of *S*-reticuline was 55 mg/L using dopamine as precursor. Furthermore, for the production of magnoflorine and scoulerine, from dopamine via reticuline, genes encoding BBE and CYP80G2 from *Coptis japonica* were cloned in *S. cerevisiae* (Minami et al. 2008). The yields obtained were 7.2 and 8.3 mg/L. These results indicate that the production via microbes incorporating plant genes not only boost mass production but also unlock the gateways for the unique benzylisoquinoline alkaloids having pharmaceutical prospective. Further, for the improved production of reticuline and its further BIA metabolites, commercially available substrate i.e. norlaudanosoline is used as a precursor instead of dopamine (Minami et al. 2008). Recombinant enzymes NCS, OMT, and CNMT from *Thalictrum flavum* and *Papaver somniferum* were cloned in *S. cerevisiae* to produce reticuline from norlaudanosoline (Hawkins and Smolke

2008). To this engineered strain, reductase enzymes from *Arabidopsis thialiana* to produce S-scoulerine and human Cyt P450 for the production of Salutaridine were also cloned. It was demonstrated that a human P450 enzyme has a unique activity in converting (R)-reticuline to salutaridine. The production titer of BIA reported here was ~150 mg/L (Hawkins and Smolke 2008). Furthermore, Nakagawa et al. (2011) focused on the (*S*)-reticuline production in *E. coli* grown on a simple medium with no use of the additional precursors like dopamine. Remodeling of biogenesis was done by incorporating tyrosinase gene from *Streptomyces* in L-tyrosine overproducing *E. coli* strain for conversion of tyrosine to dopamine. DODC from *Pseudomonas putida* was inserted in *E. coli* to catalyze subsequent steps. The improved strain was able to produce 46 mg/L of (*S*)-reticuline using glycerol as a precursor. Kim et al. (2013) worked on attuning the rate-limiting steps to achieve higher reticuline (54 mg/L) productivity. Since dopamine was the substrate for both of the MAO and NCS, NCS from *Coptis japonica* and MAO from *Micrococcus luteus* were introduced into *E. coli* to inscribe this shortcoming (Kim et al. 2013). They developed four variant strains of MAO and NCS: pCDFPL-NCS-MAO (Norcoclaurine synthetase and monoamine oxidase), pCDFPL-NCS + pMW118-MAO, pMW118-NCS + pCDFPL-MAO, and pMW118-NCS-MAO. Strain carrying pCDFPL-MAO-NCS shows the highest productivity, which shows that amount of NCS was more critical in comparison to MAO for reticuline production. The reconstitution of a complex biosynthetic pathway for BIA synthesis in *S. cerevisiae* represents an innovative advancement for the production of multiple alkaloids. Fossati et al. (2014) rebuild a ten gene plant pathway in *Saccharomyces cerevisiae* for the production of different alkaloids using different precursors. In reconstitution of the pathway, because of low activity of dihydrobenzophenanthridine oxidase in *S. cerevisiae*, it was omitted and the remaining nine genes were cloned into different plasmids and the cytochrome P450 reductase was cloned into single individual plasmid. Plasmids were individually transformed into *S. cerevisiae* for the expression and cultures were supplied individually with (*R,S*)-norlaudanosoline, *S*-reticuline, *S*-scoulerine, or *S*-stylopine as a precursors. The production of dihydrosanguinarine was 1900 μg/L with stylopine precursor, 257 with scoulerine, 147 with S-reticuline, and 50 with norlaudanosoline as a precursor. Additionally, the production of S-reticuline and stylopine was 659 and 614 μg/L with norlaudanosoline and scoulerine as a precursor. The same work was done for morphian alkaloids in 2015 again by Fossati et al. Here production of morphine was 143 μg/L with codeine as a precursor, neopine was 9 μg/L externally fed with salutaridine, and production of salutaridine was 4911 μg/L with R-reticulin. The sanguinarine pathway was previously reconstituted by Fossati et al., but the optimization of pathways and engineered strains was not done, because of multistep enzymatic pathways and the challenging job of expression of cytochrome P450. So, Trenchard and Smolke (2015), reconstruct the sanguinarine branch of the BIA pathway in *S. cerevisiae* by improving the various enzymatic steps and their conditions. The achieved titers were 676 μg/L stylopine, 252 μg/L protopine, and 80 μg/L sanguinarine from the engineered yeast strains. Further, the production of S-reticulin from starting precursor tyrosine in yeast has been a challenging task. By

co-culturing of yeast with S-reticulin producing *E. coli*, downstream BIA pathway products such as morphine, sanguinarine have been produced previously. So yeast is the suitable host for downstream BIA products. But the recent advancements demand monoculture approach. Deloache et al. (2015), by taking into account the difficulties faced at two key steps, one is conversion of tyrosine to DOPA and second is poor activity of NCS, cloned Cyt P450 from *Beta vulgaris* and NCS from *Opium poppy* in *S. cerevisiae* for the production of the key intermediate (*S*)-reticuline by using glucose. The production was 80.6 μg/L of S-reticulin and 104.6 μg/L of Norcoclaurine. Further, the improvement in the microbial system producing (*S*)-reticuline was obtained by using the tyrosine overproducing *E. coli* strain. The previous pathways showed degradative effects of some intermediates like DOPA and dopamine, thus tyrosine hydroxylase (TH) from *Drosophila* was cloned in *E. coli* (Matsumura et al. 2018). TH generally requires a cofactor BH4. For the expression of TH in *E. coli*, BH4 biosynthetic genes were separately cloned. S-reticulin production requires the fourteen genes to obtain 160 mg/L of S-reticulin, which was quadruple fold higher than the previously reported. Subsequently, the synthesis of sulfated (*S*)-reticulines by introduction of human sulfotransferases resulted into the improved (*S*)-reticuline production system (Matsumura et al. 2018).

11.5.2 Monoterpeneindole Alkaloids (MIA)

MIA's are nitrogen-containing plant-derived specialized metabolites. Approximately, 1800 compounds of monoterpene alkaloids are known, e.g., vinblastine, ajmaline, ajmalicine, vincamine, quinine, and vincristine. Medical uses include anticancerous properties of vinblastine and vincristine, as they can inhibit nucleic acid synthesis and antiarrhythmic properties. Extraction from the plant confers difficulty, as they are produced in small quantities making isolation challenging. Microbial production strategy involves decarboxylation of tryptophan by TDC (tryptophan decarboxylase) to tryptamine and condensation of tryptamine and secologanin by STR (Strictocidine synthase) to form strictosidine which is further reconstituted into other major products. Secologanin is produced from GPP (geranyl pyrophosphate) via nine steps encoded by nine enzymes GES (Geranoil synthase), G8H (Geraniol hydroxylase), GOR (Geraniol oxidoreductase), ISY (Iridoid synthase), IO (Iridoid oxidase), DLGT (Deoxyloganetic acid glycosyltransferase), DLH (Deoxyganic acid hydroxylase), LAMT (Loganic acid methyl transferase), and SLS (Secologanin synthase). Due to the importance of strictosidine as a regulatory element for all other known MIAs, major focus is on its mass production by metabolic engineering. For this, the first de novo production of Strictosidine in *S. cerevisiae* was determined. Engineered strains have three gene deletions for *ERG20* (encoded for farnesyl pyrophosphate synthase), *ATF1* (alcohol acetyl transferase), *OYE2* (encoded for NADPH oxidoreductase), 15 plant-derived biosynthetic genes (14 plant biosynthetic pathway genes from *Catharanthus roseus*, one GPPS2 from *Abies grandis*), one animal-derived gene mFPS144 (mutant Farnesyl pyrophosphate

from *Gallus gallus*), and five additional copies of yeast genes like HMGCoA reductase, IDI (IPP-DMAPP isomerase), MAF1 (inhibitor of tRNA, as IPP is common substrate for both GPP and tRNA so to reduce the competition, MAF1 is added), SAM2 (S-adenosyl methionine), ZWF (glucose-6-phosphate dehydrogenase to increase NADPH availability).The obtained titer was 0.53 mg/L using glucose as a precursor (Brown et al. 2015).

11.5.3 Indolylglucosinolate (Glucosinolate)

The natural homogenous class of secondary metabolites known to be present in Brassicaceae family of plants, more importantly in vegetables like broccoli and cabbages. They have anticarcinogenic property, activity attributed due to specific Glucosinolates. There are around 30 glucosinolate, out of which, indolylglucosinolate have major clinical implications. It contains central carbon atom, sulfur atom, and nitrogen atom. It is known to have properties like inducing apoptosis by promoting caspase pathway, cell cycle arrest by accumulating the cells at G2/M stage of cell cycle which results in decreasing the formation of DNA, antibacterial and nematocidal properties. They are synthesized from methionine, tyrosine, tryptophan, and phenylalanine. The first reactive hydrolysis product of glucosinolate is isothiocyanate, possessing the nutraceutical potential and regarded as good anticancerous agent (Villarreal-Garcia and Jacobo-Velazquez 2016). Biosynthesis of Indole Glucosinolates starts with the conversion of tryptophan to S-alkyl thiohydroximate via CYP79B2 and CYP83B1 (Cytochrome P_{450}). C-S-Lyase converts S-alkyl thiohydroximate to desulfoglucosinolate by enzyme UGT (S-glucosyltransferase) and finally converted to indole glucosinolate by sulfotransferase. Biosynthetic pathway genes of indolylglucosinolate, CYP79B2, CYP83B1, GST (Glutathione S-transferase), g-glutamylpeptidase, C-S lyase, UGT (S-glycosyltransferase), sulfotransferase which are well characterized in *A. thaliana* were introduced in *S. cerevisiae* and their functional expression in host resulted in the production of glucosinolates. The titer obtained was 1.03 mg/L of indolylglucosinolate using glucose as an externally fed precursor. The first successful production provides a platform for extensive microbial production of indole specific glucosinolates (Mikkelsen et al. 2012).

11.6 Non-Proteinogenic Amino Acid

Besides proteinogenic amino acids that can be assembled into proteins by translational machinery, hundreds of non-proteinogenic amino acids are known from natural sources that are non-coded and not incorporated into natural proteins. Although not found in proteins, many non-proteinogenic amino acids are metabolic intermediates, post-translationally formed in proteins, building blocks of several natural bioactive peptides, and have role in important physiological processes. Some of the non-proteinogenic amino acids like β-alanine, γ-aminobutyrate

(GABA), and 4-hydroxyisoleucine (4-HIL) are known to have several health beneficial effects. β-alanine also known as 3-aminopropionic acid is a naturally occurring β-amino acid which is a structural intermediate between neurotransmitters α-amino acid (glycine) and γ-amino acid (GABA). β-alanine is found in human central nervous system (CNS) and suggested to be a small molecule neurotransmitter (Tiedje et al. 2010). This molecule has a physiological significance as it forms pantothenic acid (vitamin B_5) which is a part of coenzyme A. Moreover, in muscles, β-alanine is found as cytoplasmic dipeptide carnosine (β-alanyl-L-histidine) which is an antioxidant and buffers exercise-induced acidosis. Therefore, β-alanine is an evidence-based supplement that has gained popularity as sports nutrition and its global market has reached $64 million in 2017 and will be going to reach $91 million by 2025 (Hub 2018). GABA, an inhibitory neurotransmitter in the human central nervous system (CNS), is majorly produced by pancreatic β-cells. It has been shown to have role in various physiological functions other than CNS, like lowering blood pressure, inhibition metastasis of cancer cells, improvement in immunity, anti-diabetic, anti-anxiety, diuretic, tranquilizing, and relaxation effects in humans. Moreover, GABA and GABA-enhancing compounds like acid L-theanine could play critical role in brain functions and neurological diseases, and stabilizing mood disorders. These beneficial properties of GABA have made it a popular food supplement with a global market of $64 million by 2025 (Reports 2018) and therefore a strategy is required for its economical production. Other important non-proteinogenic amino acid is 4-hydroxyisoleucine (4-HIL), which was first found in *Trigonella foenum-graecum* (fenugreek) seeds having a unique glucose-dependent insulinotropic activity in type 2 diabetes mellitus rat models. 4-HIL has three chiral centers, therefore, it can exist in eight stereoisomers, among which two stereoisomers have been identified in fenugreek seeds, one of which has 2*S*,3*R*,4*S* configuration that accounts for 90% of total 4-HIL and the other one has a 2*R*,3*R*,4*S* configuration. 4-HIL with 2*S*,3*R*,4*S* configuration exhibits insulinotropic and insulin-sensitizing effects. Insulinotropic effect of 4-HIL is strictly dependent on glucose concentration that is why it prevents undesirable side effects, such as hypoglycemia unlike the current therapies for type 2 diabetes (Maurya et al. 2014). Moreover, 4-HIL has also been found to be effective in treatment of type1 diabetes mellitus model without insulin (Haeri et al. 2012). 4-HIL has exhibited antiobesity activity and is also effective in controlling glycemia, insulinemia and decreased plasma triglyceride and total cholesterol levels in rodent models. Therefore, 4-HIL could be a promising orally active drug candidate for type 1 and type 2 diabetes and diabetic nephropathy.

11.6.1 β-Alanine

Currently, β-alanine is majorly produced by chemical conversions that generally need harsh conditions. Therefore, its green production through enzymatic catalysis is required as it is sustainable and more eco-friendly. Biologically, β-alanine is produced by enzymatic conversion of L-aspartate to β-alanine by decarboxylation in the

presence of enzyme L-aspartate-α-decarboxylase (ADC) (Konst et al. 2009), as a byproduct during conversion of L-alanine to pyruvate and by deamination and carboxylation of the uracil. Another approach is the bioconversion of β-aminopropionitrile to β-alanine using microorganisms like *Alcaligenes* sp. OMT-MY14, *Aminobacter aminobrance* ATCC23314 (Toshihiro et al. 1998), and *Rhodococcus* sp. G20 (Liang et al. 2008). Among these pathways, enzymatic synthesis of β-alanine by ADC from wide variety of microorganisms is the most exploited as it produces less byproducts and is more environment friendly. These microbes included *Bacillus subtilis*, *Corynebacterium glutamicum*, *Escherichia coli,* and *Mycobacterium tuberculosis*, but the production of ADC by these microbes is comparatively low and thereby increasing the production cost of β-alanine. Several studies have reported the recombinant expression of ADC in *E. coli* to increase its yield for β-alanine production. *PanD* gene from *C. glutamicum* and from *E. coli* encoding ADC were overexpressed in both the organisms and the construct of *E. coli* harboring *panD* from *C. glutamicum* showed maximum production of pantothenate with 140 ng of pantothenate mg (dry weight) $^{-1}$ h^{-1} (Dusch et al. 1999). In another study, *panD* gene from *C. glutamicum* expressed in *E. coli* with a specific activity of 103 U/mg showed conversion rate of 97.2% and 12.85 g/l of β-alanine yield after 36 h (Shen et al. 2014). An efficient *E. coli* strain has been developed by expressing *panD* genes encoding ADC from different donor organisms including *E. coli*, *C. glutamicum,* and *B. subtilis*, and optimized to produce 24.8 g/L of β-alanine from 40 g/L L-aspartate after 20 h (Li et al. 2018a). These methods require expensive precursor L-aspartate and large quantity of efficient enzyme for the production of β-alanine and hence are not cost effective. Song et al. (2015) developed a strain for efficient production of β-alanine by cloning the *C. glutamicum panD* gene in fumaric acid-producing *E. coli* along with the strong trc promoter instead of native promoter of ADC. Fumaric acid-producing *E. coli* has an additional advantage of having aspartase activity that can convert fumarate into aspartate thereby increasing the substrate for β-alanine production (Chao et al. 2000). Further, *aspA* gene encoding L-aspartase and *ppc* gene encoding phosphoenolpyruvate carboxylase was also overexpressed along with synthetic promoters and RBS sequences and resulted in production of 3.94 g/L of β-alanine which further increased to 32.3 g/L by fed-batch culture in 39 h. To overcome the high cost of substrate L-aspartate, a dual enzyme cascade route for β-alanine production was employed with ribosome binding site regulation of L-aspartase (*AspA*) from *E. coli* and duplication of L-aspartate-α-decarboxylase (*PanD*) from *C. glutamicum* and an *E. coli* strain was developed that can produce 80.4 gL^{-1} β-alanine from fumaric acid with a conversion rate of 95.3% (Qian et al. 2018). A recombinant strain of *E. coli* was developed by overexpressing L-aspartate aminotransferase (AspC) to produce L-aspartate from oxaloacetic acid and *panD* gene to produce β-alanine from L-aspartate. A titer of 1.01 mol L-aspartate/mol glucose and 1.52 mol β-alanine/mol glucose was achieved by blocking the competitive pathways (Piao et al. 2019). Moreover, recently extracellular production of ADC in *E. coli* using the ADC-encoding gene from an efficient strain of *Bacillus tequilensis* was achieved by using signal peptides and

co-expressing the gene with cutinase from *Thermobifida fusca* (Feng et al. 2019). Signal peptides transport the protein towards periplasmic space from where it can be secreted into medium through leakage, while cutinases help in extracellular production by increasing membrane permeability by catalyzing the hydrolysis of membrane phospholipids. The construct was capable of secreting more than 40% of the L-aspartate-α-decarboxylase extracellularly with total production of 20.3 U/mL. Using this extracellular enzyme >99% mole conversion rate was achieved with 1.5 M substrate concentration.

11.6.2 γ-Aminobutyric Acid (GABA)

GABA has been reported to be produced by plants, animals, and many microbes including fungi, yeast, and bacteria that majorly include lactic acid bacteria. Although various chemical or biological methods have been tried to synthesize GABA, biosynthetic methods could be more promising due to their simplicity, high efficiency, cost effectiveness, and environmental compatibility. GABA is biologically synthesized in a single decarboxylation step from L-glutamate catalyzed by glutamate decarboxylase (GAD) with consumption of a proton in a pyridoxal 5′-phosphate (PLP)-dependent manner. Various attempts have been made to produce GABA using purified GAD enzyme or by whole-cell biotransformation of monosodium glutamate (MSG) into GABA using natural or recombinant microorganisms expressing GAD. The key obstacle in the recombinant production of GABA was low optimal pH range of GAD which is not suitable for growth of host organisms like *E. coli*. Mutation in the Glu_{89} and His_{465} residues (Glu89Gln/Δ452–466) of GadB isomer from *E. coli* through rational mutagenesis resulted in a GAD enzyme that is active up to neutral pH (Ho et al. 2013). Recently, glutamate producing *Corynebacterium glutamicum* has been identified as an efficient host strain for the production of GABA through direct fermentation using renewable carbon sources. Cloning and expression of GAD from *Lactobacillus brevis* in *C. glutamicum* resulted in 26 g/L GABA production in 120 h, while cloning of GAD from *E. coli* in *C. glutamicum* synthesized 31 g/L GABA in 120 h after deleting *pknG* gene for serine/threonine protein kinase G (Okai et al. 2014; Shi et al. 2013). As these GAD enzymes were active only in acidic pH while the host organism grows and produces glutamate at neutral pH, therefore, synthesis of GABA starts in stationary growth phase rather than in exponential growth phase thereby delaying the cultivation time over 120 h. Therefore, Choi et al. (2015) have cloned the mutated *E. coli* GAD (Glu89Gln/Δ452–466) with activity in broad pH range in *C. glutamicum* under the strong synthetic P_{H36} promoter for increased production of GABA from glucose in comparatively less time. After optimization, GABA concentration reached 38.6 g/L in 72 h fed-batch fermentation with 0.536 g/L/h productivity. Similarly, the mutated *E. coli gad* gene expressed in *E. coli* resulted in direct production of 1.08 g/L GABA using 10 g/L glucose in 48 h batch cultivation (Pham et al. 2016). An engineered *E. coli* overexpressing GadB mutant along with conditional interruption of the TCA and glyoxylate cycles and a bypass for precursor

supply, and upregulation of GABA transporter was capable of producing 4.8 g/L of GABA from glucose in batch fermentation with threefold improvement (Soma et al. 2017). Coexpression of *E. coli gadB* mutant gene and *E. coli xylAB* genes in *C. glutamicum* under the control of a synthetic H36 promoter synthesized 35.47 g/L of GABA in batch fermentation using empty fruit bunch (EFB) solution as carbon source (glucose and xylose) (Baritugo et al. 2018). Heterologous expression of a C-terminally truncated GadA mutant ($GadA_{\Delta C14}$) into *Lactobacillus brevis* under the control of the nisin-inducible nisA promoter leads to production of 87.56 g/L GABA by the immobilized cells under the optimum conditions (Lyu et al. 2019).

11.6.3 4-Hydroxyisoleucine (4-HIL)

4-HIL obtained from fenugreek seeds has a low yield of about 150 mg from 1 kg of seeds and thus is not suitable for large scale industrial production (Jette et al. 2009). Other than extraction from fenugreek seeds, insulinotropic stereoisomer (2*S*,3*R*,4*S*)-4-HIL is also synthesized from chemical and chemoenzymatic methods but with the limitations of low yield, high cost, and heavy pollution as these methods employ multiple steps and require expensive substrates and coenzymes. Another method utilizes two enzymes, 4-hydroxy-3-methyl-2-keto-pentanoate aldolase (HPAL) and branched-chain amino acid aminotransferase (BCAT) for 4-HIL production from acetaldehyde, α-ketobutyric acid, and L-glutamate. Although this method synthesizes absolute stereoisomer of 4-HIL in just two steps, but has limitation of low yield as well as production of large quantities of byproduct- α-aminobutyric acid (Smirnov et al. 2007; Ogawa et al. 2007). In fenugreek seedlings, L-isoleucine hydroxylation activity of L-isoleucine dioxygenase (L-isoleucine-4-hydroxylase, IDO) was detected which catalyzes the synthesis of 4-HIL from L-isoleucine. Kodera et al. (2009) have discovered the Fe(II)/α-ketoglutarate-dependent IDO in *Bacillus thuringiensis* 2e2 AKU 0251 which is capable of catalyzing the C-4 hydroxylation of L-isoleucine to form only bioactive (2S,3R,4S)-4-HIL isomer and succinate. The cloning of gene for this stereo-specific IDO in the *E. coli* 2Δ strain lacking the α-ketoglutarate dehydrogenase, isocitrate lyase, and isocitrate dehydrogenase kinase/phosphatase activities leads to complement the destroyed TCA thereby resulting in 82% yield of 4-HIL from L-isoleucine (Smirnov et al. 2010). Coexpression of a gene brnQ encoding branched-chain amino acids (L-valine, L-leucine, and L-isoleucine) along with *ido* gene in an *E. coli* strain with interrupted TCA and glyoxylate cycles resulted in production of 22.96 g/L 4-HIL from 26.20 g/L L-isoleucine (Kivero et al. 2012). The *ido* gene from *B. thuringiensis* YBT-1520 cloned into an L-isoleucine-producing strain, *Corynebacterium glutamicum* ssp. *lactofermentum* SN01 for a single step conversion of its endogenous L-isoleucine to 4-HIL produced 65.44 ± 2.27 mM 4-HIL after 144 h of fermentation and the conversion ratio was 0.85 mol/mol (Shi et al. 2015). Further, coexpression of *ppc* gene for phosphoenolpyruvate carboxylase to enhance oxaloacetate (OAA), a common precursor for L-isoleucine and α-ketoglutarate, increased the production to 95.72 ± 1.52 mM (Shi et al. 2016). The group also found that

coexpression of the aspartate kinase gene *lysC* with *ido-ppc* improved the concentration of isoleucine and other L-aspartate family amino acids but decreased the 4-HIL production. Similarly, expression of an NADH kinase gene from *Saccharomyces cerevisiae* BY4742 for NADPH production increased the L-isoleucine supply but decreased the 4-HIL production which was further increased to 84.14 ± 6.38 mM by improving the supply of α-ketoglutarate by a ΔpknG::ido substitution leading to a conclusion that synergistic improvement in supply of L-isoleucine and α-ketoglutarate is required to increase the yield of 4-HIL (Shi et al. 2018). *C. glutamicum* ssp. *lactofermentum* SN01 was also engineered by overexpressing one *ido* gene from *B. thuringiensis* YBT-1520 and one *ido* gene from *B. weihenstephanensis* KBAB4 to produce 4-HIL, the *mqo* gene for Malate: quinone oxidoreductase to enhance OAA supply from malate and the *vgb* gene encoding *Vitreoscilla* hemoglobin to increase the oxygen uptake rate in cells, and deleting *aceA* gene to enhance α-ketoglutarate supply by blocking glyoxylate cycle (Shi et al. 2019). This strain was capable of producing 112–117 mM 4-HIL after optimization. Zhang et al. (2018a) has also demonstrated the significance of α-ketoglutarate supply for 4-HIL production by developing a recombinant *C. glutamicum* YI capable of producing 5.12 g/L 4-HIL after increasing the supply of OAA via *ppc* overexpression and *pyk2* deletion, and α-ketoglutarate via *gltA* and *icd* overexpression and *aceA* deletion, and reducing the consumption of α-ketoglutarate via glutamate dehydrogenase *gdh2* deletion. The production was further increased to 34.21 g/L by modulating α-ketoglutarate dehydrogenase complex (ODHC) activity (Zhang et al. 2018a). In another strategy, error prone PCR was employed to mutate *ido* gene and the mutated genes were transformed into an *E. coli* strain deleted with the genes *sucA*-encoding α-ketoglutarate dehydrogenase and *aceA*-encoding isocitrate lyase. The resulting transformant was capable of synthesizing 151.9 mmol of 4-HIL/L (22.4 g/L) in 12 h using resting cells (frozen at −80 °C) in the presence of substrates L-isoleucine and α-ketoglutarate (Zhang et al. 2018b).

11.7 Poly Amino Acids

Poly amino acids, a class of polymer, are composed of one or two types of amino acids linked together by amide bonds that unlike proteins are synthesized by ribosome-independent enzymatic processes. Natural poly amino acids including multi-*L*-arginyl-poly (*L*-aspartic acid) (cyanophycin), poly(ε-*L*-lysine), and poly-γ-glutamic acid are produced by various microorganisms. These biosynthetic poly amino acids have gained attention due to their unique and beneficial properties with vast applications in food, agriculture, pharmaceuticals, healthcare, cosmetics, nutrition, and water treatment. Cyanophycin (CGP, cyanophycin granule peptide) is known to be synthesized by most of the cyanobacteria and some heterotrophic bacteria as an intracellular reserve polymer for nitrogen/carbon in nutrition deprived conditions (Du et al. 2017; Altun et al. 2018). These bacteria harbor *cphA* gene encoding cyanophycin synthetase for catalyzing the synthesis of CGP. Naturally, CGP is composed of equimolar amount of two amino acids having a backbone of

poly-L-aspartic acid linked via forming isopeptide bond between its β-carboxyl group and α-amino group of arginine side chains. CGP can be utilized as a precursor for dipeptides and amino acids to achieve nutritional and therapeutics requirements. In addition to aspartic acid (non-essential) and arginine (semi-essential), CGP synthesized in recombinant organisms also contains some amount of an essential amino acid lysine. Since cyanobacteria are not suitable for large scale industrial level cyanophycin production due to their less growth rate, therefore researchers have cloned various cyanobacterial *cphA* gene into well established host strains including *Corynebacterium glutamicum*, *E. coli*, *Pichia pastoris*, *Ralstonia eutropha*, *Rhizopus oryzae, Saccharomyces cerevisiae,* and *Pseudomonas putida* (Aboulmagd et al. 2001; Frey et al. 2002; Steinle et al. 2008, 2010; Meussen et al. 2012; Du et al. 2017; Tseng et al. 2017; Wiefel et al. 2011; Raberg et al. 2018). The first recombinant strain for cyanophycin production was developed by cloning $cphA_{6803}$ gene from *Synechocystis* sp. PCC 6803 into *E. coli* (Ziegler et al. 1998). The cyanophycin produced was rich in aspartate and arginine with minor amount of lysine. An engineered *E. coli* harboring *cphA* gene from *Synechocystis* sp. PCC 6803 was capable of producing 120 mg cyanophycin/gram dry cell weight that contains both soluble and insoluble forms (Tseng et al. 2012). The study revealed that insoluble cyanophycin contained less amount of lysine than the soluble form. Other than $cphA_{6803}$, $cphA_{6308}$ from *Synechocystis* sp. PCC 6308 was also cloned in *E. coli* for heterologous production of cyanophycin resulting on both soluble (11.1% w/w) and insoluble fractions (25.1% w/w) (Frommeyer et al. 2014; Wiefel and Steinbuchel 2014). The $cphA_{6308}$ gene when cloned into *C. glutamicum* synthesized 3.6% (w/w) of cyanophycin, while, in *P. putida* and *R. eutropha* produced 11% and 7% of cell dry matter, respectively, when grown in a medium supplemented with aspartate and arginine (Aboulmagd et al. 2001). The comparison of *P. putida*, *R. eutropha*, *C. glutamicum,* and *B. megaterium* for recombinant production of cyanophycin revealed that the latter two strains were not suitable for heterologous production (Voss et al. 2004). Further, the overexpression of different *cphA* genes including $cphA_{7120}$, $cphA_{6308}$, $cphA_{6803}$, and $cphA_{MA19}$ individually in polyhydroxyalkanoate-negative (PHA) mutants of *P. putida* and *R. eutropha* enhanced the production of cyanophycin to 24% and 22% of cell dry matter, respectively (Voss et al. 2004). Cloning of the $cphA_{49}$ gene encoding cyanophycin with homogenous quality from uncultured bacterium in *E. coli* produced 16% (w/w) cyanophycin of cell dry matter (Du et al. 2013). To increase the production of cyanophycin in *Synechocystis* sp. PCC 6803 strain, arginine supply was improved to a tenfold higher by activation of *N*-acetylglutamate kinase (NAGK), using a mutated PII signaling protein (PII-I86N). This led to accumulation of 57% (w/w) cyanophycin per cell dry mass in the engineered strain designated as *Synechocystis* BW86 with a PII-I86N mutation (Watzer et al. 2015). The cyanobacterial *cphA* genes were also studied for their expression in eukaryotic hosts. Two transgenic strains of *P. pastoris* (GS115 and KM71H) harboring $cphA_{6308}$ gene produced cyanophycin having both soluble and insoluble fractions with higher content of lysine in soluble cyanophycin produced by *P. pastoris* KM71H (Steinle et al. 2010). The $cphA_{6308}$ gene cloned and expressed in *S. cerevisiae* was capable of synthesizing

both soluble and insoluble cyanophycin (Steinle et al. 2008). Furthermore, this gene when cloned in metabolically engineered *S. cerevisiae* strain Car1 (with deleted arginase) and strain Car2 (with deleted ornithine aminotransferase) produced up to 4% (w/w) cyanophycin, while its cloning in strains Arg1 (with deleted argininosuccinate synthetase), Arg3 (with deleted ornithine carbamoyltransferase), and Arg4 (with deleted argininosuccinate lyase) led to production of up to 15.3% (w/w) cyanophycin. The cyanophycin produced by the genetically modified strains Arg1, Arg3, and Arg4 also contains citrulline, ornithine, and lysine, respectively (Steinle et al. 2009; Steinle and Steinbuchel 2010).

References

Abedi E, Sahari MA (2014) Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr 2(5):443–463

Aboulmagd E, Voss I, Oppermann-Sanio FB et al (2001) Heterologous expression of cyanophycin synthetase and cyanophycin synthesis in the industrial relevant bacteria *Corynebacterium glutamicum* and *Ralstonia eutropha* and in *Pseudomonas putida*. Biomacromolecules 2:1338–1342

Albrecht M, Misawa N, Sandmann G (1999) Metabolic engineering of the terpenoid biosynthetic pathway of *Escherichia coli* for production of the carotenoids β-carotene and zeaxanthin. Biotechnol Lett 21(9):791–795

Alper H, Miyaoku K, Stephanopoulos G (2005) Construction of lycopene-overproducing *E. coli* strains by combining systematic and combinatorial gene knockout targets. Nat Biotechnol 23:612–616

Altun M, Wiefel L, Steinbuchel A (2018) Cyanophycin production from feather hydrolysate using biotechnological methods. Prep Biochem Biotechnol 48:589

Amiri-Jami M, Griffiths MW (2010) Recombinant production of omega-3 fatty acids in *Escherichia coli* using a gene cluster isolated from *Shewanella baltica* MAC1. J Appl Microbiol 109:1897–1905

Amiri-Jami M, LaPointe G, Griffiths MW (2014) Engineering of EPA/DHA omega-3 fatty acid production by *Lactococcus lactis* subsp. *cremoris* MG1363. Appl Microbiol Biotechnol 98:3071–3080

Andrew P, DeFilippis MD, Laurence S et al (2006) Understanding omega-3′s. Am Heart J 151:564–570

Araya-Garay JM, Feijoo-Siota L, Rosa-Dos-Santos F et al (2011) Construction of new *Pichia pastoris* X-33 strains for production of lycopene and β-carotene. Appl Microbiol Biotechnol 93 (6):2483–2492

Ashihara H, Deng WW, Mullen W et al (2010) Distribution and biosynthesis of flavan-3-ols in *Camellia sinensis* seedlings and expression of genes encoding biosynthetic enzymes. Phytochemistry 71(5–6):559–566

Backes J, Anzalone D, Hilleman D et al (2016) The clinical relevance of omega-3 fatty acids in the management of hypertriglyceridemia. Lipids Health Dis 15(1):118–129

Baritugo K-A, Kim HT, Yokimiko David Y et al (2018) Enhanced production of gamma-aminobutyrate (GABA) in recombinant *Corynebacterium glutamicum* strains from empty fruit bunch biosugar solution. Microb Cell Factories 17:129

BCC Research Staff (2018) Nutraceuticals: global markets to 2023. https://www.bccresearch.com/market-research/food-and-beverage/nutraceuticals-global-markets.html

Beekwilder J, Wolswinkel R, Jonker H et al (2006) Production of resveratrol in recombinant microorganisms. Appl Environ Microbiol 72:5670–5672

Beuttler H, Hoffmann J, Jeske M et al (2011) Biosynthesis of zeaxanthin in recombinant *Pseudomonas putida*. Appl Microbiol Biotechnol 89(4):1137–1147

Braga A, Oliveira J, Silva R et al (2018) Impact of the cultivation strategy on resveratrol production from glucose in engineered *Corynebacterium glutamicum*. J Biotechnol 265:70–75

Breitenbach J, Pollmann H, Sandmann G (2019) Genetic modification of the carotenoid pathway in the red yeast *Xanthophyllomyces dendrorhous*: engineering of a high-yield zeaxanthin strain. J Biotechnol 289:112–117

Brown S, Clastre M, Courdavault V et al (2015) De novo production of the plant-derived alkaloid strictosidine in yeast. Proc Natl Acad Sci U S A 112:3205–3210

Chao YP, Lo TE, Luo NS (2000) Selective production of L-aspartic acid and L-phenylalanine by coupling reactions of aspartase and aminotransferase in *Escherichia coli*. Enzym Microb Technol 27:19–25

Chen YY, Shen HJ, Cui YY et al (2013) Chromosomal evolution of *Escherichia coli* for the efficient production of lycopene. BMC Biotechnol 13:6

Cheng A-X, Han X-J, Wu Y-F et al (2014) The function and catalysis of 2-oxoglutarate-dependent oxygenases involved in plant flavonoid biosynthesis. Int J Mol Sci 15:1080–1095

Choi JW, Yim SS, Lee SH et al (2015) Enhanced production of gamma-aminobutyrate (GABA) in recombinant *Corynebacterium glutamicum* by expressing glutamate decarboxylase active in expanded pH range. Microb Cell Factories 14:1–11

Choi O, Wu C-Z, Kang SY et al (2011) Biosynthesis of plant-specific phenylpropanoids by construction of an artificial biosynthetic pathway in *Escherichia coli*. J Ind Microbiol Biotechnol 38:1657–1665

Chu LL, Dhakal D, Shin HJ et al (2018) Metabolic engineering of *Escherichia coli* for enhanced production of Naringenin 7-sulfate and its biological activities. Front Microbiol 9:1671

Comalada M, Ballester I, Bailon E et al (2006) Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. Biochem Pharmacol 72:1010–1021

Cory H, Passarelli S, Szeto J et al (2018) The role of polyphenols in human health and food systems: a mini-review. Front Nutr 5:87

Cutrim CS, Cortez MAS (2018) A review on polyphenols: classification, beneficial effects and their application in dairy products. Int J Dairy Technol 71:564–578

Daliu P, Santini A, Novellino E (2018) A decade of nutraceutical patents: where are we now in 2018? Expert Opin Ther Pat 28(12):875–882

Damude HG, Macool DJ, Picataggio SK et al (2009) Docosahexaenoic acid producing strains of *Yarrowia lipolytica*. US Patent 2009, US7,550,286

Das A, Yoon SH, Lee SH et al (2007) An update on microbial carotenoid production: application of recent metabolic engineering tools. Appl Microbiol Biotechnol 77(3):505–512

Defelice SL (1995) The nutraceutical revolution: its impact on food industry R&D. Trends Food Sci Technol 6:59–61

DeLoache WC, Russ ZN, Narcross L et al (2015) An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. Nat Chem Biol 11:465–471

Du J, Li L, Ding X et al (2013) Isolation and characterization of a novel cyanophycin synthetase from a deep-sea sediment metagenomic library. Appl Microbiol Biotechnol 97:8619–8628

Du J, Li L, Zhou S (2017) Enhanced cyanophycin production by *Escherichia coli* overexpressing the heterologous *cphA* gene from a deep sea metagenomic library. J Biosci Bioeng 123:239–244

Dusch N, Puhler A, Kalinowski J (1999) Expression of the *Corynebacterium glutamicum* panD gene encoding L-aspartate-alpha-decarboxylase leads to pantothenate overproduction in *Escherichia coli*. Appl Environ Microbiol 65:1530–1539

Feng Z, Zhang J, Chen G et al (2019) Extracellular expression of L-aspartate-α-decarboxylase from *Bacillus tequilensis* and its application in the biosynthesis of β-alanine. Appl Biochem Biotechnol 189:273

Fossati E, Ekins A, Narcross L et al (2014) Reconstitution of a 10-gene pathway for synthesis of the plant alkaloid dihydrosanguinarine in *Saccharomyces cerevisiae*. Nat Commun 5:3283

Fossati E, Narcross L, Ekins A et al (2015) Synthesis of morphinan alkaloids in *Saccharomyces cerevisiae*. PLoS One 10:e0124459

Frey KM, Oppermann-Sanio FB, Schmidt H et al (2002) Technical-scale production of cyanophycin with recombinant strains of *Escherichia coli*. Appl Environ Microbiol 68:3377–3384

Frommeyer M, Bergander K, Steinbuchel A (2014) Guanidination of soluble lysine-rich cyanophycin yields a homoarginine-containing polyamide. Appl Environ Microbiol 80:2381–2389

Gao S, Tong Y, Zhu L et al (2017) Iterative integration of multiple-copy pathway genes in *Yarrowia lipolytica* for heterologous beta-carotene production. Metab Eng 41:192–201

Haeri MR, Limaki HK, White CJ et al (2012) Non-insulin dependent anti-diabetic activity of (2S 3R, 4S) 4-hydroxyisoleucine of fenugreek (*Trigonella foenum graecum*) in streptozotocin-induced type I diabetic rats. Phytomedicine 19:571–574

Hawkins KM, Smolke CD (2008) Production of benzylisoquinoline alkaloids in *Saccharomyces cerevisiae*. Nat Chem Biol 4:564–573

Hayashi S, Satoh Y, Ujihara T et al (2016) Enhanced production of polyunsaturated fatty acids by enzyme engineering of tandem acyl carrier proteins. Sci Rep 6:35441

Ho NAT, Hou CY, Kim WH et al (2013) Expanding the active pH range of *Escherichia coli* glutamate decarboxylase by breaking the cooperativeness. J Biosci Bioeng 115(2):154e158

Holton TA, Brugliera F, Tanaka Y (1993) Cloning and expression of flavonol synthase from *Petunia hybrida*. Plant J 4:1003–1010

Hub MR (2018) Global beta-alanine sales market report 2018. https://www.marketresearchstore.com/report/global-beta-alanine-market-report-2018-by-manufacturer-279649

Industry Experts (2014) Omega-3 polyunsaturated fatty acids (PUFAs)—a global market overview. http://industry-experts.com/verticals/healthcare-and-pharma/omega-3-polyunsatur ated-fatty-acids-pufas-a-global-market-overview

Jackson H, Braun CL, Ernst H (2008) The chemistry of novel xanthophylls carotenoids. AmJ Cardiol 101(10A):50D–57D

Jette L, Harvey L, Eugeny K et al (2009) 4-Hydroxyisoleucine: a plant-derived treatment for metabolic syndrome. Curr Opin Investig Drugs 10(4):353–358

Jin YS, Stephanopoulos G (2007) Multi-dimensional gene target search for improving lycopene biosynthesis in *Escherichia coli*. Metab Eng 9:337–347

Jones JA, Vernacchio VR, Collins SM et al (2017) Complete biosynthesis of anthocyanins using *E. coli* polycultures. mBio 8:e621–e617

Kajiwara S, Fraser P, Kondo K et al (1997) Expression of an exogenous isopentenyl diphosphate isomerase gene enhances isoprenoid biosynthesis in *Escherichia coli*. Biochem J 324:421–426

Kalra EK (2003) Nutraceutical definition and introduction. AAPS PharmSci 5:27–28

Kang MJ, Lee YM, Yoon SH et al (2005) Identification of genes affecting lycopene accumulation in *Escherichia coli* using a shot-gun method. Biotechnol Bioeng 91:636–642

Katsuyama Y, Funa N, Horinouchi S (2007a) Precursor-directed biosynthesis of stilbene methyl ethers in *Escherichia coli*. Biotechnol J 2:1286–1293

Katsuyama Y, Funa N, Miyahisa I et al (2007b) One-pot synthesis of genistein from tyrosine by coincubation of genetically engineered *Escherichia coli* and *Saccharomyces cerevisiae* cells. Appl Microbiol Biotechnol 73(5):1143–1149

Kim J-D, Liu L, Guo W et al (2006) Chemical structure of flavonols in relation to modulation of angiogenesis and immune-endothelial cell adhesion. J Nutr Biochem 17:165–176

Kim JS, Nakagawa A, Yamazaki Y et al (2013) Improvement of reticuline productivity from dopamine by using engineered *Escherichia coli*. Biosci Biotechnol Biochem 77:2166–2168

Kim SW, Keasling J (2001) Metabolic engineering of the nonmevalonate isopentenyldiphosphate synthesis pathway in *Escherichia coli* enhances lycopene production. Biotechnol Bioeng 72:408–415

Kim SW, Kim JB, Ryu JM et al (2009) High-level production of lycopene in metabolically engineered *E. coli*. Process Biochem 44:899–905

Kivero AD, Novikova AE, Smirnov SV (2012) Modification of *E. coli* central metabolism to optimize the biotransformation of L-isolecucine into 4-hydroxyisoleucine by enzymatic hydroxylation. Appl Biochem Microbiol 48:639–644

Kodera T, Smirnov SV, Samsonova NN et al (2009) A novel L-isoleucine hydroxylating enzyme, L-isoleucine dioxygenase from *Bacillus thuringiensis*, produces (2S, 3R, 4S)-4-hydroxyisoleucine. Biochem Biophys Res Commun 390(3):506–510

Konst PM, Franssen MCR, Scott EL et al (2009) A study on the applicability of L-aspartate alpha-decarboxylase in the biobased production of nitrogen containing chemicals. Green Chem 11:1646–1652

Koopman F, Beekwilder J, Crimi B et al (2012) De novo production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. Microb Cell Factories 11:155

Landolfi R, Mower RL, Steiner M (1984) Modification of platelet function and arachidonic acid metabolism by bioflavonoids. Structure-activity relations. Biochem Pharmacol 33:1525–1530

Lange N, Steinbuchel A (2011) β-Carotene production by *Saccharomyces cerevisiae* with regard to plasmid stability and culture media. Appl Microbiol Biotechnol 91:1611–1622

Larroude M, Celinska E, Back A et al (2018) A synthetic biology approach to transform *Yarrowia lipolytica* into a competitive biotechnological producer of beta-carotene. Biotechnol Bioeng 115:464–472

Lee H, Kim B-G, Ahn J-H (2014) Production of bioactive hydroxyflavones by using monooxygenase from *Saccharothrix espanaensis*. J Biotechnol 176:11–17

Lee H, Kim BG, Kim M et al (2015) Biosynthesis of two flavones, apigenin and genkwanin, in *Escherichia coli*. J Microbiol Biotechnol 25:1442–1448

Leonard E, Yan Y, Fowler Z et al (2008) Strain improvement of recombinant *Escherichia coli* for efficient production of plant flavonoids. Mol Pharm 5:257–265

Leonard E, Yan Y, Koffas MAG (2006) Functional expression of a P450 flavonoid hydroxylase for the biosynthesis of plant-specific hydroxylated flavonols in *Escherichia coli*. Metab Eng 8:172–181

Leonard E, Yan Y, Lim KH et al (2005) Investigation of two distinct flavone synthases for plant-specific flavone biosynthesis in *Saccharomyces cerevisiae*. Appl Environ Microbiol 71:8241–8248

Li H, Lu X, Chen K et al (2018a) β-Alanine production using whole-cell biocatalysts in recombinant *Escherichia coli*. Mol Catal 449:93–98

Li M, Schneider K, Kristensen M et al (2016) Engineering yeast for high level production of stilbenoid antioxidants. Sci Rep 6:36827

Li XR, Tian GQ, Shen HJ et al (2015a) Metabolic engineering of *Escherichia coli* to produce zeaxanthin. J Ind Microbiol Biotechnol 42:627–636

Li Y, Lin Z, Huang C et al (2015b) Metabolic engineering of *Escherichia coli* using CRISPR–Cas9 meditated genome editing. Metab Eng 31:13–21

Li ZP, Meng T, Ling XP et al (2018b) Overexpression of malonyl-CoA: ACP transacylase in *Schizochytrium* sp. to improve polyunsaturated fatty acid production. J Agric Food Chem 66 (21):5382–5391

Liang LY, Zheng YG, Shen YC (2008) Optimization of beta-alanine production from beta-aminopropionitrile by resting cells of *Rhodococcus* sp G20 in a bubble column react or using response surface methodology. Process Biochem 43:758–764

Lim CG, Fowler ZL, Hueller T et al (2011) High-yield resveratrol production in engineered *Escherichia coli*. Appl Environ Microbiol 77:3451–3460

Lim CG, Wong L, Bhna N et al (2015) Development of a recombinant Escherichia coli strain for overproduction of the plant pigment anthocyanin. Appl Environ Microbiol 81(18):6276–6284

Lukacin R, Wellmann F, Britsch L et al (2003) Flavonol synthase from *Citrus unshiu* is a bifunctional dioxygenase. Phytochemistry 62:287–292

Lyu C-J, Liu L, Huang J et al (2019) Biosynthesis of g-aminobutyrate by engineered lactobacillus brevis cells immobilized in gellan gum gel beads. J Biosci Bioeng 128(2):123–128

Lyu X, Ng KR, Lee JL et al (2017) Enhancement of naringenin biosynthesis from tyrosine by metabolic engineering of *Saccharomyces cerevisiae*. J Agric Food Chem 65(31):6638–6646
Ma SM, Garcia DE, Redding-Johanson AM et al (2011) Optimization of a heterologous mevalonate pathway through the use of variant HMG-CoA reductases. Metab Eng 13:588–597
Ma T, Shi B, Ye Z et al (2019) Lipid engineering combined with systematic metabolic engineering of *Saccharomyces cerevisiae* for high-yield production of lycopene. Metab Eng 52:134–142
Marin L, Guitierrez-del-Rio I, Yague P et al (2017) Denovo synthesis of apigenin, luteolin, and eriodictyol in the actinomycete *Streptomyces albus* and production improvement by feeding and spore conditioning. Front Microbiol 8:921
Marın L, Gutierrez-del-Rio I, Entrialgo-Cadierno R et al (2018) *De novo* biosynthesis of myricetin, kaempferol and quercetin in *Streptomyces albus* and *Streptomyces coelicolor*. PLoS One 13 (11):e0207278
Matsumura E, Nakagawa A, Tomabechi Y et al (2018) Microbial production of novel sulphated alkaloids for drug discovery. Sci Rep 8(1):7980
Matthaus F, Ketelhot M, Gatter M et al (2014) Production of lycopene in the noncarotenoid-producing yeast *Yarrowia lipolytica*. Appl Environ Microbiol 80(5):1660e9
Maurya CK, Singh R, Jaiswal N et al (2014) 4-Hydroxyisoleucine ameliorates fatty acid-induced insulin resistance and inflammatory response in skeletal muscle cells. Mol Cell Endocrinol 395:51–60
McWilliams A (2018) The global market for carotenoids. https://www.bccresearch.com/market-research/food-and-beverage/the-global-market-for-carotenoids-fod025f.html
Meussen BJ, Weusthuis RA, Sanders JPM et al (2012) Production of cyanophycin in *Rhizopus oryzae* through the expression of a cyanophycin synthetase encoding gene. Appl Microbiol Biotechnol 93:1167–1174
Mikkelsen MD, Buron LD, Salomonsen B et al (2012) Microbial production of indolylglucosinolate through engineering of a multi-gene pathway in a versatile yeast expression platform. Metab Eng 14:104–111
Minami H, Kim J-S, Ikezawa N et al (2008) Microbial production of plant benzylisoquinoline alkaloids. Proc Natl Acad Sci U S A 105:7393–7398
Miyahisa I, Funa N, Ohnishi Y et al (2006) Combinatorial biosynthesis of flavones and flavonols in *Escherichia coli*. Appl Microbiol Biotechnol 71:53–58
Nakagawa A, Minami H, Kim J-S et al (2011) A bacterial platform for fermentative production of plant alkaloids. Nat Commun 2:326
Ogawa J, Yamanaka H, Mano J et al (2007) Synthesis of 4-hydroxyisoleucine by the aldolase-transaminase coupling reaction and basic characterisation of the aldolase from *Arthrobacter simplex* AKU 626. Biosci Biotechnol Biochem 71:1607–1615
Okai N, Takahashi C, Hatada K et al (2014) Disruption of pknG enhances production of gamma-aminobutyric acid by *Corynebacterium glutamicum* expressing glutamate decarboxylase. AMB Express 4:20
Orikasa Y, Tanaka M, Sugihara S et al (2009) pfaB products determine the molecular species produced in bacterial polyunsaturated fatty acid biosynthesis. FEMS Microbiol Lett 295 (2):170–176
Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47
Pandey M, Verma RK, Saraf SA (2010) Nutraceuticals: new era of medicine and health. Asian J Pharm Clin Res 3(1):11–15
Pandey RP, Parajuli P, Koffas MAG et al (2016) Microbial production of natural and non-natural flavonoids: pathway engineering, directed evolution and systems/synthetic biology. Biotechnol Adv 34:634–662
Park SR, Ahn MS, Han AR et al (2011) Enhanced flavonoid productionin *Streptomyces venezuelae* via metabolic engineering. J Microbiol Biotechnol 21:1143–1146
Peng Y-F, Chen W-C, Xiao K et al (2016) DHA production in *Escherichia coli* by expressing reconstituted key genes of polyketide synthase pathway from Marine Bacteria. PLoS One 11(9): e0162861

Pham VD, Somasundaram S, Hwan S et al (2016) Efficient production of gamma-aminobutyric acid using *Escherichia coli* by co-localization of glutamate synthase, glutamate decarboxylase, and GABA transporter. J Ind Microbiol Biotechnol 43:79–86

Piao X, Wang L, Lin B et al (2019) Metabolic engineering of *Escherichia coli* for production of L-aspartate and its derivative β-alanine with high stoichiometric yield. Metab Eng 54:244–254

Pitera DJ, Paddon CJ, Newman JD et al (2007) Balancing a heterologous mevalonate pathway for improved isoprenoid production in *Escherichia coli*. Metab Eng 9:193–207

Pollmann H, Breitenbach J, Sandmann G (2017) Engineering of the carotenoid pathway in *Xanthophyllomyces dendrorhous* leading to the synthesis of zeaxanthin. Appl Microbiol Biotechnol 101(1):103–111

Prasad E (2017) Polyphenol market by product type (apples, green tea, grape seed, and others) and application (functional beverages, functional food, dietary supplements, and others)—global opportunity analysis and industry forecast, 2014–2022. https://www.alliedmarketresearch.com/polyphenol-market

Qian Y, Liu J, Song W et al (2018) Production of β-alanine from fumaric acid using a dual-enzyme cascade. ChemCatChem 10:4984–4991

Raberg M, Volodina E, Lin K et al (2018) *Ralstonia eutropha* H16 in progress: applications beside PHAs and establishment as production platform by advanced genetic tools. Crit Rev Biotechnol 38:494–510

Rasouli H, Farzaei MH, Khodarahmi R (2017) Polyphenols and their benefits: a review. Int J Food Prop 20(2):1700–1741

Ren LJ, Zhuang XY, Chen SL et al (2015) Introduction of omega-3 desaturase obviously changed the fatty acid profile and sterol content of *Schizochytrium* sp. J Agric Food Chem 63 (44):9770–9776

Reports TM (2018) Global GABA (CAS 56-12-2) Market Insights, Forecast to 2025. https://www.themarketreports.com/report/global-gaba-cas-56-12-52-market-insights-forecast-to-2025

Rodriguez A, Strucko T, Stahlhut SG et al (2017) Metabolic engineering of yeast for fermentative production of flavonoids. Bioresour Technol 245:1645–1654

Salehi B, Fokou PVT, Sharifi-Rad M et al (2019) The therapeutic potential of naringenin: a review of clinical trials. Pharmaceuticals (Basel) 12(1):11

Santos CN, Koffas M, Stephanopoulos G (2011) Optimization of a heterologous pathway for the production of flavonoids from glucose. Metab Eng 13(4):392–400

Sarnaika A, Nambissana V, Pandita R et al (2018) Recombinant *Synechococcus elongatus* PCC 7942 for improved zeaxanthin production under natural light conditions. Algal Res 36:139–151

Sharpe PL, Ye RW, Zhu Q (2014) Carotenoid production in a recombinant oleaginous yeast. US Patent 2014, US8,846,374

Shen HJ, Cheng BY, Zhang YM et al (2016) Dynamic control of the mevalonate pathway expression for improved zeaxanthin production in *Escherichia coli* and comparative proteome analysis. Metab Eng 38:180–190

Shen Y, Zhao L, Li Y et al (2014) Synthesis of beta-alanine from L-aspartate using L-aspartate-alpha-decarboxylase from *Corynebacterium glutamicum*. Biotechnol Lett 36:1681–1686

Shi F, Huimin Fang H, Niu T et al (2016) Overexpression of ppc and lysC to improve the production of 4-hydroxyisoleucine and its precursor l-isoleucine in recombinant *Corynebacterium glutamicum* ssp. *lactofermentum*. Enzym Microb Technol 87-88:79–85

Shi F, Jiang J, Li Y et al (2013) Enhancement of gamma-aminobutyric acid production in recombinant *Corynebacterium glutamicum* by co-expressing two glutamate decarboxylase genes from *Lactobacillus brevis*. J Ind Microbio Biotechnol 40:1285–1296

Shi F, Niu T, Fang H (2015) 4-Hydroxyisoleucine production of recombinant *Corynebacterium glutamicum* ssp. *lactofermentum* under optimal corn steep liquor limitation. Appl Microbiol Biotechnol 99:3851–3863

Shi F, Zhang M, Li Y et al (2018) Sufficient NADPH supply and pknG deletion improve 4-hydroxyisoleucine production in recombinant *Corynebacterium glutamicum*. Enzym Microb Technol 115:1–8

Shi F, Zhang S, Li T et al (2019) Enhancement of substrate supply and ido expression to improve 4-hydroxyisoleucine production in recombinant *Corynebacterium glutamicum* ssp. *lactofermentum*. Appl Microbiol Biotechnol 103:4113–4124

Smirnov SV, Kodera T, Samsonova NN et al (2010) Metabolic engineering of *Escherichia coli* to produce (2S,3R,4S)-4-hydroxyisoleucine. Appl Microbiol Biotechnol 88:719–726

Smirnov SV, Samsonova NN, Novikova AE et al (2007) A novel strategy for enzymatic synthesis of 4-hydroxyisoleucine: identification of an enzyme possessing HMKP (4-hydroxy-3-methyl-2-ketopentanoate) aldolase activity. FEMS Microbiol Lett 273:70–77

Sokoła-Wysoczańska E, Wysoczański T, Wagner J et al (2018) Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders-a review. Nutrients 10 (10):1561–1581

Soma Y, Fujiwara Y, Nakagawa T et al (2017) Reconstruction of a metabolic regulatory network in *Escherichia coli* for purposeful switching from cell growth mode to production mode in direct GABA fermentation from glucose. Metab Eng 43:54–63

Song CW, Lee J, Ko YS et al (2015) Metabolic engineering of *Escherichia coli* for the production of 3-aminopropionic acid. Metab Eng 30(3):121–129

Steinle A, Bergander K, Steinbuchel A (2009) Metabolic engineering of *Saccharomyces cerevisiae* for production of novel cyanophycins with an extended range of constituent amino acids. Appl Environ Microbiol 75:3437–3446

Steinle A, Oppermann-Sanio FB, Reichelt R et al (2008) Synthesis and accumulation of cyanophycin in transgenic strains of *Saccharomyces cerevisiae*. Appl Environ Microbiol 74:3410–3418

Steinle A, Steinbuchel A (2010) Establishment of a simple and effective isolation method for cyanophycin from recombinant *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 85:1393–1399

Steinle A, Witthoff S, Krause JP et al (2010) Establishment of cyanophycin biosynthesis in *Pichia pastoris* and optimization by use of engineered cyanophycin synthetases. Appl Environ Microbiol 76:1062–1070

Suh W (2012) High isoprenoid flux *Escherichia coli* as a host for carotenoids production. Methods Mol Boil 834:49–62

Tavares S, Grotkjaer T, Obsen T et al (2011) Metabolic engineering of *Saccharomyces cerevisiae* for production of eicosapentaenoic acid, using a novel Δ5-desaturase from *Paramecium tetraurelia*. Appl Environ Microbiol 77:1854–1861

Tereshina V, Memorskaya A, Feofilova E (2010) Lipid composition of the mucoraceous fungus *Blakeslea trispora* under lycopene formation-stimulating conditions. Microbiology 79(1):34–39

Tiedje KE, Stevens K, Barnes S et al (2010) β-Alanine as a small molecule neurotransmitter. Neurochem Int 57:177–188

Toshihiro O, Yasuko M, Akio S et al (1998) Production of beta-alanine by microorganism. JP Patent 10042886

Trantas E, Panopoulos N, Ververidis F (2009) Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. Metab Eng 11:355–366

Trenchard IJ, Smolke CD (2015) Engineering strategies for the fermentative production of plant alkaloids in yeast. Metab Eng 30:96–104

Tsao R (2010) Chemistry and biochemistry of dietary polyphenols. Nutrients 2:1231–1246

Tseng WC, Fang TY, Cho CY et al (2012) Assessments of growth conditions on the production of cyanophycin by recombinant *Escherichia coli* strains expressing cyanophycin synthetase gene. Biotechnol Prog 28:358–363

Tseng WC, Fang TY, Hsieh YC et al (2017) Solubility and thermal response of fractionated cyanophycin prepared with recombinant *Escherichia coli*. J Biotechnol 249:59–65

Umar KM, Abdulkarim SM, Radu S et al (2012) Engineering the production of major catechins by *Escherichia coli* carrying metabolite genes of *Camellia sinensis*. Sci World J 2012:529031

Verwaal R, Wang J, Meijnen JP et al (2007) High-level production of beta-carotene in *Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from *Xanthophyllomyces dendrorhous*. Appl Environ Microbiol 73:4342–4350

Villarreal-Garcia D, Jacobo-Velazquez DA (2016) Glucosinolates from broccoli: nutraceutical properties and their purification. J Neutraceutical Food Sci 1(1):5

Voss I, Diniz SC, Aboulmagd E et al (2004) Identification of the *Anabaena* sp strain PCC7120 cyanophycin synthetase as suitable enzyme for production of cyanophycin in gram-negative bacteria like *Pseudomonas putida* and *Ralstonia eutropha*. Biomacromolecules 5:1588–1595

Wan X, Peng Y, Zhou X et al (2016) Effect of cerulenin on fatty acid composition and gene expression pattern of DHA-producing strain *Colwellia psychrerythraea* strain 34H. Microb Cell Factories 15:30–42

Wang F, Bi Y, Diao J et al (2019) Metabolic engineering to enhance biosynthesis of both docosahexaenoic acid and odd-chain fatty acids in *Schizochytrium* sp. S31. Biotechnol Biofuels 12:141

Watts KT, Lee PC, Schmidt-Dannert C (2006) Biosynthesis of plant-specific stilbene polyketides in metabolically engineered *Escherichia coli*. BMC Biotechnol 6:1–12

Watzer B, Engelbrecht A, Hauf W et al (2015) Metabolic pathway engineering using the central signal processor PII. Microb Cell Factories 14:192

Wiefel L, Broker A, Steinbuchel A (2011) Synthesis of a citrulline-rich cyanophycin by use of *Pseudomonas putida* ATCC 4359. Appl Microbiol Biotechnol 90:1755–1762

Wiefel L, Steinbuchel A (2014) Solubility behavior of cyanophycin depending on lysine content. Appl Environ Microbiol 80:1091–1096

Wildman REC (ed) (2001) Handbook of nutraceuticals and functional foods. CRC Press, Boca Raton, pp 13–30

Williams CM, Burdge G (2006) Long-chain n-3 PUFA: plant versus marine sources. Proc Nutr Soc 65:42–50

Wu J, Liu P, Fan Y et al (2013) Multivariate modular metabolic engineering of *Escherichia coli* to produce resveratrol from l-tyrosine. J Biotechnol 167:404–411

Wu J, Zhou P, Zhang X et al (2017) Efficient *de novo* synthesis of resveratrol by metabolically engineered *Escherichia coli*. J Ind Microbiol Biotechnol 44:1083–1095

Wu J, Zhou T, Du G et al (2014) Modular optimization of heterologous pathways for de novo synthesis of (2S)-naringenin in *Escherichia coli*. PLoS One 9:e101492

Xie D, Miller E, Tyreus B (2016) Sustainable production of omega-3 eicosapentaenoic acid by fermentation of metabolically engineered *Yarrowia lipolytica*. In: Lau CK, Berlin P (eds) Quality living through chemurgy and green chemistry. Springer, Heidelberg, pp 17–33

Xie W, Liu M, Lv X et al (2014) Construction of a controllable β-carotene biosynthetic pathway by decentralized assembly strategy in *Saccharomyces cerevisiae*. Biotechnol Bioeng 111:125–133

Xu F, Li L, Zhang W et al (2012) Isolation, characterization, and function analysis of a flavonol synthase gene from *Ginkgo biloba*. Mol Biol Rep 39:2285–2296

Xu P, Qiao K, Ahn WS et al (2016) Engineering *Yarrowia lipolytica* as a platform for synthesis of drop-in transportation fuels and oleochemicals. Proc Natl Acad Sci U S A 113:10848–10853

Xue Z, Sharpe PL, Hong S-P et al (2013) Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. Nat Biotechnol 31:734–740

Yabuzaki J (2017) Carotenoids database: structures, chemical fingerprints and distribution among organisms. Database 2017:bax004. https://doi.org/10.1093/database/bax004

Yamano S, Ishii T, Nakagawa M et al (1994) Metabolic engineering for production of beta-carotene and lycopene in *Saccharomyces cerevisiae*. Biosci Biotechnol Biochem 58:1112–1114

Yan GL, Wen KR, Duan CQ (2011) Enhancement of β-carotene production by over-expression of HMG-CoA reductase coupled with addition of ergosterol biosynthesis inhibitors in recombinant *Saccharomyces cerevisiae*. Curr Microbiol 64(2):159–163

Yan JF, Cheng RB, Lin XZ et al (2013) Overexpression of acetyl-CoA synthetase increased the biomass and fatty acid proportion in microalga *Schizochytrium*. Appl Microbiol Biotechnol 97 (5):1933–1939

Yan Y, Chemler J, Huang L et al (2005) Metabolic engineering of anthocyanin biosynthesis in *Escherichia coli*. Appl Environ Microbiol 71:3617–3623
Yang JM, Guo LZ (2014) Biosynthesis of beta-carotene in engineered *E. coli* using the MEP and MVA pathways. Microb Cell Factories 13:160
Yazawa K (1996) Production of eicosapentaenoic acid from marine bacteria. Lipids 31(Suppl): S297–S300
Ye RW, Sharpe PL, Zhu Q (2012) Bioengineering of oleaginous yeast *Yarrowia lipolytica* for lycopene production. Methods Mol Biol 898:153–159
Yoon SH, Lee SH, Das A et al (2009) Combinatorial expression of bacterial whole mevalonate pathway for the production of β-carotene in *E. coli*. J Biotechnol 140:218–226
Yoon SH, Park HM, Kim JE et al (2007) Increased β-carotene production in recombinant Escherichia coli harboring an engineered isoprenoid precursor pathway with mevalonate addition. Biotechnol Prog 23:599–605
Zha J, Zhang Y, Mattozzi M et al (2018) Metabolic engineering of *Corynebacterium glutamicum* for anthocyanin production. Microb Cell Factories 17(1):143
Zhang C, Li Y, Ma J et al (2018a) High production of 4-hydroxyisoleucine in *Corynebacterium glutamicum* by multistep metabolic engineering. Metab Eng 49:287–298
Zhang C, Ma J, Li Z et al (2018b) A strategy for L-isoleucine dioxygenase screening and 4-hydroxyisoleucine production by resting cells. Bioengineered 9(1):72–79
Zhang S, He Y, Sen B et al (2018c) Alleviation of reactive oxygen species enhances PUFA accumulation in *Schizochytrium* sp. through regulating genes involved in lipid metabolism. Metab Eng Commun 6:39–48
Zhang W, Liu H, Li X et al (2017) Production of naringenin from D-xylose with co-culture of *E. coli* and *S. cerevisiae*. Eng Life Sci 17:1021–1029
Zhao J, Li Q, Sun T et al (2013) Engineering central metabolic modules of *Escherichia coli* for improving β-carotene production. Metab Eng 17:42–50
Zhao S, Jones JA, Lachance DM et al (2015) Improvement of catechin production in *Escherichia coli* through combinatorial metabolic engineering. Metab Eng 28:43–53
Zhu F, Lu L, Fu S et al (2015) Targeted engineering and scale up of lycopene overproduction in *Escherichia coli*. Process Biochem 50:341–346
Zhu Q, Jackson EN (2015) Metabolic engineering of *Yarrowia lipolytica* for industrial applications. Curr Opin Biotechnol 36:65–72
Ziegler K, Diener A, Herpin C et al (1998) Molecular characterization of cyanophycin synthetase, the enzyme catalyzing the biosynthesis of the cyanobacterial reserve material multi-L-arginyl-poly-L-aspartate (cyanophycin). Eur J Biochem 254:154–159

Nutrigenomics Approaches to Control Metabolic Diseases and Challenges to Personalized Nutritional Intervention

12

Chandrajeet Singh, Arpana Sharma, Mahendra Bishnoi, Kanthi K. Kondepudi, and Ravindra Pal Singh

Abstract

Concept of applying nutrients as a medical therapy arose around 2000 years ago by Hippocrates, however, only after the development of sequencing technologies and completion of Human Genome Project have aided the knowledge regarding the interaction of selected nutrients, genes, and specific organ/tissue of the host. Particularly, extensive evaluation of human gut microbiota drastically provided significant insights about the dynamic nature of residential microbial communities under several endogenous and exogenous factors including dietary nutrients. Amidst them, dietary fibers recognize as a key determinant for defining microbial spatial structures, and their functionality and interactions with host genes that directly or indirectly dictate detrimental or beneficial outcomes to host health. It has been increasingly recognized that each individual differentially responses to the same dietary nutrient intake due to the intrinsic nature of gut and polymorphism of genes, thus it led to a concept of personalized nutrition. Such a scenario raised a question of how a nutrient influences the individual's genetic makeup/homeostasis and vice versa. In order to answer this question and develop targeted nutritional therapy, we need better sympathetic of what we eat and our risk and response to metabolic diseases (cancer, cardio-metabolic diseases, allergies, and obesity), as well as the molecular mediators such as

C. Singh
National Agri-Food Biotechnology Institute (NABI), SAS Nagar, Punjab, India

Central University of Gujarat, Gandhinagar, Gujarat, India

A. Sharma
Department of Biotechnology, Mehsana Urban Institute of Sciences, Ganpat University, Kherva, Gujarat, India

M. Bishnoi · K. K. Kondepudi · R. P. Singh (✉)
National Agri-Food Biotechnology Institute (NABI), SAS Nagar, Punjab, India
e-mail: r.p.singh@nabi.res.in

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_12

genes and their expression and metabolic biomarkers. Taken together, in the current chapter, we will summarize the impact of dietary nutrients on metabolic diseases and underlying challenges in the nutrigenomics branch.

Keywords

Nutrigenomics · Gut microbiome · Dietary fibers · Genes · Metabolites

12.1 Introduction

Nutrigenomics is one of the fascinating streams of the meta-omics tools, which consisted of an integrated interaction of diet, genetic constitution, morphological and physiological responses at a genetic level in an organized and efficient manner (Sales et al. 2014). Furthermore, the nutrigenomics aids on the underlying information of nutritional structures, such as structures of dietary glycans, and their impacts on certain biomarkers linking to metabolic diseases (Rana et al. 2016). Eventually, it assists in diminishing nutritional severity (including malnutrition) and postponing the diseases embarking under changing lifestyle (Phillips 2013). In last couple of decades, multiple bioactive compounds such as polyphenols (Watjen et al. 2005), rutin (Cai and Lin 2009), dietary fibers (Rose et al. 2007), and other epi-nutrients (Gaziev et al. 1996; Eseberri et al. 2019) have been impacted positively on health. The information generated from these integrated approaches proves better nutritional assistance for the individual's health status.

The amalgamation of many fields like knowledge of phenotypic responses shown as a consequence of individual's genetic background (nutrigenetics), the expression of genes (epigenetics and transcriptomics), modifications in functioning of proteins (proteomics), and changes in small compounds (metabolomics) help in categorizing responders from non-responders (Trujillo et al. 2006; Davis 2007; Davis and Milner 2007). Generally, dietary signals detected by our cellular sensor systems influence the expression of different genes and proteins, subsequently leading to a wide variety of metabolites networking associated with diverse cellular pathways (Mead 2007). For example, Fenech et al. (2005) identified nine key nutrients that can alter genomic integrity in various ways relating to cancer onset. The study found that riboflavin, pantothenic acid, and biotin are allied with a rise in DNA damage in conjugation with occupational exposure to genotoxic and carcinogenic, whereas vitamin B12, vitamin E, folate, niacin, calcium, and retinol are related with a decrease in DNA damage to some degree when consumed in increasing quantities in food ingredients. The study highlights that different components present in food can bring positive and negative consequences with their mechanisms, which can be exploited to decrease the risk of cancer. Similarly, diabetes and obesity are resultant of an imbalanced diet and genetic susceptibility differences among individuals (Doo and Kim 2015).

Functional food and nutraceutical are growing areas, which can augment or supplement our diet for maintaining the better health status of an individual (Alkhatib et al. 2017). Nowadays, personalized genetic testing is also becoming popular among consumers for health and well-being (Ordovas et al. 2018). The

commercial companies are performing genetic analysis in a cost-effective manner and also provide lifestyle advice, which is another new and developing area in the health industry (Phillips et al. 2018). As per our latest understanding, nearly all diseases have a genetic link, and a technique "called genome-wide association studies (GWAS)" are determining genetic variations of individuals such as the presence of single-nucleotide polymorphisms (SNPs) (Forte et al. 2016; Coltell et al. 2019). The SNPs mainly alter the function of housekeeping genes that involve in the elementary maintenance of a cell, and are expected to modify the peril of emerging diseases (such as obesity) on the basis of dietary factors as environmental signals (Frayling et al. 2007; Pausova et al. 2009). Additionally, chromatin remodeling and DNA methylation/histone acetylation are the most generic epigenetic systems that determine the functional status of cells,—are under the control of environmental factors, such as dietary factors (Meyer and Jaffrey 2014; Zhou et al. 2015).

Nevertheless, the area of research faces many challenges ranging from an understanding of elements and fibers present in the food materials to properly inform consumers and healthcare practitioners. For example, the SNP–nutrient and SNP–selenium metabolism interactions of chronic disease are very complex because of the intrinsic complexities in studying genotypes and analyzing dietary composition and amounts of the nutrient that was ingested (Han 2013; Meplan 2015). In terms of gene sequencing, the development of sequencing and metabolic tools (meta-omics) in the current decade has drastically improved performing genomic and spectral analyses on large scale (Singh et al. 2017). These meta-omics methodologists produce vast numbers of data, though we could not be yet able to store them in proper manner and also lack some useful software that can compile and analyze diverse data obtained from different sources (such as protein–protein interaction database, miRNA and transcriptional inference) in an integrated form (Raghupathi and Raghupathi 2014). Another major challenge is analyzing assessments of dietary intake of daily life. This is majorly suffering because of lacking tools that can precisely define carbohydrate contained in a given diet.

For the success of nutrition research, the above-mentioned challenges have to be addressed to facilitate the personalized nutrition approach. This helps in studying the quantitative effects of genes in their environment on disease and wellness conditions. A concerted effort for making standardized technology, methodology, and data format techniques for handling these data is being generated in the current time. Therefore, in the present study, we are summarizing different approaches and current challenges of nutrigenomics that are being known to improve the health and well-being of the individual.

12.2 Nutrigenomics in Controlling Metabolic Diseases

As nutrigenomics refers to the interaction of dietary molecules to the genetic expression of responsive genes, several food components directly or indirectly affect an individual' gene profile through differential regulation by modulating their

expression in both ways—positively and negatively (van Ommen 2004). Nutrition such as proteins, carbohydrates, fats, vitamins, minerals, macro- and micro-nutrients, flavonoids, and other bioactive compounds of our diet are responsible for such cause. Research in recent years divulged that food plays an important role in improving life and associated with many diseases (Ohlhorst et al. 2013). Reformulate food contents for use as a medicine is a new strategy to treat a disease. This formulated diet as modulatory roles in the medical field for mitigating several metabolic diseases and suggesting diet as nutritional therapy for improving health and well-being (van Ommen et al. 2017). Therefore, there has been a recommendation for using a proper and balancing diet on the basis of the genetic profiling of an individual (discussed in the following section). Here, we have discussed how the various food contents affect profiling genes and subsequently their protein product while focusing on some metabolic diseases.

12.2.1 Nutrigenomics in Cancer

According to GLOBOCAN database, there were 18.1 million new cancer cases in 2018 worldwide, of which 9.6 million cancer deaths occurred. The most common types of cancers are colorectal, lung, breast, prostate, and stomach. It suggested that 21.7 million new cancer cases and 13 million cancerous deaths will occur by 2030 (Bray et al. 2018). Lifestyle and dietary habits are the major factors for cancer death. Therefore, nutrigenomics, the modern era, is growing fast in aspect to cancer prevention. Nutrients such as proteins, carbohydrates, lipids, vitamins, minerals, micro- and macro-nutrients, flavonoids, and alkaloids have bioactive compound and shown anti-cancer property on several occasions (Michaud et al. 2000; Davis and Milner 2011; Fenech et al. 2011; Zhao et al. 2013; Vinceti et al. 2014). Phytochemicals are major components of the study in nutrigenomics as many active compounds have anti-cancer and anti-inflammatory property (Zubair et al. 2017; Chirumbolo et al. 2018; Phan et al. 2018; Zhu et al. 2018). Many clinical studies have shown that phytochemicals have been studied on cancer cells, and shown their modulatory effect on cancerous properties (Howes and Simmonds 2014; Kotecha et al. 2016). Many cellular processes such as apoptosis, autophagy, reactive oxygen species, anoikis, adhesion, and angiogenesis are affected by various phytochemicals (Tsai et al. 2018; Shen et al. 2016; Wu et al. 2016; Byun et al. 2017). Nutrients and bioactive food components are directly or indirectly involved in gene and protein expression, which are eventually involved in many biological functions. Also, various signaling pathways are controlled by a proper diet. Cancer cell metabolites are different from normal cells and modulate several pathways that engage in cancer growth and proliferation (Table 12.1). Dysregulated metabolites are one of the major hallmarks of cancer and diet components that are more energetically active towards growth and proliferation (Hirschey et al. 2015; Pavlova and Thompson 2016; Fouad and Aanei 2017).

Several bioactive products of nutrition have benefits for human health and help in cancer prevention or treatment. Many phytochemicals have the ability to moderate

Table 12.1 List of plant flavonoids regulates cancer-associated proteins and involved mechanisms

Product	Flavonoid	Mechanism	Targeted molecule	Reference
Flavan-3-ols	Catechin	Epigenetic modification	TIMP-3, MMP-2 and MMP-9	Deb et al. (2015)
	Epigallocatechin gallate	Cell cycle arrest, apoptosis, reduced mitochondria membrane potential	HSP90 Bcl-2, Bcl-xl, xIAP, Apaf1, cytochrome C, Caspase-8, 9, 3, 7	Wu et al. (2009), Srividhya and Kalaiselvi (2013), Tsai et al. (2018)
Flavonols	Kaempferol	Cell growth, apoptosis, and migration	EGFR, Src, ERK1/2, AKT, BAX, SIRT3, PTEN	Marfe et al. (2009), Li et al. (2011), Xie et al. (2013), Yao et al. (2016)
	Quercetin	Cell cycle arrest, induced DNA damage, and apoptosis	p21, CDK, pRb, chk2, CyclinB1, PI3K, AKT	Jeong et al. (2009), Shen et al. (2016)
	Myricetin	Cell proliferation, apoptosis, migration, cell cycle arrest	Bax, Bcl-2, caspase-3, MDR-1, cyclinB1, cyclinD1, CDK1, and CDC25C, p38 MAPK, MMP-9	Sun et al. (2012), Feng et al. (2015), Zheng et al. (2017)
	Fisetin	Cell proliferation, cell cycle arrest, reduced ROS, anti-angiogenesis, promotes apoptosis, autophagy, necrosis, migration, invasion	FAK, uPA, NF-kB, PI3K, ERK1/2, AKT, Bax, Bcl-2, caspase-3, p38 MAPK, MMP-9	Tsai et al. (2018), Fu et al. (2019), Guo et al. (2019), Park et al. (2019)
Flavones	Apigenin	Cell proliferation, epithelial-mesenchymal transition, cell cycle arrest, promotes apoptosis, autophagy, migration, invasion	IL-6, Bax, Bcl-2, caspase-3, snail, twist, cyclinB1, cyclinD1, CDK1, and CDC25C, p38 MAPK, PTEN, MMP-9, NF-kB, YAP/TAZ	Qin et al. (2016), Li et al. (2018), Erdogan et al. (2020), Ittiudomrak et al. (2019), Tong et al. (2019)

(continued)

Table 12.1 (continued)

Product	Flavonoid	Mechanism	Targeted molecule	Reference
	Luteolin	Cell proliferation, apoptosis, epithelial-mesenchymal transition, cell cycle arrest, promotes apoptosis, autophagy, migration, invasion	p21, p53, Bim, CYT-c and cPARP, EGFR, PI3k/Akt, STAT3, HIF-1α/VEGF, β-catenin, β3-integrin, β1-integrin, FAK	Ruan et al. (2012), Chen et al. (2013), Sui et al. (2016), Chen et al. (2017), Wheeler et al. (2017), Li et al. (2019)
Flavonones	Hesperetin	ROS, cell cycle arrest, migration, invasion	FAK, Cyt-C, Bax, Bcl-2, caspase-3, cyclin B1, CDK1, and p21, p38 MAPK, PI3K/AKT, ERK, TGF-β, Smad3	Yang et al. (2012), Wu et al. (2016), Li et al. (2020)
	Naringenin	Cell proliferation migration, invasion, apoptosis, reduced ROS, apoptosis, epithelial-mesenchymal transition, cell cycle arrest, promotes apoptosis, migration, invasion	MMP-2, MMP-9, AKT, PI3K/AKT, ASK1, Bax, Bcl-2, caspase3, 9, p53, p38, JNK, TGF-β	Chen et al. (2019), Klaver et al. (2019), Wang et al. (2019)
Isoflavones	Genistein	Cell proliferation, angiogenesis, epigenetic regulation, apoptosis, epithelial-mesenchymal transition, cell cycle arrest, promotes apoptosis, migration, invasion, and glycolysis	HIF-α1, MGMT, RARβ, p21, E-cadherin, DAPK1, DMNTs, HDACs, MYC, PTEN, Gli1, AKT, ERK, NF-kB	Pavese et al. (2014), Liu et al. (2015), Li et al. (2017), Ning et al. (2017), Sundaram et al. (2018), Zhao et al. (2018a)

(continued)

Table 12.1 (continued)

Product	Flavonoid	Mechanism	Targeted molecule	Reference
	Daidzein	Cell proliferation, apoptosis, epithelial-mesenchymal transition, cell cycle arrest, promotes, apoptosis, migration, invasion	FGFR3, BFR2, BCRP/ABCG2, Akt/FOXO3a, FAK, PI3K/AKT, p21, cyclinD, MMP-2, mTOR, Cytochrome C, Bax, Bcl2, Bcl-xL Apaf-1, Caspase 9 and 3	Park et al. (2013), Magee et al. (2014), He et al. (2016), Kaushik et al. (2018)
Flavonolignan	Silibinin	Cell proliferation, ROS, angiogenesis apoptosis, epithelial-mesenchymal transition, cell cycle arrest, promotes apoptosis, autophagy, and migration, invasion	HIF-α, ERK1/2, Bim, PI3K/Akt, EGFR, ERK1/2. p21, p27 PTEN, Bcl-2, Jak/STAT3, NF-kB,	Bayram et al. (2017), Byun et al. (2017), Choi et al. (2017), Imai-Sumida et al. (2017), Wheeler et al. (2017)

gene profiles and alter their expression in many cancers. Available cancer treatment strategies are not so helpful to treat cancer, therefore there is a need to develop a new strategy to target cancer cells and improvement in the immune system against cancer conditions. Some of them are summarized in Table 12.1.

Myriad research articles demonstrated that plant flavonoids, which are components of our diet, have anti-cancer properties through modulating cancer-associated gene expression. Epigenetics modification plays an imperative role in gene regulation and necessary for normal growth, development, and disease (Portela and Esteller 2010; Hussain 2012). Several flavonoids regulate this modification through modulating DNA methylation and histone acetylation and play crucial roles in the maintenance of gene activity (Lee et al. 2013; Shukla et al. 2014; Shankar et al. 2016). Therefore, aberrant epigenetic modification is one of the best therapeutic targets for preventing cancer. Many fruits and vegetables are major sources for flavonoids and have anti-cancer, anti-diabetes, anti-inflammatory, anti-bacterial, and anti-fungal properties (Ren et al. 2003; Harnly et al. 2006; Friedman 2007; Orhan et al. 2010; Chahar et al. 2011; Kawser Hossain et al. 2016; Akhlaghi et al. 2018). Along with flavonoids, vitamins, minerals, and micronutrients are also essential components of food and necessary for a healthier

life (Shenkin 2006; Kennedy 2016; Rautiainen et al. 2016; Pullar et al. 2017; Thakur et al. 2017). Such as vitamins act as a cofactor and these cofactors are part of enzymes (Tutelyan et al. 2013; Okano 2016). Without cofactor, enzymes are not properly active, and not carry out their desired functions. Thus, many flavonoids and alkaloids inhibit cancer progression through modulating protein functionality (Parry et al. 2010; Chen et al. 2015; Kim et al. 2015; Kurosu 2018; Klaver et al. 2019). The impact of some flavonoids is summarized in Table 12.1.

Micronutrients such as selenium regulates cell cycle and apoptosis (Xiao et al. 2008; Zeng and Combs Jr. 2008; de Miranda et al. 2014). Humans consumed selenium from food containing bread, cereals, meat, fish, and poultry. Selenium involves in many biological activities such as DNA hypomethylation (Davis and Uthus 2003), cell cycle arrest, promotes cell deaths, inhibits cell proliferation, and increased glutathione peroxidase (Ip et al. 2002) that promotes DNA repair (Bera et al. 2013). Selenium also plays essential roles in the immune system (Rayman 2012) and development (Kohrle 2000). Some human cells' growth also increases in the presence of selenium (Zeng 2002). Selenium deficiency has a direct impact on lymphocyte proliferation towards mitogens (Hoffmann and Berry 2008). Carnitine is another form of micronutrients, obtained by the consumption of red meat, has reduced the cancer growth through inhibiting HDAC I/II activities (Huang et al. 2012). It involves in fatty acid metabolism by dietary intake and synthesized by lysine and methionine (Need ref). Micronutrients such as vitamins C, D, E, B6, folic acid, omega-3, and calcium are found very less in cancer patients as compared to healthy individuals (Zastre et al. 2013; Campbell 2017; Lettieri-Barbato and Aquilano 2018)**.** For that reason, micronutrients increase responses for cancer prevention.

12.2.2 Aging and Life Span

The aging process is a common phenomenon in which cells unable to perform the normal functions due to loss in homeodynamics behavior as they become older with time (Fedarko 2011). Aging is a complex phenomenon where many factors are involved in this process. Such as cellular senescence, DNA damage, loss of proteostasis, mitochondria dysfunctions, telomerase shortening, epigenetic alterations, deregulation of metabolism, stem cell exhaustion, loss in cellular communication (López-Otín et al. 2013). Diets are vital environmental factors that consider its development, prevention, and treatment of disease. For example, olive oil, the major food component of Mediterranean, increases life span by controlling inflammation and oxidation, which further recover cardiovascular disease and other life-threatening diseases. Study shows that olive oil also enhances motor coordination and contextual memory in mice (Pitozzi et al. 2012).

Moreover, it has been demonstrated that the younger one has more active genes related to DNA repair, replication, anti-oxidative stress, and protein metabolism than the older one (Gorbunova et al. 2007). Micronutrients such as zinc, copper, and selenium affect gene expression and improve the immune system (Monteiro et al.

2015). Zinc is one of the micronutrients and plays an important role in the aging process and age-related genes (Chasapis et al. 2012). It also regulates many enzyme activities as it is necessary for the catalytic activity of enzymes (Maret 2013). Many age-related diseases such as atherosclerosis, degenerative diseases of the nervous system, immunosenescence, and cancer are associated with deficiency of zinc (Prasad et al. 2009).

A proper diet containing fruits and vegetables has many antioxidants and improves human health. Some components of food can be beneficial to and improve the immune system and enhance innate and adaptive immunity (Grimble 2001). Intake of anti-cancer, anti-diabetes, anti-inflammation, and anti-oxidative agents keeps the cells healthy and makes them more capable and adjustable for connecting to their adjacent cells (Leone et al. 2012). Therefore, well-connected cells in a tissue or organ control all signaling pathways in the proper direction. Well-established communication of cells talks to each other and gives a signal to complete a task in an appropriate way. In contrast, cells with loose connections are not communicated well and go to in stress so that cells are going to die in the early stage. Many flavonoids such as resveratrol, epicatechin, quercetin, curcumin, and others have control ROS and maintain homeodynamics behavior of cells resulting in the increased life span of many organisms included humans (Si and Liu 2014). Some bioactive molecules work at a molecule level and regulate many age-related pathways. Disease-associated molecules targeted by many phytochemicals and improved life span of individuals (Si and Liu 2014).

12.2.3 Cardiovascular Disease

Cardiovascular disease (CVD) is a leading reason for death worldwide. More than 50% deaths are occurring due to heart-related diseases (Mc Namara et al. 2019). CVD is affected by both nutritional and genetic factors. Mutation in CVD-causing genes is responsible for the changes in metabolic rate (Kathiresan and Srivastava 2012; Eilat-Adar et al. 2013). Nutrigenomics and nutrigenetics are capable to improve many diseases including CVD. Nowadays, diet or nutrition is being used for modulating gene to improve CVD (peripheral cardiovascular, cerebrovascular, and coronary heart diseases) (Hare et al. 2014; Wu et al. 2015; Kuehl et al. 2016; Xu et al. 2017; Le Bras 2018). The causes of CVD are high cholesterol, high blood pressure, weight depression diabetes, and family history. Fatty deposits inside the artery block the blood flow and low-density cholesterol (LDL) build up in the artery and decrease their diameter so that oxygen supply is limited results in stroke (Linton et al. 2000). To avoid heart disease, a balanced diet is necessary with a low level of saturated fat, salt, and sugar, intake of fibers, fruits, and vegetables. This diet based spectacular treatment is very effective to cure CVD at the gene level. The genetic variance is not subject to a risk for particular disease but bioactive components present in a diet can alter gene polymorphism and can cause a disease (Barnes 2008). Many studies have shown the importance of nutrigenomics to improve health and prevent disease. Polymorphism in a gene is responsible for the modification of

a gene activity and can effect several metabolites pathways, involving in the basic functions of particular gene or/and protein (Fenech et al. 2011; Ahmed et al. 2016). We have summarized nutrition-based polymorphism of a gene and affected phenotype in various types of CVD (Table 12.2).

12.2.4 Diabetes

Diabetes is one of the major causes of death globally. In this multifactorial disease, many metabolic disorders are responsible for increased blood sugar for a long time. It has estimated that 415 million suffer from diabetes according to the International Diabetes Federation Atlas 2015 (Cho et al. 2018). Among three (gestational diabetes, type 1 and type 2), type 2 diabetes (T2DM) is the most general kind of diabetes. T2DM is very complex disease and several factors are involved such as epigenetic, genetic, lifestyle, and environmental factors (Ling and Ronn 2019). We are still unknown to the pathogenesis of T2DM. But numerous studies published and showed that the gene–nutrition interaction has play a role in the prevention of T2DM (Ortega et al. 2017).

Many genes are involved in the controlling of glucose levels and mutation in these genes has affected glucose metabolism and insulin function (Taneera et al. 2015). Several polyphenols involve in diabetes-related gene interaction and regulated T2DM. Such as polyphenols (flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins) maintain glucose levels (Russo et al. 2019). Polyphenolic compounds of diet can exert hypoglycemic effects in numerous ways, such as inhibition of glucose release, glucose absorption, and diminished carbohydrate digestion, protection of pancreatic β-cells against glucotoxicity, and stimulation of insulin secretion. They can also increase glucose uptake in peripheral tissues by controlling intracellular signaling, assisting in antioxidant activity, and preventing advanced glycation end product development (Aryaeian et al. 2017).

12.2.5 Obesity

At present, obesity is one of the major problems and health issue in worldwide. More death is occurred in overweight and obese people than underweight. In 2016, 650 million adults are obese out of more than 1.9 million of total obese according to WHO. Women (15%) are more obese than men (11%) (WHO 2018). Obesity is a multifactorial disease that is affected by the food components presence in our diet. Low cost of food content with low nutritional value and high energy responsible for obesity. Many other diseases are also associated with obesity such as cardiovascular, many cancers, and diabetes (Nakamura et al. 2014; Cifarelli and Hursting 2015). Genetic analyses showed that 40–70% risk of obesity is caused by genetic factors affect body mass index (BMI) (Maes et al. 1997; Herrera and Lindgren 2010).

Table 12.2 Different genes of a cardiovascular system that can be modulated by dietary nutrient

Gene symbol and their protein name	Chromosome location	Function	Associated diseases	Reference
Low density lipoprotein receptor (LDLR)	19p13.2	It binds to the major cholesterol-carrying lipoprotein called LDL. LDL internalized by endocytosis once recognized by LDLR	Alzheimer's and cardiovascular diseases, hypercholesterolemia, familial and carotid artery occlusion, skeleton	Bursill et al. (2001), Muller and Kersten (2003), Lamsa et al. (2008), Al-Naqeep et al. (2009)
Methylenetetrahydrofolate reductase (MTHFR)	1p36.22	It converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. It is the rate-limiting biocatalyst of the methyl cycle, which maintains methyl pool, required for controlling functions	Cancer, cardiovascular diseases, psychiatric diseases, schizophrenia, psoriasis	Ulrich (2005), Vasku et al. (2009), Almen et al. (2010), Alshatwi (2010), Moustafa et al. (2014), Xuan et al. (2014)
Apolipoprotein E (APOE)	19q13.32	It binds to fat molecules such as triglyceride-rich lipoprotein and involves in catabolism of them in liver specifically	Cancer, cardiovascular diseases, Alzheimer's disease, lipoprotein glomerulopathy	Saito et al. (2014), Xu et al. (2016), Kodera et al. (2017), Mahley (2017), Brandon et al. (2018), Zhao et al. (2018b)
Cholesteryl ester transfer protein (CETP)	16q13	It involves in transferring triglycerides from LDL or VLDL with cholesteryl esters from HDL, and vice versa	Hyperalphalipoproteinaemia and coronary stenosis	Hirano et al. (2014), Iwanicka et al. (2018)
Apolipoprotein A5 (APOA5)	11q23.3	Associated with HDL and VLDL. May also be associated with chylomicrons	Hyperlipoproteinemia, type v and hypertriglyceridemia, familial	Evans et al. (2011), Kim et al. (2017), Treviño-Villarreal et al. (2018)
Transcription factor 7 like 2 (TCF7L2)	10q25.2-q25.3	It is transcription protein and it has been suggested to maintain blood glucose homeostasis through Wnt signaling pathway	Obesity, noninsulin-dependent and gestational diabetes, and cancer	Florez et al. (2006), Grant et al. (2006), Frayling et al. (2007), Torres et al. (2016)

(continued)

Table 12.2 (continued)

Gene symbol and their protein name	Chromosome location	Function	Associated diseases	Reference
Arachidonate 5-lipoxygenase (ALOX5)	10q11.21	This enzyme involves in the synthesis of leukotrienes, which play a significant role in inflammatory and allergic conditions	Mutation of this genes can cause several metabolic diseases including asthma and atherosclerosis susceptibility	Assimes et al. (2008), Liu et al. (2017), Bruno et al. (2018)
Lipase C, hepatic type (LIPC)	15q21.3	It involves in lipoprotein uptake besides functioning as triglyceride hydrolase	Modulation of its activity can cause hepatic lipase deficiency	Iijima et al. (2008), Brunzell et al. (2012), Khera et al. (2017)
Apolipoprotein C3 (APOC3)	11q23.3	Liver secreted small size protein and associated with metabolism VLDL, LDL, and HDL	Overexpression of this protein can be linked with coronary heart disease	Xiong et al. (2015), Novoselova et al. (2016)
Perilipin 1 (*PLIN)*	15q26.1	Associated with lipolysis	Lipodystrophy, familial partial, type 4 and perianal hematoma, obesity, and type-2 diabetes	Laclaustra et al. (2007)
Apolipoprotein A5 (APOA5)	11q23.3	The protein plays a vital role in regulating the plasma triglyceride levels, in association with HDL	Mutation of these genes are associated with hyperlipoproteinemia, type v and hypertriglyceridemia	Szalai et al. (2004), Kim et al. (2017)
Serpin family e member 1 (SEPRIN1)	7q22.1	It is serine proteinase inhibitor (serpin) superfamily. It is known as principal inhibitor of urokinase and tissue plasminogen activation	Plasminogen activator inhibitor-1 deficiency, hepatic veno-occlusive disease, Parkinson's disease	Jesse et al. (2012), Kim et al. (2018), Mauro et al. (2018)
Angiotensinogen (AGT)		It is maintaining blood pressure when converts into angiotensin I by enzyme renin	Mutations in this gene are associated with renal tubular dysgenesis, hypertension, cardiovascular disease, and diabetes	Renner et al. (2005), Kobori and Urushihara (2013), Gubler (2014), Lu et al. (2016), Mauro et al. (2018), Zhai et al. (2019)

Cytochrome p450 family 1 subfamily a member 2 (CYP1A2)	15q24.1	It oxidizes a number of structurally different substances, including fatty acids, steroids, and xenobiotics. It acts via NADPH dependent electron transport pathway	Myocardial infarction, Parkinson's disease	Forsyth et al. (2000), Cornelis et al. (2004)
Paraoxonase 1 (PON1)	7q21.3	It is connected with HDL particles, and transforms xenobiotics and thioacetones through hydrolysis	It shows polymorphisms, which can be associated with diabetic retinopathy, and coronary artery disease	Mackness et al. (2004), Ticozzi et al. (2010), Jamuna Rani et al. (2014), Hernández-Díaz et al. (2016)
Glutathione S-transferase (GST)		It mitigates the effect of ROS species and reduces oxidative stress	Cancer, diabetes type 2, hepatocellular damage, and coronary artery disease	Ketterer et al. (1992), Tamer et al. (2004), Pahwa et al. (2017)

Obesity susceptible loci are identified by GWAS. Several obesity-related genes are affected by nutrition through alteration in their gene expression and hormone level (Martínez 2014; Nakamura et al. 2014). These genes are associated with BMI and obesity. Fat mass and obesity-associated gene (*FTO*) is a strongest gene, which is associated with food intake and appetite (Olszewski et al. 2009). *FTO* SNP is more susceptible to the risk of obesity (Speliotes et al. 2010; Chasapis et al. 2012). Enhancement of obesity risk in *FTO* SNP is improved by the intake of dietary fiber. The study has shown that *FTO* SNP and dietary fiber interaction reduced the risk of obesity (Neale et al. 2014).

Adiponectin is encoded by *ADIPOQ* gene secreted by adipocytes and involved in the maintenance of body size. It has been demonstrated that in *ADIPOQ* SNP modulates adiponectin function and susceptible to obesity (Khabour et al. 2018). A diet containing a low level of monounsaturated fatty acids (MUFAs) has a high risk of obesity in *ADIPOQ* SNP. Some components of food have decreased the risk of obesity through regulation of the obesity-related genes. Such as beta-glucan, a dietary fiber present in oat and barley bran control obesity by controlling the body weight and BMI. Additionally, beta-glucan reduces low-density lipoprotein (LDL) cholesterol in hypercholesterolemia (Anderson et al. 2000). Beta-glucan also helps in improving metabolic impair in obese and has immunomodulatory effect in obese (Sun et al. 2017; Straczkowski et al. 2018). It has been demonstrated that some dietary fibers controlled obese and diabetes, such as *Plantago Ovata* husks (psyllium) positively regulates metabolic syndrome, tumor necrosis factor–α (TNF-α), and secretion of adiponectin in obese Zucker rats (Galisteo et al. 2010). Some plant rhizome, such as ginger also improves obese and inflammation by regulating the adenosine monophosphate-activated protein kinase (AMPK) activity as a study has been shown in high fat-fed rats (Kim et al. 2018). Energy intake is directly associated with different polymorphisms in obesity-related genes. We have summarized of well-known associated genes with BMI in Table 12.3.

12.3 Challenges in Nutrigenomics

Nutrigenomics has shown the impact of dietary components (some of them can work as dietary signatures) on specific cells, tissues, and organisms to comprehend how these signals manipulate signaling and metabolic homeostasis for controlling health and diet-related several chronic diseases, like obesity, metabolic diseases, and cancer as aforementioned (Palou and Bonet 2013). These dietary signatures are now being explored as biomarkers for disease prevention in the context of public health strategies (Palou and Bonet 2013). The main goal of nutrition research is diminution of the austerity of these diseases by optimizing the health risks by preventing, interrupting, or reducing their prevalence using a dietary means in an individual.

The very first challenge the area of research encounters is that how we can determine the optimal dietary intake, which can have various food-compounds like various micro-nutrients, macro-nutrients, non-nutritional bioactive mixtures for maintaining the health requirements for every individual. Although there has been

Table 12.3 Different genes of obesity associated that can be modulated by dietary nutrient

Gene symbol and their protein name	Chromosome location	Function	Reference
Adipocyte-, C1q-, and collagen domain-containing (ADIPOQ)	3q27.3	Secretes by adipocytes and enhances energy expenditure	AlSaleh et al. (2011), Hjort et al. (2017), de Luis et al. (2019)
Fat mass- and obesity-associated gene (FTO)	16q12.2	Associated with food intake and appetite	Church et al. (2009), Fischer et al. (2009), Olszewski et al. (2009)
Leptin (LEP)	7q32.1	Associated with energy intake	Lubkowska et al. (2015)
Leptin receptor (LEPR)	1p31.3	It inhibits appetite when leptin binds to this receptor	Dubern and Clement (2012), Wasim et al. (2016), Olczyk et al. (2017)
Insulin-induced gene 2 (INSIG2)	2q14.1-q14.2	Regulation of fatty acid synthesis and cholesterol	Krapivner et al. (2008), Scioli et al. (2014), Liu et al. (2015)
Melanocortin 4 receptor (MC4R)	18q31.32	It is a G protein-coupled receptor and activates by α-melanocyte, which is stimulating hormone, linking to appetite	Fani et al. (2014), Koochakpoor et al. (2016), Krashes et al. (2016), Huang et al. (2017)
Proprotein convertase subtilisin/kexin type 1 (PCSK1)	5q15	Regulates insulin biosynthesis	Hsiao et al. (2014), Stijnen et al. (2016), Ramos-Molina et al. (2018)
Peroxisome proliferator-activated receptor gamma (PPARG)	3p25.2	It induces development of fat tissue and stimulates lipid uptake	Sharma and Staels (2007), Donma and Donma (2016), Polvani et al. (2016), Shao et al. (2016), Abbasi et al. (2017), Wheeler et al. (2017)
Neural growth regulator 1 (NEGR1)	1p31	Regulation of BMI	Vassy et al. (2014), Hyde et al. (2016), Ni et al. (2018)
Transmembrane protein 18 (TMEM18)	2p25	Control appetite and body weight	Almen et al. (2010), Jurvansuu and Goldman (2011), Larder et al. (2017)
Melanocortin 2 receptor accessory protein 2 (MRAP2)	6q14.2	Interact with all known melanocortin receptors, and may regulate both receptor trafficking and activation in response to ligands A mutation in this gene can be allied with severe obesity	Asai et al. (2013), Liu et al. (2013), Jackson et al. (2015), Novoselova et al. (2016), Bruschetta et al. (2018)

(continued)

Table 12.3 (continued)

Gene symbol and their protein name	Chromosome location	Function	Reference
Transcription factor AP-2 beta (TFAP2B)	6p12	Associated with body weight and shape	Tao et al. (2006), Tsukada et al. (2006), Stocks et al. (2012), Stocks et al. (2013)
Phosphotriesterase related (PTER)	10p13	Obesity, adolescent idiopathic scoliosis, urate measurement, body mass index, bone fracture, osteoporosis, eye morphology measurement	Morgan et al. (2010), Bernhard et al. (2013)
Prolactin (PRL)	6p22.2-p31.3	Chronic obstructive pulmonary disease, pulmonary function measurement, smoking behavior measurement, obesity, osteitis deformans	Carre and Binart (2014), Ben-Jonathan and Hugo (2015), Bernard et al. (2019)
Methionine sulfoxide reductase A (MSRA)	8p23.1	Systolic blood pressure, alcohol drinking, neuroticism measurement, systolic blood pressure, mathematical ability, smoking status measurement	Lindgren et al. (2009), Scherag et al. (2010), Bille et al. (2011)
Mitochondria carrier 2 (MTCH2)	11p11.2	Play a governing role in adipocyte differentiation and its biology	Heid et al. (2010), Kulyte et al. (2011), Fall et al. (2012), Cheng and Almeida (2014)
Glucoseamine-6-phosphate deaminase2 (GNPDA2)	4p12	Body mass index	Li et al. (2010), Ouyang et al. (2016)
SH2B1 Adaptor protein (SH2B1)	16p11.2	Involved in many signaling pathways	Bachmann-Gagescu et al. (2010), Herrera and Lindgren (2010), Doche et al. (2012), Zheng et al. (2013), Mansego et al. (2015)
Neuropeptide Y (NPY)	7p15.3	Regulation of energy balance by stimulating food intake	Crescenti et al. (2013), Zain et al. (2015), Gotthardt et al. (2016), Kim and Bi (2016), Alkan et al. (2019)
Beta-carotene oxygenase 1 (BCO1)	16q23.2	Involved in waist-hip ratio	Hessel et al. (2007), Arias et al. (2009), Ford et al. (2013)

strong relationship and understanding established between the basic nutrients required for the health and quantifying the nutrition present in given diet (van Ommen et al. 2010). Many modern approaches and technologies have been used by the researchers to define beneficial diet for refining the nutritional guidance on the basis of nutrigenomics, which can help to lead a disease-free life, but two major important axes of nutrition specific research which are far from perfectness yet. Firstly, a major challenge is the characterization and quantification of the nutrients with perfect accuracy on large population studies that have not been established yet. However, it is important that we should know how much dietary exposure is given very precisely to an individual, which is termed as "input" or exposure axis (Herrera et al. 2011).

The second difficulty is the level of biomarkers that have been recognized to quantify the phenotypic outcome of dietary exposure is suboptimal. Good health is identified as a condition where there is no evidence of diseases, though it has nowadays been widely documented that this definition is inadequate. Many experiments performed with nutrition studies have shown that even though there was no phenotypic recognition of the disease, but their genetic makeup was shown the disease risks at their endpoints. These altered markers have very important implications in nutritional studies, but very few of these markers are authenticated. Another factor that should be taken into consideration is the homeostatic robustness, a phenomenon by which an individual meets to its nutritional requirements. Besides these two axes, another factor that has to be considered is the genotypic variation, which includes genetic and epigenetic changes upon time duration. These variations further pose difficulties to establish that the relationship between input and output axis is complicated (Herrera et al. 2011).

Eating is a psychosocial behavior that involves many aspects like habits, tastes of the material and environmental factors. So, one of the greatest challenges faced by many dietary management approaches is to encourage individuals to modify their dietary habits. With this known fact, the consumption of food is a complicated area which comprises both emotional, taste, and pleasure-based reaction, thus a multifaceted path which must be taken in account while working with a person's inspiration to pursue the dietary recommendations. The success of personalized and customized diets is remarkably dependent on the sole factor of the individual's will-power and motivation, only then the prospective benefits of this nutrigenomics can be achieved (Fallaize et al. 2013).

Moreover, this personalized nutrition advancement based on genetic information can pose ethical and functional controversies. All the health experts previously suggested the personalized diet plan to an individual, by routinely evaluating many biological data like height, weight, gender, and status of many biomarkers like cholesterol, vitamin, hormonal, and mineral deficiency. But with the coming of nutrigenomics, it has been well validated that genotype is one of the key factors, which is the determinant, has to be included. Also, the recent trends suggested that nutrigenomics advices are better comprehended, and are followed than general dietary instructions due to their genetic modulation (Nielsen and El-Sohemy 2014; Nielsen et al. 2014).

Those individuals identified with a nutrigenetic test at a possibility of higher disease risk may be encouraged more to follow dietic orders. In this context, widespread issues of dietary changes are addressed and interlinked with changing eating behavior to genetic changes (Joost et al. 2007). Nutrigenomics is confirmed to be advantageous in controlling the long-standing weight problems (Arkadianos et al. 2007), as verified with the Food4me project. Food4me speaks of the broader and complex portion of ethical aspects of food consumption, evaluating the effectiveness of personalized nutrition not only with the changing activities but also with business connotation, consumer needs, and awareness (Markovina et al. 2015).

Nutrigenomics is significant for recognizing obesity with its linked diseases. All the dietary chemicals from the food act together with the biochemical pathways, which are concerned with the control of body fat (lipogenesis, nutrient assimilation in the intestine, heat generation, and finally white adipose tissue (WAT) browning), together with inflammation and stress pathways. Through these studies, it has been shown that specific food chemicals alleviate obesity in animals by molecularly distinct machinery (Kim and Park 2011; Bonet et al. 2012; Bonet et al. 2013). With above-gained knowledge, nutraceuticals have proposed functional foods for weight management. Our existing knowledge is just based on animal models and in vitro cell studies (Palou and Bonet 2013). Human intervention studies are very limited, thus proposed most of the "anti-obesity" foods have remained a challenge till date to determine their efficacy.

The major target of nutrigenomics studies is the public health intervention and awareness at the population and individual levels (Gibney and Gibney 2004; Cambon-Thomsen et al. 2007). At this stage, the application of genetic knowledge at the population level is a very critical issue. There has been much advancement in the human genetic study through meta-omics tools for the modification of the public health nutrition policy (Zeisel 2013b). But this framework is way behind at a functional perspective when it comes to using genetic studies on the improvements of public health. For example, a researcher found that a specific polymorphism can alter the risk of diabetes in individuals (Zeisel 2013b), which further suggests modifying the requirements of the nutrient composition at an individual level. Also, with the application of genetic knowledge, it is known that low dietary fibers can lower the risk of diabetes, however, when it comes to giving personalized nutritional recommendation–it again poses the difficulty of recommending to intake five times fruit or vegetables in a single day (Zeisel 2013b). Such a scenario warrants further studies at gene–nutrient interaction association.

Dietary habits and their components change the molecular targets, which are present in a specific pathway of cell control, and any disruption in them can increase cancer risk. These dietary components may exert additive or synergistic behavior like they interfere with the different phases of the cell cycle and inhibit tumor progression. Furthermore, these nutritional habits are important environmental factors, which can be easily modified cancerous conditions. Such as, it is estimated that around 30–40% of cancer cases are linked to changes in dietary practices (Wiseman 2008). As similar to other metabolic conditions, there are challenges to find accurate and economical methods for assessing the specific nutrients (essential

and non-essential) present in the diet, which can reduce the cancer risk are still in infancy. For instance, there are many incomplete error-prone results available that estimate the content of nutrients, interactions of different food components with metabolic pathways (Olafsdottir et al. 2006). All these data limit our knowledge in estimating exposure/biomarker dependent on the fact, obtained from all the data on different content of nutrients. In such circumstances, there are two major data compilation tools, namely food frequency questioners (FFQ) and 24-h recalls that help in guesstimate exposure of nutrients (Olafsdottir et al. 2006).

The nutrition-specific requirements are adopted by proposing certain strategies that address data handling challenges. It is a very important area, which explains the difficulties faced in data processing for nutrigenomics research. A well-established infrastructure list of data processing is existing called "A nutrigenomics research infrastructure." And another crucial database, called the nutritional phenotype database (dbNP), along with nutrigenomics research infrastructure provides open access to detailed accounts of information that link relationships between diet, lifestyle, and health. The Organisation, who maintains a sustainable model for the database, is "Nutrigenomics Organisation (NuGO, http://www.nugo.org)" and focuses on joint research activity related to molecular and personalized nutrition, nutrigenomics, and nutritional systems biology. There are six major areas where nutrition research has shown revolutionary changes, as shown in Fig. 12.1 (van Ommen et al. 2010).

1. Data density—there is a large amount of data production as a result of the new technological progress in genomics, storage, and handling of information. However, assessment of the immensity of data should be considered as noise or sound solid information platform. Usually, we do nowadays rely on multiple genes or whole cell proteomics assays that ultimately produce a vast number of information in forms of bytes. For example, we perform whole genome sequencing of gut microbes, magnetic resonance imaging (MRI), mRNA expression of cellular pathways, and multiplex protein studies to understand the impact of a specific nutrient, which was given to an individual (Claus and Swann 2013; Swann and Claus 2014). These techniques ultimately produce a large amount of data in routine practices in nutrition research. Recently transcriptome analysis is ongoing in large human cohorts (Li et al. 2014; Sweet et al. 2018; Ray et al. 2019).
2. Accessibility—Due to the establishment of several biobanks, the introduction of Web 2.0, journal policies, and legislative practices conveniently allow sharing the data for a scientific perspective. And these accessibilities provide great help in analyzing data for mining biomarkers of diseases. Such as a recent study mined some cancer protective gene signature (Martin-Hernandez et al. 2018). Some important data sources are relied by many nutritional research scientists, such as dbGAP database (Mailman et al. 2007). Besides those, strict regulation is maintained to preserve the privacy policy related to genetic information (Cupples et al. 2007).
3. Scale expansion—due to widespread research empowerment and investment by the public on nutrition research and development of meta-omics technologies, we have observed an increasing trend of sizes and cohorts through genome-wide

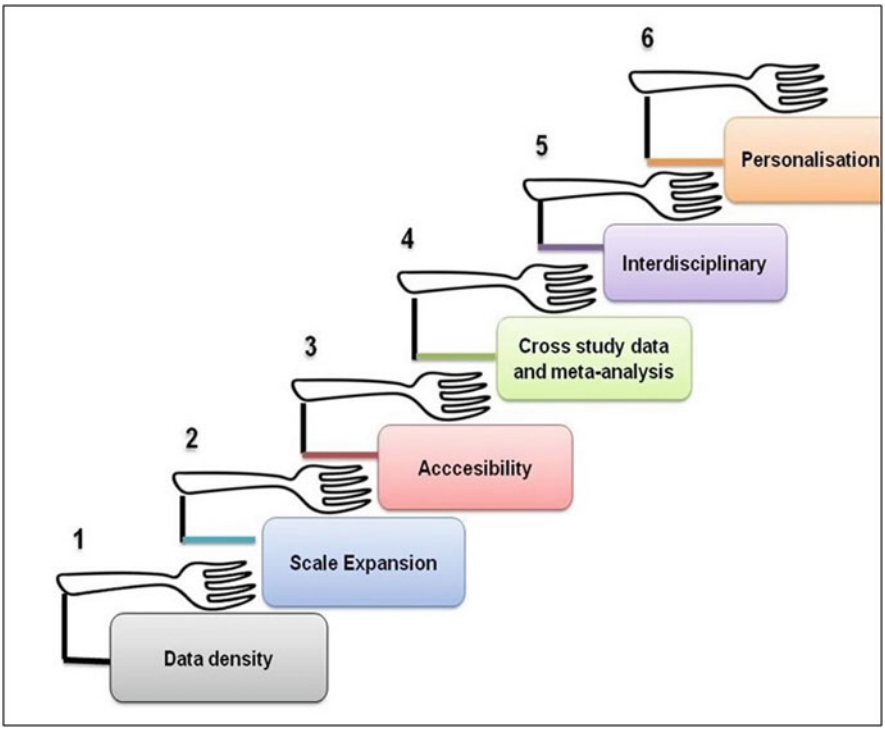

Fig. 12.1 Major 6 areas of data handling challenges for nutrition research (van Ommen et al. 2010)

geno-/haplotyping studies (Yuille et al. 2008; Takahashi et al. 2014). Large repositories called biobanks have been established with the help of improved protocols and methodologies as well as stored the data (Szczepek et al. 2019), Biobanking Trends, Challenges, and Opportunities (Paskal et al. 2018).

4. Meta-analysis and cross-study validation—Huge number of nutrigenomics data can be accessed, handled, organized, and analyzed easily by the development of several softwares. In regard to this, it will be handy to dig out significant information of specific trade with robustness. This has been demonstrated by Pico et al. (2019).
5. Interdisciplinary—in order to get significant information on any metabolic diseases, we need to have a team of scientists that should have complementary expertise of interdisciplinary subjects ranging of biochemistry, analytical chemistry, and molecular biology. Having these combinations would provide a detailed picture of metabolic disease (such as obesity or diabetes) from the structuring of diet ingredients to gene expression. The study will also need a bioinformatician who can rationally compare the results of a study to publicly available data.
6. Personalization—with the advancement in resolving the database challenges, it has become possible to get access to accurate clues or biomarkers through diverse

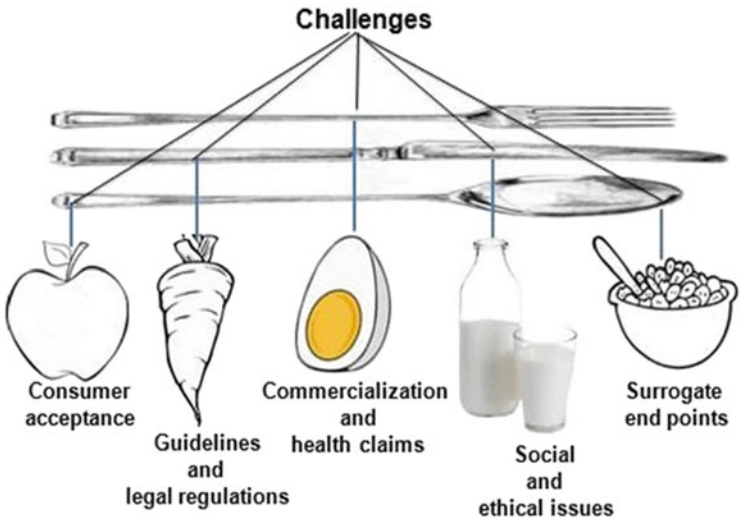

Fig. 12.2 Challenging areas of nutrigenomics

diagnostic applications tools/softwares, which as assemble genotyping and phenotyping data on a blank canvas, such as PheGenI. Henceforth, we have become proficient enough to divide the population into subclasses based on the specific nutritional requirements and genetically response.

Over the years, we have made some advancements due to the invention of metaomics techniques and softwares. Nevertheless, taking the next level of this area, it is very important that we recognize the complexities faced by the discipline from every aspect. If left unchecked, it may damage the credibility of the complete field. So, it is mandatory to manage these difficulties for the smooth functioning of the field with full integrity. We summarize the major challenges of the area which has to be answered for its advancement (Fig. 12.2).

12.3.1 Consumer Acceptance: A Challenge in Tailoring Diets for Public Service Delivery a Question Still Unanswered

Any new technology is acceptable to the public with awareness, which influences the recipient's approvals. Furthermore, public acceptance highly depends on the benefits, expenses, risks, moral beliefs, and personal attitudes that are associated (Sjöberg 2004; Ronteltap et al. 2009). There have been many studies that show the widespread use of genetic tests for customized nutritional approach, while a widespread positive attitude shown by the public concerning to personalized nutrition (Roosen et al. 2008; Stewart-Knox et al. 2009; Ahlgren et al. 2013). The huge innovations in food technology and nutrigenomic research have led individuals to

react regarding conceptions of health benefits with lowered chances of nutrition-related diseases (Costa-Font and Mossialos 2007). But, these genotype-based dietary advice can bring a large-scale behavioral change to those who are still with the uncertainty of nutrient benefits. In a particular study, 149 individuals have participated, and out of them who received detail information regarding genetic benefits on the basis of personalized nutrition, the intervention was highly responded as compared to others who got information that given nutrition would be useful (Nielsen and El-Sohemy 2012).

The privacy of genetic information is a big concern because it can influence the insurance companies, selection of athletic teams, and appointment of employers (Roche and Annas 2001). With that regard, putting the genetic profile information of an individual publicly may not legal or may not be accepted by the individual, which could hamper this area of research. Another concern is the genetic profiling of family history. As a matter of fact, we do share many genes with our family members. So, health professionals should engage our family members in performing genetic profiling and share the individual's private genetic information or keep it confidential. This is relevant because many hereditable diseases are in family history, which may help in predicting future disease risk (Camp and Trujillo 2014).

Overall, detailed genetic tests one hand help in clearing the doubts of individuals, while another side it can cause unnecessary misinterpretation. As genetic test may give an ample amount of information that may not be important to a person but can deliver many unwanted results—create anxiety in the individuals (Zeisel 2013a). So, it must be the job of health professionals to address the issues connecting the opportunistic screening and should provide only that much information that is asked or needed. The current ethically right of an individual for knowing the information about genetic profiling is summarized by Allyse et al. (2018), and also suggested about direct-to-consumer (DTC) genetic testing.

For a long time, genetic tests are provided to the patients with the consent of medical referrals and genetic counselors for health-related problems. But, with the commercialization and increasing curiosity of the individuals, there is an advertisement boom of a genetic test in the markets with varying involvement of the medical professionals (Sanfilippo et al. 2015). In some cases, even though the DTC is advertised by health professionals, but the service providers are sold resultant to recipients without any participation of medical white-collars (Hogarth et al. 2008). The problem arises with the latter cases, which is a very controversial topic among the different stakeholders. Nevertheless, a solid criticism exists to this model of easy availability of genetic test provisions without any medical supervision and genetic counseling (Allyse et al. 2018). Many of the commercial stakeholders and medical professionals have raised concerns about the possibilities of misjudgment of test results by consumers, which may be creating unnecessary confusion, distress, and culminating in error-prone healthcare systems liabilities (Howard and Borry 2012).

12.3.2 Guidelines and Legal Regulations

Every country should have regulatory agencies, which can formulate regulations for genetic testing. It is the moral duty of the producers of genetic testing that they should follow the strict guidelines laid according to the ethical/legal rules established in their own country (Annas and Elias 2014). These guidelines are very important as it aims to safeguard the individuals from the false, dangerous services, provide accuracy of the genetic test, interpretation validity of clinical data, and its clinical utility. All these considerations when taken into account will help in identifying the genes and polymorphisms, and authentication of the data, which will ultimately lead to the positive consumer adherence to nutrigenomics (Castle and Ries 2007). However, it is depressing that legal regulations are insufficient, and no countries have strict or fully functional guidelines documented yet. But with the recent understanding, progress has been made by many countries to publish information and guidelines after discussing with various government agencies and organizations regarding genetic testing to have more in-depth clarifications to alert the individuals (Liu and Qian 2011; Kalokairinou et al. 2018). Previously nutrigenomics tests were categorized under "lifestyle test" which does not require any legal regulations as proposed by the Medicine and Healthcare Products Agency (MHPA) and Nuffield Council of Bioethics. But this was strongly opposed by Human Genetic Commission, who disapproved and recommended, these tests are similar to other normal routine diagnostic tests; hence they should strictly follow all the laid rules and legislation for the same (Borry 2009).

Presently, three major organizations are involved in making legislative measures in the USA are the Food and Drug Administration (FDA), the centers for Medicare & Medicaid Services (CMS), and the Federal Trade Commission (FTC) https://www.genome.gov/about-genomics/policy-issues/Regulation-of-Genetic-Tests. All these agencies have the authority to regulate all the legislative measures but then also some of the important points are still compromised. FDA regulates a test on its entrance in the market, and when it is sold to multiple laboratories as kits. But no announcement on regulation has still made when it comes to a small number of tests or confining to the single laboratory, as is the case in nutrigenomics FDA has no regulation on this. The CMS regulates compliance of genetic testing, with the Clinical Laboratory Improvement Amendments (CLIA) of 1988. But rather than investigating clinically meaningfulness of these genetic analyses, it only focuses on experience, credentials of technicians, and quality control. The FTC is not only restricted on advertising the test on just guaranteeing that information is not ambiguous (European Commission 2004), but also looks on advertising claims for genetic testing products and services as well as protect genetic data of personal (Wagner 2014).

The different countries have formulated their national laws in providing DTC genetic testing. But these regulations appropriateness is complicated as they are formulated outside the jurisdiction of traditional healthcare systems. Also, with the applicability of legal regulations, a complex heterogeneity prevails, in order to govern these DTC tastings. These regulations are not sturdy and the genetic testing

approaches show the obscurity in formulating these legal regulations, which blur the lines between medical, legal, and consumer framework (Kalokairinou et al. 2018). Some countries are on the verge of banning DTC testing, whereas some others have a very fragile regulatory structure (Kalokairinou et al. 2018). However, many specific laws are made to protect the consumer's protection in regard to the use of in vitro diagnostics.

12.3.3 Commercialization: Building Health Professional Capacity from Academics to Industries

Many online companies have come into the markets offering genetic tests, but these offered tests are subjected to strong criticisms of misleading the consumers. Also, there is an imbalance in the promises posed that have far-reaching claims, but whatever is received is under contradictories (Ahlgren et al. 2013). The offered genetic tests are a matter of discussion, as they can give ambiguous information to the consumers, which may harm their right to get information autonomy (Vayena 2015). Such as direct-to-consumer genetic testing (DTC GT) is under threat because they perform limited validation of the tests and there is a risk to increase psychosocial stress (Schaper and Schicktanz 2018). FDA has approved only one test called Personal Genome Service (PGS), which gives the status of the carrier, familial reports, and wellness traits. Also, it has been mandatory to ask health professionals if any major change in lifestyle has to be undertaken, e.g. the press release report in 2015 of "23andMe" says that they cannot anticipate or diagnose any disease conditions, which may not have relevant to nutrition (Annas and Elias 2014; Annas and Elias 2014). Such strong legislative measures approval for the commercialization of genetic tests will make a founding milestone and safeguarding on consumer health (Darnovsky and Cussins 2014).

12.3.4 Premature Health Claims

The major challenge is the exploitation of the enormous amount of information, which is a consequence of the weakness in current practices. These ample amounts of data may be misused as they can be directly utilized by the financial companies, consequently, they can rush into the markets for promoting their interests. Industries are playing major roles in providing infrastructure, sound technology, and scientific research for the betterment of the public, but on the other side they can manipulate available data and disseminate partial evidences in wrong hands for their advantage. Such condition can create problems to the consumers (Hunter et al. 2008).

12.3.5 Social, Ethical, and Legal Implications

Nutrigenomics research has transformed food into medication, but this raised tension with the socio-cultural aspects. Food in totality is the sum of active and inactive

ingredients, which interacts with our genes and genomes. Nevertheless, according to social aspects food has a deeper insight to play in a community rather than just to support health and disease reduction (Korthals 2011; Nordstrom et al. 2013).

The field of nutrigenomics, which is full of genetic information, is burdened with complexity and intricacy. With this field are attached many legal, ethical, and social implications that should be properly known and followed for well awareness of patients (Capron 2009). The main obscurity is needed to manage the genetic information, confidentiality of information with familial consequences, and protection from insurers (Castle and Ries 2007). We should be clear with the fact that a healthy diet and lifestyle are the keys to a disease-free life, and we should not dilute this message with any unrealistic expectations. It is also advisable that the consumers should not be frightened of the results of genetic testing and start speculating future dangers. This will unnecessarily give the advantages to non-medical group people to modify us by creating a condition, which can be cured using the expensive but still unproven "personalized diet."

So, we should look at what we have in our hands and continue looking at things which are within our reach, as there are many dietary choices like organic foods, low fat and glycemic products, roughage containing fruits and vegetable known. These are healthier to combat the deficiency and, very importantly, free of any independent knowledge of genetic makeup (De Caterina 2010a). Contrastingly, in order to properly implementation of personalized diet to an individual for the medical purpose, it should be clearly discussed with the patient about the risk and benefits associated with this therapy. Genetic profiling of individuals should be kept secret even from an immediate family member unless it is not necessary to disclose with other members of the family in critical condition. For minimizing social issues, health professionals must respect cultural and societal values and must associate with a patient to get him aware of the social consequences of genetic information and look after through follow-up care (www.ornl.gov/sci/techresources/human_genome/research/elsi.shtml). Health professionals can be made referrals to psychologists, genetic counselors, or social workers if needed. All these immutable rigid considerations must be answered. Otherwise, it may lead to reductionist approaches towards nutritional sciences which continuously concretize food as only explicating factor for disease preventions (Lang and Barling 2013).

12.3.6 Surrogate Endpoints: A Double-Edged Sword Showing Optimism and Skepticism of Nutrigenomics Science

Every clinical investigation should have readily measurable and accessible parameters, which will help to find the way where the research has started, and where that research topic is going, i.e. we should have surrogate endpoints. For example, high-density lipoprotein (HDL) cholesterol is characterized as good cholesterol and can be used to measure the status of coronary heart disease death (Zeng and Gao 2017). By measuring such a parameter, it can be judged an impact of a nutrient that is given to a patient in negative and positive ways. So that if any health

issue arises, can be prevented or any alternative nutrient can be prescribed (De Caterina 2010b). To the patients, it may be of non-significant importance; however, surrogates can be misleading and deriving erroneous conclusions if proper personalized nutrition care was not adopted (Collins et al. 2008). Surrogate endpoints also involve clinical biomarkers that give the intimation about status of a disease and can be categorized into different types and classes and can be used for enrichment, predictive, and prognostic for clinical trials to support drug and biologic approvals (Cagney et al. 2018). The surrogate endpoints have always been the discussion of debates and lead to differences in opinions and present conflicting viewpoints in the researcher's community itself (Medeiros 2017). Hence, it is very important that all the researches should put their perspectives of the research trials with proper endpoints.

12.3.7 Newer Upcoming Challenges and Our Expectations

There has been lots of advancement in meta-omic technologies, despite that we cannot evaluate the dietary intake of volunteers, which is the first step challenge in nutrigenomics. Most of the tools developed so far are less reliable, so the goal of the future should be the development of improved tools, which will help in quantifying the food intakes (Tucker et al. 2013). The nutrition-related study is complicated and encompasses the multigenic factor, so just studying the genotype will not solve the purpose. We should know much more about the polymorphisms and interaction between the genes of the pathways, which in today's time has got limited information. So, we need to advance technologies and tools that can help us to understand the entire interactive gene influencing under nutritional metabolism. Such as the SNPsnap (a web-based tool) is new software, which helps us in analyzing the quantity of trait-associated single-nucleotide polymorphisms (SNPs), and linkage disequilibrium among individual of a population. But for firmer knowledge, we need more such programs which can aid us to discover the nutritional metabolic pathways which affect the nutrition requirement (Pers et al. 2015).

One of the remarkable future perspectives of personalized nutrition is the identification and evaluation of novel biomarkers, which can be successfully used in metabolic interference in order to reduce the risk of diseases (Lu et al. 2011; Belongie et al. 2017). Three major fields that are still in developing stages—lipidomics, proteomics, and metabolomics. These areas show promising future to be applied as biomarkers, programming the course of metabolism and food utilization in dietary interventions, which are the main challenges for personalized nutrition (Hyotylainen et al. 2013). Nutrimetabolomics targets various biomarker profiles of various food constituents, gut microbiota, and their distinct molecular signatures (Claus and Swann 2013; Mancano et al. 2018).

Our gut microbiota has the potentiality to modify our dietary nutrients, and their responses by changing the dietary compounds and their composition (Singh 2019), hence assessing the functional human microbiome is progressing very fast (Almeida et al. 2019). Therefore, we need an integrated study of dietary nutrients with the gut

flora, and responses of host cells (including immune cells), which covers all the biotransformation, ingestion, absorption, and digestion of food (Dutton and Turnbaugh 2012). Consequently, investigating the genomic profiling of the intestinal gut microbiota is an efficient tool for diagnostics and personalized metabolic interventions in nutrigenomics (Leshem et al. 2019).

Many dieticians and physicians lack the education and information needed to infer the results of either nutrition or genetic so that they can give personal advice. Also, many dieticians do not regard themselves suitable to take nutrigenomics into their practice, while many others lack curiosity in taking personalized nutrition into practice (Cormier et al. 2014). With this kind of confusion, it is very difficult to build the trust of the consumers, as they should be offered reliable services. So, it is very important for the success of nutrigenomics that there should be a great understanding about the subject among dieticians to build the confidence of the consumer (Stewart-Knox et al. 2013).

Another hurdle in applying the nutrigenomics technologies on large scale clinical practices is expenses, which are not within reach of the majority of the population. This fact of high cost limits the use of personalized nutrition. So, one of the aims of scientists and industries should be to decrease the expenses of personalized genetic examining programs and make products affordable for individuals. Not only this, it is crucial that advisory services should be also economical to interpret these tests, otherwise it would be hampered additional cost for consumers (Brunham and Hayden 2012).

Meta-omics technologies are useful in validating the hypothesis or without having any pre-determined hypothesis. The advantage of these technologies is the production of a huge amount of data, which is publicly available. Nevertheless, they have raised the biggest challenge, mainly on how to organize this boom of knowledge into powerful decision-making or recommending a diet to patients. We need strong statistical methodologies to transfer the extensive genetic knowledge into useful information (Lundstrom 2013; Phillips 2013). These mathematical models should provide integrated information on the exploration of the genotypic knowledge and surrounding environment, which can be extracted into useful, applicable forms (Rimbach and Minihane 2009; Ong et al. 2015). It will not be exaggerated, if we pose that future of all genomic sciences is dependent on the power and speedy use of newer efficient bioinformatics tools and how these tools will support the nutritional approaches, strategies, and interventions at public health level or individual level (Rimbach and Minihane 2009).

12.3.8 How to Fight These Challenges

We summarized the major hurdles in the field of nutrigenomics in the current chapter. Overcoming these challenges is difficult but not impossible. Also, fighting this battle will become furthermore difficult if we have only individualistic approaches. Rather, we should have multidisciplinary team approaches that can synchronize as an intermediate between the consumers, scientists, and industries.

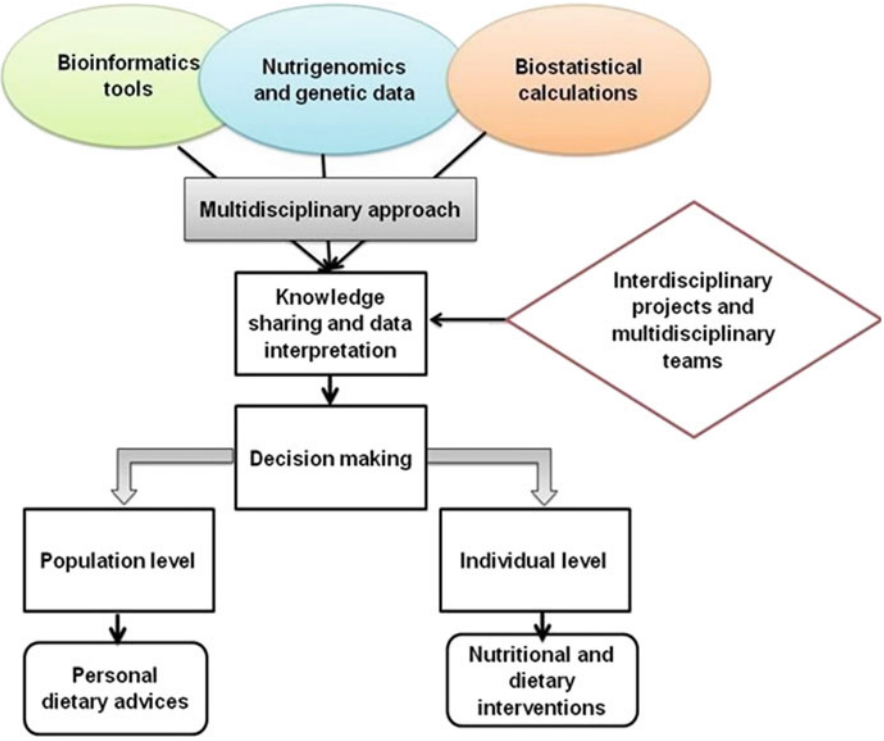

Fig. 12.3 The protocol of translating nutrigenomics information to the public. Nutrigenomics study is a collaborative approach to encompassing diverse stakeholders. So, for the smooth functioning of these nutrition sciences, it is fundamental to have expertise collaborations among different stakeholders, socioeconomic inclusion, appreciating the limitations. Taking all these points into considerations initial design of effective research protocols should be prepared, which can be helpful in fulfilling the benefits and assessing the risks of nutrigenomics (Hurlimann et al. 2014)

These daunting challenges will be overcome by having an increased amount of understanding with sound scientific knowledge, and all the informations should be positioned in the correct place with the individual. The nutrigenetics approaches should be culminated in such a manner that greater perception of information should be passed on to the public. So that they know the role of genetic variation and response to different dietary nutrients in the sense of gene expression (Kelder et al. 2009). Overall, we can summarize an accurate way of translating nutrigenomics studies to the public through Fig. 12.3.

12.4 Conclusion

The future of nutrition and genes is highly promising. It explains that the future will entail a better understanding of the full connection between genes and health. By identifying these connections, nutrigenomics may help to provide an explanation between chronic disease development and diet or even a specific food, built on the person's DNA testing. Furthermore, this advancement will positively affect the food industries, as they will start manufacturing the designed fortified foods. With these insights, it is probable that new nutritional policies will be achieved for large populations. Therefore, nutritional supplementation and disease prevention programs will be implemented to suitable individuals who are found to be at risk. It is further realized that it will require extensive clinical trials to appreciate the facts for which group of the population can be considered for nutritional interventional implementation. Outcomes of the clinical trial will help in personal nutritional counseling rather than typical nutritional diets that was being given for several years. Personal nutritional counseling will assist in improving lifestyle through changing diet habits; thus, it will allow an improved diagnostic of certain diseases and disregard the development of chronic illnesses.

Acknowledgments R. P. Singh would like to thank the Department of Biotechnology, India for providing Ramalingaswami Re-entry Fellowship.

References

Abbasi A, Moghadam AA, Kahrarian Z, Abbsavaran R, Yari K, Alizadeh E (2017) Molecular effects of leptin on peroxisome proliferator activated receptor gamma (PPAR-gamma) mRNA expression in rat's adipose and liver tissue. Cell Mol Biol (Noisy-le-Grand) 63:89–93

Ahlgren J, Nordgren A, Perrudin M, Ronteltap A, Savigny J, van Trijp H, Nordstrom K, Gorman U (2013) Consumers on the internet: ethical and legal aspects of commercialization of personalized nutrition. Genes Nutr 8:349–355

Ahmed S, Zhou Z, Zhou J, Chen SQ (2016) Pharmacogenomics of drug metabolizing enzymes and transporters: relevance to precision medicine. Genomics Proteomics Bioinformatics 14:298–313

Akhlaghi M, Ghobadi S, Mohammad Hosseini M, Gholami Z, Mohammadian F (2018) Flavanols are potential anti-obesity agents, a systematic review and meta-analysis of controlled clinical trials. Nutr Metab Cardiovasc Dis 28:675–690

Alkan I, Altunkaynak BZ, Altun G, Erener E (2019) The investigation of the effects of topiramate on the hypothalamic levels of fat mass/obesity-associated protein and neuropeptide Y in obese female rats. Nutr Neurosci 22:243–252

Alkhatib A, Tsang C, Tiss A, Bahorun T, Arefanian H, Barake R, Khadir A, Tuomilehto J (2017) Functional foods and lifestyle approaches for diabetes prevention and management. Nutrients 9: E1310

Allyse MA, Robinson DH, Ferber MJ, Sharp RR (2018) Direct-to-consumer testing 2.0: emerging models of direct-to-consumer genetic testing. Mayo Clin Proc 93:113–120

Almeida A, Mitchell AL, Boland M, Forster SC, Gloor GB, Tarkowska A, Lawley TD, Finn RD (2019) A new genomic blueprint of the human gut microbiota. Nature 568:499–504

Almen MS, Jacobsson JA, Shaik JH et al (2010) The obesity gene, TMEM18, is of ancient origin, found in majority of neuronal cells in all major brain regions and associated with obesity in severely obese children. BMC Med Genet 11:58

Al-Naqeep G, Ismail M, Allaudin Z (2009) Regulation of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression by thymoquinone-rich fraction and thymoquinone in HepG2 cells. J Nutrigenet Nutrigenomics 2:163–172

AlSaleh A, O'Dell SD, Frost GS, Griffin BA, Lovegrove JA, Jebb SA, Sanders TA (2011) Single nucleotide polymorphisms at the ADIPOQ gene locus interact with age and dietary intake of fat to determine serum adiponectin in subjects at risk of the metabolic syndrome. Am J Clin Nutr 94:262–269

Alshatwi AA (2010) RETRACTED: breast cancer risk, dietary intake, and methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphisms. Food Chem Toxicol 48:1881–1885

Anderson JW, Davidson MH, Blonde L, Brown WV, Howard WJ, Ginsberg H, Allgood LD, Weingand KW (2000) Long-term cholesterol-lowering effects of psyllium as an adjunct to diet therapy in the treatment of hypercholesterolemia. Am J Clin Nutr 71:1433–1438

Annas GJ, Elias S (2014) 23andMe and the FDA. N Engl J Med 370:985–988, 2248–2249

Arias E, Gonzalez A, Shimada A, Varela-Echavarria A, Ruiz-Lopez F, During A, Mora O (2009) Beta-carotene is incorporated or mobilized along with triglycerides in bovine adipose tissue in response to insulin or epinephrine. J Anim Physiol Anim Nutr 93:83–93

Arkadianos I, Valdes AM, Marinos E, Florou A, Gill RD, Grimaldi KA (2007) Improved weight management using genetic information to personalize a calorie controlled diet. Nutr J 6:29

Aryaeian N, Sedehi SK, Arablou T (2017) Polyphenols and their effects on diabetes management: a review. Med J Islam Repub Iran 31:134

Asai M, Ramachandrappa S, Joachim M et al (2013) Loss of function of the melanocortin 2 receptor accessory protein 2 is associated with mammalian obesity. Science 341:275–278

Assimes TL, Knowles JW, Priest JR et al (2008) Common polymorphisms of ALOX5 and ALOX5AP and risk of coronary artery disease. Hum Genet 123:399–408

Bachmann-Gagescu R, Mefford HC, Cowan C et al (2010) Recurrent 200-kb deletions of 16p11.2 that include the SH2B1 gene are associated with developmental delay and obesity. Genet Med 12:641–647

Barnes S (2008) Nutritional genomics, polyphenols, diets, and their impact on dietetics. J Am Diet Assoc 108:1888–1895

Bayram D, Cetin ES, Kara M, Ozgocmen M, Candan IA (2017) The apoptotic effects of silibinin on MDA-MB-231 and MCF-7 human breast carcinoma cells. Hum Exp Toxicol 36:573–586

Belongie KJ, Ferrannini E, Johnson K, Andrade-Gordon P, Hansen MK, Petrie JR (2017) Identification of novel biomarkers to monitor beta-cell function and enable early detection of type 2 diabetes risk. PLoS One 12:e0182932

Ben-Jonathan N, Hugo E (2015) Prolactin (PRL) in adipose tissue: regulation and functions. Adv Exp Med Biol 846:1–35

Bera S, De Rosa V, Rachidi W, Diamond AM (2013) Does a role for selenium in DNA damage repair explain apparent controversies in its use in chemoprevention? Mutagenesis 28:127–134

Bernard V, Young J, Binart N (2019) Prolactin - a pleiotropic factor in health and disease. Nat Rev Endocrinol 15:356–365

Bernhard F, Landgraf K, Kloting N, Berthold A, Buttner P, Friebe D, Kiess W, Kovacs P, Bluher M, Korner A (2013) Functional relevance of genes implicated by obesity genome-wide association study signals for human adipocyte biology. Diabetologia 56:311–322

Bille DS, Banasik K, Justesen JM et al (2011) Implications of central obesity-related variants in LYPLAL1, NRXN3, MSRA, and TFAP2B on quantitative metabolic traits in adult Danes. PLoS One 6:e20640

Bonet ML, Ribot J, Palou A (2012) Lipid metabolism in mammalian tissues and its control by retinoic acid. BBA-Mol Cell Biol L 1821:177–189

Bonet ML, Oliver P, Palou A (2013) Pharmacological and nutritional agents promoting browning of white adipose tissue. Biochim Biophys Acta 1831:969–985

Borry P (2009) Conference scene: direct-to-consumer genetic testing in the age of personalized medicine. Per Med 6:617–619

Brandon JA, Farmer BC, Williams HC, Johnson LA (2018) APOE and Alzheimer's disease: neuroimaging of metabolic and cerebrovascular dysfunction. Front Aging Neurosci 10:180
Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424
Brunham LR, Hayden MR (2012) Whole-genome sequencing: the new standard of care? Science 336:1112–1113
Bruno F, Spaziano G, Liparulo A, Roviezzo F, Nabavi SM, Sureda A, Filosa R, D'Agostino B (2018) Recent advances in the search for novel 5-lipoxygenase inhibitors for the treatment of asthma. Eur J Med Chem 153:65–72
Brunzell JD, Zambon A, Deeb SS (2012) The effect of hepatic lipase on coronary artery disease in humans is influenced by the underlying lipoprotein phenotype. Biochim Biophys Acta 1821:365–372
Bruschetta G, Kim JD, Diano S, Chan LF (2018) Overexpression of melanocortin 2 receptor accessory protein 2 (MRAP2) in adult paraventricular MC4R neurons regulates energy intake and expenditure. Mol Metab 18:79–87
Bursill C, Roach PD, Bottema CDK, Pal S (2001) Green tea upregulates the low-density lipoprotein receptor through the sterol-regulated element binding protein in HepG2 liver cells. J Agric Food Chem 49:5639–5645
Byun HJ, Darvin P, Kang DY, Sp N, Joung YH, Park JH, Kim SJ, Yang YM (2017) Silibinin downregulates MMP2 expression via Jak2/STAT3 pathway and inhibits the migration and invasive potential in MDA-MB-231 cells. Oncol Rep 37:3270–3278
Cagney DN, Sul J, Huang RY, Ligon KL, Wen PY, Alexander BM (2018) The FDA NIH biomarkers, EndpointS, and other tools (BEST) resource in neuro-oncology. Neuro-Oncology 20:1162–1172
Cai EP, Lin JK (2009) Epigallocatechin gallate (EGCG) and rutin suppress the glucotoxicity through activating IRS2 and AMPK signaling in rat pancreatic beta cells. J Agric Food Chem 57:9817–9827
Cambon-Thomsen A, Rial-Sebbag E, Knoppers BM (2007) Trends in ethical and legal frameworks for the use of human biobanks. Eur Respir J 30:373–382
Camp KM, Trujillo E (2014) Position of the academy of nutrition and dietetics: nutritional genomics. J Acad Nutr Diet 114:299–312
Campbell TC (2017) Cancer prevention and treatment by wholistic nutrition. J Nat Sci 3:e448
Capron AM (2009) Learning from the past and looking to the future. J Nutrigenet Nutrigenomics 2:85–90
Carre N, Binart N (2014) Prolactin and adipose tissue. Biochimie 97:16–21
Castle D, Ries NM (2007) Ethical, legal and social issues in nutrigenomics: the challenges of regulating service delivery and building health professional capacity. Mutat Res 622:138–143
Chahar MK, Sharma N, Dobhal MP, Joshi YC (2011) Flavonoids: a versatile source of anticancer drugs. Pharmacogn Rev 5:1–12
Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME (2012) Zinc and human health: an update. Arch Toxicol 86:521–534
Chen KC, Chen CY, Lin CR, Yang TY, Chen TH, Wu LC, Wu CC (2013) Luteolin attenuates TGF-beta1-induced epithelial-mesenchymal transition of lung cancer cells by interfering in the PI3K/Akt-NF-kappaB-snail pathway. Life Sci 93:924–933
Chen CM, Hsieh YH, Hwang JM, Jan HJ, Hsieh SC, Lin SH, Lai CY (2015) Fisetin suppresses ADAM9 expression and inhibits invasion of glioma cancer cells through increased phosphorylation of ERK1/2. Tumour Biol 36:3407–3415
Chen P, Zhang JY, Sha BB, Ma YE, Hu T, Ma YC, Sun H, Shi JX, Dong ZM, Li P (2017) Luteolin inhibits cell proliferation and induces cell apoptosis via down-regulation of mitochondrial membrane potential in esophageal carcinoma cells EC1 and KYSE450. Oncotarget 8:27471–27480

Chen YY, Chang YM, Wang KY, Chen PN, Hseu YC, Chen KM, Yeh KT, Chen CJ, Hsu LS (2019) Naringenin inhibited migration and invasion of glioblastoma cells through multiple mechanisms. Environ Toxicol 34:233–239
Cheng Z, Almeida FA (2014) Mitochondrial alteration in type 2 diabetes and obesity: an epigenetic link. Cell Cycle 13:890–897
Chirumbolo S, Bjorklund G, Lysiuk R, Vella A, Lenchyk L, Upyr T (2018) Targeting cancer with phytochemicals via their fine tuning of the cell survival signaling pathways. Int J Mol Sci 19 (11):3568
Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B (2018) IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138:271–281
Choi ES, Oh S, Jang B et al (2017) Silymarin and its active component silibinin act as novel therapeutic alternatives for salivary gland cancer by targeting the ERK1/2-Bim signaling cascade. Cell Oncol (Dordr) 40:235–246
Church C, Lee S, Bagg EA et al (2009) A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene. PLoS Genet 5:e1000599
Cifarelli V, Hursting SD (2015, 2015) Obesity, diabetes and Cancer: a mechanistic perspective. Int J Diabetol Vasc Dis Res (Suppl 4). https://doi.org/10.19070/2328-353X-SI04001
Claus SP, Swann JR (2013) Nutrimetabonomics: applications for nutritional sciences, with specific reference to gut microbial interactions. Annu Rev Food Sci Technol 4:381–399
Collins N, Tighe AP, Brunton SA, Kris-Etherton PM (2008) Differences between dietary supplement and prescription drug omega-3 fatty acid formulations: a legislative and regulatory perspective. J Am Coll Nutr 27:659–666
Coltell O, Asensio EM, Sorli JV et al (2019) Genome-wide association study (GWAS) on bilirubin concentrations in subjects with metabolic syndrome: sex-specific GWAS analysis and gene-diet interactions in a Mediterranean population. Nutrients 11:E90
Cormier H, Tremblay BL, Paradis AM, Garneau V, Desroches S, Robitaille J, Vohl MC (2014) Nutrigenomics - perspectives from registered dietitians: a report from the Quebec-wide e-consultation on nutrigenomics among registered dietitians. J Hum Nutr Diet 27:391–400
Cornelis MC, El-Sohemy A, Campos H (2004) Genetic polymorphism of CYP1A2 increases the risk of myocardial infarction. J Med Genet 41:758–762
Costa-Font J, Mossialos E (2007) Are perceptions of 'risks' and 'benefits' of genetically modified food (in)dependent? Food Qual Prefer 18:173–182
Crescenti A, Sola R, Valls RM, Anguera A, Arola L (2013) Polymorphisms in LEP and NPY genes modify the response to soluble fibre Plantago ovata husk intake on cardiovascular risk biomarkers. Genes Nutr 8:127–136
Cupples LA, Arruda HT, Benjamin EJ et al (2007) The Framingham heart study 100K SNP genome-wide association study resource: overview of 17 phenotype working group reports. BMC Med Genet 8(Suppl 1):S1
Darnovsky M, Cussins J (2014) FDA halts 23andMe personal genetic tests. What might this mean for the future of direct-to-consumer testing? MLO Med Lab Obs 46:33
Davis CD (2007) Nutrigenomics and the prevention of colon cancer. Pharmacogenomics 8:121–124
Davis CD, Milner JA (2007) Biomarkers for diet and cancer prevention research: potentials and challenges. Acta Pharmacol Sin 28:1262–1273
Davis CD, Milner JA (2011) Nutrigenomics, vitamin D and cancer prevention. J Nutrigenet Nutrigenomics 4:1–11
Davis CD, Uthus EO (2003) Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats. J Nutr 133:2907–2914
De Caterina R (2010a) Opportunities and challenges in nutrigenetics/nutrigenomics and health. World Rev Nutr Diet 101:1–7

De Caterina R (2010b) Opportunities and challenges in nutrigenetics/nutrigenomics and health. J Nutrigenet Nutrigenomics 3:137–143
de Luis DA, Izaola O, Primo D, Aller R (2019) Single nucleotide polymorphisms at the ADIPOQ gene locus rs1501299 interact with different type of dietary fatty acids in two hypocaloric diets. Eur Rev Med Pharmacol Sci 23:2960–2970
de Miranda JX, Andrade Fde O, Conti A, Dagli ML, Moreno FS, Ong TP (2014) Effects of selenium compounds on proliferation and epigenetic marks of breast cancer cells. J Trace Elem Med Biol 28:486–491
Deb G, Thakur VS, Limaye AM, Gupta S (2015) Epigenetic induction of tissue inhibitor of matrix metalloproteinase-3 by green tea polyphenols in breast cancer cells. Mol Carcinog 54:485–499
Doche ME, Bochukova EG, Su HW et al (2012) Human SH2B1 mutations are associated with maladaptive behaviors and obesity. J Clin Invest 122:4732–4736
Donma MM, Donma O (2016) Promising link between selenium and peroxisome proliferator activated receptor gamma in the treatment protocols of obesity as well as depression. Med Hypotheses 89:79–83
Doo M, Kim Y (2015) Obesity: interactions of genome and nutrients intake. Prev Nutr Food Sci 20:1–7
Dubern B, Clement K (2012) Leptin and leptin receptor-related monogenic obesity. Biochimie 94:2111–2115
Dutton RJ, Turnbaugh PJ (2012) Taking a metagenomic view of human nutrition. Curr Opin Clin Nutr 15:448–454
Eilat-Adar S, Sinai T, Yosefy C, Henkin Y (2013) Nutritional recommendations for cardiovascular disease prevention. Nutrients 5:3646–3683
Erdogan S, Turkekul K, Dibirdik I, Doganlar ZB, Doganlar O, Bilir A (2020) Midkine silencing enhances the anti-prostate cancer stem cell activity of the flavone apigenin: cooperation on signaling pathways regulated by ERK, p 38, PTEN, PARP, and NF-κB. Investig New Drugs 38 (2):246–263
Eseberri I, Miranda J, Lasa A, Mosqueda-Solis A, Gonzalez-Manzano S, Santos-Buelga C, Portillo MP (2019) Effects of quercetin metabolites on triglyceride metabolism of 3T3-L1 preadipocytes and mature adipocytes. Int J Mol Sci 20(2):264
European Commission (2004) The 25 recommendations on ethical, legal and social implications of genetic testing. Office for Official Publications of the European Communities, Luxembourg
Evans D, Bode A, von der Lippe G, Beil FU, Mann WA (2011) Cerebrovascular atherosclerosis in type III hyperlipidemia is modulated by variation in the apolipoprotein A5 gene. Eur J Med Res 16:79–84
Fall T, Arnlov J, Berne C, Ingelsson E (2012) The role of obesity-related genetic loci in insulin sensitivity. Diabet Med 29:e62–e66
Fallaize R, Macready AL, Butler LT, Ellis JA, Lovegrove JA (2013) An insight into the public acceptance of nutrigenomic-based personalised nutrition. Nutr Res Rev 26:39–48
Fani L, Bak S, Delhanty P, van Rossum EF, van den Akker EL (2014) The melanocortin-4 receptor as target for obesity treatment: a systematic review of emerging pharmacological therapeutic options. Int J Obes 38:163–169
Fedarko NS (2011) The biology of aging and frailty. Clin Geriatr Med 27:27–37
Fenech M, Baghurst P, Luderer W, Turner J, Record S, Ceppi M, Bonassi S (2005) Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability--results from a dietary intake and micronucleus index survey in South Australia. Carcinogenesis 26:991–999
Fenech M, El-Sohemy A, Cahill L et al (2011) Nutrigenetics and nutrigenomics: viewpoints on the current status and applications in nutrition research and practice. J Nutrigenet Nutrigenomics 4:69–89

Feng J, Chen X, Wang Y, Du Y, Sun Q, Zang W, Zhao G (2015) Myricetin inhibits proliferation and induces apoptosis and cell cycle arrest in gastric cancer cells. Mol Cell Biochem 408:163–170

Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Bruning JC, Ruther U (2009) Inactivation of the Fto gene protects from obesity. Nature 458:894–898

Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D (2006) TCF7L2 polymorphisms and progression to diabetes in the diabetes prevention program. N Engl J Med 355:241–250

Ford NA, Elsen AC, Erdman JW Jr (2013) Genetic ablation of carotene oxygenases and consumption of lycopene or tomato powder diets modulate carotenoid and lipid metabolism in mice. Nutr Res 33:733–742

Forsyth JT, Grunewald RA, Rostami-Hodjegan A, Lennard MS, Sagar HJ, Tucker GT (2000) Parkinson's disease and CYP1A2 activity. Br J Clin Pharmacol 50:303–309

Forte TM, Sharma V, Ryan RO (2016) Apolipoprotein A-V gene therapy for disease prevention/treatment: a critical analysis. J Biomed Res 30:88–93

Fouad YA, Aanei C (2017) Revisiting the hallmarks of cancer. Am J Cancer Res 7:1016–1036

Frayling TM, Timpson NJ, Weedon MN et al (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316:889–894

Friedman M (2007) Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res 51:116–134

Fu C-Y, Chen M-C, Tseng Y-S, Chen M-C, Zhou Z, Yang J-J, Lin Y-M, Viswanadha VP, Wang G, Huang C-Y (2019) Fisetin activates hippo pathway and JNK/ERK/AP-1 signaling to inhibit proliferation and induce apoptosis of human osteosarcoma cells via ZAK overexpression. Environ Toxicol 34:902–911

Galisteo M, Moron R, Rivera L, Romero R, Anguera A, Zarzuelo A (2010) Plantago ovata husks-supplemented diet ameliorates metabolic alterations in obese Zucker rats through activation of AMP-activated protein kinase. Comparative study with other dietary fibers. Clin Nutr 29:261–267

Gaziev AI, Sologub GR, Fomenko LA, Zaichkina SI, Kosyakova NI, Bradbury RJ (1996) Effect of vitamin-antioxidant micronutrients on the frequency of spontaneous and in vitro gamma-ray-induced micronuclei in lymphocytes of donors: the age factor. Carcinogenesis 17:493–499

Gibney MJ, Gibney ER (2004) Symposium on 'Implications for dietary guidelines of genetic influences on requirements' - diet, genes and disease: implications for nutrition policy. Proc Nutr Soc 63:491–500

Gorbunova V, Seluanov A, Mao Z, Hine C (2007) Changes in DNA repair during aging. Nucleic Acids Res 35:7466–7474

Gotthardt JD, Verpeut JL, Yeomans BL, Yang JA, Yasrebi A, Roepke TA, Bello NT (2016) Intermittent fasting promotes fat loss with lean mass retention, increased hypothalamic norepinephrine content, and increased neuropeptide Y gene expression in diet-induced obese male mice. Endocrinology 157:679–691

Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 38:320–323

Grimble RF (2001) Nutritional modulation of immune function. Proc Nutr Soc 60:389–397

Gubler MC (2014) Renal tubular dysgenesis. Pediatr Nephrol 29:51–59

Guo G, Zhang W, Dang M, Yan M, Chen Z (2019) Fisetin induces apoptosis in breast cancer MDA-MB-453 cells through degradation of HER2/neu and via the PI3K/Akt pathway. J Biochem Mol Toxicol 33:e22268

Han TK (2013) Effects Ala54Thr polymorphism of FABP2 on obesity index and biochemical variable in response to a aerobic exercise training. J Exerc Nutr Biochem 17:209–217

Hare DL, Toukhsati SR, Johansson P, Jaarsma T (2014) Depression and cardiovascular disease: a clinical review. Eur Heart J 35:1365–1372

Harnly JM, Doherty RF, Beecher GR, Holden JM, Haytowitz DB, Bhagwat S, Gebhardt S (2006) Flavonoid content of U.S. fruits, vegetables, and nuts. J Agric Food Chem 54:9966–9977

He Y, Wu X, Cao Y, Hou Y, Chen H, Wu L, Lu L, Zhu W, Gu Y (2016) Daidzein exerts anti-tumor activity against bladder cancer cells via inhibition of FGFR3 pathway. Neoplasma 63:523–531

Heid IM, Jackson AU, Randall JC et al (2010) Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet 42:949–960

Hernández-Díaz Y, Tovilla-Zárate CA, Juárez-Rojop IE, González-Castro TB, Rodríguez-Pérez C, López-Narváez ML, Rodríguez-Pérez JM, Cámara-Álvarez JF (2016) Effects of paraoxonase 1 gene polymorphisms on heart diseases: systematic review and meta-analysis of 64 case-control studies. Medicine 95:e5298

Herrera BM, Lindgren CM (2010) The genetics of obesity. Curr Diab Rep 10:498–505

Herrera BM, Keildson S, Lindgren CM (2011) Genetics and epigenetics of obesity. Maturitas 69:41–49

Hessel S, Eichinger A, Isken A et al (2007) CMO1 deficiency abolishes vitamin a production from beta-carotene and alters lipid metabolism in mice. J Biol Chem 282:33553–33561

Hirano KI, Nagasaka H, Kobayashi K, Yamaguchi S, Suzuki A, Toda T, Doyu M (2014) Disease-associated marked hyperalphalipoproteinemia. Mol Genet Metab Rep 1:264–268

Hirschey MD, DeBerardinis RJ, Diehl AME et al (2015) Dysregulated metabolism contributes to oncogenesis. Semin Cancer Biol 35(Suppl):S129–s150

Hjort L, Jorgensen SW, Gillberg L, Hall E, Brons C, Frystyk J, Vaag AA, Ling C (2017) 36 h fasting of young men influences adipose tissue DNA methylation of LEP and ADIPOQ in a birth weight-dependent manner. Clin Epigenetics 9:40

Hoffmann PR, Berry MJ (2008) The influence of selenium on immune responses. Mol Nutr Food Res 52:1273–1280

Hogarth S, Javitt G, Melzer D (2008) The current landscape for direct-to-consumer genetic testing: legal, ethical, and policy issues. Annu Rev Genomics Hum Genet 9(1):161–182

Howard HC, Borry P (2012) Is there a doctor in the house? : The presence of physicians in the direct-to-consumer genetic testing context. J Community Genet 3:105–112

Howes MJ, Simmonds MS (2014) The role of phytochemicals as micronutrients in health and disease. Curr Opin Clin Nutr Metab Care 17:558–566

Hsiao TJ, Hwang Y, Chang HM, Lin E (2014) Association of the rs6235 variant in the proprotein convertase subtilisin/kexin type 1 (PCSK1) gene with obesity and related traits in a Taiwanese population. Gene 533:32–37

Huang H, Liu N, Guo H et al (2012) L-carnitine is an endogenous HDAC inhibitor selectively inhibiting cancer cell growth in vivo and in vitro. PLoS One 7:e49062

Huang H, Wang W, Tao YX (2017) Pharmacological chaperones for the misfolded melanocortin-4 receptor associated with human obesity. Biochim Biophys Acta Mol basis Dis 1863:2496–2507

Hunter DJ, Khoury MJ, Drazen JM (2008) Letting the genome out of the bottle - will we get our wish? N Engl J Med 358:105–107

Hurlimann T, Menuz V, Graham J, Robitaille J, Vohl MC, Godard B (2014) Risks of nutrigenomics and nutrigenetics? What the scientists say. Genes Nutr 9(1):370

Hussain N (2012) Epigenetic influences that modulate infant growth, development, and disease. Antioxid Redox Signal 17:224–236

Hyde CL, Nagle MW, Tian C, Chen X, Paciga SA, Wendland JR, Tung JY, Hinds DA, Perlis RH, Winslow AR (2016) Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. Nat Genet 48:1031–1036

Hyotylainen T, Bondia-Pons I, Oresic M (2013) Lipidomics in nutrition and food research. Mol Nutr Food Res 57:1306–1318

Iijima H, Emi M, Wada M et al (2008) Association of an intronic haplotype of the LIPC gene with hyperalphalipoproteinemia in two independent populations. J Hum Genet 53:193–200

Imai-Sumida M, Chiyomaru T, Majid S, Saini S, Nip H, Dahiya R, Tanaka Y, Yamamura S (2017) Silibinin suppresses bladder cancer through down-regulation of actin cytoskeleton and PI3K/Akt signaling pathways. Oncotarget 8:92032–92042

Ip C, Dong Y, Ganther HE (2002) New concepts in selenium chemoprevention. Cancer Metastasis Rev 21:281–289

Ittiudomrak T, Puthong S, Roytrakul S, Chanchao C (2019) α-Mangostin and apigenin induced cell cycle arrest and programmed cell death in SKOV-3 ovarian cancer cells. Toxicol Res 35:167–179

Iwanicka J, Iwanicki T, Niemiec P, Balcerzyk A, Krauze J, Górczyńska-Kosiorz S, Ochalska-Tyka A, Grzeszczak W, Żak I (2018) Relationship between CETP gene polymorphisms with coronary artery disease in polish population. Mol Biol Rep 45:1929–1935

Jackson DS, Ramachandrappa S, Clark AJ, Chan LF (2015) Melanocortin receptor accessory proteins in adrenal disease and obesity. Front Neurosci 9:213

Jamuna Rani A, Mythili SV, Nagarajan S (2014) Study on paraoxonase 1 in type 2 diabetes mellitus. Indian J Physiol Pharmacol 58:13–16

Jeong JH, An JY, Kwon YT, Rhee JG, Lee YJ (2009) Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. J Cell Biochem 106:73–82

Jesse S, Lehnert S, Jahn O et al (2012) Differential sialylation of serpin A1 in the early diagnosis of Parkinson's disease dementia. PLoS One 7:e48783

Joost HG, Gibney MJ, Cashman KD, Gorman U, Hesketh JE, Mueller M, van Ommen B, Williams CM, Mathers JC (2007) Personalised nutrition: status and perspectives. Br J Nutr 98:26–31

Jurvansuu JM, Goldman A (2011) Obesity risk gene TMEM18 encodes a sequence-specific DNA-binding protein. PLoS One 6:e25317

Kalokairinou L, Howard HC, Slokenberga S et al (2018) Legislation of direct-to-consumer genetic testing in Europe: a fragmented regulatory landscape. J Community Genet 9:117–132

Kathiresan S, Srivastava D (2012) Genetics of human cardiovascular disease. Cell 148:1242–1257

Kaushik S, Shyam H, Sharma R, Balapure AK (2018) Dietary isoflavone daidzein synergizes centchroman action via induction of apoptosis and inhibition of PI3K/Akt pathway in MCF-7/MDA MB-231 human breast cancer cells. Phytomedicine 40:116–124

Kawser Hossain M, Abdal Dayem A, Han J, Yin Y, Kim K, Kumar Saha S, Yang GM, Choi HY, Cho SG (2016) Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. Int J Mol Sci 17:569

Kelder T, Pico AR, Hanspers K, van Iersel MP, Evelo C, Conklin BR (2009) Mining biological pathways using WikiPathways web services. PLoS One 4:e6447

Kennedy DO (2016) B vitamins and the brain: mechanisms, dose and efficacy--a review. Nutrients 8:68

Ketterer B, Harris JM, Talaska G, Meyer DJ, Pemble SE, Taylor JB, Lang NP, Kadlubar FF (1992) The human glutathione S-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. Environ Health Perspect 98:87–94

Khabour OF, Alomari MA, Abu Obaid AA (2018) The relationship of adiponectin level and ADIPOQ gene variants with BMI among young adult women. Dermatoendocrinol 10:e1477902

Khera AV, Won HH, Peloso GM et al (2017) Association of rare and common variation in the lipoprotein lipase gene with coronary artery disease. JAMA 317:937–946

Kim YJ, Bi S (2016) Knockdown of neuropeptide Y in the dorsomedial hypothalamus reverses high-fat diet-induced obesity and impaired glucose tolerance in rats. Am J Physiol Regul Integr Comp Physiol 310:R134–R142

Kim KH, Park Y (2011) Food components with anti-obesity effect. Annu Rev Food Sci Technol 2:237–257

Kim YS, Kim J, Kim KM, Jung DH, Choi S, Kim CS, Kim JS (2015) Myricetin inhibits advanced glycation end product (AGE)-induced migration of retinal pericytes through phosphorylation of ERK1/2, FAK-1, and paxillin in vitro and in vivo. Biochem Pharmacol 93:496–505

Kim M, Kim M, Yoo HJ, Lee E, Chae JS, Lee SH, Lee JH (2017) A promoter variant of the APOA5 gene increases atherogenic LDL levels and arterial stiffness in hypertriglyceridemic patients. PLoS One 12:e0186693

Kim S, Lee MS, Jung S, Son HY, Park S, Kang B, Kim SY, Kim IH, Kim CT, Kim Y (2018) Ginger extract ameliorates obesity and inflammation via regulating MicroRNA-21/132 expression and AMPK activation in white adipose tissue. Nutrients 10:E1567

Klaver E, Zhao P, May M, Flanagan-Steet H, Freeze HH, Gilmore R, Wells L, Contessa J, Steet R (2019) Selective inhibition of N-linked glycosylation impairs receptor tyrosine kinase processing. Dis Model Mech 12:dmm039602

Kobori H, Urushihara M (2013) Augmented intrarenal and urinary angiotensinogen in hypertension and chronic kidney disease. Pflugers Arch 465:3–12

Kodera H, Mizutani Y, Sugiyama S, Miyata T, Ehara T, Matsunaga A, Saito T (2017) A case of lipoprotein glomerulopathy with apoE Chicago and apoE (Glu3Lys) treated with fenofibrate. Case Rep Nephrol Dial 7:112–120

Kohrle J (2000) The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. Cell Mol Life Sci 57:1853–1863

Koochakpoor G, Hosseini-Esfahani F, Daneshpour MS, Hosseini SA, Mirmiran P (2016) Effect of interactions of polymorphisms in the Melanocortin-4 receptor gene with dietary factors on the risk of obesity and Type 2 diabetes: a systematic review. Diabet Med 33:1026–1034

Korthals M (2011) Coevolution of nutrigenomics and society: ethical considerations. Am J Clin Nutr 94:2025s–2029s

Kotecha R, Takami A, Espinoza JL (2016) Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. Oncotarget 7:52517–52529

Krapivner S, Popov S, Chernogubova E, Hellenius ML, Fisher RM, Hamsten A, van't Hooft FM (2008) Insulin-induced gene 2 involvement in human adipocyte metabolism and body weight regulation. J Clin Endocrinol Metab 93:1995–2001

Krashes MJ, Lowell BB, Garfield AS (2016) Melanocortin-4 receptor-regulated energy homeostasis. Nat Neurosci 19:206–219

Kuehl LK, Muhtz C, Hinkelmann K, Dettenborn L, Wingenfeld K, Spitzer C, Otte C (2016) Association between major depression and cardiovascular risk: the role of antidepressant medication. Psychopharmacology 233:3289–3295

Kulyte A, Ryden M, Mejhert N, Dungner E, Sjolin E, Arner P, Dahlman I (2011) MTCH2 in human white adipose tissue and obesity. J Clin Endocrinol Metab 96:E1661–E1665

Kurosu M (2018) Inhibition of N-glycosylation towards novel anti-cancer chemotherapeutics. J Mol Pharm Org Process Res 6:141

Laclaustra M, Corella D, Ordovas JM (2007) Metabolic syndrome pathophysiology: the role of adipose tissue. Nutr Metab Cardiovasc Dis 17:125–139

Lamsa R, Helisalmi S, Herukka SK, Tapiola T, Pirttila T, Vepsalainen S, Hiltunen M, Soininen H (2008) Genetic study evaluating LDLR polymorphisms and Alzheimer's disease. Neurobiol Aging 29:848–855

Lang T, Barling D (2013) Nutrition and sustainability: an emerging food policy discourse. Proc Nutr Soc 72:1–12

Larder R, Sim MFM, Gulati P et al (2017) Obesity-associated gene TMEM18 has a role in the central control of appetite and body weight regulation. Proc Natl Acad Sci U S A 114:9421–9426

Le Bras A (2018) Public health: light smoking and CVD risk. Nat Rev Cardiol 15:136

Lee JH, Khor TO, Shu L, Su ZY, Fuentes F, Kong AN (2013) Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression. Pharmacol Ther 137:153–171

Leone A, Longo C, Trosko JE (2012) The chemopreventive role of dietary phytochemicals through gap junctional intercellular communication. Phytochem Rev 11:285–307

Leshem A, Horesh N, Elinav E (2019) Fecal microbial transplantation and its potential application in cardiometabolic syndrome. Front Immunol 10:1341

Lettieri-Barbato D, Aquilano K (2018) Pushing the limits of cancer therapy: the nutrient game. Front Oncol 8:148
Li S, Zhao JH, Luan J, Luben RN, Rodwell SA, Khaw KT, Ong KK, Wareham NJ, Loos RJ (2010) Cumulative effects and predictive value of common obesity-susceptibility variants identified by genome-wide association studies. Am J Clin Nutr 91:184–190
Li RJ, Mei JZ, Liu GJ (2011) Kaempferol-induced apoptosis of human esophageal squamous carcinoma Eca-109 cells and the mechanism. Nan Fang Yi Ke Da Xue Xue Bao 31:1440–1442
Li X, Battle A, Karczewski KJ, Zappala Z, Knowles DA, Smith KS, Kukurba KR, Wu E, Simon N, Montgomery SB (2014) Transcriptome sequencing of a large human family identifies the impact of rare noncoding variants. Am J Hum Genet 95:245–256
Li S, Li J, Dai W et al (2017) Genistein suppresses aerobic glycolysis and induces hepatocellular carcinoma cell death. Br J Cancer 117:1518–1528
Li YW, Xu J, Zhu GY, Huang ZJ, Lu Y, Li XQ, Wang N, Zhang FX (2018) Apigenin suppresses the stem cell-like properties of triple-negative breast cancer cells by inhibiting YAP/TAZ activity. Cell Death Discov 4:105
Li C, Wang Q, Shen S, Wei X, Li G (2019) HIF-1alpha/VEGF signaling-mediated epithelial-mesenchymal transition and angiogenesis is critically involved in anti-metastasis effect of luteolin in melanoma cells. Phytother Res 33:798–807
Li Q, Miao Z, Wang R, Yang J, Zhang D (2020) Hesperetin induces apoptosis in human glioblastoma cells via p38 MAPK activation. Nutr Cancer 72(3):538–545
Lindgren CM, Heid IM, Randall JC et al (2009) Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet 5:e1000508
Ling C, Ronn T (2019) Epigenetics in human obesity and type 2 diabetes. Cell Metab 29:1028–1044
Linton MRF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, Doran AC, Vickers KC (2000) The role of lipids and lipoproteins in atherosclerosis. In: Feingold KR, Anawalt B, Boyce A et al (eds) Endotext. MDText.com, South Dartmouth
Liu B, Qian SB (2011) Translational regulation in nutrigenomics. Adv Nutr 2:511–519
Liu T, Elmquist JK, Williams KW (2013) Mrap2: an accessory protein linked to obesity. Cell Metab 18:309–311
Liu FH, Song JY, Zhang YN, Ma J, Wang HJ (2015) Gender-specific effect of -102G>a polymorphism in insulin induced gene 2 on obesity in Chinese children. Int J Endocrinol 2015:872506
Liu D, Liu L, Song Z, Hu Z, Liu J, Hou D (2017) Genetic variations of oxidative stress related genes ALOX5, ALOX5AP and MPO modulate ischemic stroke susceptibility through main effects and epistatic interactions in a Chinese population. Cell Physiol Biochem 43:1588–1602
López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153:1194–1217
Lu JP, Knezevic A, Wang YX et al (2011) Screening novel biomarkers for metabolic syndrome by profiling human plasma N-glycans in Chinese Han and Croatian populations. J Proteome Res 10:4959–4969
Lu H, Cassis LA, Kooi CW, Daugherty A (2016) Structure and functions of angiotensinogen. Hypertens Res 39:492–500
Lubkowska A, Radecka A, Bryczkowska I, Rotter I, Laszczynska M, Dudzinska W (2015) Serum adiponectin and leptin concentrations in relation to body fat distribution, hematological indices and lipid profile in humans. Int J Environ Res Public Health 12:11528–11548
Lundstrom K (2013) Past, present and future of nutrigenomics and its influence on drug development. Curr Drug Discov Technol 10:35–46
Mackness MI, Durrington PN, Mackness B (2004) The role of paraoxonase 1 activity in cardiovascular disease: potential for therapeutic intervention. Am J Cardiovasc Drugs 4:211–217
Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. Behav Genet 27:325–351

Magee PJ, Allsopp P, Samaletdin A, Rowland IR (2014) Daidzein, R-(+)equol and S-(−)equol inhibit the invasion of MDA-MB-231 breast cancer cells potentially via the down-regulation of matrix metalloproteinase-2. Eur J Nutr 53:345–350
Mahley RW (2017) Apolipoprotein E: remarkable protein sheds light on cardiovascular and neurological diseases. Clin Chem 63:14–20
Mailman MD, Feolo M, Jin Y et al (2007) The NCBI dbGaP database of genotypes and phenotypes. Nat Genet 39:1181–1186
Mancano G, Mora-Ortiz M, Claus SP (2018) Recent developments in nutrimetabolomics: from food characterisation to disease prevention. Curr Opin Food Sci 22:145–152
Mansego ML, Milagro FI, Zulet MA, Martinez JA (2015) SH2B1 CpG-SNP is associated with body weight reduction in obese subjects following a dietary restriction program. Ann Nutr Metab 66:1–9
Maret W (2013) Zinc biochemistry: from a single zinc enzyme to a key element of life. Adv Nutr 4:82–91
Marfe G, Tafani M, Indelicato M, Sinibaldi-Salimei P, Reali V, Pucci B, Fini M, Russo MA (2009) Kaempferol induces apoptosis in two different cell lines via Akt inactivation, Bax and SIRT3 activation, and mitochondrial dysfunction. J Cell Biochem 106:643–650
Markovina J, Stewart-Knox BJ, Rankin A, Gibney M, de Almeida MDV, Fischer A, Kuznesof SA, Poinhos R, Panzone L, Frewer LJ (2015) Food4Me study: validity and reliability of food choice questionnaire in 9 European countries. Food Qual Prefer 45:26–32
Martínez JA (2014) Perspectives on personalized nutrition for obesity. J Nutrigenet Nutrigenomics 7:I–III
Martin-Hernandez R, Reglero G, Davalos A (2018) Data mining of nutrigenomics experiments: identification of a cancer protective gene signature. J Funct Foods 42:380–386
Mauro M, Saggiorato G, Sartori MT et al (2018) Venoocclusive disease due to chemotherapy for pediatric acute lymphoblastic leukemia is associated with increased levels of plasminogen-activator inhibitor-1. Pediatr Blood Cancer 65:e26963
Mc Namara K, Alzubaidi H, Jackson JK (2019) Cardiovascular disease as a leading cause of death: how are pharmacists getting involved? Integr Pharm Res Pract 8:1–11
Mead MN (2007) Nutrigenomics: the genome--food interface. Environ Health Perspect 115:A582–A589
Medeiros FA (2017) Biomarkers and surrogate endpoints: lessons learned from glaucoma. Invest Ophthalmol Vis Sci 58:Bio20–Bio26
Meplan C (2015) Selenium and chronic diseases: a nutritional genomics perspective. Nutrients 7:3621–3651
Meyer KD, Jaffrey SR (2014) The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat Rev Mol Cell Biol 15:313–326
Michaud DS, Spiegelman D, Clinton SK, Rimm EB, Willett WC, Giovannucci E (2000) Prospective study of dietary supplements, macronutrients, micronutrients, and risk of bladder cancer in US men. Am J Epidemiol 152:1145–1153
Monteiro JP, Kussmann M, Kaput J (2015) The genomics of micronutrient requirements. Genes Nutr 10:466
Morgan AR, Thompson JM, Murphy R, Black PN, Lam WJ, Ferguson LR, Mitchell EA (2010) Obesity and diabetes genes are associated with being born small for gestational age: results from the Auckland birthweight collaborative study. BMC Med Genet 11:125
Moustafa AA, Hewedi DH, Eissa AM, Frydecka D, Misiak B (2014) Homocysteine levels in schizophrenia and affective disorders-focus on cognition. Front Behav Neurosci 8:343
Muller M, Kersten S (2003) Nutrigenomics: goals and strategies. Nat Rev Genet 4:315–322
Nakamura K, Fuster JJ, Walsh K (2014) Adipokines: a link between obesity and cardiovascular disease. J Cardiol 63:250–259
Neale BM, Corvin A, Walters JT (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Ni H, Xu M, Zhan GL et al (2018) The GWAS risk genes for depression may be actively involved in Alzheimer's disease. J Alzheimers Dis 64:1149–1161
Nielsen DE, El-Sohemy A (2012) A randomized trial of genetic information for personalized nutrition on behaviour outcomes. FASEB J 26:380–388
Nielsen DE, El-Sohemy A (2014) Disclosure of genetic information and change in dietary intake: a randomized controlled trial. PLoS One 9:e112665
Nielsen DE, Shih S, El-Sohemy A (2014) Perceptions of genetic testing for personalized nutrition: a randomized trial of DNA-based dietary advice. J Nutrigenet Nutrigenomics 7:94–104
Ning Y, Xu M, Cao X, Chen X, Luo X (2017) Inactivation of AKT, ERK and NF-kappaB by genistein derivative, 7-difluoromethoxyl-5,4′-di-n-octylygenistein, reduces ovarian carcinoma oncogenicity. Oncol Rep 38:949–958
Nordstrom K, Coff C, Jonsson H, Nordenfelt L, Gorman U (2013) Food and health: individual, cultural, or scientific matters? Genes Nutr 8:357–363
Novoselova TV, Larder R, Rimmington D et al (2016) Loss of Mrap2 is associated with Sim1 deficiency and increased circulating cholesterol. J Endocrinol 230:13–26
Ohlhorst SD, Russell R, Bier D, Klurfeld DM, Li Z, Mein JR, Milner J, Ross AC, Stover P, Konopka E (2013) Nutrition research to affect food and a healthy lifespan. Adv Nutr (Bethesda) 4:579–584
Okano T (2016) A new horizon in vitamin K research. Yakugaku Zasshi 136:1141–1159
Olafsdottir AS, Thorsdottir I, Gunnarsdottir I, Thorgeirsdottir H, Steingrimsdottir L (2006) Comparison of women's diet assessed by FFQs and 24-hour recalls with and without underreporters: associations with biomarkers. Ann Nutr Metab 50:450–460
Olczyk P, Koprowski R, Komosinska-Vassev K, Jura-Poltorak A, Winsz-Szczotka K, Kuznik-Trocha K, Mencner L, Telega A, Ivanova D, Olczyk K (2017) Adiponectin, leptin, and leptin receptor in obese patients with type 2 diabetes treated with insulin detemir. Molecules 22 (8):1274
Olszewski PK, Fredriksson R, Olszewska AM, Stephansson O, Alsio J, Radomska KJ, Levine AS, Schioth HB (2009) Hypothalamic FTO is associated with the regulation of energy intake not feeding reward. BMC Neurosci 10:129
Ong ML, Lin X, Holbrook JD (2015) Measuring epigenetics as the mediator of gene/environment interactions in DOHaD. J Dev Orig Health Dis 6:10–16
Ordovas JM, Ferguson LR, Tai ES, Mathers JC (2018) Personalised nutrition and health. BMJ 361: bmj.k2173
Orhan DD, Ozcelik B, Ozgen S, Ergun F (2010) Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res 165:496–504
Ortega Á, Berná G, Rojas A, Martín F, Soria B (2017) Gene-diet interactions in type 2 diabetes: the chicken and egg debate. Int J Mol Sci 18:E1188
Ouyang H, Zhang H, Li W, Liang S, Jebessa E, Abdalla BA, Nie Q (2016) Identification, expression and variation of the GNPDA2 gene, and its association with body weight and fatness traits in chicken. PeerJ 4:e2129
Pahwa S, Sharma R, Singh B (2017) Role of glutathione S-transferase in coronary artery disease patients with and without type 2 diabetes mellitus. J Clin Diagn Res 11:Bc05–Bc08
Palou A, Bonet ML (2013) Challenges in obesity research. Nutr Hosp 28(Suppl 5):144–153
Park HJ, Jeon YK, You DH, Nam MJ (2013) Daidzein causes cytochrome c-mediated apoptosis via the Bcl-2 family in human hepatic cancer cells. Food Chem Toxicol 60:542–549
Park BS, Choi NE, Lee JH, Kang HM, Yu SB, Kim HJ, Kang HK, Kim IR (2019) Crosstalk between fisetin-induced apoptosis and autophagy in human oral squamous cell carcinoma. J Cancer 10:138–146
Parry D, Guzi T, Shanahan F et al (2010) Dinaciclib (SCH 727965), a novel and potent cyclin-dependent kinase inhibitor. Mol Cancer Ther 9:2344–2353
Paskal W, Paskal AM, Debski T, Gryziak M, Jaworowski J (2018) Aspects of modern biobank activity - comprehensive review. Pathol Oncol Res 24:771–785

Pausova Z, Syme C, Abrahamowicz M et al (2009) A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in French Canadians. Circ Cardiovasc Genet 2:260–269

Pavese JM, Krishna SN, Bergan RC (2014) Genistein inhibits human prostate cancer cell detachment, invasion, and metastasis. Am J Clin Nutr 100(Suppl 1):431s–436s

Pavlova NN, Thompson CB (2016) The emerging hallmarks of cancer metabolism. Cell Metab 23:27–47

Pers TH, Timshel P, Hirschhorn JN (2015) SNPsnap: a web-based tool for identification and annotation of matched SNPs. Bioinformatics 31:418–420

Phan MAT, Paterson J, Bucknall M, Arcot J (2018) Interactions between phytochemicals from fruits and vegetables: effects on bioactivities and bioavailability. Crit Rev Food Sci Nutr 58:1310–1329

Phillips CM (2013) Nutrigenetics and metabolic disease: current status and implications for personalised nutrition. Nutrients 5:32–57

Phillips KA, Deverka PA, Hooker GW, Douglas MR (2018) Genetic test availability and spending: where are we now? where are we going? Health Aff (Millwood) 37:710–716

Pico C, Serra F, Rodriguez AM, Keijer J, Palou A (2019) Biomarkers of nutrition and health: new tools for new approaches. Nutrients 11:E1092

Pitozzi V, Jacomelli M, Catelan D, Servili M, Taticchi A, Biggeri A, Dolara P, Giovannelli L (2012) Long-term dietary extra-virgin olive oil rich in polyphenols reverses age-related dysfunctions in motor coordination and contextual memory in mice: role of oxidative stress. Rejuvenation Res 15:601–612

Polvani S, Tarocchi M, Tempesti S, Bencini L, Galli A (2016) Peroxisome proliferator activated receptors at the crossroad of obesity, diabetes, and pancreatic cancer. World J Gastroenterol 22:2441–2459

Portela A, Esteller M (2010) Epigenetic modifications and human disease. Nat Biotechnol 28:1057–1068

Prasad AS, Beck FW, Snell DC, Kucuk O (2009) Zinc in cancer prevention. Nutr Cancer 61:879–887

Pullar JM, Carr AC, Vissers MCM (2017) The roles of vitamin C in skin health. Nutrients 9:866

Qin Y, Zhao D, Zhou HG et al (2016) Apigenin inhibits NF-kappaB and snail signaling, EMT and metastasis in human hepatocellular carcinoma. Oncotarget 7:41421–41431

Raghupathi W, Raghupathi V (2014) Big data analytics in healthcare: promise and potential. Health Inf Sci Syst 2:3

Ramos-Molina B, Molina-Vega M, Fernandez-Garcia JC, Creemers JW (2018) Hyperphagia and obesity in Prader(−)Willi syndrome: PCSK1 deficiency and beyond? Genes 9:E288

Rana S, Kumar S, Rathore N, Padwad Y, Bhushan S (2016) Nutrigenomics and its impact on life style associated metabolic diseases. Curr Genomics 17:261–278

Rautiainen S, Manson JE, Lichtenstein AH, Sesso HD (2016) Dietary supplements and disease prevention - a global overview. Nat Rev Endocrinol 12:407–420

Ray PR, Khan J, Wangzhou A, Tavares-Ferreira D, Akopian AN, Dussor G, Price TJ (2019) Transcriptome analysis of the human tibial nerve identifies sexually dimorphic expression of genes involved in pain, inflammation, and neuro-immunity. Front Mol Neurosci 12:37

Rayman MP (2012) Selenium and human health. Lancet 379:1256–1268

Ren W, Qiao Z, Wang H, Zhu L, Zhang L (2003) Flavonoids: promising anticancer agents. Med Res Rev 23:519–534

Renner W, Nauck M, Winkelmann BR, Hoffmann MM, Scharnagl H, Mayer V, Boehm BO, Marz W (2005) Association of angiotensinogen haplotypes with angiotensinogen levels but not with blood pressure or coronary artery disease: the Ludwigshafen risk and cardiovascular health study. J Mol Med (Berl) 83:235–239

Rimbach G, Minihane AM (2009) Nutrition society silver medal lecture nutrigenetics and personalised nutrition: how far have we progressed and are we likely to get there? Proc Nutr Soc 68:162–172

Roche PA, Annas GJ (2001) Opinion - protecting genetic privacy. Nat Rev Genet 2:392–396
Ronteltap A, van Trijp JCM, Renes RJ (2009) Consumer acceptance of nutrigenomics-based personalised nutrition. Br J Nutr 101:132–143
Roosen J, Bruhn M, Mecking RA, Drescher LS (2008) Consumer demand for personalized nutrition and functional food. Int J Vitam Nutr Res 78:269–274
Rose DJ, Demeo MT, Keshavarzian A, Hamaker BR (2007) Influence of dietary fiber on inflammatory bowel disease and colon cancer: importance of fermentation pattern. Nutr Rev 65:51–62
Ruan JS, Liu YP, Zhang L, Yan LG, Fan FT, Shen CS, Wang AY, Zheng SZ, Wang SM, Lu Y (2012) Luteolin reduces the invasive potential of malignant melanoma cells by targeting beta3 integrin and the epithelial-mesenchymal transition. Acta Pharmacol Sin 33:1325–1331
Russo B, Picconi F, Malandrucco I, Frontoni S (2019) Flavonoids and insulin-resistance: from molecular evidences to clinical trials. Int J Mol Sci 20(9):2061
Saito T, Matsunaga A, Ito K, Nakashima H (2014) Topics in lipoprotein glomerulopathy: an overview. Clin Exp Nephrol 18:214–217
Sales NMR, Pelegrini PB, Goersch AC (2014) Nutrigenomics: definitions and advances of this new science. J Nutr Metab 2014:202759
Sanfilippo PG, Kearns LS, Wright P, Mackey DA, Hewitt AW (2015) Current landscape of direct-to-consumer genetic testing and its role in ophthalmology: a review. Clin Exp Ophthalmol 43:578–590
Schaper M, Schicktanz S (2018) Medicine, market and communication: ethical considerations in regard to persuasive communication in direct-to-consumer genetic testing services. BMC Med Ethics 19:56
Scherag A, Dina C, Hinney A et al (2010) Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and German study groups. PLoS Genet 6:e1000916
Scioli MG, Cervelli V, Arcuri G, Gentile P, Doldo E, Bielli A, Bonanno E, Orlandi A (2014) High insulin-induced down-regulation of Erk-1/IGF-1R/FGFR-1 signaling is required for oxidative stress-mediated apoptosis of adipose-derived stem cells. J Cell Physiol 229:2077–2087
Shankar E, Kanwal R, Candamo M, Gupta S (2016) Dietary phytochemicals as epigenetic modifiers in cancer: promise and challenges. Semin Cancer Biol 40–41:82–99
Shao X, Wang M, Wei X, Deng S, Fu N, Peng Q, Jiang Y, Ye L, Xie J, Lin Y (2016) Peroxisome proliferator-activated receptor-gamma: master regulator of adipogenesis and obesity. Curr Stem Cell Res Ther 11:282–289
Sharma AM, Staels B (2007) Review: peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. J Clin Endocrinol Metab 92:386–395
Shen X, Si Y, Wang Z, Wang J, Guo Y, Zhang X (2016) Quercetin inhibits the growth of human gastric cancer stem cells by inducing mitochondrial-dependent apoptosis through the inhibition of PI3K/Akt signaling. Int J Mol Med 38:619–626
Shenkin A (2006) Micronutrients in health and disease. Postgrad Med J 82:559–567
Shukla S, Meeran SM, Katiyar SK (2014) Epigenetic regulation by selected dietary phytochemicals in cancer chemoprevention. Cancer Lett 355:9–17
Si H, Liu D (2014) Dietary antiaging phytochemicals and mechanisms associated with prolonged survival. J Nutr Biochem 25:581–591
Singh RP (2019) Glycan utilisation system in Bacteroides and Bifidobacteria and their roles in gut stability and health. Appl Microbiol Biotechnol 103(18):7287–7315
Singh R, Kothari R, Koringa PG, Singh SP (2017) Understanding host-microbiome interactions - an omics approach. Springer, Singapore
Sjöberg L (2004) Gene Technology in the eyes of the public and experts. Moral opinions, attitudes and risk perception. In: SSE/EFI working paper series in business administration, vol 7. Stockholm School of Economics, Stockholm
Speliotes EK, Willer CJ, Berndt SI et al (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42:937–948

Srividhya R, Kalaiselvi P (2013) Neuroprotective potential of epigallo catechin-3-gallate in PC-12 cells. Neurochem Res 38:486–493

Stewart-Knox BJ, Bunting BP, Gilpin S, Parr HJ, Pinhao S, Strain JJ, de Almeida MD, Gibney M (2009) Attitudes toward genetic testing and personalised nutrition in a representative sample of European consumers. Br J Nutr 101:982–989

Stewart-Knox B, Kuznesof S, Robinson J et al (2013) Factors influencing European consumer uptake of personalised nutrition. Results of a qualitative analysis. Appetite 66:67–74

Stijnen P, Brouwers B, Dirkx E, Ramos-Molina B, Van Lommel L, Schuit F, Thorrez L, Declercq J, Creemers JW (2016) Endoplasmic reticulum-associated degradation of the mouse PC1/3-N222D hypomorph and human PCSK1 mutations contributes to obesity. Int J Obes 40:973–981

Stocks T, Angquist L, Banasik K et al (2012) TFAP2B influences the effect of dietary fat on weight loss under energy restriction. PLoS One 7:e43212

Stocks T, Angquist L, Hager J, Charon C, Holst C, Martinez JA, Saris WH, Astrup A, Sorensen TI, Larsen LH (2013) TFAP2B -dietary protein and glycemic index interactions and weight maintenance after weight loss in the DiOGenes trial. Hum Hered 75:213–219

Straczkowski M, Nikolajuk A, Majewski R, Filarski R, Stefanowicz M, Matulewicz N, Karczewska-Kupczewska M (2018) The effect of moderate weight loss, with or without (1, 3) (1, 6)-beta-glucan addition, on subcutaneous adipose tissue inflammatory gene expression in young subjects with uncomplicated obesity. Endocrine 61:275–284

Sui JQ, Xie KP, Xie MJ (2016) Inhibitory effect of luteolin on the proliferation of human breast cancer cell lines induced by epidermal growth factor. Sheng Li Xue Bao 68:27–34

Sun F, Zheng XY, Ye J, Wu TT, Wang J, Chen W (2012) Potential anticancer activity of myricetin in human T24 bladder cancer cells both in vitro and in vivo. Nutr Cancer 64:599–606

Sun Q, Li M, Yang X, Xu X, Wang J, Zhang J (2017) Dietary salecan reverts partially the metabolic gene expressions and NMR-based metabolomic profiles from high-fat-diet-induced obese rats. J Nutr Biochem 47:53–62

Sundaram MK, Ansari MZ, Al Mutery A, Ashraf M, Nasab R, Rai S, Rais N, Hussain A (2018) Genistein induces alterations of epigenetic modulatory signatures in human cervical cancer cells. Anti Cancer Agents Med Chem 18:412–421

Swann JR, Claus SP (2014) Nutrimetabonomics: nutritional applications of metabolic profiling. Sci Prog 97:41–47

Sweet ME, Cocciolo A, Slavov D et al (2018) Transcriptome analysis of human heart failure reveals dysregulated cell adhesion in dilated cardiomyopathy and activated immune pathways in ischemic heart failure. BMC Genomics 19:812

Szalai C, Keszei M, Duba J, Prohaszka Z, Kozma GT, Csaszar A, Balogh S, Almassy Z, Fust G, Czinner A (2004) Polymorphism in the promoter region of the apolipoprotein A5 gene is associated with an increased susceptibility for coronary artery disease. Atherosclerosis 173:109–114

Szczepek AJ, Frejo L, Vona B, Trpchevska N, Cederroth CR, Caria H, Lopez-Escamez JA (2019) Recommendations on collecting and storing samples for genetic studies in hearing and tinnitus research. Ear Hear 40:219–226

Takahashi S, Saito K, Jia HJ, Kato H (2014) An integrated multi-omics study revealed metabolic alterations underlying the effects of coffee consumption. PLoS One 9:e91134

Tamer L, Ercan B, Camsari A, Yildirim H, Cicek D, Sucu N, Ates NA, Atik U (2004) Glutathione S-transferase gene polymorphism as a susceptibility factor in smoking-related coronary artery disease. Basic Res Cardiol 99:223–229

Taneera J, Fadista J, Ahlqvist E, Atac D, Ottosson-Laakso E, Wollheim CB, Groop L (2015) Identification of novel genes for glucose metabolism based upon expression pattern in human islets and effect on insulin secretion and glycemia. Hum Mol Genet 24:1945–1955

Tao Y, Maegawa H, Ugi S et al (2006) The transcription factor AP-2beta causes cell enlargement and insulin resistance in 3T3-L1 adipocytes. Endocrinology 147:1685–1696

Thakur K, Tomar SK, Singh AK, Mandal S, Arora S (2017) Riboflavin and health: a review of recent human research. Crit Rev Food Sci Nutr 57:3650–3660

Ticozzi N, LeClerc AL, Keagle PJ et al (2010) Paraoxonase gene mutations in amyotrophic lateral sclerosis. Ann Neurol 68:102–107

Tong J, Shen Y, Zhang Z, Hu Y, Zhang X, Han L (2019) Apigenin inhibits epithelial-mesenchymal transition of human colon cancer cells through NF-kappaB/snail signaling pathway. Biosci Rep 39:BSR20190452

Torres K, Labrador L, Valderrama E, Chiurillo MA (2016) TCF7L2 rs7903146 polymorphism is associated with gastric cancer: a case-control study in the Venezuelan population. World J Gastroenterol 22:6520–6526

Treviño-Villarreal JH, Reynolds JS, Bartelt A et al (2018) Dietary protein restriction reduces circulating VLDL triglyceride levels via CREBH-APOA5-dependent and -independent mechanisms. JCI Insight 3:e99470

Trujillo E, Davis C, Milner J (2006) Nutrigenomics, proteomics, metabolomics, and the practice of dietetics. J Am Diet Assoc 106:403–413

Tsai CF, Chen JH, Chang CN, Lu DY, Chang PC, Wang SL, Yeh WL (2018) Fisetin inhibits cell migration via inducing HO-1 and reducing MMPs expression in breast cancer cell lines. Food Chem Toxicol 120:528–535

Tsukada S, Tanaka Y, Maegawa H, Kashiwagi A, Kawamori R, Maeda S (2006) Intronic polymorphisms within TFAP2B regulate transcriptional activity and affect adipocytokine gene expression in differentiated adipocytes. Mol Endocrinol 20:1104–1111

Tucker KL, Smith CE, Lai CQ, Ordovas JM (2013) Quantifying diet for nutrigenomic studies. Annu Rev Nutr 33(33):349–371

Tutelyan VA, Kravchenko LV, Aksenov IV, Trusov NV, Guseva GV, Kodentsova VM, Vrzhesinskaya OA, Beketova NA (2013) Activity of xenobiotic-metabolizing enzymes in the liver of rats with multi-vitamin deficiency. Int J Vitam Nutr Res 83:5–13

Ulrich CM (2005) Nutrigenetics in cancer research—folate metabolism and colorectal Cancer. J Nutr 135:2698–2702

van Ommen B (2004) Nutrigenomics: exploiting systems biology in the nutrition and health arenas. Nutrition 20:4–8

van Ommen B, Bouwman J, Dragsted LO et al (2010) Challenges of molecular nutrition research 6: the nutritional phenotype database to store, share and evaluate nutritional systems biology studies. Genes Nutr 5:189–203

van Ommen B, van den Broek T, de Hoogh I et al (2017) Systems biology of personalized nutrition. Nutr Rev 75:579–599

Vasku V, Bienertova-Vasku J, Necas M, Vasku A (2009) MTHFR (methylenetetrahydrofolate reductase) C677T polymorphism and psoriasis. Clin Exp Med 9:327–331

Vassy JL, Hivert MF, Porneala B et al (2014) Polygenic type 2 diabetes prediction at the limit of common variant detection. Diabetes 63:2172–2182

Vayena E (2015) Direct-to-consumer genomics on the scales of autonomy. J Med Ethics 41:310–314

Vinceti M, Dennert G, Crespi CM, Zwahlen M, Brinkman M, Zeegers MP, Horneber M, D'Amico R, Del Giovane C (2014) Selenium for preventing cancer. Cochrane Database Syst Rev 2014:Cd005195

Wagner JK (2014) FTC takes action to protect consumers from false genetic advertising claims. Federal Trade Commission, Washington, DC

Wang R, Wang J, Dong T, Shen J, Gao X, Zhou J (2019) Naringenin has a chemoprotective effect in MDA-MB-231 breast cancer cells via inhibition of caspase-3 and -9 activities. Oncol Lett 17:1217–1222

Wasim M, Awan FR, Najam SS, Khan AR, Khan HN (2016) Role of leptin deficiency, inefficiency, and leptin receptors in obesity. Biochem Genet 54:565–572

Watjen W, Michels G, Steffan B, Niering P, Chovolou Y, Kampkotter A, Tran-Thi QH, Proksch P, Kahl R (2005) Low concentrations of flavonoids are protective in rat H4IIE cells whereas high concentrations cause DNA damage and apoptosis. J Nutr 135:525–531

Wheeler E, Leong A, Liu CT et al (2017) Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: a transethnic genome-wide meta-analysis. PLoS Med 14:e1002383

WHO (2018) Obesity and overweight. WHO, Geneva

Wiseman M (2008) The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Proc Nutr Soc 67:253–256

Wu PP, Kuo SC, Huang WW, Yang JS, Lai KC, Chen HJ, Lin KL, Chiu YJ, Huang LJ, Chung JG (2009) (-)-Epigallocatechin gallate induced apoptosis in human adrenal cancer NCI-H295 cells through caspase-dependent and caspase-independent pathway. Anticancer Res 29:1435–1442

Wu CY, Hu HY, Chou YJ, Huang N, Chou YC, Li CP (2015) High blood pressure and all-cause and cardiovascular disease mortalities in community-dwelling older adults. Medicine 94:e2160

Wu D, Zhang J, Wang J, Li J, Liao F, Dong W (2016) Hesperetin induces apoptosis of esophageal cancer cells via mitochondrial pathway mediated by the increased intracellular reactive oxygen species. Tumour Biol 37:3451–3459

Xiao HJ, Huang CY, Wang HY, Li M (2008) Effect of selenium and zinc on the proliferation of human esophageal cancer Eca109 cell line in vitro. Nan Fang Yi Ke Da Xue Xue Bao 28:2117–2120

Xie F, Su M, Qiu W, Zhang M, Guo Z, Su B, Liu J, Li X, Zhou L (2013) Kaempferol promotes apoptosis in human bladder cancer cells by inducing the tumor suppressor, PTEN. Int J Mol Sci 14:21215–21226

Xiong X, Liu H, Hua L, Zhao H, Wang D, Li Y (2015) The association of HDL-apoCIII with coronary heart disease and the effect of statin treatment on it. Lipids Health Dis 14:127

Xu M, Zhao J, Zhang Y, Ma X, Dai Q, Zhi H, Wang B, Wang L (2016) Apolipoprotein E gene variants and risk of coronary heart disease: a meta-analysis. Biomed Res Int 2016:3912175

Xu M, Xu J, Yang X (2017) Asthma and risk of cardiovascular disease or all-cause mortality: a meta-analysis. Ann Saudi Med 37:99–105

Xuan C, Li H, Zhao JX, Wang HW, Wang Y, Ning CP, Liu Z, Zhang BB, He GW, Lun LM (2014) Association between MTHFR polymorphisms and congenital heart disease: a meta-analysis based on 9,329 cases and 15,076 controls. Sci Rep 4:7311

Yang Y, Wolfram J, Shen H, Fang X, Ferrari M (2012) Hesperetin: an inhibitor of the transforming growth factor-beta (TGF-beta) signaling pathway. Eur J Med Chem 58:390–395

Yao S, Wang X, Li C, Zhao T, Jin H, Fang W (2016) Kaempferol inhibits cell proliferation and glycolysis in esophagus squamous cell carcinoma via targeting EGFR signaling pathway. Tumour Biol 37:10247–10256

Yuille M, van Ommen GJ, Brechot C et al (2008) Biobanking for Europe. Brief Bioinform 9:14–24

Zain SM, Mohamed Z, Jalaludin MY, Fauzi F, Hamidi A, Zaharan NL (2015) Comprehensive evaluation of the neuropeptide-Y gene variants in the risk of obesity: a case-control study and meta-analysis. Pharmacogenet Genomics 25:501–510

Zastre JA, Sweet RL, Hanberry BS, Ye S (2013) Linking vitamin B1 with cancer cell metabolism. Cancer Metab 1:16

Zeisel SH (2013a) Keeping genetic information safe. In: Nutrigenetics: applying the science of personal nutrition. Academic Press, Boston, pp 335–353

Zeisel SH (2013b) Practical uses of nutrigenetics. In: Nutrigenetics: applying the science of personal nutrition. Academic Press, Boston, pp 307–333

Zeng H (2002) Selenite and selenomethionine promote HL-60 cell cycle progression. J Nutr 132:674–679

Zeng H, Combs GF Jr (2008) Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion. J Nutr Biochem 19:1–7

Zeng N, Gao Z (2017) Health wearable devices and physical activity promotion. In: Technology in physical activity and health promotion. Routledge, London, pp 148–164

Zhai C, Cong H, Zhang H, Hou K, Zhang Y, Zhang Y (2019) M235T polymorphism in the angiotensinogen gene and cardiovascular disease: An updated meta-analysis of 39 case-control comparisons. Anatol J Cardiol 21:222–232

Zhao J, Lee SH, Huss M, Holme P (2013) The network organization of cancer-associated protein complexes in human tissues. Sci Rep 3:1583

Zhao D, Yin J, Wu S, Wu Z, Pan H (2018a) Effects of genistein on proliferation and invasion of human thyroid squamous cell carcinoma SW579 cells. Wei Sheng Yan Jiu 47:984–992

Zhao Z, Zou S, Guan X et al (2018b) Apolipoprotein E overexpression is associated with tumor progression and poor survival in colorectal cancer. Front Genet 9:650

Zheng Z, Hong L, Huang X et al (2013) Screening for coding variants in FTO and SH2B1 genes in Chinese patients with obesity. PLoS One 8:e67039

Zheng AW, Chen YQ, Zhao LQ, Feng JG (2017) Myricetin induces apoptosis and enhances chemosensitivity in ovarian cancer cells. Oncol Lett 13:4974–4978

Zhou XH, Chen JQ, Chen J, Wu WC, Wang XX, Wang YZ (2015) The beneficial effects of betaine on dysfunctional adipose tissue and N6-methyladenosine mRNA methylation requires the AMP-activated protein kinase alpha 1 subunit. J Nutr Biochem 26:1678–1684

Zhu F, Du B, Xu B (2018) Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: a review. Crit Rev Food Sci Nutr 58:1260–1270

Zubair H, Azim S, Ahmad A, Khan MA, Patel GK, Singh S, Singh AP (2017) Cancer chemoprevention by phytochemicals: nature's healing touch. Molecules 22:E395

Diagnostic Tools for Food Safety

13

Saloni Sharma, Satveer Kaur, Aman Kumar, Natasha Sharma, Payal Kapoor, Venkatesh Chunduri, Anita Kumari, and Monika Garg

Abstract

Issues regarding food safety have become a threat to human health in both developed and developing countries. Demand to ensure the safe food supply is increasing day by day. A wide array of molecular detection methods with high sensitivity, specificity, and rapidity are considered as the feasible approaches for the recognition of various foodborne pathogens and toxic chemicals to manage and prevent the health hassles. Old-traditional microbiological detection methods are often time-consuming, less-efficient, and laborious, which makes them inadequate to achieve the requirement of rapid food testing. In order to mitigate this problem, a wide variety of rapid and efficient detection methods such as immunological, nucleic acid, and biosensor-based methods have been emerged out in order to detect, identify, and enumerate various food-borne pathogens. So, this chapter intends to delve into various traditional, advanced, and rapid techniques with regard to their principles, applications, pros, and cons that have been implicated in determining various foodborne pathogens to ensure food safety in the near future.

Keywords

Diagnostic tools · Human health · Foodborne pathogen · Immunology · Allergens · Food adultration · Gas chromatography

S. Sharma · S. Kaur · A. Kumar · N. Sharma · P. Kapoor · V. Chunduri · A. Kumari · M. Garg (✉)
National Agri-Food Biotechnology Institute, Mohali, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_13

13.1 Introduction

In the present scenario, food safety issues are considered as a matter of grave concern for human illness and death. According to WHO, food safety is defined as the approach of preparing, managing, and storing food in order to prevent contamination and to facilitate that food retains enough nutrients to support a healthy diet. Many consumers, retailers, and regulatory authorities have been confronting the problem of food safety in terms of food poisoning and adulteration (El Sheikha 2019). Organoleptic changes in food due to the growth of various microorganisms make it unacceptable to the consumer with regard to their health (Yager et al. 2008). There are a wide array of harmful microorganisms such as *Vibrio cholera, Staphylococcal aureus, S. enteritis, Bacillus cereus, Escherichia coli O157;H7, Listeria monocytogenes, Salmonella* spp., and *Campylobacter jejuni* which are responsible for food-borne illness and intoxication (Scallan et al. 2011; Oliver et al. 2005; Zhao et al. 2014b). Many diseases emanate from these pathogens by transmitting through polluted water, air, and food as well as during growing, harvesting, processing, and food preparation stage of crop production, which may lead to serious repercussions to human health (Umesha et al. 2016). Food adulteration is also considered as one of the major factors which lead to a reduction in food quality and imparts a negative impact on human health. For example, in China, a mass adulteration of infant food with melamine affected a large number of children (MacMahon et al. 2012; Qin et al. 2013; Xin and Stone 2008). In order to prevent the dissemination of food-borne pathogens, there is an imperative requirement for a more accurate, rapid, and efficient method for identification and enumeration of pathogenic microorganisms at all levels of food and crop production before it enters the body to cause a serious outbreak. Nowadays, a host of promising techniques are available to detect harmful toxins and food-borne microorganisms in different foods to ensure food safety and shelf-life stability. Various detection methods have been categorized into different groups with regard to their principles, advantages, and disadvantages with suitable examples for better comprehending the gradual improvement in these detection systems (Bansal et al. 2017). So, the prime objective of this chapter is to shed light on an overall summary of various detection methods which enable us to detect various foodborne microorganisms and chemical adulterants in a wide variety of food samples (Fig. 13.1).

13.1.1 Current Challenges: The Necessity of Efficient Methods for Ensuring Food Safety

Nowadays, food safety is a global health concern. It is an all-pervading problem that is intensifying day by day, and many countries such as Germany, France, USA, and North Europe have been grappling with this issue (Bernard et al. 2014; Frundt et al. 2013). Microbiology analysis and testing of food, especially for particular pathogenic species and adulterants remain a challenging task due to some constraints such as:

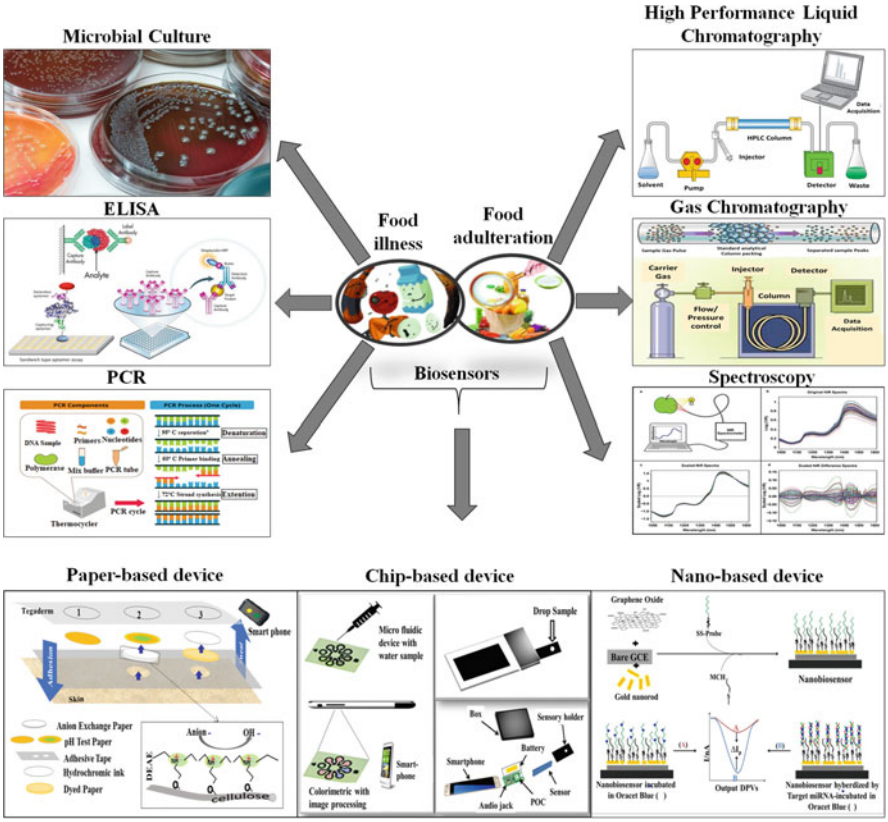

Fig. 13.1 Overview of various conventional and analytical methods in order to determine food allergens and adulterants in various foods for food safety analysis. (Images modified from the webpage and Mu et al. 2015; Yang et al. 2016; Azimzadeh et al. 2016)

1. The distribution of bacteria is not uniform in food samples.
2. Variability in various food matrices in terms of ingredients, physical nature, and thickness which may act as a hurdle inappropriate blending (Mandal et al. 2011).

In order to lessen problems regarding health and food toxicity, there is an utmost necessity in enhancement in analytical methodologies, which are novel, rapid, specific, sensitive, easy, and effective to analyze the pathogens and adulterants detection (El Sheikha 2019). Recently, different advance techniques have assured the recognition and identification of pathogens rapidly and conveniently in comparison to traditional assays (Mangal et al. 2016). The layout of various diagnostic tools (from conventional to advance and then to rapid diagnostic methods) used for food safety analysis is elucidated in Fig. 13.2.

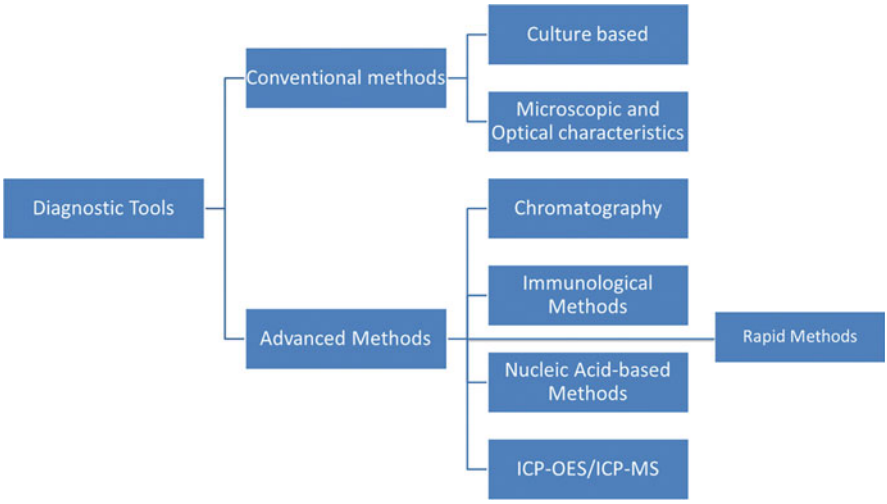

Fig. 13.2 The figure demonstrates the various conventional and advanced methods for food analysis

13.2 Conventional Methods

13.2.1 Culture-Based Methods

Culturing of pathogens on selective media is recognized as one of the oldest methods which are based upon the culturing of microorganisms on a selective enrichment medium followed by standard biochemical identifications (Mandal et al. 2011). There are different selective media which are known for the selection of different microbes (Some examples in Table 13.1). Culturing method is highly reliable, accurate, and cost-effective. However, the major drawback of this method is that it is time-consuming and requires 2–3 days for preliminary identification and the next 7–10 days for further confirmation of the pathogens (Adzitey et al. 2013). In order to get more efficient results, these methods can be combined with other detection methods such as mechanical or semi-mechanical DNA amplification, antibody or biochemical-based methods. There is an improvement in traditional methods and laboratories have begun to use advanced techniques in order to identify the pathogens in a variety of foods (Lopez-Campos et al. 2012).

13.2.2 Microscopic and Optical Characteristics-Based Method

For assuring microbe-free food, microscopic and optical methods, viz., Direct Epifluorescent Filter Technique (DEFT), Flow Cytometry, and Solid Phase laser Cytometry (SPC) have been developed (Examples: Table 13.1) (Mangal et al. 2016).

Table 13.1 Cultural-based, microscopic and optical characteristics-based evaluated examples

Sample type	Media/technique	Targets	Reference
Cultural-based methods			
Any food sample type	Sorbitol MacConkey agar (SMAC)	*E. coli O157:H7*	Hirvonen et al. (2012) and March and Ratnam (1986)
	Cefsulodin-Irgasan-Novobiocin (CIN) agar	Discrimination between *Yersinia enterocolitica* and *non Y. enterocolitica*	Tan et al. (2014)
	Cetrimide agar	*Pseudomonas aeruginosa*	Brown and Lowbury (1965)
	Xylose-lysine-deoxycholate agar	*Salmonella.* spp.	Miller et al. (1991)
	Mannitol salt agar	*S. aureus*	Kateete et al. (2010)
Microscopic and optical characteristics based			
Milk and milk products, beverages, foods	DEFT	Food-borne pathogens	Hermida et al. (2000)
Spinch	Flow cytometry	*E. coli* O157:H7	Williams et al. (2017) and Buzatu et al. (2014)
–	Solid phase cytometry	*E. coli* O157:H7	Pyle et al. (1999)

The DEFT technique involves the arresting of pathogens on polycarbonate membrane filters, fluorochrome staining, and visualization using epifluorescence microscopy (Pettipher et al. 1992; Lopez-Campos et al. 2012). This is a very accurate method for enumerating microbes but labor-intensive. On the other hand, flow cytometry is a rapid, specific, and automatic method that quantitatively measures optical characteristics of viable bacteria cells, when they are passed through a beam of light individually (Lopez-Campos et al. 2012). However, it is unable to measure the microbes at the lower detection level (Mangal et al. 2016). The solid phase laser cytometry technique is also used to detect fluorescently stained microbes that are arrested on membrane filters, with high rapidity, specificity, and sensitivity (Pyle et al. 1999).

13.3 Advance Methods

13.3.1 Chromatography

13.3.1.1 High Performance Liquid Chromatography (HPLC)

HPLC considered as the most operative analytical technique having high separation efficiency and sensitivity for the determination of structural characterization and analysis of food adulterants (Examples: Table 13.2). It is considered much more versatile and rapid over immunoassays because of a wide option of stationary phases and may also be used to resolve small molecules at non-physiological pH, thus, it has become the most preferred technology. A schematic of a HPLC system includes a pump, injector, degasser, sampler, column, detector, and data system. The mobile phase containing the sample mixture relies on the pump in order to deliver through the system. The major drawback of liquid chromatography is the consumption of a high volume of solvent; among used solvents, some are toxic in nature which further reduces the qualitative power of chromatography (Silva et al. 2000).

13.3.1.2 Gas Chromatography (GC)

GC is preferably suitable for analyzing thermally stable non-polar, semi-polar, and other substances that can be volatile without decomposition (Table 13.2). GC is a highly accurate technique that can measure various food additives and toxins even in picomolar concentration. The basic gas chromatography system comprised of gas supply with regulators, autosamplers, inlets, and detectors for data recording and processing systems (Esteki et al. 2017).

13.3.2 Immunology-Based Methods

The antibody-based method recognized as one of the well-known biochemical techniques which couples an immunoassay with an enzymatic analysis for detection of food-borne pathogens and toxins in various foodstuffs. ELISA is a plate-based technique that is highly precise, sensitive, easy, and cost-effective which can be

Table 13.2 Food samples evaluated by HPLC and GC method

Sample type	Pathogens/adulterants	Reference
	HPLC	
Ovine and caprine cheese	β-Lactoglobulin of bovine and caprine milk	Ferreira and Caçote (2003)
Olive oil	Hazelnut oil	Blanch et al. (1998)
Quince jams	Apple or pear puree	Silva et al. (2000)
	GC	
Processed meets	Polycyclic aromatic hydrocarbons (PAHs)	Olatunji et al. (2014)

Table 13.3 Food pathogens evaluated by immunological-based assays

Sample type	Pathogens/adulterants	Reference
Vegetables, milk and various foods, beef	*Salmonella.* spp.	Fusco et al. (2011)
	S. enterotoxins A, B, C, and E	Schlosser et al. (2007) and Shen et al. (2014)
	E. coli O157:H7	Hibi et al. (2006)
	L. monocytogenes	Bolton et al. (2000)

mechanized for an extensive approach. The basic principle of ELISA includes the binding of the antibody to a specific antigen on a 96-well plate, followed by visualization of the antigen-antibody complex. The advantage of the antibody-based detection method is that it gives results within 2–3 days instead of one week, which is required by conventional culture-based methods. This method employs rapid and accurate identification of contamination and adulteration which cannot be easily recognized by traditional cultural approaches (Table 13.3). Contrarily, the major limitation of this method is the low affinity of the antibody towards the pathogen and intervention of other contaminants (Meng and Doyle 2002).

13.3.3 Nucleic Acid-Based Methods

Nucleic acid-based method relies on the identification of definite nucleic acid sequences in the target pathogen through cycling amplification. In this method targeted DNA is hybridized with primers having complementary sequences to their target pathogen (Zhao et al. 2014a, b). The recent nucleic acid-based methods (Table 13.4) include several advanced techniques such as conventional polymerase chain reaction (PCR), multiplex polymerase chain reaction (mPCR), loop-mediated isothermal amplification (LAMP), quantitative/real-time polymerase chain reaction (qPCR), nucleic acid sequence-based amplification (NASBA), and DNA microarray technology (Law et al. 2015).

13.3.3.1 Cycling Amplification Method-Polymerase Chain Reaction (PCR)

PCR is an in-vitro method which involves the exponential amplification of the specific DNA sequences in a cyclical process by using a thermostable DNA polymerase in order to detect foodborne pathogens (Mandal et al. 2011;Olsen et al. 1995).

The results can be visualized on gel electrophoresis by staining the bands with EtBr dye (Zhao et al. 2014a, b). This technique also involves the use of multiple primers (multiplex PCR) which enables us to differentiate the wide variety of foodborne pathogens. This method especially employed for the detection of *Salmonella* in seafood in comparison to traditional and immunology-based methods (Murphy et al. 2007). A new approach, qRT-PCR has greatly enhanced the accuracy and

Table 13.4 Examples of various nucleic acid-based techniques for the identification of a wide variety of food-borne microorganisms present in different foodstuffs

Food sample types	Targets	References
Polymerase chain reaction		
Seafood and various food samples	*E. coli*O157:H7	Tsai et al. (1993)
	Salmonella spp.	Naravaneni and Jamil (2005)
	Shigella	Rahn et al. (1992)
	Y. enterocolitica	Frankel et al. (1990)
	V. cholera	Ibrahim et al. (1992)
	L.monocytogenes	Shangkuan et al. (1995)
	Clostridium botulinum	Simon et al. (1996)
Multiplex PCR		
Chicken, beef and artificially pork, apple cider, cantaloupe, lettuce, tomato, watermelon, pork	*S. enteritidis, S. aureus, S. flexneri, L. monocytogenes, and E. coli O157:H7*	Chen et al. (2012) Silva et al. (2011) and Guan et al. (2013) Verstraete et al. (2012)
Real-time PCR		
Chicken, vegetables, peanut butter, pork, fruits, meat, eggs and various other cooked dishes	*S. enterica subsp., L. monocytogenes, E. coli O157, V. parahaemolyticus, V. vulnificus, C. jejuni, Enterobacter sakazakii and Shigella*	Chen et al. (2010), Suo et al. (2010), Kawasaki et al. (2010), Ma et al. (2014), and Ruiz-Rueda et al. (2010)
DNA microarray		
Various food such as meat, milk, eggs and vegetables	*S.aureus*, *L. monocytogenes*, *V. parahaemolyticus*, *V. cholerae*, C.*jejuni*, *C. perfringens*, *Shigella* spp., *Salmonella* spp., and *Bacillus cereus*	Suo et al. (2010)

specificity of PCR-based detection methods. The basic principle of qRT-PCR includes the measurement of a fluorescence during the PCR reaction which allows both detection as well as the quantification of actual number of the amplified target pathogen. The real time help to monitor the intensity of fluorescence which directly proportional to the quantity of amplicon products. The real-time PCR cut the time required in order to obtain accurate results in comparison to conventional PCR and it also does not require any visualization of results on gel. This method also makes possible for the identification of various RNA viruses (Hanna et al. 2005).

13.3.3.2 DNA Microarray

DNA microarray considered an emerging molecular technique that helps to detect a large number of genes with high accuracy and specificity (Call et al. 2001). Microarrays are made up of solid matrix or nylon membrane chips, which are coated by single-stranded oligonucleotide probes (Severgnini et al. 2011). An array of

Table 13.5 Food-borne pathogens evaluated by nucleic acid hybridization method

Targets in food samples	References
Fluorescent in-situ *Hybridization*	
Salmonella spp.	Bisha and Brehm-Stecher (2010)
Chlamydia spp.	Poppert et al. (2002)
P. aeruginosa, Helicobacter spp., *Streptococcus* spp.	Moter and Gobel (2000) and Trebesius et al. (2001)
Line probe assay	
Mycobacterium spp	El-Etr et al. (2004)
Hybridization protection assay	
M. avium, M. intracellulare, and *M. Gordonae*	Lindholm and Sarkkinen (2004)

single-stranded oligonucleotides probes exposed to labeled target DNA (Wang et al. 2007). DNA microarray enables the recognition of food-borne bacterial pathogens, subsequently followed by their characterization and molecular identification. The advantage of using microarray technology is that it possesses high throughput capability and multiple pathogens can be detected in a single assay (Al-Khaldi et al. 2002; De Boer and Lopez 2012).

There are several other advanced techniques such as Loop Mediated Isothermal Amplification (LAMP), Ligase Chain Reaction (LCR), Ligase Detection Reaction (LDR), Low Stringency Single-Specific-Primer (LSSCP), and Single Stand Conformation Polymorphism (SSCP), etc. which are potent for the identification of food-borne pathogens (Umesha and Manukumar 2018).

13.3.3.3 Nucleic Acid Hybridization

Nucleic acid hybridization is a widely known technique for the identification, differentiation, and relatedness of the DNA sequence of multiple pathogens. Nucleic acid hybridization technologies depend upon observation of a specific target pathogen by fluorescent, luminescence, and chemiluminescence, as examples depicted in Table 13.5.

13.3.4 ICP-OES/ICP-MS

The inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) techniques include the spectrometry. These techniques are well-known for the metal determination and for the evaluation of heavy metal contamination or the excess dose of metals in diverse food items.

ICP-OES also better-known as inductively coupled plasma-atomic emission spectrometry (ICP-AES) works on the characteristics of target elements that gets excited into atoms and ions at a particular wavelength by plasma (Tyler 1994). It is efficiently analyzes up to 60 elements per minute simultaneously with a detection limit in ppb. It requires an average amount of sample for analysis. After calibration, it

Table 13.6 Food samples analyzed by ICP-OES/ICP-MS

Sample types	Pre-treatment requirement[a]	
	ICP-OES	ICP-MS
Water	–	–
Fruit juices and beverages	–	Yes
Wine	–	Yes
Milk	–	Yes
Seafood, beef, meat	Yes	Yes
Fruits and vegetables	Yes	Yes
Flours and bakery products	Yes	Yes

[a]Digestion with high-purity acids followed by dilution (Pizzolon and Hoenig 2005)

can be operated by a common laboratory person. This tool is best suited for the evaluation of fruit juices and beverages or any other liquid sample which is suspended with solid components (Table 13.6).

Contrarily, ICP-MS is a more advanced version which utilizes the induced coupled plasma (ICP) to dissociate sample into atoms and ions and analyze their mass-to-charge ratios (MS). It is a rapid semi-quantitative method, covers a wide dynamic range of elements and provides valuable isotopic information with lowest detection limit up to parts per trillion (ppt.) (Ammann 2007; Beauchemin 2017). That is why in the last two decades, ICP-MS became the most preferred technology for metal determination in a wide variety of products. For analysis, the product must be in the liquid digested form (Table 13.6). For analyzing solid samples, laser ablation-ICP-MS (LA-ICP-MS), an alternative form of ICP-MS which measures the small number of solid samples without using any chemical treatment. This technique also reduces the risk of contamination and also helpful in in-situ determination of spatial distribution (Fig. 13.3) (Mokgalaka and Gardea-Torresdey 2006).

13.3.5 Spectroscopy

Spectroscopy is a non-destructive technique based on the principle of spectrum generation as a wavelength response obtained by the electrochemical radiation bombardment onto a sample, for instance, it is dependent upon the structure and properties of matter (Penner 2017). In recent years, combining spectroscopy with chemometric data analysis is an attractive option for the recognition of various adulterants in foods (Reid et al. 2006; Zhang et al. 2011). Being an easy and rapid technique, spectroscopy can be practically and effectively used by researchers and the food industry (Valous et al. 2010; El Masry et al. 2012).

There are various other methods like UV-VIS (ultraviolet and visible), NIR (Near-infrared), and MIR (Mid-infrared) based on the assimilation and release of radiation in the respective frequency range, universally used for food analysis and each method records numerous types of molecular and atomic transitions (Penner 2017).

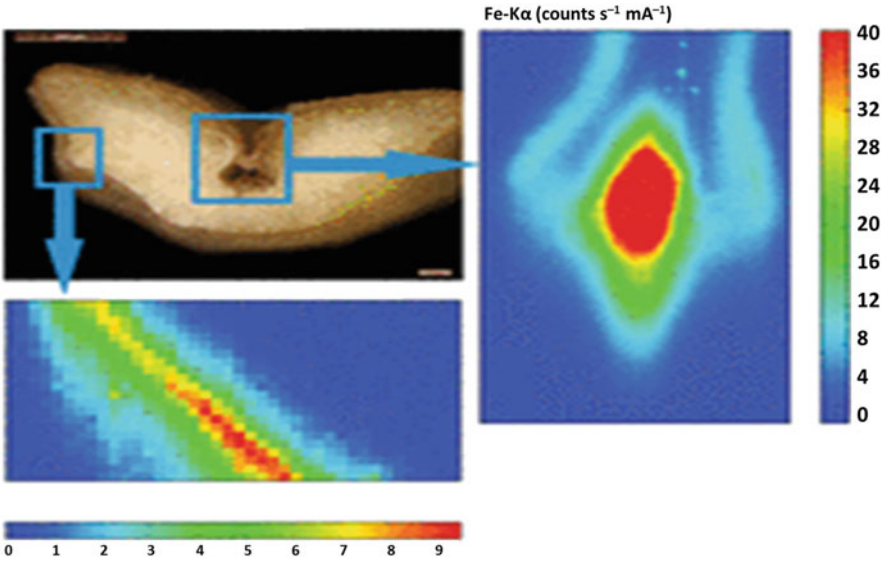

Fig. 13.3 LA-ICP-MS used in-situ to quantify iron (Fe) content in wheat seed (adapted from Singh et al. 2013)

Table 13.7 Food samples analyzed by UV-VIS/NIR/MIR

Sample types	UV-VIS	NIR/MIR
Water	✓	✓
Fruit juices and beverages	✓	✓
Wine	✓	✓
Oil	✓	✓
Dairy and dairy products	NA	✓
Seafood, beef, meat	NA	✓
Fruits and vegetables	NA	✓
Flours and bakery products	NA	✓

NA not applicable (Palacios-Morillo et al. 2013; Prisna et al. 2018); *NA* not applicable

13.3.5.1 UV-VIS

UV-VIS spectrometer covers a wide range of wavelengths (200–800 nm) spectra. It is a simple, flexible, and best suited for rapid quantitative and qualitative analysis. Visible ultraviolet region spectroscopy is specifically used for the characterization of organic and inorganic components in various food matrices (Prisna et al. 2018). This technique can be used to screen plenty of samples at once, thus recommended and used by several quality control authorities of food and beverages for regulating possible contamination in food products (Callao and Ruisanchez 2018). It is a non-destructive method for liquid samples and has been used to differentiate caffeinated and decaffeinated coffee, origin and variety of wine and olive oil, and to check the purity of water and juices (Table 13.7) (Palacios-Morillo et al. 2013;

Prisna et al. 2018). However, for solid food samples, it is a destructive method and less specific.

13.3.5.2 NIR/MIR

Near-infrared (NIR) and mid-infrared (MIR) spectroscopy based on the vibrations of the atoms of a molecule by absorption of radiation in 780 nm–5 μm and 5–30 μm wavelength, respectively (Nawrocka and Lamorska 2013). In NIR, analysis of food samples done on the basis of vibration and combination overtones of the fundamental bonds of molecules, while MIR gives additional particulars of the molecules involved in the general stretching, twisting, and oscillating motions of functional groups of molecules (Workman 2000). NIR/MIR has a significant ability to determine several components in a food sample simultaneously without destructing its integrity (non-destructive method). It has been predominantly used for the quantitative analysis of food properties or adulterant contamination in foods (Nawrocka and Lamorska 2013). In comparison to UV-VIS spectroscopy, NIR and MIR illustrate fast and satisfactory results for all types of food samples. NIR can also be used with the help of fiber-optic probe to evaluate the samples by surface contacts (Qu et al. 2015) and thus can be used for preliminary screening of a large number of food samples, reducing costs and time. For the last 15 years this tool has been used for the food safety evaluation like to differentiate meat and fish species, to check adulteration and quality in fruits & vegetables, cereals and cereals products, dairy and dairy products, bakery products, distilled alcoholic beverages, etc. (Table 13.8) (Qu et al. 2015; Karunathilaka et al. 2018).

NIR/MIR has several advantages like it is a non-destructive, non-invasive, speedy, portable, and cost-effective equipment, and less pre-treatment condition is required. But the disadvantage is that it requires a significant amount of time and expense for calibration, which is essential frequently and also has less sensitive for minor components in food samples (Nawrocka and Lamorska 2013; Qu et al. 2015).

13.4 Rapid Diagnostic Methods

As described above, conventional and advanced analytical methods like culturing techniques, ELISA, and various nucleic acid-based techniques used for the detection of foodborne pathogens are very reliable methods but have their disadvantages (Table 13.8). Similarly, methods (HPLC, GC, ICP-OES/ICP-MS, UV-VIS, and NIR/MIR) use for the detection of food chemicals/adulterants/toxic metals also have disadvantages (Table 13.8). Overall, these tools for the diagnosis of food samples are not accessible to everyone and can be used by only by trained persons. Thus, there is an urgent need of tools or methods which are rapid, sensitive, and specifically monitor the food contaminants. In recent years with the advancement of biosensors and nanotechnology, it is possible to have devices that are rapid and robust, inexpensive, sensitive, specific, adaptable, and easy deliverable to end-users (Valderrama et al. 2016; Chen et al. 2016a). These rapid diagnostic devices are also

Table 13.8 Tabular data illustrates the positive and negative aspects of conventional and advanced methods use for food contaminants

For foodborne pathogens		
Advantages	Disadvantages	References
Culturing methods		
Inexpensive and simple	Time-consuming, more laborious, and low sensitivity	Lee et al. (2014) and Hameed et al. (2018)
Immunological-based methods (ELISA)		
Specific towards targets More number of samples can be handle	Lower specificity than other methods low sensitivity and time-consuming	Ito et al. (2016)
	Cross-reactivity	Zhao et al. (2014a, b)
	Need proper labeling of antigens and antibodies	Park et al. (2014)
Nucleic acid-based techniques		
PCR		
High sensitivity, precision, accuracy, rapidity, and even detect low quantity of samples	Tedious and numerous operation steps	Bavisetty et al. (2018)
	Unable to distinguish between live and dead cells	Toze (1999)
	Complex procedure	Biswas et al. (2008)
Real-time PCR		
Rapid, contamination reduced, and easy handling	mRNA stability issue at RT	Mackay et al. (2002)
	Prone to ribonucleases	Maibach and Altwegg (2003)
	Change with pH	
DNA microarray		
High sensitivity and specificity in a single reaction for diverse pathogens	Unable to differentiate between viable and non-viable cells	Park et al. (2014)
	Expensive	Lauri and Mariani (2009)
For food toxic chemicals/adulterants		
HPLC and GC		
Rapid method, high separation efficiency, and sensitivity	It requires special equipment systems which are costly, time-consuming calibration, and standardization protocols	Weng and Neethirajan (2017)
	Labor intensive	
	Trained workers required	
IC-OES/ICP-MS		
Detection limit is ppt Screen large number of samples	Costly benchtop equipment systems, time-consuming calibration method	Beauchemin (2017)
UV-VIS spectroscopy		
Rapid method for quantitative and qualitative analysis	Specificity reduces for solid food samples	Prisna et al. (2018)

(continued)

Table 13.8 (continued)

For foodborne pathogens		
Advantages	Disadvantages	References
NIR/MIR		
Non-destructive method Once calibrated very fast screening	Costly and time-consuming calibration required	Qu et al. (2015)

known as point-of-care (POC) devices based upon paper, chip, thread, cuvette, glass slide, and nano-material which are generally work upon principals of colorimetric, fluorescent, chemiluminescence, electrochemical and surface plasmon resonance signals. These are also considered as rapid and simple monitoring methods (Choi et al. 2017).

13.4.1 Biosensors

Biosensor device contains a bio-recognition element that might be aptamer, antibody, enzyme, etc. specific to target molecule in a sample and produce a physiological or biological signal. These signals are convertible to measurable quantity by transducers and recorded by the computer. Signals are either in the form of optical, viz., colorimetric, fluorescence, chemiluminescence, and surface plasmon resonance or electrical, viz., voltammetry, impedance, and capacitance and might be magneto-electric or piezoelectric (Neethirajan et al. 2018). The only drawback of biosensors is that it needs expensive instruments compatible with computer software to evaluate the resultant signals, which prove to be costly sometimes. To overcome this drawback, point-of-care (POC) devices are discovered for the on-site availability and cost-effective applicability for food diagnosis (Vashist et al. 2015).

13.4.2 Paper-Based Devices

These are the most attractive and widely used devices for the analysis of various foods specifically in developing and underdeveloped countries. It provides several advantages over other materials as the paper is a simple, thin, light, inexpensive, flexible, biodegradable material and can be easily fabricated, altered, and functionalized (Yetisen et al. 2013; Martinez et al. 2010; Shafiee et al. 2015). The best advantage of paper-based devices is that it can be used by any inexpert users. However, further, for the improvement of sensitivity of paper strips, they are modified to test strips with the lateral flow and microfluidic paper-based emerging devices (μPAD) (Fig. 13.4), whereas for improving specificity, molecular imprinted polymers (MIP) are incorporated (Uzun and Turner 2016; Chen et al. 2016a, b).

There are various types of detection methods, viz., calorimetric, fluorescence, chemiluminescence, electrochemical, and surface plasmon resonance utilized for paper-based platforms for food safety analysis.

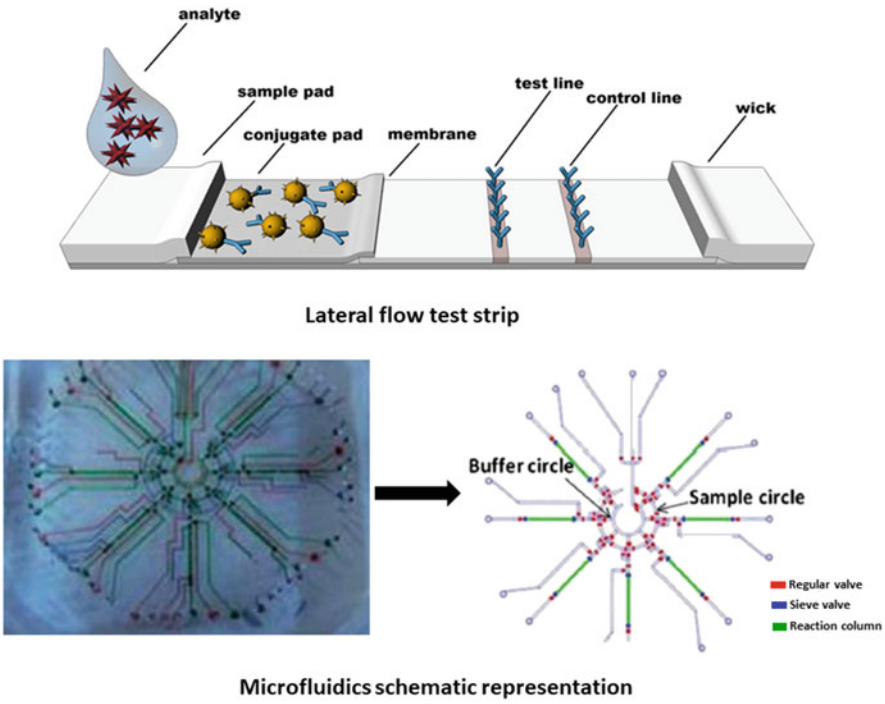

Fig. 13.4 Diagrammatic representation of lateral flow strip and microfluidic chip (modified image from Miocevic et al. 2017; Neethirajan et al. 2018)

13.4.2.1 Calorimetric

This detection method is the most commonly used for monitoring food by paper-based material as color changes can be easily observed by naked eyes (Choi et al. 2017). Generally, in the colorimetric method, chemical and biochemical signals are monitored, which are produced by the interaction between target molecules and respective probes (Piriya et al. 2017). It can be utilized for the detection of pathogens as well as for food chemicals (Table 13.9). These devices are also integrated with Smartphone's to provide quick responses and analysis.

13.4.2.2 Fluorescence

Fluorescent detection methods are also used in most of the paper-based devices as it produces more sensitive signals compared to colorimetric ones. Although, fluorescent-based paper strips are highly sensitive and specific, to further enhance its sensitivity and specificity fluorescent quantum dots (QD) or graphene oxide (GO) are combined with MIPs in paper-based devices (Noor and Krull 2014; Fronczek et al. 2014). These integrated paper-based devices provide several additional advantages like highly sensitive and selective multiplexed analysis, easy fabrication, and low cost for the wide range of food contaminants (Table 13.9). No doubt, fluorescent signals are more sensitive than colorimetric signals, but for

Table 13.9 Different methods utilized for analysis of various food samples by paper-based devices

Types	Sample types	Targets	References
Calorimetric			
Food-borne microorganisms	Chinese cabbage, PBS, milk, water and apple juice	*E.coli* O157:H7	Pang et al. (2018) and Wu et al. (2015)
	Water	*Cronobacter* spp. and *P. aeruginosa*	Sun et al. (2018) and Chen et al. (2016a, b)
	Milk, Juice, PBS	*Salmonella typhimurium*	Tang et al. (2017)
Food chemicals/ adulterants	Milk	Clenbuterol	Ma et al. (2018a)
	Water	Nitrite ion	Wang et al. (2017)
		Benzoic acid	Liu et al. (2018)
	Tomato juices	Copper ions	Chaiyo et al. (2015)
	Milk	17β-estradiol	Xiao et al. (2017)
Fluorescence			
Food-borne pathogens	Milk, water, poultry packing liquid dairy products, marine products, beverages, snacks, and meats	*E. coli* O157:H7, *S. typhimurium, S. paratyphi A, S. paratyphi*	Xing et al. (2018), Zhao et al. (2016), Morales-Narvaez et al. (2015), and Connelly et al. (2015)
		S. paratyphi C, S. typhi, S. enteritidis, S. choleraesuis, V. cholera O1, V. cholera O139, and V. Parahaemolyticus, Phycocyanin	
	Water		Fronczek et al. (2014)
Food chemicals/ adulterants	Water	Thiram	Mei et al. (2016)
		Mercury (II) ion	Zhang et al. (2015)
		Silver (I) ion	
		Neomycin	
Electrochemical			
Food-borne pathogens	Water	*E. coli and Bacillus* sp.	Wang et al. (2017)
Food chemicals/ adulterants	Water	Nitrite	Rengaraj et al. (2018)
	Beer	Ethanol	Cinti et al. (2017)
	Rice, water and fish	Lead (II) ion	Chaiyo et al. (2016)
	Samples	Cadmium (II) ion	
		Copper (II) ion	
Chemiluminescence			
Food-borne pathogens	Water	*Salmonella* sp.	Jin et al. (2015)
Food chemicals/ adulterants	–	–	–

(continued)

Table 13.9 (continued)

Types	Sample types	Targets	References
Surface-enhanced raman scattering			
Food-borne pathogens	–	–	–
Food chemicals/ adulterants	Apples, oranges, tomatoes, and green vegetables, water	Thiram	Ma et al. (2018a, b)
		Thiabendazole	Lee et al. (2018)
		Methyl parathion	
		Ferbam	

accurate quantification, specifically in multiplexed devices, they need to be integrated with smartphones so it also acts as POC.

13.4.2.3 Electrochemical

Electrochemical detection method provides excellent sensitivity and selectivity and has been broadly used by μPAD for the detection of food contaminants (Table 13.9). Paper-based electrochemical devices (ePAD) also provide quantitative measures even with low detection limits (Mettakoonpitak et al. 2016).

13.4.2.4 Chemiluminescence and Surface-Enhanced Raman Scattering (SERS)

Other than colorimetric, fluorescence, and electrochemical methods there are two other methods such as chemiluminescence and surface-enhanced Raman scattering (SERS) have also been utilized by μPAD for the detection of food pathogens and chemicals (Table 13.9). One highly sensitive and reproducible paper-based SERS device also emerged out in which modified hydrophobic filter paper is being used (Lee et al. 2018).

Currently, all these paper-based devices are integrated with smartphones for specific and sensitive quantification (Yu et al. 2015). But further, there is a need to develop reliable apps for smartphones that can record signals in image form and perform rapid quantification and analysis of data. There is also a necessity to develop multiplexed paper-based devices that can detect multiple targets or analyze multiple samples at once (Wang and Duncan 2017).

13.4.3 Chip-Based Devices

These devices are developed from PDMS (Polydimethylsiloxane) or PMMA (polymethyl methacrylate) polymers fabricated into microfluidic chips (Weng and Neethirajan 2017). Similar to μPAD, chip-based devices are also widely used for the recognition of both foodborne microorganisms and harmful chemicals detection correspondingly by colorimetric, fluorescent, electrochemical, and surface plasmon resonance methods (Table 13.10). Chip-based devices can detect a minute amount of samples with precise control and high efficient analysis (Choi et al. 2019).

Table 13.10 Different methods utilized for analysis of various food samples by chip-based devices

Types	Sample types	Targets	References
Calorimetric			
Food-borne pathogens	Corn	Aflatoxin B1	Li et al. (2017a)
Food chemicals/ adulterants	Wheat	Gluten	Weng et al. (2016)
	Peanut	Ara h1	Zhao et al. (2014a, b)
		Lead (II) ion	
		Aluminum (III) ion	
	Apple	Malathion	Meng et al. (2015)
	Water	Tetrabromodiphenyl ether	Chen et al. (2014)
Fluorescence			
Food-borne pathogens	Shrimp	*S. aureus*	Pang et al. (2018)
	Serum	*V. parahaemolyticus*	Kim et al. (2015)
	Chicken extract	*S. typhimurium*	
	Beef filtrate	*L. monocytogenes*	Malic et al. (2015)
	Serum	*E. coli*	Chen et al. (2017)
	Pork meat	*Proteus hauseri*	Sun et al. (2015)
		S. enterica	
Food chemicals/ adulterants	Biscuit	Ara h1	Weng et al. (2016)
	Milk	Anti-recombinant bovine somatropin antibody	Ludwig et al. (2014)
Electrochemical			
Food-borne pathogens	Water	*E. coli*	Kim et al. (2015) and Tian et al. (2016)
	PBS	*E. coli* O157:H7	
	Milk	*S. aureus*	Yao et al. 2018;
		S. typhimurium	De Oliveira et al. 2018
Food chemicals/ adulterants	Water	Clenbuterol	Dou et al. (2016)
Surface plasmon resonance			
Food-borne pathogens	Cucumber	*E. coli* O157:H7	Vaisocherova-Lisalova et al. (2016)
	Hamburger	*Salmonella* sp.	Tokel et al. (2015)
	PBS	*E. coli*	
		Lactobacillus acidophilus	
		S. typhimurium	Yoo et al. (2015)
		P. aeruginosa	
Food chemicals/ adulterants	Wine and peanut oil	Ochratoxin A	Zhu et al. (2015)

Colorimetric chip-based devices are also integrated with smartphone apps for on-site quantification. But here, again colorimetric detection has poor sensitivity and it is challenging to develop multiplexed colorimetric chip-based devices. On the other hand, fluorescent devices have superior detection sensitivity and specificity which can be further enhanced by replacing fluorescent dyes with Quantum dots. But it proves to be unsuitable for POC devices as its quantification is equipment dependent (Weng et al. 2016; Malic et al. 2015; Chen et al. 2017).

Chip-based electrochemical devices are cost-effective, rapid, and highly sensitive and can easily develop by the integration of microfluidic chip with microelectronics (Rateni et al. 2017). They are also integrated with smart phone to quantify the target and to improve its applicability. Additional to colorimetric, fluorescence, and electrochemical detection method surface plasmon resonance (SPR) has been also used broadly with microfluidic chips for the detection of food contaminants (Table 13.10).

All existing chips are integrated with multiple micro-channels or micro-wells to achieve multiplexed detection for food contaminants at once.

13.4.4 Other Material-Based Devices

Food contaminants detection are also possible by using thread, tube, cuvette, well plate, disc, and glass-based biosensor devices (Table 13.11) providing significant sensitivity and functionality in comparison to paper-based and chip-based devices (Choi et al. 2018; Caetano et al. 2018; Levin et al. 2016; Xiao et al. 2016; Li et al. 2017a; Su et al. 2017; Sayad et al. 2018; Ludwig et al. 2014). These respective materials have various advantages over paper and chip-based devices such as threads have higher mechanical strength under wet conditions, can easily form micro-channels and diverse types of fiber material provide different functionalities to threads (Agustini et al. 2016). They all are also linked with smartphone software to use as POC devices.

13.4.5 Nanomaterial-Based Devices

To detect trace amount (up to nanoscale) of chemicals and microbiological contaminants in food, nanotechnology provides the best sensing technique (Wang and Duncan 2017). This sensing technique has grown exponentially and incorporated to every portable and lab-on-chip device. In the above described POC devices there are many different types of nonmaterial used to enhance their efficiency like QDS or graphene oxide in fluorescence assay, the gold, and silver nanoparticles (AuNPs and AgNPs) or gold-coated magnetic beads to enhance electrochemical conductivity. Graphene oxides also possess excellent electric conductivity and high surface area proving to be an excellent material for sensing and detection. Overall nanoparticles have the capacity to produce enormous signals, selective amplification, and strong structural stability and they are verified to be an excellent material for bio-sensing devices. There are also many examples in which solely nanomaterial-based devices are used for food contaminant detection (Table 13.12).

Table 13.11 Different methods utilized for analysis of various food samples by other material-based devices

Types	Sample types	Targets	References
Thread-based device			
Calorimetric			
Food-borne pathogens	Milk, Orange juice, lettuce	*S. enterica*	Choi et al. (2018)
Food chemicals/ adulterants	–	–	–
Electrochemical			
Food-borne pathogens	–	–	–
Food chemicals/ adulterants	Water	Phenol	Caetano et al. (2018)
Tube-based devices			
Calorimetric			
Food-borne pathogens	–	–	–
Food chemicals/ adulterants	Water	Fluoride	Levin et al. (2016)
		Mercury (II) ion	
Cuvette-based devices			
Calorimetric			
Food borne pathogens	–	–	–
Food chemicals/ adulterants	Water	Fluoride	Xiao et al. (2016)
		Mercury (II) ion	
Well plate-based devices			
Calorimetric			
Food-borne pathogens	–	–	–
Food chemicals/ adulterants	Milk Shellfish	Tetracyclines, quinolones	Li et al. (2017b)
		Saxitoxin, okadaic acid	Su et al. (2017)
Disc-based devices			
Fluorescence			
Food-borne pathogens	Chicken meat	*E. coli, Salmonella* sp.	Sayad et al. (2018)
		V. cholerae	
Food chemicals/ adulterants	–	–	–
Glass slide-based devices			
Fluorescence			
Food-borne pathogens	–	–	–
Food chemicals/ adulterants	Milk	Anti-recombinant bovine	Ludwig et al. (2014)
		Somatropin antibody	

Table 13.12 Nano-material-based devices utilized different methods for the analysis of various food samples

Types	Sample types	Targets	References
Fluorescence			
Food-borne pathogens	Milk	*S. Typhimurium*	Duan et al. (2014)
Food chemicals/adulterants	–	–	–
Electrochemical			
Food-borne pathogens	Water, fruit juice, milk	*E. coli O157:H7*	Pandey et al. (2017)
Food chemicals/adulterants	–	–	–
Chemiluminescence			
Food-borne pathogens	Milk	*S. typhimurium*	Pal et al. (2017)
Food chemicals/adulterants	–	–	–
Gas-pressure induced ink bar advancement			
Food-borne pathogens	Milk	*S. enteriditis*	Bu et al. (2019)
		E. coli O157:H7	
Food chemicals/adulterants	–	–	–

13.5 Conclusion

To encapsulate, it is explicitly observed that a wide variety of conventional and advanced detection methods proved to be potent diagnostic tools for food safety in terms of asepticism as well as quality control. There are several factors such as accuracy, reproducibility, sensitivity, validation, speed, costs, and simplicity that must be taken into consideration before adopting new technology or technique (Mandal et al. 2011). These days, food is not only known to provide energy but also plays a significant role to prevent lifestyle related disorders. In order to live a healthy life, there is an imperative need to consume food in fresh (pathogen-free) and natural form (adulterant free). Since every technique has its positive and negative aspects and it is impossible to fulfill the criteria of the best diagnostic tool for food analysis with the help of any single method. So, it is up to the user's wise choice and priorities, which method is practically feasible and best for respective food items. To sum up, there are a number of rapid and automated detection methods with extensive applications that have been utilized to detect food-borne pathogens and food adulterants (Table 13.13). Moreover, with the development of point of care (POC) like devices that offer wide applicability, researchers are still developing sophisticated methods with regard to rapidity, sensitivity, and specificity to mitigate the problems associated with food safety in the near future (Zhao et al. 2014a, b).

Table 13.13 Various diagnostic tools comparison for food safety analysis

Diagnostic tests/devices/tools		Principal	Time	Specificity (%)	Sensitivity (%)	Sample cost ($)
(A) Food-borne pathogens						
Destructive	Bacterial culture	Culturing	~1 to 2 h	100 (gold standard)	100	3–6
	ELISA	Immunoassay	~ 6 h	70–90	60–99	10
	PCR types	Nucleic acid hybridization	4 h	100	80–100	20
(B) Adulterant identification/food chemicals						
Destructive	HPLC	Chromatography	~30 min to 1 h[a]	95	99	20–30
	GC		~30 min to 1 h[a]	95	99	20–30
	ICOP-MS/ICP-MS	Mass spectroscopy	~ 6 h	100	100	50
Non-destructive	Spectroscopic					
	UV-VIS	Spectroscopy	5 min to 1 h[a]	100	100	8–10
	NIR/MIR		5 min	100[b]	100	20
Biosensors/Nano-sensors: Both A and B identifier						
Paper-based devices		Calorimetric	~5 min to 1 h[a]	~80–100	>80	2
		Fluorescence	~5 min to 1 h[a]	~100	90–100	
		Electrochemical	~10 min to 1 h[a]	~100	100	
		Chemiluminescence	~35 min	~100	100	
		Surface-enhanced Raman scattering	~5 min	~100	100	
Chip-based devices		Calorimetric	~10 min to 1 h[a]	~80–100	>80	
		Fluorescence	~1 to 2.5 h[a]	~100	90–100	
		Electrochemical	~1 to 30 min[a]	~100	100	
		Surface plasmon resonance	~2.5 min to 1 h[a]	~100	100	
		Turbidity	~1.75 h	~100	100	

Thread-based devices	Calorimetric	~10 min	~80–100	80–100	
	Electrochemical	–	~100	100	
Cuvette-based devices	Calorimetric	~1 to 20 min[a]	~80–100	80–100	
Well plated-based devices	Calorimetric	~1 h	~80–100	80–100	
Disc-based devices	Fluorescence	~1 h	~100	100	
Glass slide-based devices	Fluorescence	~2.5 h	~100	100	
Nanomaterial-based devices	Fluorescence	~45 min	100	100	
	Electrochemical	–	100	100	
	Chemiluminescence	~2.5 h	100	100	

[a]Time can be variable
[b]Specificity varies with the sample

References

Adzitey F, Huda N, Ali GR (2013) Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. 3 Biotech 3(2):97–107. https://doi.org/10.1007/s13205-012-0074-4

Agustini D, Bergamini MF, Marcolino-Junior LH (2016) Low cost microfluidic device based on cotton threads for electro analytical application. Lab Chip 16(2):345–352. https://doi.org/10.1039/c5lc01348h

Al-Khaldi SF, Martin SA, Rasooly A et al (2002) DNA microarray technology used for studying foodborne pathogens and microbial habitats: mini review. J AOAC Int 85(4):906–910

Ammann AA (2007) Inductively coupled plasma mass spectrometry (ICP MS): a versatile tool. Int J Mass Spectrom 42(4):419–427. https://doi.org/10.1002/jms.1206

Azimzadeh M, Rahaie M, Nasirizadeh N et al (2016) An electrochemical nanobiosensor for plasma miRNA-155, based on graphene oxide and gold nanorod, for early detection of breast cancer. Biosens Bioelectron 77:99–106. https://doi.org/10.1016/j.bios.2015.09.020

Bansal S, Singh A, Mangal M et al (2017) Food adulteration: sources, health risks, and detection methods. Crit Rev Food Sci Nutr 57(6):1174–1189. https://doi.org/10.1080/10408398.2014.967834

Bavisetty SC, Vu HT, Benjakul S et al (2018) Rapid pathogen detection tools in seafood safety. Curr Opin Food Sci 20:92–99. https://doi.org/10.1016/j.cofs.2018.05.013

Beauchemin D (2017) Inductively coupled plasma mass spectrometry methods. In: Encyclopedia of spectroscopy and spectrometry. Academic Press, San Diego, pp 236–245. https://doi.org/10.1016/b978-0-12-409547-2.11222-3

Bernard H, Faber M, Wilking H et al (2014) Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. Euro Surveill 19(8). https://doi.org/10.2807/1560-7917.es2014.19.8.20719

Bisha B, Brehm-Stecher BF (2010) Combination of Adhesive-tape-based Sampling and Fluorescence *in situ* Hybridization for Rapid Detection of *Salmonella* on Fresh Produce. J Vis Exp (44). https://doi.org/10.3791/2308

Biswas A, Kondaiah N, Bheilegaonkar K et al (2008) Microbial profiles of frozen trimmings and silver sides prepared at Indian buffalo meat packing plants. Meat Sci 80(2):418–422. https://doi.org/10.1016/j.meatsci.2008.01.004

Blanch GP, Caja MDM, Castillo MLRD, Herraiz M (1998) Comparison of different methods for the evaluation of the authenticity of olive oil and hazelnut oil. J Agric Food Chem 46:3153–3157. https://doi.org/10.1021/jf9800209

Bolton FJ, Fritz E, Poynton S et al (2000) Rapid enzyme-linked immunoassay for detection of Salmonella in food and feed products: performance testing program. J AOAC Int 83:299–303

Brown VI, Lowbury EJL (1965) Use of an improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa*. J Clin Pathol 18:752–756. https://doi.org/10.1136/jcp.18.6.752

Bu S, Wang K, Ju C et al (2019) Point-of-care assay to detect foodborne pathogenic bacteria using a low-cost disposable medical infusion extension line as readout and MnO_2 nanoflowers. Food Control 98:399–404. https://doi.org/10.1016/j.foodcont.2018.11.053

Buzatu DA, Moskal TJ, Williams AJ et al (2014) An integrated flow cytometry-based system for real-time, high sensitivity bacterial detection and identification. PLoS One 9(4):e94254. https://doi.org/10.1371/journal.pone.0094254

Caetano F, Carneiro E, Agustini D et al (2018) Combination of electrochemical biosensor and textile threads: a microfluidic device for phenol determination in tap water. Biosens Bioelectron 99:382–388. https://doi.org/10.1016/j.bios.2017.07.070

Call DR, Brockman FJ, Chandler DP (2001) Detecting and genotyping *Escherichia coli* O157:H7 using multiplexed PCR and nucleic acid microarrays. Int J Food Microbiol 67:71–80. https://doi.org/10.1016/s0168-1605(01)00437-8

Callao MP, Ruisanchez I (2018) An overview of multivariate qualitative methods for food fraud detection. Food Control 86:283–293. https://doi.org/10.1016/j.foodcont.2017.11.034

Chaiyo S, Apiluk A, Siangproh W et al (2016) High sensitivity and specificity simultaneous determination of lead, cadmium and copper using μPAD with dual electrochemical and colorimetric detection. Sens Actuators B Chem 233:540–549. https://doi.org/10.1016/j.snb.2016.04.109

Chaiyo S, Siangproh W, Apilux A et al (2015) Highly selective and sensitive paper-based colorimetric sensor using thiosulfate catalytic etching of silver nano plates for trace determination of copper ions. Anal Chim Acta 866:75–83. https://doi.org/10.1016/j.aca.2015.01.042

Chen A, Wang R, Bever CRS et al (2014) Smartphone-interfaced lab-on-a-chip devices for field-deployable enzyme-linked immunosorbent assay. Biomicrofluidics 8:064101. https://doi.org/10.1063/1.4901348

Chen C, Liu P, Zhao X et al (2017) A self-contained microfluidic in-gel loop-mediated isothermal amplification for multiplexed pathogen detection. Sens Actuators B Chem 239:1–8. https://doi.org/10.1016/j.snb.2016.07.164

Chen J, Tang J, Liu J et al (2012) Development and evaluation of a multiplex PCR for simultaneous detection of five foodborne pathogens. J Appl Microbiol 112:823–830. https://doi.org/10.1111/j.1365-2672.2012.05240.x

Chen L, Wang X, Lu W et al (2016a) Molecular imprinting: perspectives and applications. Chem Soc Rev 45(8):2137–2211. https://doi.org/10.1039/c6cs00061d

Chen Y, Cheng N, Xu Y et al (2016b) Point-of-care and visual detection of *P. aeruginosa* and its toxin genes by multiple LAMP and lateral flow nucleic acid biosensor. Biosens Bioelectron 81:317–323. https://doi.org/10.1016/j.bios.2016.03.006

Chen J, Zhang L, Paoli GC et al (2010) A real-time PCR method for the detection of *Salmonella enterica* from food using a target sequence identified by comparative genomic analysis. Int J Food Microbiol 137(2–3):168–174. https://doi.org/10.1016/j.ijfoodmicro.2009.12.004

Choi J, Yong K, Choi J et al (2019) Emerging point-of-care technologies for food safety analysis. Sensors 19(4):817. https://doi.org/10.3390/s19040817

Choi JR, Nilghaz A, Chen L et al (2018) Modification of thread-based microfluidic device with polysiloxanes for the development of a sensitive and selective immunoassay. Sens Actuators B Chem 260:1043–1051. https://doi.org/10.1016/j.snb.2018.01.102

Choi JR, Yong KW, Tang R et al (2017) Advances and challenges of fully integrated paper-based point-of-care nucleic acid testing. Trends Anal Chem 93:37–50. https://doi.org/10.1016/j.trac.2017.05.007

Cinti S, Basso M, Moscone D et al (2017) A paper-based nanomodified electrochemical biosensor for ethanol detection in beers. Anal Chim Acta 960:123–130. https://doi.org/10.1016/j.aca.2017.01.010

Connelly JT, Rolland JP, Whitesides GM (2015) "Paper machine" for molecular diagnostics. Anal Chem 87(15):7595–7601. https://doi.org/10.1021/acs.analchem.5b00411

De Boer SH, Lopez MM (2012) New grower-friendly methods for plant pathogen monitoring. Annu Rev Phytopathol 50(1):197–218. https://doi.org/10.1146/annurev-phyto-081211-172942

De Oliveira TR, Martucci DH, Faria RC (2018) Simple disposable microfluidic device for *Salmonella typhimurium* detection by magneto-immunoassay. Sens Actuators B Chem 255:684–691. https://doi.org/10.1016/j.snb.2017.08.075

Dou Y, Jiang Z, Deng W et al (2016) Portable detection of clenbuterol using a smartphone-based electrochemical biosensor with electric field-driven acceleration. J Electroanal Chem 781:339–344. https://doi.org/10.1016/j.jelechem.2016.04.022

Duan YF, Ning Y, Song Y et al (2014) Fluorescent apta sensor for the determination of *Salmonella typhimurium* based on a graphene oxide platform. Microchim Acta 181(5–6):647–653. https://doi.org/10.1007/s00604-014-1170-4

El Sheikha AF (2019) DNA foil: novel technology for the rapid detection of food adulteration. Trends Food Sci Technol 86:544–552. https://doi.org/10.1016/j.tifs.2018.11.012

El-Etr SH, Subbian S, Cirillo SL et al (2004) Identification of two *Mycobacterium marinum* loci that affect interactions with macrophages. Infect Immun 72(12):6902–6913. https://doi.org/10.1128/iai.72.12.6902-6913.2004

El Masry G, Barbin DF, Sun D et al (2012) Meat quality evaluation by hyper spectral imaging technique: an overview. Crit Rev Food Sci Nutr 52(8):689–711. https://doi.org/10.1080/10408398.2010.507908

Esteki M, Farajmand B, Kolahderazi Y et al (2017) Chromatographic fingerprinting with multivariate data analysis for detection and quantification of apricot kernel in almond powder. Food Anal Methods 10(10):3312–3320. https://doi.org/10.1007/s12161-017-0903-5

Ferreira IM, Caçote H (2003) Detection and quantification of bovine, ovine and caprine milk percentages in protected denomination of origin cheeses by reversed-phase high-performance liquid chromatography of beta-lactoglobulins. J Chromatogr A 1015:111–118. https://doi.org/10.1016/s0021-9673(03)01261-5

Frankel G, Riley L, Giron JA et al (1990) Detection of Shigella in feces using DNA amplification. J Infect Dis 161(6):1252–1256. https://doi.org/10.1093/infdis/161.6.1252

Fronczek CF, Park TS, Harshman DK et al (2014) Paper microfluidic extraction and direct smartphone-based identification of pathogenic nucleic acids from field and clinical samples. RSC Adv 4(22):11103. https://doi.org/10.1039/c3ra47688j

Frundt T, Hopker W-W, Hagel C et al (2013) EHEC-O104:H4-Ausbruch im Sommer 2011. Rechtsmedizin 23(5):374–382. https://doi.org/10.1007/s00194-013-0910-9

Fusco V, Quero GM, Morea M et al (2011) Rapid and reliable identification of *Staphylococcus aureus* harbouring the enterotoxin gene cluster (egc) and quantitative detection in raw milk by real time PCR. J Microbiol Methods 144(3):528–537. https://doi.org/10.1016/j.ijfoodmicro.2010.11.016

Guan ZP, Jiang Y, Gao F et al (2013) Rapid and simultaneous analysis of five foodborne pathogenic bacteria using multiplex PCR. Eur Food Res Technol 237(4):627–637. https://doi.org/10.1007/s00217-013-2039-1

Hameed S, Xie L, Ying Y (2018) Conventional and emerging detection techniques for pathogenic bacteria in food science: a review. Trends Food Sci Technol 81:61–73. https://doi.org/10.1016/j.tifs.2018.05.020

Hanna SE, Connor CJ, Wang HH (2005) Real-time polymerase chain reaction for the food microbiologist: technologies, applications, and limitations. J Food Sci 70(3):R49–R53. https://doi.org/10.1111/j.1365-2621.2005.tb07149.x

Hermida M, Taboada M, Menendez S et al (2000) Semi-automated direct epifluorescent filter technique for total bacterial count in raw milk. J AOAC Int 83(6):1345–1348

Hibi K, Abe A, Ohashi E et al (2006) Combination of immunomagnetic separation with flow cytometry for detection of *Listeria monocytogenes*. Anal Chim Acta 573-574:158–163. https://doi.org/10.1016/j.aca.2006.03.022

Hirvonen JJ, Siitonen A, Kaukoranta S-S (2012) Usability and performance of CHROM agar STEC medium in detection of Shiga toxin-producing *Escherichia coli* strains. J Clin Microbiol 50(11):3586–3590. https://doi.org/10.1128/jcm.01754-12

Ibrahim A, Liesack W, Stackebrandt E (1992) The polymerase chain reaction: an epidemiological tool to differentiate between two clusters of pathogenic *Yersinia enterocolitica* strains. FEMS Microbiol Lett 97(1–2):63–66. https://doi.org/10.1016/0378-1097(92)90364-t

Ito K, Yamamoto T, Oyama Y et al (2016) Food allergen analysis for processed food using a novel extraction method to eliminate harmful reagents for both ELISA and lateral-flow tests. Anal Bioanal Chem 408(22):5973–5984. https://doi.org/10.1007/s00216-016-9438-7

Jin S, Guo S, Zuo P et al (2015) A cost-effective Z-folding controlled liquid handling microfluidic paper analysis device for pathogen detection via ATP quantification. Biosens Bioelectron 63:379–383. https://doi.org/10.1016/j.bios.2014.07.070

Karunathilaka SR, Yakes BJ, He K et al (2018) Non-targeted NIR spectroscopy and SIMCA classification for commercial milk powder authentication: a study using eleven potential adulterants. Heliyon 4(9):e00806. https://doi.org/10.1016/j.heliyon.2018.e00806

Kawasaki S, Fratamico PM, Horikoshi N et al (2010) Multiplex real-time polymerase chain reaction assay for simultaneous detection and quantification of *Salmonella* species, *Listeria monocytogenes*, and *Escherichia coli O157:H7* in ground pork samples. Foodborne Pathog Dis 7(5):549–554. https://doi.org/10.1089/fpd.2009.0465

Kateete DP, Kimani CN, Katabazi FA et al (2010) Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob 9(1):23

Kim M, JungT KY et al (2015) A microfluidic device for label-free detection of *Escherichia coli* in drinking water using positive dielectrophoretic focusing, capturing, and impedance measurement. Biosens Bioelectron 74:1011–1015. https://doi.org/10.1016/j.bios.2015.07.059

Lauri A, Mariani PO (2009) Potentials and limitations of molecular diagnostic methods in food safety. Genes Nutr 4(1):1–12. https://doi.org/10.1007/s12263-008-0106-1

Law JW-F, Mutalib N-SA, Chan K-G et al (2015) Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol 5:770. https://doi.org/10.3389/fmicb.2014.00770

Lee M, Oh SK, Choi H et al (2018) Sub nanomolar sensitivity of filter paper-based SERS sensor for pesticide detection by hydrophobicity change of paper surface. ACS Sens 3(1):151–159. https://doi.org/10.1021/acssensors.7b00782

Lee N, Kwon KY, Oh SK et al (2014) A multiplex PCR assay for simultaneous detection of *Escherichia coli O157:H7*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella spp*., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-eat food. Foodborne Pathog Dis 11(7):574–580. https://doi.org/10.1089/fpd.2013.1638

Levin S, Krishnan S, Rajkumar S et al (2016) Monitoring of fluoride in water samples using a smartphone. Sci Total Environ 551-552:101–107. https://doi.org/10.1016/j.scitotenv.2016.01.156

Li Y, Sun Y, Beier RC et al (2017a) Immunochemical techniques for multianalyte analysis of chemical residues in food and the environment: a review. Trends Anal Chem 88:25–40. https://doi.org/10.1016/j.trac.2016.12.010

Li Z, Li Z, Zhao D et al (2017b) Smartphone-based visualized microarray detection for multiplexed harmful substances in milk. Biosens Bioelectron 87:874–880. https://doi.org/10.1016/j.bios.2016.09.046

Lindholm L, Sarkkinen H (2004) Direct identification of gram-positive cocci from routine blood cultures by using Accu Probe tests. J Clin Microbiol 42(12):5609–5613. https://doi.org/10.1128/jcm.42.12.5609-5613.2004

Liu C, Wang Y, Fu L et al (2018) Microfluidic paper-based chip platform for benzoic acid detection in food. Food Chem 2(49):162–167. https://doi.org/10.1016/j.foodchem.2018.01.004

Lopez-Campos G, Martinez-Suarez JV, Aguado-Urda M et al (2012) Applications of DNA microarrays to study bacterial foodborne pathogens. In: Microarray detection and characterization of bacterial foodborne pathogens. Springer, Boston, pp 93–114. https://doi.org/10.1007/978-1-4614-3250-0_5

Ludwig SK, Tokarski C, Lang SN et al (2014) Calling biomarkers in Milk using a protein microarray on your smartphone. PLoS One 10(8):e0134360. https://doi.org/10.1371/journal.pone.0134360

Ma L, Nilghaz A, Choi JR et al (2018a) Rapid detection of clenbuterol in milk using microfluidic paper-based ELISA. Food Chem 246:437–441. https://doi.org/10.1016/j.foodchem.2017.12.022

Ma Y, Wang Y, Luo Y et al (2018b) Rapid and sensitive on-site detection of pesticide residues in fruits and vegetables using screen-printed paper-based SERS swabs. Anal Methods 10(38):4655–4664. https://doi.org/10.1039/c8ay01698d

Ma K, Deng Y, Bai Y et al (2014) Rapid and simultaneous detection of *Salmonella*, *Shigella* and *Staphylococcus aureus* in fresh pork using a multiplex real-time PCR assay based on immunomagnetic separation. Food Control 42:87–93. https://doi.org/10.1016/j.foodcont.2014.01.042

Mackay IM, Arden KE, Nitsche A (2002) Real-time PCR in virology. Nucleic Acids Res 30 (6):1292–1305. https://doi.org/10.1093/nar/30.6.1292

MacMahon S, Begley TH, Diachenko GW et al (2012) A liquid chromatography–tandem mass spectrometry method for the detection of economically motivated adulteration in protein-containing foods. J Chromatogr A 1220:101–107. https://doi.org/10.1016/j.chroma.2011.11.066

Maibach RC, Altwegg M (2003) Cloning and sequencing an unknown gene of *Tropheryma whipplei* and development of two LightCycler® PCR assays. Diagn Microbiol Infect Dis 46 (3):181–187. https://doi.org/10.1016/s0732-8893(03)00063-4

Malic L, ZhangX BD et al (2015) Polymer-based microfluidic chip for rapid and efficient immunomagnetic capture and release of *Listeria monocytogenes*. Lab Chip 15 (20):3994–4007. https://doi.org/10.1039/c5lc00852b

Mandal P, BiswasA CK et al (2011) Methods for rapid detection of foodborne pathogens: an overview. Am J Pharmacol Toxicol 6(2):87–102. https://doi.org/10.3923/ajft.2011.87.102

Mangal M, Bansal S, Sharma SK et al (2016) Molecular detection of foodborne pathogens: a rapid and accurate answer to food safety. Crit Rev Food Sci Nutr 56(9):1568–1584. https://doi.org/10.1080/10408398.2013.782483

March SB, Ratnam S (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157: H7 associated with hemorrhagic colitis. J Clin Microbiol 23(5):869–872

Martinez AW, Phillips ST, Whitesides GM et al (2010) Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal Chem 82(1):3–10. https://doi.org/10.1021/ac9013989

Mei Q, Jing H, Li Y et al (2016) Smartphone based visual and quantitative assays on up conversional paper sensor. Biosens Bioelectron 75:427–432. https://doi.org/10.1016/j.bios.2015.08.054

Meng J, Doyle MP (2002) Introduction. Microbiological food safety. Microbes Infect 4 (4):395–397. https://doi.org/10.1016/s1286-4579(02)01552-6

Meng X, Schultz CW, Cui C et al (2015) On-site chip-based colorimetric quantitation of organo phosphorus pesticides using an office scanner. Sens Actuators B Chem 215:577–583. https://doi.org/10.1016/j.snb.2015.04.011

Mettakoonpitak J, Boehle K, Nantaphol S et al (2016) Electrochemistry on paper-based analytical devices: a review. Electroanalysis 28(7):1420–1436. https://doi.org/10.1002/elan.201501143

Miller RG, Tate CR, Mallinson ET et al (1991) Xylose-lysine-Tergitol 4: an improved selective agar medium for the isolation of Salmonella. Poult Sci 70(12):2429–2432. https://doi.org/10.3382/ps.0702429

Miocevic O, Cole CR, Laughlin MJ et al (2017) Quantitative lateral flow assays for salivary biomarker assessment: a review. Front Public Health 5. https://doi.org/10.3389/fpubh.2017.00133

Mokgalaka NS, Gardea-Torresdey JL (2006) Laser ablation inductively coupled plasma mass spectrometry: principles and applications. Appl Spectrosc Rev 41(2):131–150. https://doi.org/10.1080/05704920500510703

Morales-Narvaez E, Naghdi T, Zor E et al (2015) Photoluminescent lateral-flow immunoassay revealed by graphene oxide: highly sensitive paper-based pathogen detection. Anal Chem 87 (16):8573–8577. https://doi.org/10.1021/acs.analchem.5b02383

Moter A, Gobel UB (2000) Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. J Microbiol Methods 41(2):85–112. https://doi.org/10.1016/s0167-7012(00)00152-4

Mu X, Xin X, Fan C et al (2015) A paper-based skin patch for the diagnostic screening of cystic fibrosis. Chem Commun 51(29):6365–6368. https://doi.org/10.1039/c5cc00717h

Murphy N, McLauchlinn J, Ohain C et al (2007) Construction and evaluation of a microbiological positive process internal control for PCR-based examination of food samples for *Listeria monocytogenes* and *Salmonella enterica*. Int J Food Microbiol 120(1–2):110–119. https://doi.org/10.1016/j.ijfoodmicro.2007.06.006

Naravaneni R, Jamil K (2005) Rapid detection of food-borne pathogens by using molecular techniques. J Med Microbiol 54(1):51–54. https://doi.org/10.1099/jmm.0.45687-0

Nawrocka A, Lamorska J (2013) Determination of food quality by using spectroscopic methods. In: Avdvances in agro physical research. IntechOpen Limited, London. https://doi.org/10.5772/52722

Neethirajan S, Ragavan V, Weng X et al (2018) Biosensors for sustainable food engineering: challenges and perspectives. Biosensors 8(1):23. https://doi.org/10.3390/bios8010023

Noor MO, Krull UJ (2014) Camera-based ratiometric fluorescence transduction of nucleic acid hybridization with reagentless signal amplification on a paper-based platform using immobilized quantum dots as donors. Anal Chem 86(20):10331–10339. https://doi.org/10.1021/ac502677n

Olatunji OS, Fatoki OS, Opeolu BO et al (2014) Determination of polycyclic aromatic hydrocarbons [PAHs] in processed meat products using gas chromatography – flame ionization detector. Food Chem 156:296–300. https://doi.org/10.1016/j.foodchem.2014.01.120

Oliver S, Jayarao B, Almeida R (2005) Foodborne pathogens in Milk and the dairy farm environment: food safety and public health implications. Foodborne Pathogens Dis 2(2):115–129. https://doi.org/10.1089/fpd.2005.2.115

Olsen J, Aabo S, Hill W et al (1995) Probes and polymerase chain reaction for detection of food-borne bacterial pathogens. Int J Food Microboil 28(1):1–78. https://doi.org/10.1016/0168-1605(94)00159-4

Pal M, Lee S, Kwon D et al (2017) Direct immobilization of antibodies on Zn-doped Fe_3O_4 nano clusters for detection of pathogenic bacteria. Anal Chim Acta 952:81–87. https://doi.org/10.1016/j.aca.2016.11.041

Palacios-Morillo A, Alcazar A, Pablos FD et al (2013) Differentiation of tea varieties using UV–Vis spectra and pattern recognition techniques. Spectrochim Acta A 103:79–83. https://doi.org/10.1016/j.saa.2012.10.052

Pandey CM, Tiwari I, Singh VN et al (2017) Highly sensitive electrochemical immunosensor based on graphene-wrapped copper oxide-cysteine hierarchical structure for detection of pathogenic bacteria. Sens Actuator B Chem 238:1060–1069. https://doi.org/10.1016/j.snb.2016.07.121

Pang B, FuK LY et al (2018) Development of a self-priming PDMS/paper hybrid microfluidic chip using mixed-dye-loaded loop-mediated isothermal amplification assay for multiplex foodborne pathogens detection. Anal Chim Acta 1040:81–89. https://doi.org/10.1016/j.aca.2018.07.024

Park SH, Aydin M, Khatiwara A et al (2014) Current and emerging technologies for rapid detection and characterization of *Salmonella* in poultry and poultry products. Food Microbiol 38:250–262. https://doi.org/10.1016/j.fm.2013.10.002

Penner MH (2017) Basic principles of spectroscopy. In: Food analysis, Food science text series, pp 79–88. https://doi.org/10.1007/978-3-319-45776-5_6

Pettipher G, Watts Y, Langford S et al (1992) Preliminary evaluation of COBRA, an automated DEFT instrument, for the rapid enumeration of micro-organisms in cultures, raw milk, meat and fish. Lett Appl Microbiol 14(5):206–209. https://doi.org/10.1111/j.1472-765x.1992.tb00686.x

Piriya VSA, Joseph P, Daniel SCG et al (2017) Colorimetric sensors for rapid detection of various analytes. Mater Sci Eng C 78:1231–1245. https://doi.org/10.1016/j.msec.2017.05.018

Pizzolon JC, Hoenig M (2005) Analysis of food samples by ICP-MS. development and routine: where are the differences? Can J Anal Sci Spectrosc 50(5):221–227

Poppert S, Essig A, Marre R et al (2002) Detection and differentiation of Chlamydiae by fluorescence in situ hybridization. Appl Environ Microbiol 68(8):4081–4089. https://doi.org/10.1128/aem.68.8.4081-4089.2002

Prisna JS, Leder O, Ornella Maria Porcu (2018) The importance of UV-Vis spectroscopy: application in food products characterization. Scholarly J Food Nutr 1(3). https://doi.org/10.32474/sjfn.2018.01.000111

Pyle BH, Broadaway SC, McFeters GA (1999) Sensitive detection of Escherichia coli O157: H7 in food and water by immunomagnetic separation and solid-phase laser cytometry. Appl Environ Microbiol 65(5):1966–1972

Qin J, Chao K, Kim MS (2013) Simultaneous detection of multiple adulterants in dry milk using macro-scale Raman chemical imaging. Food Chem 138(2–3):998–1007. https://doi.org/10.1016/j.foodchem.2012.10.115

Qu J, Liu D, Cheng J et al (2015) Applications of near-infrared spectroscopy in food safety evaluation and control: a review of recent research advances. Crit Rev Food Sci Nutr 55 (13):1939–1954. https://doi.org/10.1080/10408398.2013.871693

Rahn K, De Grandis S, Clarke R et al (1992) Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol Cell Probes 6(4):271–279. https://doi.org/10.1016/0890-8508(92)90002

Rateni G, Dario P, Cavallo F (2017) Smartphone-based food diagnostic technologies: a review. Sensors 17(6):1453. https://doi.org/10.3390/s17061453

Reid LM, O'Donnell CP, Downey G (2006) Recent technological advances for the determination of food authenticity. Trends Food Sci Technol 17(7):344–353. https://doi.org/10.1016/j.tifs.2006.01.006

Rengaraj S, Cruz-Izquierdo A, Scott JL et al (2018) Impedimetric paper-based biosensor for the detection of bacterial contamination in water. Sens Actuators B Chem 265:50–58. https://doi.org/10.1016/j.snb.2018.03.020

Ruiz-Rueda O, Soler M, Calvo L et al (2010) Real-time PCR for the simultaneous detection of *Salmonella spp.* and *Listeria monocytogenes* in food samples. Food Anal Method 4(2):131–138. https://doi.org/10.1007/s12161-010-9163-3

Sayad A, Ibrahim F, Uddin SM et al (2018) A microdevice for rapid, monoplex and colorimetric detection of foodborne pathogens using a centrifugal microfluidic platform. Biosens Bioelectron 100:96–104. https://doi.org/10.1016/j.bios.2017.08.060

Scallan E, Hoekstra RM, Angulo FJ et al (2011) Foodborne illness acquired in the United States—major pathbogens. Emerg Infect Dis 17(1):7–15. https://doi.org/10.3201/eid1701.p11101

Schlosser G, Kacer P, Kuzma M et al (2007) Coupling immunomagnetic separation on magnetic beads with matrix-assisted laser desorption ionization-time of flight mass spectrometry for detection of *Staphylococcal* enterotoxin B. Appl Environ Microbiol 73(21):6945–6952. https://doi.org/10.1128/aem.01136-07

Severgnini M, Cremonesi P, Consolandi C et al (2011) Advances in DNA microarray technology for the detection of foodborne pathogens. Food Bioprocess Technol 4(6):936–953. https://doi.org/10.1007/s11947-010-0430-5

Shafiee H, Asghar W, Inci F et al (2015) Paper and flexible substrates as materials for biosensing platforms to detect multiple biotargets. Sci Rep 5(1):8719. https://doi.org/10.1038/srep08719

Shangkuan Y, Show Y, Wang T (1995) Multiplex polymerase chain reaction to detect toxigenic *Vibrio cholera* and to biotype *Vibrio cholera* O1. J Appl Bacteriol 79(3):264–273. https://doi.org/10.1111/j.1365-2672.1995.tb03136.x

Shen Z, Hou N, Jin M et al (2014) A novel enzyme-linked immunosorbent assay for detection of *Escherichia coli* O157:H7 using immune-magnetic and beacon gold nanoparticles. Gut Pathogens 6(1):14. https://doi.org/10.1186/1757-4749-6-14

Silva BM, Andrade PB, Mendes GC et al (2000) Analysis of phenolic compounds in the evaluation of commercial quince jam authenticity. J Agric Food Chem 48(7):2853–2857. https://doi.org/10.1021/jf9911040

Silva DS, Canato T, Magnani M et al (2011) Multiplex PCR for the simultaneous detection of *Salmonella spp*. and *Salmonella enteritidis* in food. J Food SciTechnol 46(7):1502–1507. https://doi.org/10.1111/j.1365-2621.2011.02646.x

Simon MC, Gray DI, Cook N (1996) DNA extraction and PCR methods for the detection of *Listeria monocytogenes* in cold-smoked Salmon. Appl Environ Microbiol 62(3):822–824

Singh SP, Vogel-Mikus K, Arcon I et al (2013) Pattern of iron distribution in maternal and filial tissues in wheat grains with contrasting levels of iron. J Exp Bot 64(11):3249–3260. https://doi.org/10.1093/jxb/ert160

Sun L, Jiang Y, Pan R et al (2018) A novel, simple and low-cost paper-based analytical device for colorimetric detection of *Cronobacter* spp. Anal Chim Acta 1036:80–88. https://doi.org/10.1016/j.aca.2018.05.061

Sun Y, Quyen TL, Hung TQ et al (2015) A lab-on-a-chip system with integrated sample preparation and loop-mediated isothermal amplification for rapid and quantitative detection of *Salmonella spp*. in food samples. Lab Chip 15(8):1898–1904. https://doi.org/10.1039/c4lc01459f

Suo B, He Y, Paoli G et al (2010) Development of an oligonucleotide-based microarray to detect multiple foodborne pathogens. Mol Cell Probes 24(2):77–86. https://doi.org/10.1016/j.mcp.2009.10.005

Su K, Pan Y, Wan Z et al (2017) Smartphone-based portable biosensing system using cell viability biosensor for okadaic acid detection. Sens Actuators B Chem 251:134–143. https://doi.org/10.1016/j.snb.2017.04.036

Tan LK, Ooi PT, Carniel E et al (2014) Evaluation of a modified cefsulodin-irgasan-novobiocin agar for isolation of Yersinia spp. PLoS One 9(8):e106329. https://doi.org/10.1371/journal.pone.0106329

Tang R, Yang H, Gong Y et al (2017) A fully disposable and integrated paper-based device for nucleic acid extraction, amplification and detection. Lab Chip 17(7):1270–1279. https://doi.org/10.1039/c6lc01586g

Tian F, Lyu J, Shi J et al (2016) A polymeric microfluidic device integrated with nanoporous alumina membranes for simultaneous detection of multiple foodborne pathogens. Sens Actuators B Chem 225:312–318. https://doi.org/10.1016/j.snb.2015.11.059

Tokel O, Yildiz UH, Inci F et al (2015) Portable microfluidic integrated plasmonic platform for pathogen detection. Sci Rep 5(1). https://doi.org/10.1038/srep09152

Toze S (1999) PCR and the detection of microbial pathogens in water and waste water. Water Res 33(17):3545–3556. https://doi.org/10.1016/s0043-1354(99)00071-8

Trebesius K, Adler K, Vieth M et al (2001) Specific detection and prevalence of helicobacter heilmannii-like organisms in the human gastric mucosa by fluorescent in situ hybridization and partial 16S ribosomal DNA sequencing. J Clin Microbiol 39(4):1510–1516. https://doi.org/10.1128/jcm.39.4.1510-1516.2001

Tsai YL, Palmer CJ, Sangermano LR (1993) Detection of *Escherichia coli* in sewage and sludge by polymerase chain reaction. Appl Environ Microbiol 59(2):353–357

Tyler G (1994) ICP-MS, or ICP-AES and AAS?—a comparison. Varian, Belrose

Umesha S, Manukumar HM, Chandrasekhar B et al (2016) Aflatoxins and food pathogens: impact of biologically active aflatoxins and their control strategies. J Sci Food Agric 97(6):1698–1707. https://doi.org/10.1002/jsfa.8144

Umesha S, Manukumar HM (2018) Advanced molecular diagnostic techniques for detection of food-borne pathogens: current applications and future challenges. Crit Rev Food Sci Nutr 58(1):84–104. https://doi.org/10.1080/10408398.2015.1126701

Uzun L, Turner AP (2016) Molecularly-imprinted polymer sensors: realising their potential. Biosens Bioelectron 76:131–144. https://doi.org/10.1016/j.bios.2015.07.013

Vaisocherova-Lisalova H, Visova I, Ermini ML et al (2016) Low-fouling surface plasmon resonance biosensor for multi-step detection of foodborne bacterial pathogens in complex food samples. Biosens Bioelectron 80:84–90. https://doi.org/10.1016/j.bios.2016.01.040

Valderrama WB, Dudley EG, Doores S et al (2016) Commercially available rapid methods for detection of selected food-borne pathogens. Crit Rev Food Sci Nutr 56(9):1519–1531. https://doi.org/10.1080/10408398.2013.775567

Valous NA, Mendoza F, Sun D (2010) Emerging non-contact imaging, spectroscopic and colorimetric technologies for quality evaluation and control of hams: a review. Trends Food SciTechnol 21(1):26–43. https://doi.org/10.1016/j.tifs.2009.09.003

Vashist SK, Luppa PB, Yeo LY et al (2015) Emerging technologies for next-generation point-of-care testing. Trends Biotechnol 33(11):692–705. https://doi.org/10.1016/j.tibtech.2015.09.001

Verstraete K, Robyn J, Del-Favero J et al (2012) Evaluation of a multiplex-PCR detection in combination with an isolation method for STEC O26, O103, O111, O145 and sorbitol

fermenting O157 in food. Food Microbiol 29(1):49–55. https://doi.org/10.1016/j.fm.2011.08.017

Wang P, Wang M, Zhou F et al (2017) Development of a paper-based, inexpensive, and disposable electrochemical sensing platform for nitrite detection. Electrochem Commun 81:74–78. https://doi.org/10.1016/j.elecom.2017.06.006

Wang X, Zhang L, Jin L et al (2007) Development and application of an oligonucleotide microarray for the detection of food-borne bacterial pathogens. Appl Microbiol Biotechnol 76(1):225–233. https://doi.org/10.1007/s00253-007-0993-x

Wang Y, Duncan TV (2017) Nanoscale sensors for assuring the safety of food products. Curr Opin Biotechnol 44:74–86. https://doi.org/10.1016/j.copbio.2016.10.005

Weng X, Gaur G, Neethirajan S (2016) Rapid detection of food allergens by microfluidics ELISA-based optical sensor. Biosensors 6(2):24. https://doi.org/10.3390/bios6020024

Weng X, Neethirajan S (2017) Ensuring food safety: quality monitoring using microfluidics. Trends Food Sci Technol 65:10–22. https://doi.org/10.1016/j.tifs.2017.04.015

Williams AJ, Cooper WM, Ramsaroop S et al (2017) Rapid flow cytometry detection of a single viable *Escherichia coli* O157:H7 cell in raw spinach using a simplified sample preparation technique. Front Microbiol 8:1493. https://doi.org/10.3389/fmicb.2017.01493

Workman JJ (2000) Near-infrared spectroscopy of polymers and rubbers. Enc Anal Chem. https://doi.org/10.1002/9780470027318.a2021

Wu W, Zhao S, Mao Y et al (2015) A sensitive lateral flow biosensor for Escherichia coli O157:H7 detection based on aptamer mediated strand displacement amplification. Anal Chim Acta 861:62–68. https://doi.org/10.1016/j.aca.2014.12.041

Xiao L, Zhang Z, Wu C et al (2017) Molecularly imprinted polymer grafted paper-based method for the detection of 17β-estradiol. Food Chem 221:82–86. https://doi.org/10.1016/j.foodchem.2016.10.062

Xiao W, Xiao M, Fu Q et al (2016) A portable smart-phone readout device for the detection of mercury contamination based on an aptamer-assay nanosensor. Sensors 16(11):1871. https://doi.org/10.3390/s16111871

Xin H, Stone R (2008) Tainted milk scandal: Chinese probe unmasks high-tech adulteration with melamine. Science 322(5906):1310–1311. https://doi.org/10.1126/science.322.5906.1310

Xing K, Peng J, Liu D et al (2018) Novel immune-chromatographic assay based on Eu (III)-doped polystyrene nanoparticle-linker-monoclonal antibody for sensitive detection of *Escherichia coli* O157:H7. Anal Chim Acta 998:52–59. https://doi.org/10.1016/j.aca.2017.10.027

Yager P, Domingo GJ, Gerdes J (2008) Point-of-care diagnostics for global health. Annu Rev Biomed Eng 10(1):107–144

Yang K, Peretz-Soroka H, Liu Y et al (2016) Novel developments in mobile sensing based on the integration of microfluidic devices and smartphones. Lab Chip 16(6):943–958. https://doi.org/10.1039/c5lc01524c

Yao L, Wang L, Huang F et al (2018) A microfluidic impedance biosensor based on immunomagnetic separation and urease catalysis for continuous-flow detection of *E. coli* O157:H7. Sens Actuators B:Chem 259:1013–1021. https://doi.org/10.1016/j.snb.2017.12.110

Yetisen AK, Akram MS, Lowe CR (2013) Paper-based microfluidic point-of-care diagnostic devices. Lab Chip 13(12):2210. https://doi.org/10.1039/c3lc50169h

Yoo SM, Kim D, Lee SY (2015) Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species. Talanta 132:112–117. https://doi.org/10.1016/j.talanta.2014.09.003

Yu L, Shi Z, Fang C et al (2015) Disposable lateral flow-through strip for smartphone-camera to quantitatively detect alkaline phosphatase activity in milk. Biosens Bioelectron 69:307–315. https://doi.org/10.1016/j.bios.2015.02.035

Zhang J, Zhang X, Dediu L et al (2011) Review of the current application of fingerprinting allowing detection of food adulteration and fraud in China. Food Control 22(8):1126–1135. https://doi.org/10.1016/j.foodcont.2011.01.019

Zhang Y, Zuo P, Ye B (2015) A low-cost and simple paper-based microfluidic device for simultaneous multiplex determination of different types of chemical contaminants in food. Biosens Bioelectron 68:14–19. https://doi.org/10.1016/j.bios.2014.12.042

Zhao C, Zhong G, Kim D et al (2014a) A portable lab-on-a-chip system for gold-nanoparticle-based colorimetric detection of metal ions in water. Biomicrofluidics 8(5):052107. https://doi.org/10.1063/1.4894244

Zhao X, Lin C, Wang J et al (2014b) Advances in rapid detection methods for foodborne pathogens. J Microbiol Biotechnol 24(3):297–312. https://doi.org/10.4014/jmb.1310.10013

Zhao Y, Wang H, Zhang P et al (2016) Rapid multiplex detection of 10 foodborne pathogens with an up-converting phosphor technology-based 10-channel lateral flow assay. Sci Rep 6(1). https://doi.org/10.1038/srep21342

Zhu Z, Feng M, Zuo L et al (2015) An aptamer based surface plasmon resonance biosensor for the detection of ochratoxin A in wine and peanut oil. Biosens Bioelectron 65:320–326. https://doi.org/10.1016/j.bios.2014.10.059

Nanotechnology for Food: Regulatory Issues and Challenges

14

Sandhya Sharma, Nidhi Rawat, Sandeep Kumar, Zahoor Mir, and Kishor Gaikwad

Abstract

Nanotechnology holds great promise in various fields ranging from household to industry. Apart from the engineering and medical sector, they are also being employed in agriculture and food production. Potential applications in the food industry include pathogen detection sensors, efficient food processing, and packaging, etc. In this chapter, we have discussed several wide-ranging applications of nanotechnology and new advances in this area. Further, it also throws light on how nanomaterials are released from food contact materials and reach to the various cellular organelles. What are the different ways to enter and how to assess the biosafety of these nanomaterials prior to use? Besides, regulatory aspects of nanomaterials have also been explained.

Keywords

Nanotechnology · Smart packaging · Food science · Regulatory issue · Nanomaterial biosafety

14.1 Introduction

Nanotechnology involves the application of principles of matter at the nanolevel in biological systems. It is majorly an interdisciplinary field involving applied physics, device physics, mechanical engineering, biological engineering, supramolecular

S. Sharma (✉) · Z. Mir · K. Gaikwad
National Institute for Plant Biotechnology, New Delhi, India

N. Rawat
Central Institute for Research on Buffalo, Hisar, Haryana, India

S. Kumar
Xcelris Lab Pvt Ltd, Ahmedabad, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_14

chemistry, chemical engineering, interface and colloid science, material science, electrical engineering, interface science, self-replicating machines, microfabrication technologies, and robotics, etc. Today nanotechnology is revolutionizing world agricultural practices, food production, and food processing (Carmen et al. 2003).

Nanotechnology has a huge number of applications in several fields ranging from household to industry. Their uses are not limited to the engineering and medical sector only, but they are also being introduced to agriculture, e.g., as sensors for detecting harmful microorganisms, efficient food packaging and processing, and targeted delivery of prodrugs. The consumer demands fresh, authentic, convenient, and flavorful foods. To meet the consumer's needs in today's competitive world, one requires keeping abreast with the latest technologies in the agriculture and food processing industry. Reserach methods are being targeted at producing safer, nutritious, and more appealing foods, so as to cater to the desires of the consumer. The future belongs to new products, new processes, and new technologies that hold the capability to enhance shelf life and quality of the stored food items, and to improve the safety standards (Shefer 2008). Such technologies hold great importance for developing countries like India where still a large proportion of children are undernourished (Kadiyala et al. 2014; Headey et al. 2012).

14.2 Properties of Nanomaterials

On the basis of their surface characteristics, nanomaterials have been divided into several categories. The major features which are considered with respect to the safety of nanomaterials are given below:

Physical properties

- Surface features such as shape, morphology, surface area, size, etc.
- Whether soluble or not.
- Aggregated or dispersed.
- Whether crystalline or amorphous.
- Size distribution of nanomaterials.

Chemical properties

- Molecular structure of nanomaterials including presence/absence of special groups.
- Extent of purity.
- Presence of charged molecules, reactive sites, catalytic properties, etc.
- Hydrophilic or hydrophobic nature.

While introducing these nanomaterials in food systems, one should know the property, reactivity, and compatibility of nanomaterials with surrounding media,

Table 14.1 Different methods used for characterization of nanomaterials

	Properties	Analytical tools/techniques
Physical properties	Specific surface area Aspect ratio	BET (dry powder), ASTM, NSAM, SAXS
	Shape	SAXS, SEM, AFM
	Particle size	DLS, NTA, AFM, ATFMS or TOF-AMS, PCS, DMA, SMPS, XRD, SAXS, XPS, STM
	State agglomeration/ aggregation	DLS, SNOM, UV-Vis spectroscopy
	Structure	XRD, EDX, SEM, STEM, HRTEM, XRR.IR
	Surface morphology/ tomography	SEM, STEM, HR TEM
Chemical properties	Chemical composition	TOF-AMS, EDX
	Surface chemistry/ surface charge	AES, XPS, BET, NMR, XPS/SAM, ZETA potential
	Photo catalytic property	Optical microscopy
	Hydrophobicity	DLS, ICP-AES, ICPMS, colorimetric, UV-Vis spectroscopy

because of their properties changes with the test systems, and suspension medium. Various tools (Table 14.1) have been used to characterize these nanomaterials.

14.3 Characterization of Nanomaterials

After synthesis, nanomaterials are characterized so as to know whether they conform to the desired shape, size, etc. Further, the need to characterize nanomaterials had arisen because researchers found that sometimes nanomaterials of the same size of a different class, or same class of different size, same class, and same size and shape of nanomaterials behave differently. Table 14.1 lists some of the methods used for physicochemical characterization of nanomaterials used in the food and other industries.

14.4 Types of Nanomaterials in Food

Broadly, the nanoparticles are categorized as organic or inorganic depending on the basis of their composition. Commonly used inorganic nanomaterials in the food industry include silver, iron oxide, silicon dioxide, zinc oxide, titanium dioxide, etc. Different nanomaterials have different surface characteristics, comes in varying shapes and sizes, have different chemical properties such as solubility, pH, and ionic strength. The organic nanomaterials consist of biomolecules, including lipids, peptides, and carbohydrates. Most of the organic nanoparticles are spherical in shape

Table 14.2 Uses of inorganic and organic nanomaterials in the food industry

Inorganic nanomaterials	Use	Reference
Silver	Antimicrobial agent	Deshmukh et al. (2019) and Wang et al. (2018)
	Determination of sugar content in food matrices	Della et al. (2019)
	Biosensors	Meng et al. (2019), Zhai et al. (2019), and Yaseen et al. (2019)
Zinc oxide	Food supplement	Pei et al. (2019) and Kumar et al. (2018)
	Antimicrobial	Sun et al. (2018), Król et al. (2017), Shabib et al. (2016), Venkatasubbu et al. (2016), and Suo et al. (2017)
Iron oxide	Colorant	Chai et al. (2016) and Askri et al. (2019)
	Food supplement	Fernandez et al. (2018) and Kim et al. (2017)
Titanium dioxide	Lightening/coloring agent	Lim et al. (2018), Kim et al. (2018), Chen et al. (2018), Rompelberg et al. (2016), and Grande et al. (2016)
	Antimicrobial	Venkatasubbu et al. (2016)
Silicon dioxide	Food additive	Echegoyen et al. (2016) and Lee et al. (2017)

Organic nanomaterials	Inorganic	Function	Reference
Cellulose acetate	AgNPs-organoclay	Packaging	Dairi et al. (2019)
Chitosan	ZnO/TiO2 NPs	Preservation	Wei et al. (2018)
	Silver	Anti-bacterial	Santiago et al. (2019)
	ZnO	Packaging	Saral et al. (2019)
	Cellulose/zinc oxide	Packaging	Youssef et al. (2016)
Curcumin	Mesoporous silica	Packaging	Wu et al. (2019)
Cysteine	Iron oxide	Food supplement	Mohammadi et al. (2017)
Polydopamine-nisin	Iron oxide	Antimicrobial	Song et al. (2019)
Poly lactic acid	TiO_2 n silver	Packaging	Li et al. (2017)
Soyabean polysaccharide	TiO_2	Packaging	Salarbashi et al. (2018)
PVA	Iron oxide	Biosensor	Sanaeifar et al. (2017)
Whey protein	TiO_2	Packaging	Feng et al. (2019)

and have liquid or gel consistency at ambient temperature. The organic nanoparticles are considered to be less toxic as compared to inorganic nanoparticles as they are easily dissolved or digested in various parts of the digestive tract. Nowadays, these organic and inorganic nanoparticles are combined together in a more effective form to be used in the food industry. The inorganic nanoparticles are encapsulated by organic nanomaterials to form hybrid/ composite materials which show low toxicity and high proficiency. Tables 14.2 and 14.3 summarize the use of various organic and inorganic nanomaterials in the food industry.

Table 14.3 Different types of organic nanomaterials used in food industry

Type of organic nanomaterials	Example	Reference
Polymer based	Poly vinyl alcohol (PVA)	Sarwar et al. (2018) and Achabyet al. (2017)
	Polylactic acid (PLA)	Rezaeigolestani et al. (2017), Shavisi et al. (2017), and Wen et al. (2016)
	Poly-3-hydroxybutyrate (PHB)	Mayorga et al. (2018a) and Kuntzler et al. (2018)
	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	Mayorga et al. (2018b) and Shakil et al. (2017)
Polysaccharide based	Starch based	Iamareerat et al. (2018), Requena et al. (2017), Silva et al. (2012), and Memiş et al. (2017)
	Cellulose based	Liu et al. (2018a), Lavoine et al. (2016), Shankar et al. (2016), and Tayeb et al. (2018)
	Chitosan based	Buslovich et al. (2017), Liang et al. (2017), Wang et al. (2019), and Saral et al. (2019)
Protein based	Zein based	Aytac et al. (2017), Oymaci et al. (2016), and Gilbert et al. (2018)
	Whey protein based	Qazanfarzadeh et al. (2016) and Kolaee et al. (2016)
	Casein based	Pardo et al. (2015) and Pan et al. (2014)

14.5 Role of Nanomaterials in the Food Industry

With the introduction of newer and broadly safe nanomaterials, their demand in the food industry has also raised, as they help to offer solutions from food manufacturing to packaging. Besides, some of them are also used as nutrient supplements to improve the organoleptic characteristics of food products, viz. flavor and texture, enhance the quality of frozen products, and protect them from microbes, owing to their antibacterial properties. Nanomaterials are also being used to sense contamination in food products, thus improving food storage. Figure 14.1 provides an overview of the applications of nanotechnology in the food industry.

14.5.1 Use of Nanomaterials in Food Processing

Food processing involves converting raw ingredients into edible and marketable form. It improves the shelf life of the product, removes toxins, and reduces the microbial load so that it can be protected from spoilage and can be easily transported and stored for a longer time period. It also improves the taste, smell, texture, and appearance of the food products which makes it marketable. Nowadays, nanocapsule delivery systems or nanocarriers are being used to deliver food supplements to various body parts depending on the size of the nanocarriers. These nanocarriers are designed to target precise organs so that the active compound is delivered

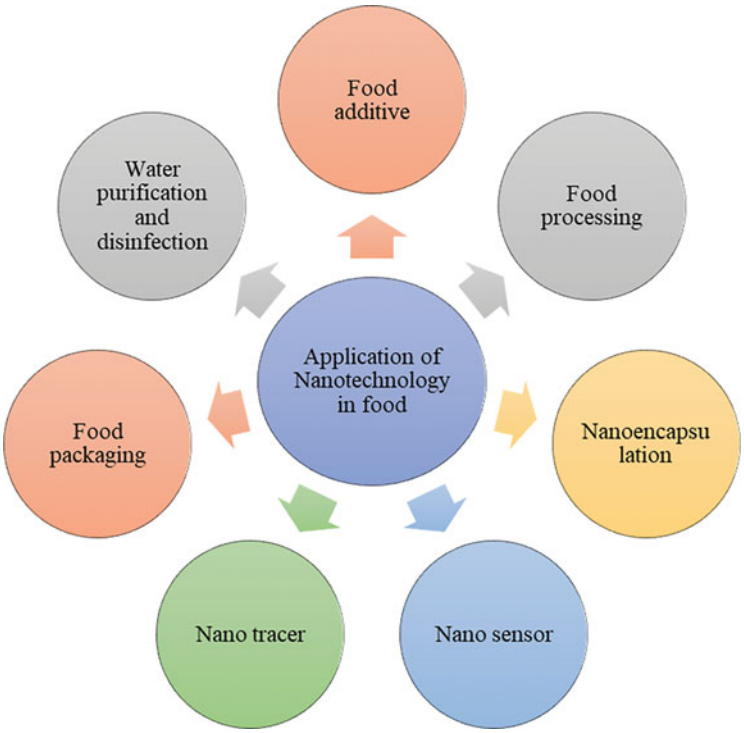

Fig. 14.1 Applications of nanotechnology in the food sector

directly to the preferred site. The organic encapsulation of these carriers helps in the slow and sustained release of the bioactive molecules. Their size allows them to penetrate deep into the tissues and reach the target sites easily. With the help of nanoencapsulation technique, the compounds which are unstable or have low shelf life can be delivered easily. Table 14.4 shows different encapsulation techniques and their pros and cons as well.

One prominent example of nanomaterial encapsulation is the delivery of carotenoids to the body. Carotenoids are highly sensitive to light, heat, and aerobic conditions, but the nanoencapsulated carotenoids have increased stability which in turn increase their bioavailability to the human body (Santos et al., 2018; İnanç Horuz et al., 2018). Similarly, nanoencapsulation of phenolic compounds as anthocyanins has many presumed health benefits. Recently, the formation of stable double emulsions by using whey protein solutions have been used to encapsulate the phenolic compounds derived from blueberry. This water-in-oil-in-water system can efficiently encapsulate the phenolic compounds and anthocyanins (Bamba et al., 2018). Another bioactive hydrophilic compound aspalathin has been nanoencapsulated using both natural as well as synthetic polymers to improve its stability and bioavailability (Human et al., 2019). Similarly, a cytoprotective effect of two flavonoids, quercetin, and baicalein were studied after encapsulation into

Table 14.4 Different encapsulation techniques and their advantages and disadvantages

	Nano-liposomes	Nano-emulsions	Nano-structures
Pros	• Controlled delivery system • Eco-friendly and nontoxic properties • Enhanced bioavailability of nutrients	• Labile at lower pH values • Transparent optical properties • Prepared using lower surfactant level • Enhances stability of product • Monodisperse deposition on substrate	• Higher available space for nutrients • Enhanced stability • Improved dispersibility • Carry both lipophilic and hydrophilic nutrients • Controlled particle size
Cons	• Lack of stability • Low loading space • Unknown interactions between the wall and core materials • High cost for nanoliposome preparation	• Liberation process is not controlled • Show coalescence, flocculation and Ostwald ripening, thus not much stable	• High raw material cost • Affected by environment conditions

chitosan-based polymers. Thus, this technique has been quite promising in using the phytochemicals for controlling bacterial infections. The high stability of encapsulated nanomaterials, slow-release, and the cytoprotective effect was studied and verified (Omwenga et al., 2018). Apart from the nutritional profile, flavor, and fragrance are the special components of food that make the food products more alluring to costumers. But, the chemicals responsible for flavor and smell are highly unstable and get deteriorated if exposed to light, heat, or oxygen. Encapsulating such components can enhance their stability and helps in controlled release (He et al. 2018). Many such examples are given in Table 14.5.

14.5.2 Application of Nanomaterials in Packaging

Our increasing dependence on ready to eat food has generated a demand to develop more smart and active food packaging materials with twin abilities of high strength and biodegradability. The conventional polymers used for packaging have several limitations, as a result, nano-based food packaging material is becoming popular these days. They possess great mechanical strength, antimicrobial properties, thereby acting as barriers for harmful microbes and improving the quality of food materials. One of the most prominent polymers used in food packaging is polyvinyl alcohol (PVA) because of its good flexibility and adhesive properties. To further improve the properties of PVA, it has been fabricated with nanomaterials such as zinc oxide. The resulting nanocomposite films have enhanced water barrier, antimicrobial properties, and more strength (Jayakumar et al., 2019). A similar nanocomposite has been prepared by using silver nanoparticles and PVA which exhibited antibacterial activity and high mechanical strength and stability (Tao et al.,

Table 14.5 Types of encapsulation of different active molecules

Active molecule	Purpose of encapsulation	Encapsulating material	Reference
Folic acid	To protect folic acid during processing	Pectin–whey protein concentrate (WPC) double emulsions	Assadpour and Jaffari (2017)
Insulin	For controlled release of insulin	Chitosan and Arabic gum	Avadi et al. (2010)
Fish oil	Protect from heat and oxidation	Sodium caseinate, Arabic gum, and sage extract	Binsi et al. (2017)
Zeaxanthin	Protection from degradation	Cactus cladode mucilage	Camilade et al. (2018)
Chia seed oil	To gain thermal and oxidative stability	Chia seed mucilage	Campo et al. (2017)
Quercetin	For pharmacological use	Almond gum and shellac	Doost et al. (2018)
Epigallocatechin gallate	For pharmacological use of bioactive compound	Chitosan and gellan gum	Dahiya et al. (2017)
Omega-3 fatty acids	Protection against oxidation	Whey protein	Eratte et al. (2014)
Vitamin A	Stability against acidic conditions	Polyvinyl alcohol	Arezoo and Fathi (2018)
Cumin seed oil	To be used as preservative and anti-cancer drug	Sodium caseinate–guar gum	Farshi et al. (2017)
Zataria multiflora essential oil	To increase the bioactivity of compound	Basil seed gum	Gahruie et al. (2017)
Orange peel oil	For controlled release	Pectin-whey complex	Ghasemi et al. (2017)
D-limonene	For protecting the volatile compound	Whey protein–pectin	Ghasemi et al. (2018)
Eucalyptus staigeriana essential oil	To be used as natural preservative	Cashew gum	Emanuele et al. (2015)
Eugenol oil	To be used as natural preservative	Arabic gum and lecithin	Qiaobin et al. (2016)
Saffron petal anthocyanin	For protecting heat sensitive compound	Cress seed gum	Jafari et al. (2016)

2017). Not only with inorganic nanoparticles, blend of PVA with organic material as chitosan also resulted in PVA-chitosan films showing lower oxygen permeability, high water barrier, and good antibacterial properties (Liu et al. 2018b). Table 14.6 shows different modifications in PVA for food packaging purposes.

Packaging and maintenance of fresh-cut commodities are much more difficult as compared to dry products. Processing of fruits and vegetables generates physiological stresses in the cut tissues which reduce their shelf life and hence deteriorate their quality. Therefore, several strategies have been implemented to reduce the

Table 14.6 Different modifications in polyvinyl alcohol for efficient food packaging

Modification	Property	Reference
SiO_2	More tensile strength and barrier properties	Yu et al. (2018)
BSA modified SiO_2	Upgraded mechanical, thermal, and optical properties	Mallakpour and Nazari (2018)
Silver NPs	Enhanced antibacterial properties	Sarwar et al. (2018)
TiO_2 NPs	More homogenous and compact nanocomposite	Youssef et al. (2018)
Hydroxytyrosol	Strong antioxidant activity	Luzi et al. (2018)
Larch bark tannin	Antioxidant and UV protection	Zhai et al. (2018)
Cellulose nanocrystals	Enhanced tensile strength and toughness	Shalom et al. (2019)
Limonene	Best degradability and bacteriostatic property	Lan et al. (2019)
Graphene modified TiO_2 NPs	Outstanding thermal and water resistance and green biodegradable material	Bi et al. (2019)
Chitosan	Better maintenance of fruit quality	Ding et al. (2019)
N-halamine and zwitterions	Antifouling and biocidal function	Ma et al. (2019)

Table 14.7 Newly introduced nanocomposites for food packaging

Product	Coating/ nanocomposite	Property	Reference
Green grapes	Agar-ZnO nanocomposite	Fresh appearance of fruits up to 21 days at ambient conditions	Kumar et al. (2019)
Strawberries and loquats	Calcium alginate and silver nanoparticles	Enhanced shelf life	Hanif et al. (2019)
Black grapes	Chitosan-cellulose acetate phthalate with ZnO NPs	Extended shelf life up to 9 days	Indumathi et al. (2019)
Tomatoes	Polyacrylonitrile and TiO_2 NPs	Exhibited degradation of ethylene and slowed down color changes shift and deterioration during storage	Zhu et al. (2019)
White button mushroom	Chitosan nanoparticles-loaded Citrus aurantium essential oil	Extended shelf life up to 15 days	Karimirad et al. (2018)
Kinnow fruit	Guar gum-based silver nanoparticle coatings	Maintained quality for 4 months, if stored at 4 °C, and for 2 months at 10 °C after coating	Shah et al. (2015)

deterioration of fresh-cut products. These strategies include coating with natural additives, nanoencapsulation, and coating with nanocomposites. The coating acts as a semipermeable barrier for atmospheric gases and water vapors and thus reduces enzymatic browning, oxidation, and tissue metabolism. Also, the coatings provide protection from microbes and are loaded with antioxidants and flavors to keep fruits fresh. Some of the recently produced nanocomposites are shown in Table 14.7.

14.5.3 Use of Nanomaterials in Smart Packaging/Biosensors

As mentioned previously, smart food packaging with nanomaterials as biosensors (nanosensors) is being used to detect food pathogens, contaminants, pesticides, and toxins in food materials. Nanomaterials offer high sensitivity in detecting pathogens in processed food materials and thus alert the costumers regarding the safety status of the food. These nanosensors work by responding to changes in storage conditions as humidity, temperature, oxygen content, or microbial load in the environment or any degradation or change in constituents of food material. For this purpose, nanosensors can be derived in various structures as nanoparticles, thin films, nanofibers, or nanorods. Natural polymer (starch and gelatin) based nanosensors offer significant advantages in terms of biodegradability, edibility, reduced cost, and abundance. A starch-based biopolymer was designed to alter surface features of gelatin films including their surface hydrophobicity, tensile strength, opacity, water vapor uptake, and anti-degradation capacity (Tao et al., 2018). Mesoporous silica nanoparticles (MSNs) based packaging material having pH sensitivity has also been designed. These nanofilms allow the delivery of active compounds depending on the environmental pH, e.g., the antimicrobial, antioxidants, or aromatic molecules get released at neutral pH (Muriel et al. 2018). Nanohydrogels showing temperature sensitivity has been synthesized by nanoemulsion polymerization in a water-in-oil emulsion system. These hydrogels are incorporated by cross-linking with acrylic acid as co-monomer. These PNIPA (N-isopropylacrylamide) nanohydrogels have been evaluated for their suitability as smart delivery systems for active packaging (Fuciños et al., 2014). An upcoming perspective to combine nanotechnology with nanophotonics to create smart packaging material was shown by Sarapulova and coworkers (Sarapulova et al., 2015). Decaying food material changes the luminescence intensity of ZnO nanoparticles. These compositions can be applied to the inner side of the packaging material and are entirely nontoxic and safe.

14.5.4 Nanomaterials in the Biomedical Industry

Nanomaterials have also found their usage in the field of tissue engineering and regenerative medicine, e.g., in construction of a 3D scaffold for cells or to mimic the cementing substance of tissues. Nanomaterials have been used to manufacture support structures for implantable organ systems (Zorlutuna et al. 2013). Further, owing to their nanosize, nanomaterials provide the required tensile strength and also help in the controlled release of bioactive reagents (Bahal et al. 2016; Mi et al. 2016). But, the use of nanomaterials is hindered due to low solubility, toxicity, and short half-life of bioactive compounds such as drugs, growth factors, proteins, cytokines, etc. Different nanomaterials with different properties make them suitable for various applications in regenerative medicine. One of the most commonly used metallic nanomaterials in regenerative medicine is gold nanoparticles (AuNPs). Kang and colleagues used AuNPs to destroy the cancer cells by inhibiting cell division in the affected cell nuclei (Kang et al. 2010). Recently, a report stated the use of AuNPs in plasmonic-based photothermal therapy in cancer (Panikkanvalappil et al. 2017). The

AuNPs protected the healthy cells by reducing the heat-induced damage. In another report, AuNPs have been used to reduce the cancer cell migration by targeting AuNPs to cell nucleus membrane, leading to increased expression of laminin A/C and nuclear rigidity (Ali et al. 2017). AuNPs promote cell differentiation which makes them a suitable candidate to be used as a framework for enhancing osteo-regeneration (Ko et al. 2015). AuNPs conjugated with 2,2,6,6-Tetramethylpiperidine-N-oxyl (TEMPO) has been reported to be used for ROS induced dysfunctions and to enhance osteogenic differentiation of human MSCs (Li et al., 2017). AuNPs have also shown high neovascularization which makes them a potential material to be used for wound healing. AuNPs containing wet electrospun silk fibroin have shown better wound closure in comparison to the control group (Akturk et al. 2016).

Another metallic nanomaterial that is widely used in the biomedical field is silver nanoparticle (AgNPs) due to its antimicrobial properties. AgNPs have shown efficient cross-linking ability with decellualrized porcine liver which helps in the maintenance of collagen fiber in the decellularized liver, thus slowing down its degradation (Saleh et al. 2018). Like AuNPs, AgNPs have also shown cytotoxic effects over cancer cells. Venkatesan and coworkers have shown the cytotoxic effect of AgNPs biosynthesized with chitosan-alginate against cancer cells (Venkatesan et al. 2017). AgNPs has been reported as a potential facilitator of the wound (Tian et al. 2007). The study also reported antimicrobial properties and healing efficiency of AgNPs on different types of wounds including burn and diabetic wounds. Scaffolds containing AgNPs have also been produced including electrospun fibers, nanofibers, etc. (Venkatesan et al. 2017; Biswas et al. 2018). The antimicrobial scaffolds containing chitin/nanosilver composite has also shown increased blood clotting efficiency by inactivating the anticoagulant molecules (Madhumathi et al. 2010).

Another category of nanomaterials used in the biomedical field is ceramic nanoparticles. Hydrogels made of bioactive glass-ceramic nanoparticles (n-BGC) and gelatin has been used in wound dressing (Wang et al. 2016). The composite possesses unique characteristics like gel form, become injectable when sheared and again form gel after some time, thus making it a good candidate for wound coverage. The glass-ceramic nanoparticles have shown potential applications in dentin regeneration, angiogenesis (Moonesi Rad et al. 2019), bone tissue engineering (Covarrubias et al. 2018; Li et al. 2018), etc. These hybrid materials show intrinsic biomineralization and proliferative effect on osteoblasts. Some nanomaterial is reported to have excellent biocompatibility and they are being used efficiently as smart nanocarriers for targeted transport and site-specific delivery of highly toxic compounds/drugs, and other chemotherapeutic agents for cancer treatment (Sanand et al. 2018b).

14.6 Food Contact Material and Their Migrations

The extensive use of nanomaterials in daily consumables mainly in the form of food contact materials (FCM) has raised the chances for the direct contact of consumers with nanomaterials, knowingly or unknowingly. The migration effect of nanoparticles has augmented the situation in the last few years and questions the

implementation of nanomaterials. Therefore, studies had been conducted to determine the migration of different types of FCM into foodstuffs under varying conditions. Huang et al. (2011) has studied the migration principles of nanosilver from commercial containers made of polyethylene plastics to the different types of food stimulating solutions. They observed that the migration increased linearly with temperature, but was least when foodstuffs had alcohol as food stimulating solution. Another group measured the migration rates of AgNPs from food packaging vessels in ultra-pure water, ethanol, and acetic acid for 10 days at 40 °C. Highest migration was observed in acetic acid which shows that the highest chances for nanomaterials to migrate into food are under acidic conditions (Mackevica et al., 2016; Osorio et al., 2019). Another study tested the release of important elements such as lead, cadmium, zinc, and copper from glassware into food stimulants (4% acetic acid) and showed that the shape and area of glassware also affect the migration. The migration was minimum for copper and maximum for lead and cadmium (Mania et al., 2018). Another commonly used biopolymer for food packaging is polylactic acid (PLA). Ubeda and coworkers studied 39 different PLA oligomers for migration analysis and observed that linear oligomers show the highest migration, while cyclic oligomers were not found in migration solutions. Another group studied the migration of carbon black from two different types of plastic materials made of polyethylene and polystyrene. The experimental findings concluded that carbon black is safer to use as it does not migrate into food from plastic material even when aqueous and fatty food was kept for an extended time period (Bott et al., 2014). The migration of phenols, bisphenol analogs, and p-tert-butylphenol present in FCMs was studied by Hwang and coworkers into food stimulants as water, 4% acetic acid, 50% ethanol, and n-heptane. They found the presence of phenols in food stimulants while bisphenol analogs did not migrate (Hwang et al., 2018).

14.7 Adverse Effect of Nanomaterials

MSNs have been used as a smart drug carrier in animals and also as an excellent nanocarrier for controlled release of insecticides, fungicide, and fertilizers. It is considered as one of the safest nanomaterials for delivery purpose and its toxicity has also been assessed in various model systems (Rawat et al. 2016). Determining the half-maximum inhibitory concentrations of different nanomaterials is an important step towards delineating their biosafety aspects. A study by Sanand et al. (2018a) determined the half-maximum inhibitory concentration (IC50) of Ag NPs and MSNs in buffalo bull spermatozoa. Many reports suggest that IC50 values vary according to the cell type, shape and nanomaterial class, nanomaterial size and also the composition of the medium in which nanomaterials are dissolved (Baslak et al., 2015, Broggi et al., 2013 and Aminzadeh et al., 2016). Limited information on these aspects is available and this infers a vital prerequisite for generating toxicokinetic/ADME/biosafety data for each class of nanomaterials (Sanand et al., 2015). The possible production, exposure, and distribution of nanomaterials are still a major constrain for successful exploration of recent advances in the field (Fig. 14.2).

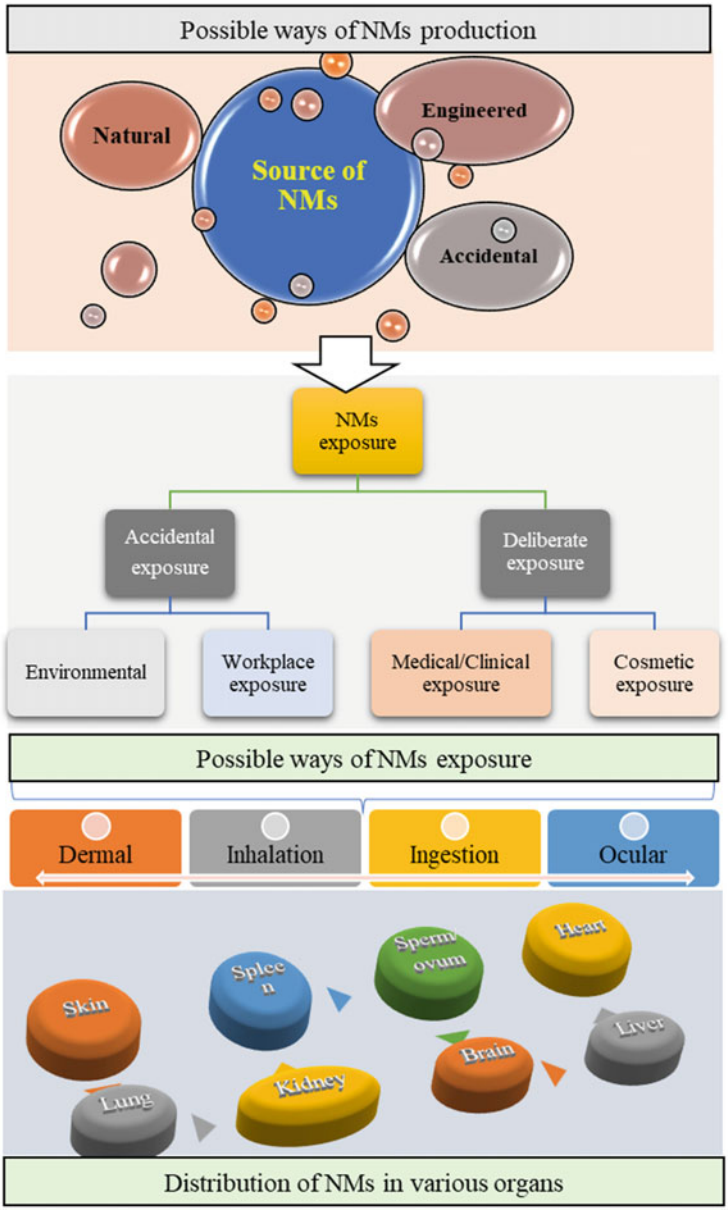

Fig. 14.2 Possible production, exposure, and distribution of nanomaterials

14.8 Regulatory Measures of Nanomaterials Used in the Food Sector

With the increasing influence of nanotechnology in agricultural and allied sectors, a pertinent need has arisen to regulate their use and frame guidelines for the same. The present legislations cover the regulations for the production of nanomaterials only. But, no legislation as of now speaks or provides rules about the regulated use of nanomaterials. Some of the leading agencies of the USA like United States Environmental Protection Agency, National Institute for Occupational Safety and Health, Food and Drug Administration (FDA), Health and Consumer Protection, Directorate of the European Commission have come forward to frame new rules and regulations for awareness and protection of consumers and environment (Fessi et al. 1989; Decher and Schlenoff 2003; Bieberstein et al., 2013). But this is not enough. A lot of information is required to assess the potential risks associated with the safe use of nanomaterials.

The Food and Drug Administration (FDA) is the prime agency to set guidelines for the use of nanomaterials in the agricultural sector (Fig. 14.3). Traditionally, FDA has listed several products containing nanosize materials and made regulations for their use, but did not focus specifically on nanomaterials-based products yet (Weiss et al., 2006). The Institute of Food Science and Technology (IFST) is another European agency working towards different safety aspects related to food

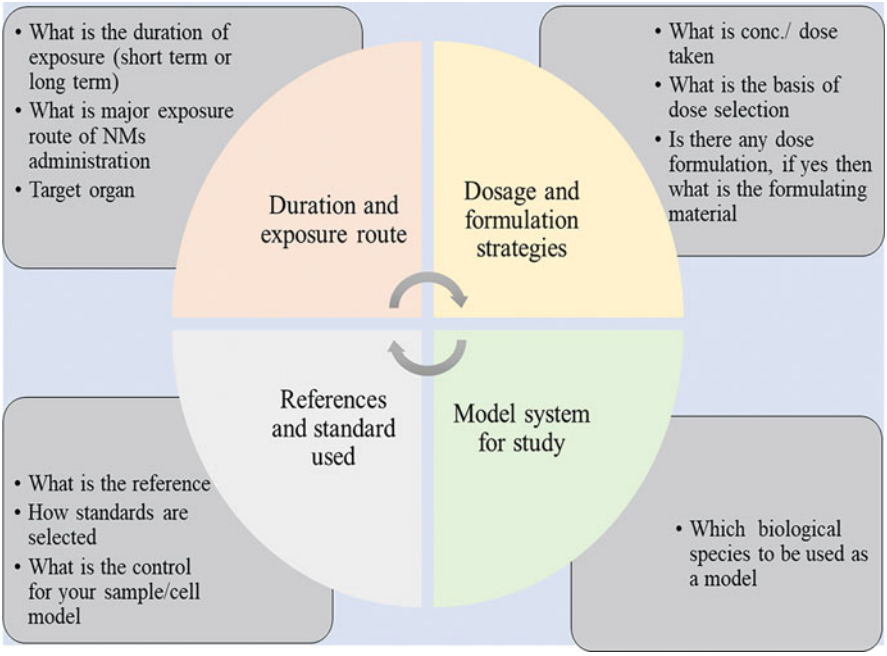

Fig. 14.3 Important points to be considered while designing the biosafety experiments

Table 14.8 List of agencies participating in the National Nanotechnological initiative

Agencies	Major focuses and area of research
NSF National Science Foundation	Toxic effects of nanomaterials in air, water
EPA Environmental Protection Agency	Toxicokinetics of synthesized nanomaterials: Fate, transport and transformation, human exposure, and bioavailability
DoD Departments of Défense	Toxicological properties of nanomaterials: Computational models that will predict toxic, salutary, and biocompatible effects based on nanostructured features
NTP National Toxicology Program	Potential toxicity of nanomaterials: Titanium dioxide, several types of quantum dots, and fullerenes
DoE Departments of Energy	Transport and transformation of nanoparticles in the environment: Exposure and risk analysis, health effects
DOJ Departments of Justice	Independent, evidence-based knowledge, and tools to meet the challenges of crime and justice, particularly at the state and local levels
DOT Department of Transportation	Transport and transformation of nanoparticles in the environment
NIH National Institute of health	Nanomaterials in the body: Cell cultures and laboratory use for diagnostic and research tools
NIST National Institute of Standards and Technology	Developing measurement tools: Tests and analytical methods

Adopted from: EPA and Nanotechnology, Strategy, responsibilities and activities, April, 2006

technology. For consumer awareness, IFST has suggested a small modification in the labeling of food materials. According to IFST, the food products containing nanomaterials as additives should have a subscript "n" along with the conventional E-numbering system for labeling (Maynard et al., 2005). IFST is funding research and studies in this direction and recently presented important data and studies regarding these issues (IFST, 2006).

World Health Organisation (WHO) and Food and Agriculture Organization (FAO) are working to keep checks on the use of nanotechnology and its future perspectives. The Codex Alimentarius also sets guidelines and rules related to safe food production. WHO and FAO have assigned Codex Alimentarius to keep updating about the current developments regarding the use of nanotechnology. In 2008, Codex committee conducted a meeting where sessions on the application of nanomaterials as food additives and contaminants were held and detail discussion was done (Maynard et al., 2005; IFST, 2006; CAC, 2007; Newsome, 2007).

Gradually, the rules and regulations are being framed on the use of nanotechnology in food sector. These regulations require studies on risk assessment of nanomaterials and will keep on changing with updated data and new experiments (Weiss et al., 2006). The Organization of Economic Cooperation and Development (OECD) has launched a program to test the safe use of manufactured nanomaterials as food additives (OECD, 2007). Based on research conducted till date, FDA has produced regulatory guidelines in the form of three guidance documents concerning

the use of nanomaterials in cosmetics and food items (U.S. FDA 2014). In the consecutive year, FDA issued one more document related to the safe use of nanomaterials for animals (U.S. FDA 2015). Same year, the US Environmental Protection Agency (EPA) proposed Toxic Substances Control Act Section 8 (a) under which they have proposed reporting and record-keeping of nanomaterial use in the food industry (U.S. EPA 2015). Table 14.8 lists different agencies working towards the safe use of nanomaterials and their efforts in this area.

References

Achaby M, El Miri N, Aboulkas A et al (2017) Processing and properties of eco-friendly bio-nanocomposite films filled with cellulose nanocrystals from sugarcane bagasse. Int J Biol Macromol 96:340–352

Akturk O, Kismet K, Yasti AC et al (2016) Wet electrospun silk fibroin/gold nanoparticle 3D matrices for wound healing applications. RSC Adv 6(16):13234–13250

Ali MRK, Wu Y, Ghosh D et al (2017) Nuclear Membrane-Targeted Gold Nanoparticles Inhibit Cancer Cell Migration and Invasion. ACS Nano 11(4):3716–3726

Aminzadeh Z, Jamalan M, Chupani L et al (2016) In vitro reprotoxicity of carboxyl-functionalised single-and multi-walled carbon nanotubes on human spermatozoa. Andrologia 49:e12741. https://doi.org/10.1111/and.12741

Arezoo F, Fathi M (2018) Development of cress seed mucilage/PVA nanofibers as a novel carrier for vitamin A delivery. Food Hydrocoll 81:31–38

Askri D, Ouni S, Galai S (2019) Nanoparticles in foods? A multiscale physiopathological investigation of iron oxide nanoparticle effects on rats after an acute oral exposure: trace element biodistribution and cognitive capacities. Food Chem Toxicol 127:173–181

Assadpour E, Jafari SM (2017) Spray drying of folic acid within nano-emulsions: optimization by Taguchi approach. Dry Technol 35(9):1152–1160

Avadi MR, Sadeghi AM, Mohammadpour N et al (2010) Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. Nanomedicine 6 (1):58–63

Aytac Z, Ipek S, Durgun E et al (2017) Antibacterial electrospun zein nanofibrous web encapsulating thymol/cyclodextrin-inclusion complex for food packaging. Food Chem 233:117–124

Bahal R, Ali MN, Quijano E et al (2016) In vivo correction of anaemia in β-thalassemic mice by γPNA-mediated gene editing with nanoparticle delivery. Nat Commun 7:13304. https://doi.org/10.1038/ncomms13304

Bamba BSB, Shi J, Tranchant CC et al (2018) Coencapsulation of polyphenols and anthocyanins from blueberry pomace by double emulsion stabilized by whey proteins: effect of homogenization parameters. Molecules 23(10):E2525

Baslak C, Kars MD, Karaman M et al (2015) Biocompatible multi-walled carbon nanotube–CdTe quantum dot–polymer hybrids for medical applications. J Lumin 160:9–15

Bi S, Zhang L, Li C (2019) Multifunctional films with a highly oriented "nano-brick wall" structure by regulating modified TiO_2@graphene oxide/poly(vinyl alcohol) nanocomposites. Nanoscale 11(15):7424–7432

Bieberstein A, Roosen J, Marette S, Blanchemanche S, Vandermoere F (2013) Consumer choices for nano-food and nanopackaging in France and Germany. Eur Rev Agric Econ 40:73e94

Binsi PK, Nayak N, Sarkar PC et al (2017) Structural and oxidative stabilization of spray dried fish oil microencapsulates with gum arabic and sage polyphenols: characterization and release kinetics. Food Chem 219:158–168

Biswas DP, Simpson NM, Reynolds EC et al (2018) Comparative study of novel in situ decorated porous chitosan-selenium scaffolds and porous chitosan-silver scaffolds towards antimicrobial wound dressing application. J Colloid Interface Sci 515:78–91

Bott J, Störmer A, Franz R (2014) Migration of nanoparticles from plastic packaging materials containing carbon black into foodstuffs. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 31(10):1769–1782

Broggi F, Ponti J, Giudetti G et al (2013) Silver nanoparticles induce cytotoxicity, but not cell transformation or genotoxicity on Balb3T3 mouse fibroblasts. BioNanoMaterials 14 (1–2):49–60

Buslovich A, Horev B, Rodov V et al (2017) One-step surface grafting of organic nanoparticles: in situ deposition of antimicrobial agents vanillin and chitosan on polyethylene packaging films. J Mater Chem B 5:2655–2661

Camilade C, Santos M, Costa P et al (2018) Zeaxanthin nanoencapsulation with Opuntia monacantha mucilage as structuring material: Characterization and stability evaluation under different temperatures. AGRIS 558(10):410–421

Campo C, Santos PP, Costa TMH et al (2017) Nanoencapsulation of chia seed oil with chia mucilage (Salvia hispanica L.) as wall material: characterization and stability evaluation. Food Chem 234:1–9

Carmen IM, Panchapakesan C, Huang Q et al (2003) Nanotechnology: a new frontier in food science. Article Food Technol 57(12):24–29

Chai W, Wang H, Zhang Y et al (2016) Preparation of polydopamine-coated magnetic nanoparticles for dispersive solid-phase extraction of water-soluble synthetic colorants in beverage samples with HPLC analysis. Talanta 149:13–20

Chen C, Marcus IM, Waller T et al (2018) Comparison of filtration mechanisms of food and industrial grade TiO2 nanoparticles. Anal Bioanal Chem 410(24):6133–6140

Codex Alimentarius Commission (2007) Report of the 39th Session of the Codex Committee of Food Additives. ALINORM 07/30/12 Rev. May 2007. http://www.codexalimentarius/.net

Control of Nanoscale Materials under the Toxic Substances Control Act. US Environmental Protection Agency; 2015. https://www.epa.gov/reviewing-new-chemicals-undertoxic-substances-control-act-tsca/control-nanoscalematerials-under

Covarrubias C, Cádiz M, Maureira M et al (2018) Bionanocomposite scaffolds based on chitosan–gelatin and nanodimensional bioactive glass particles: in vitro properties and in vivo bone regeneration. J Biomater Appl 32(9):1155–1163

Dahiya S, Rani R, Kumar S et al (2017) Chitosan-gellan gum bipolymeric nanohydrogels—a potential nanocarrier for the delivery of epigallocatechin gallate. BioNanoScience 7(3):508–520

Dairi N, Ferfera H, Ramos M et al (2019) Cellulose acetate/AgNPs-organoclay and/or thymol nano-biocomposite films with combined antimicrobial/antioxidant properties for active food packaging use. Int J Biol Macromol 121:508–523

Decher G, Schlenoff IB (2003) Multilayer thin films: sequential assembly of nanocomposite materials. Wiley-VCH, Weinheim, p 543

Della PF, Scroccarello A, Scarano S et al (2019) Silver nanoparticles-based plasmonic assay for the determination of sugar content in foodmatrices. Anal Chim Acta 21(1051):129–137

Deshmukh SP, Patil SM, Mullani SB et al (2019) Silver nanoparticles as an effective disinfectant: a review. Mater Sci Eng C Mater Biol Appl 97:954–965

Ding J, Zhang R, Ahmed S et al (2019) Effect of sonication duration in the performance of polyvinyl alcohol/chitosan bilayer films and their effect on strawberry preservation. Molecules 24(7):E1408

Doost AS, Muhammad DRA, Stevens CV (2018) Fabrication and characterization of quercetin loaded almond gum-shellac nanoparticles prepared by antisolvent precipitation. Food Hydrocoll 83:190–201

Echegoyen Y, Rodríguez S, Nerín C (2016) Nanoclay migration from food packaging materials. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 33(3):530–539

Emanuele D, Haroldo H, Paula DB et al (2015) Physicochemical and antimicrobial properties of nanoencapsulated Eucalyptus staigeriana essential oil. LWT Food Sci Technol 61(2):484–491
Eratte D, Wang B, Dowling K et al (2014) Complex coacervation with whey protein isolate and gum arabic for the microencapsulation of omega-3 rich tuna oil. Food Funct 5(11):2743–2750
Farshi P, Tabibiazar M, Ghorbani M et al (2017) Evaluation of antioxidant activity and cytotoxicity of cumin seed oil nanoemulsion stabilized by sodium caseinate- guar gum. Pharm Sci 23 (4):293–300
FDA issues guidance on the use of nanomaterials in food for animals. Division of Dockets Management, HFA-305, U.S. Food and Drug Administration; 2015. http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm457112
FDA issues guidance to support the responsible development of nanotechnology products. U.S. Food and Drug Administration; 2014. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm402499
Feng Z, Li L, Wang Q et al (2019) Effect of antioxidant and antimicrobial coating based on whey protein nanofibrils with TiO_2 nanotubes on the quality and shelf life of chilled meat. Int J Mol Sci 20(5):184–190
Fernández JG, Sánchez GC, Bettmer J et al (2018) Quantitative assessment of the metabolic products of iron oxide nanoparticles to be used as iron supplements in cell cultures. Anal Chim Acta 18(1039):24–30
Fessi HPFD, Puisieux F, Devissaguet JP et al (1989) Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 55(1):R1–R4
Fuciños C, Fuciños P, Míguez M et al (2014) Temperature- and pH-sensitive nanohydrogels of poly (N-Isopropylacrylamide) for food packaging applications: modelling the swelling-collapse behaviour. PLoS One 9(2):e87190
Gahruie H, Ziaee E, Eskandari MH et al (2017) Characterization of basil seed gum-based edible films incorporated with Zataria multiflora essential oil nanoemulsion. Carbohydr Polym 166:93–103
Ghasemi S, Mahdi S, Assadpour JE et al (2017) Production of pectin-whey protein nano-complexes as carriers of orange peel oil. Carbo polym 177:369–377
Ghasemi S, Mahdi S, Assadpour JE et al (2018) Nanoencapsulation of D-limonene within nanocarriers produced by pectin-whey protein. Food Hydrocoll 77:152–162
Gilbert J, Cheng CJ, Jones OG et al (2018) Vapor barrier properties and mechanical behaviors of composite hydroxypropyl methylcelluose/zein nanoparticle films. Food Biophys 13:25–36
Grande F, Tucci P (2016) Titanium dioxide nanoparticles: a risk for human health? Mini Rev Med Chem 16(9):762–769
Hanif J, Khalid N, Khan RS et al (2019) Formulation of active packaging system using Artemisia scoparia for enhancing shelf life of fresh fruits. Mater Sci Eng C Mater Biol Appl 100:82–93
Headey DD, Chiu A, Kadiyala S (2012) Agriculture's role in the Indian enigma: help or hindrance to the crisis of undernutrition? Food Sec 4:87–102
He L, Hu J, Deng W (2018) Preparation and application of flavor and fragrance capsules. Polym Chem 9(40):4926–4946
Horuz T, Belibağlı KB (2018) Nanoencapsulation by electrospinning to improve stability and water solubility of carotenoids extracted from tomato peels. Food Chem 268:86–93
Huang Y, Chen S, Bing X et al (2011) Nanosilver migrated into food-simulating solutions from commercially available food fresh containers. Packag Technol Sci 24(5):291–297
Human C, Beer D, Rijst M et al (2019) Electrospraying as a suitable method for nanoencapsulation of the hydrophilic bioactive dihydrochalcone, aspalathin. Food Chem 276:467–474
Hwang J, Bae IA, Lee C et al (2018) Simultaneous analysis and exposure assessment of migrated bisphenol analogues, phenol, and p-tert-butylphenol from food contact materials. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 35(11):2270–2278
Iamareerat B, Singh M, Sadiq MB et al (2018) Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. J Food Sci Technol 55(5):1953–1959

Indumathi MP, Sarojini K, Rajarajeswari GR (2019) Antimicrobial and biodegradable chitosan/cellulose acetate phthalate/ZnO nano composite films with optimal oxygen permeability and hydrophobicity for extending the shelf life of black grape fruits. Int J Biol Macromol 132:1112–1120

Institute of Food Science and Technology (2006) Nanotechnology information statement. (IFST) Trust Fund, London. http://www.ifst.org/nano.pdf

Jafari MS, Katayoun MK, Kakhki AH (2016) Microencapsulation of saffron petal anthocyanins with cress seed gum compared with Arabic gum through freeze drying. Carbohydr Polym 140:20–25

Jayakumar A, Heera KV, Sumi TS et al (2019) Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application. Int J Biol Macromol 136:395–403

Kadiyala S, Harris J, Headey D, Yosef S, Gillespie S (2014) Agriculture and nutrition in India: mapping evidence to pathways. Ann N Y Acad Sci 1331(1):43–56

Kang B, Mackey MM, Sayed M (2010) Nuclear targeting of gold nanoparticles in cancer cells induces dna damage, causing cytokinesis arrest and apoptosis. J Am Chem Soc 132 (5):1517–1519

Karimirad R, Behnamian M, Dezhsetan S et al (2018) Chitosan nanoparticles-loaded Citrus aurantium essential oil: a novel delivery system for preserving the postharvest quality of Agaricus bisporus. J Sci Food Agric 98(13):5112–5119

Kim HJ, Bae SH, Kim HJ et al (2017) Cytotoxicity, intestinal transport, and bioavailability of dispersible Iron and zinc supplements. Front Microbiol 28(8):749

Kim N, Kim C, Jung S (2018) Determination and identification of titanium dioxide nanoparticles in confectionery foods, marketed in South Korea, using inductively coupled plasma optical emission spectrometry and transmission electron microscopy. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 35(7):1238–1246

Ko WK, Heo DN, Moon HJ et al (2015) The effect of gold nanoparticle size on osteogenic differentiation of adipose-derived stem cells. J Colloid Interface Sci 438:68–76

Kolaee M, Khodaiyan F, Pourahmad R et al (2016) Development of ecofriendly bionanocomposite: whey protein isolate/pullulan films with nano-SiO2. Int J Biol Macromol 86:139–144

Król A, Pomastowski P, Rafińska K et al (2017) Zinc oxide nanoparticles: synthesis, antiseptic activity and toxicity mechanism. Adv Colloid Interf Sci 249:37–52

Kumar N, Krishnani KK, Singh NP (2018) Effect of dietary zinc-nanoparticles on growth performance, anti-oxidative and immunological status of fish reared under multiple stressors. Biol Trace Elem Res 186(1):267–278

Kumar S, Boro JC, Ray D et al (2019) Bionanocomposite films of agar incorporated with ZnO nanoparticles as an active packaging material for shelf life extension of green grape. Heliyon 5 (6):e01867

Kuntzler SG, Almeida ACA, Costa JAV et al (2018) Polyhydroxybutyrate and phenolic compounds microalgae electrospun nanofibers: a novel nanomaterial with antibacterial activity. Int J Biol Macromol 113:1008–1014

Lan W, Liang X, Lan W et al (2019) Electrospun polyvinyl alcohol/d-limonene fibers prepared by ultrasonic processing for antibacterial active packaging material. Molecules 20:24(4)

Lavoine N, Guillard V, Desloges I et al (2016) Active bio-based food-packaging: diffusion and release of active substances through and from cellulose nanofiber coating toward food-packaging design. Carbohydr Polym 149:40–50

Lee JA, Kim MK, Song JH et al (2017) Biokinetics of food additive silica nanoparticles and their interactions with food components. Colloids Surf B Biointerfaces 1(150):384–392

Li W, Zhang C, Chi H et al (2017) Development of antimicrobial packaging film made from poly (lactic acid) incorporating titanium dioxide and silver nanoparticles. Molecules 22(7):13–20

Li Y, Guo Y, Niu W et al (2018) Biodegradable multifunctional bioactive glass-based nanocomposites elastomers with controlled biomineralization activity, realtime bioimaging tracking and decreased inflammatory response. ACS Appl Mater Interfaces 10 (21):17722–17731

Liang J, Yan H, Zhang J et al (2017) Preparation and characterization of antioxidant edible chitosan films incorporated with epigallocatechin gallate nanocapsules. Carbohydr Polym 171:300–306
Lim JH, Bae D, Fong A (2018) Titanium dioxide in food products: quantitative analysis using ICP-MS and Raman spectroscopy. J Agric Food Chem 66(51):13533–13540
Liu S, Li X, Chen L et al (2018a) Tunable d-limonene permeability in starch-based nanocomposite films reinforced by cellulose nanocrystals. J Agric Food Chem 66(4):979–987
Liu Y, Wang S, Lan W et al (2018b) Fabrication of antibacterial chitosan-PVA blended film using electrospray technique for food packaging applications. Int J Biol Macromol 107:848–854
Luzi F, Fortunati E, Michele DA et al (2018) Nanostructured starch combined with hydroxytyrosol in poly(vinyl alcohol) based ternary films as active packaging system. Carbohydr Polym 193:239–248
Ma Y, Li J, Si Y et al (2019) Rechargeable antibacterial N-halamine films with antifouling function for food packaging applications. ACS Appl Mater Interfaces 11(19):17814–17822
Mackevica A, Olsson ME, Hansen SF (2016) Silver nanoparticle release from commercially available plastic food containers into food simulants. J Nanopart Res 18(5):1–11
Madhumathi K, Kumar PS, Abhilash S et al (2010) Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. J Mater Sci Mater Med 21(2):807–813
Mallakpour S, Nazari HY (2018) The influence of bovine serum albumin-modified silica on the physicochemical properties of poly(vinyl alcohol) nanocomposites synthesized by ultrasonication technique. Ultrason Sonochem 41:1–10
Mania M, Szynal T, Rebeniak M et al (2018) Exposure assessment to lead, cadmium, zinc and copper released from ceramic and glass wares intended to come into contact with food. Rocz Panstw Zakl Hig 69(4):405–411
Maynard AD, Kuempel ED (2005) Airborne nanostructured particles and occupational health. J Nanopart Res 7(6):587–614
Mayorga JL, Freitas F, Reis MAM et al (2018a) Biosynthesis of silver nanoparticles and polyhydroxybutyrate nanocomposites of interest in antimicrobial applications. Int J Biol Macromol 108:426–435
Mayorga JL, Rovira MJ, Mas L et al (2018b) Antimicrobial nanocomposites and electrospun coatings based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and copper oxide nanoparticles for active packaging and coating applications. J Appl Polym Sci 135:45673
Memiş S, Tornuk F, Bozkurt F et al (2017) Production and characterization of a new biodegradable fenugreek seed gum based active nanocomposite film reinforced with nanoclays. Int J Biol Macromol 103:669–675
Meng F, Sun H, Huang Y et al (2019) Peptide cleavage-based electrochemical biosensor coupling graphene oxide and silver nanoparticles. Anal Chim Acta 24(1047):45–51
Mi P, Kokuryo D, Cabral H et al (2016) A pH-activatable nanoparticle with signal-amplification capabilities for non-invasive imaging of tumour malignancy. Nat Nanotechnol 11(8):724–730
Mohammadi H, Farzinpour A, Vaziry A (2017) Reproductive performance of breeder quails fed diets supplemented with L-cysteine-coated iron oxide nanoparticles. Reprod Domest Anim 52 (2):298–304
Moonesi Rad R, Pazarçeviren E, Akgün E et al (2019) In vitro performance of a nanobiocomposite scaffold containing boron-modified bioactive glass nanoparticles for dentin regeneration. J Biomater Appl 33(6):834–853
Muriel GV, Perez EE, Ruiz RM et al (2018) Anchoring gated mesoporous silica particles to ethylene vinyl alcohol films for smart packaging applications. Nanomaterials (Basel) 8(10):865
Newsome R (2007) Codex vital in global harmonization. Food Technol 61:100. http://www.ift.org/
Omwenga EO, Hensel A, Shitandi A et al (2018) Chitosan nanoencapsulation of flavonoids enhances their quorum sensing and biofilm formation inhibitory activities against an E.coli Top 10 biosensor. Colloids Surf B Biointerfaces 164:125–133
Osorio J, Dreolin N, Aznar M et al (2019) Determination of volatile non intentionally added substances coming from a starch-based biopolymer intended for food contact by different gas chromatography-mass spectrometry approaches. J Chromatogr A 1599:215–222

Oymaci P, Altinkaya SA et al (2016) Improvement of barrier and mechanical properties of whey protein isolate based food packaging films by incorporation of zein nanoparticles as a novel bionanocomposite. Food Hydrocoll 54:1–9

Pan K, Luo Y, Gan Y et al (2014) pH-driven encapsulation of curcumin in self-assembled casein nanoparticles for enhanced dispersibility and bioactivity. Soft Matter 10(35):6820–6830

Panikkanvalappil SR, Hooshmand N, Sayed E et al (2017) Intracellular assembly of nuclear-targeted gold nanosphere enables selective plasmonic photothermal therapy of cancer by shifting their absorption wavelength toward near-infrared region. Bioconjug Chem 28 (9):2452–2460

Pardo G, Joye IJ, McClements DJ et al (2015) Food-grade protein-based nanoparticles and microparticles for bioactive delivery: fabrication, characterization, and utilization. Adv Protein Chem Struct Biol 98:293–325

Pei X, Xiao Z, Liu L et al (2019) Effects of dietary zinc oxide nanoparticles supplementation on growth performance, zinc status, intestinal morphology, microflora population, and immune response in weaned pigs. J Sci Food Agric 99(3):1366–1374

Qazanfarzadeh Z, Kadivar M et al (2016) Properties of whey protein isolate nanocomposite films reinforced with nanocellulose isolated from oat husk. Int J Biol Macromol 91:1134–1140

Qiaobin H, Hannah G, Upadhyaya I et al (2016) Antimicrobial eugenol nanoemulsion prepared by gum arabic and lecithin and evaluation of drying technologies. Int J Biol Macromol 87:130–140

Rawat N, Sandhya SK et al (2016) Comparative in vivo toxicity assessment places multiwalled carbon nanotubes at a higher level than mesoporous silica nanoparticles. Toxicol Ind Health 33 (2):1–11

Requena VH, Rivas BL, Pérez MA et al (2017) Thermoplastic starch/clay nanocomposites loaded with essential oil constituents as packaging for strawberries—in vivo antimicrobial synergy over Botrytis cinerea. Postharvest Biol Technol 129:29–36

Rezaeigolestani M, Misaghi A, Khanjari A et al (2017) Antimicrobial evaluation of novel poly-lactic acid based nanocomposites incorporated with bioactive compounds in-vitro and in refrigerated vacuum-packed cooked sausages. Int J Food Microbiol 260:1–10

Rompelberg C, Heringa MB, Donkersgoed G et al (2016) Oral intake of added titanium dioxide and its nanofraction from food products, food supplements and toothpaste by the Dutch population. Nanotoxicology 10(10):1404–1414

Salarbashi D, Tafaghodi M, Bazzaz BSF (2018) Soluble soybean polysaccharide/TiO2 bionanocomposite film for food application. Carbohydr Polym 15(186):384–393

Saleh T, Ahmed E, Yu L et al (2018) Silver nanoparticles improve structural stability and biocompatibility of decellularized porcine liver. Artif Cells Nanomed Biotechnol 46(Suppl. 2):273–284

Sanaeifar N, Rabiee M, Abdolrahim M et al (2017) A novel electrochemical biosensor based on Fe3O4 nanoparticles-polyvinyl alcohol composite for sensitive detection of glucose. Anal Biochem 15(519):19–26

Sanand S, Kumar S, Bara N et al (2018a) Comparative evaluation of half-maximum inhibitory concentration and cytotoxicity of silver nanoparticles and multiwalled carbon nanotubes using buffalo bull spermatozoa as a cell model. Toxicol Ind Health 34(9):640–652

Sanand S, Tyagi A, Kumar S et al (2018b) Smart mesoporous nanomaterials with improved therapeutic applications: therapeutic application of MSN. IGI global. https://doi.org/10.4018/978-1-5225-4781-5.ch016

Sanand S, Hussain A, Kaul G (2015) Biosafety of nanomaterials used in nanoceramics and nanocomposites. In: Handbook of nanoceramic and nanocomposite coatings and materials. Butterworth-Heinemann, Amsterdam, pp 471–487. https://doi.org/10.1016/b978-0-12-799947-0.00022-5

Santiago TR, Bonatto CC, Rossato M et al (2019) Green synthesis of silver nanoparticles using tomato leaf extract and their entrapment in chitosan nanoparticles to control bacterial wilt. J Sci Food Agric 99(9):4248–4259

Santos PP, Andrade LA, Flôres SH et al (2018) Nanoencapsulation of carotenoids: a focus on different delivery systems and evaluation parameters. J Food Sci Technol 55(10):3851–3860
Saral SK, Indumathi MP, Rajarajeswari GR (2019) Mahua oil-based polyurethane/chitosan/nano ZnO composite films for biodegradable foodpackaging applications. Int J Biol Macromol 1 (124):163–174
Sarapulova O, Sherstiuk V, Shvalagin V et al (2015) Photonics and nanophotonics and information and communication technologies in modern food packaging. Nanoscale Res Lett. https://doi.org/10.1186/s11671-015-0939-7
Sarwar MS, Niazi MBK, Jahan Z et al (2018) Preparation and characterization of PVA/nanocellulose/Ag nanocomposite films for antimicrobial food packaging. Carbohydr Polymr 15(184):453–464
Shefer A (2008) The application of nanotechnology in the food industry. http://www.foodtech-international.com/papers/application-nano.html
Shabib NA, Husain FM, Ahmed F et al (2016) Biogenic synthesis of zinc oxide nanostructures from Nigella sativa seed: prospective role as food packaging material inhibiting broad-spectrum quorum sensing and biofilm. Sci Rep 5(6):367–372
Shah SW, Jahangir M, Qaisar M et al (2015) Storage stability of Kinnow fruit (Citrus reticulata) as affected by CMC and guar gum-based silver nanoparticle coatings. Molecules 20 (12):22645–22661
Shakil O, Masood F, Yasin T (2017) Characterization of physical and biodegradation properties of poly-3-hydroxybutyrate-co-3-hydroxyvalerate/sepiolite nanocomposites. Mater Sci Eng C Mater Biol Appl 77:173–183
Shalom BT, Nevo Y, Leibler D et al (2019) Cellulose nanocrystals (CNCs) induced crystallization of polyvinyl alcohol (PVA) super performing nanocomposite films. Macromol Biosci. https://doi.org/10.1002/mabi.201800347
Shankar S, Rhim JW (2016) Preparation of nanocellulose from micro-crystalline cellulose: the effect on the performance and properties of agar-based composite films. Carbohydr Polym 135:18–26
Shavisi N, Khanjari A, Basti AA et al (2017) Effect of PLA films containing propolis ethanolic extract, cellulose nanoparticle and Ziziphora clinopodioides essential oil on chemical, microbial and sensory properties of minced beef. Meat Sci 124:95–104
Silva JB, Pereira FV, Druzian JI et al (2012) Cassava starch-based films plasticized with sucrose and inverted sugar and reinforced with cellulose nanocrystals. J Food Sci 77(6):14–29
Song Z, Wu H, Niu C et al (2019) Application of iron oxide nanoparticles @ polydopamine-nisin composites to the inactivation of Alicyclobacillus acidoterrestris in apple juice. Food Chem 30 (287):68–75
Sun Q, Li J, Le T (2018) Zinc oxide nanoparticle as a novel class of antifungal agents: current advances and future perspectives. J Agric Food Chem 66(43):11209–11220
Suo B, Li H, Wang Y et al (2017) Effects of ZnO nanoparticle-coated packaging film on pork meat quality during cold storage. J Sci Food Agric 97(7):2023–2029
Tao F, Shi C, Cui Y (2018) Preparation and physicochemistry properties of smart edible films based on gelatin-starch nanoparticles. J Sci Food Agric 98(14):5470–5478
Tao G, Cai R, Wang Y et al (2017) Biosynthesis and characterization of AgNPs-silk/PVA film for potential packaging application. Materials (Basel) 10(6):667
Tayeb AH, Amini E, Ghasemi S et al (2018) Cellulose nanomaterials-binding properties and applications: a review. Molecules 23(10):2684
Testing programme of manufactured nanomaterials. Organisation for Economic Co-operation and Development; 2007. http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials
Tian J, Wong KK, Ho CM et al (2007) Topical delivery of silver nanoparticles promotes wound healing. Chem Med Chem 2(1):129–136
Venkatasubbu GD, Baskar R, Anusuya T et al (2016) Toxicity mechanism of titanium dioxide and zinc oxide nanoparticles against food pathogens. Colloids Surf B Biointerfaces 1(148):600–606

Venkatesan J, Lee JY, Kang DS et al (2017) Antimicrobial and anticancer activities of porous chitosan-alginate biosynthesized silver nanoparticles. Int J Biol Macromol 98:515–525

Wang H, Gong X, Miao Y et al (2019) Preparation and characterization of multilayer films composed of chitosan, sodium alginate and carboxymethyl chitosan-ZnO nanoparticles. Food Chem 283:397–403

Wang Q, Jiang J, Chen W et al (2016) Targeted delivery of low-dose dexamethasone using PCL-PEG micelles for effective treatment of rheumatoid arthritis. J Control Release 230:64–72

Wang H, Jiang Y, Zhang Y et al (2018) Silver nanoparticles: a novel antibacterial agent for control of Cronobacter sakazakii. J Dairy Sci 101(12):10775–10791

Wei XQ, Li XP, Wu CL et al (2018) The modification of in situ SiOx chitosan coatings by ZnO/TiO2 NPs and its preservation properties to silver carp fish balls. J Food Sci 83 (12):2992–3001

Weiss J, Takhistov P, McClements DJ (2006) Functional materials in food nanotechnology. J Food Sci 71:R107

Wen P, Zhu DH, Feng K et al (2016) Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/β-cyclodextrin inclusion complex for antimicrobial packaging. Food Chem 196:996–1004

Wu C, Zhu Y, Wu T et al (2019) Enhanced functional properties of biopolymer film incorporated with curcumin-loaded mesoporous silica nanoparticles for food packaging. Food Chem 1 (288):139–145

Yaseen T, Pu H, Sun DW (2019) Rapid detection of multiple organophosphorus pesticides (triazophos and parathion-methyl) residues in peach by SERS based on core-shell bimetallic Au-Ag NPs. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 36(5):762–778

Youssef AM, Sayed SM, Sayed HS et al (2016) Enhancement of Egyptian soft white cheese shelf life using a novel chitosan/carboxymethyl cellulose/zinc oxide bionanocomposite film. Carbohydr Polym 20(151):9–19

Youssef AM, Sayed SM, Sayed HS et al (2018) Novel bionanocomposite materials used for packaging skimmed milk acid coagulated cheese (Karish). Int J Biol Macromol 115:1002–1011

Yu Z, Li B, Chu J et al (2018) Silica in situ enhanced PVA/chitosan biodegradable films for food packages. Carbohydr Polym 184:214–220

Zhai X, Li Z, Shi J et al (2019) A colorimetric hydrogen sulfide sensor based on gellan gum-silver nanoparticles bio-nanocomposite for monitoring of meat spoilage in intelligent packaging. Food Chem 30(290):135–143

Zhai Y, Wang J, Wang H et al (2018) Preparation and characterization of antioxidative and UV-protective larch bark tannin/PVA Composite membranes. Molecules 19:23(8)

Zhu Z, Zhang Y, Zhang Y et al (2019) Preparation of PAN@TiO_2 nanofibers for fruit packaging materials with efficient photocatalytic degradation of ethylene. Materials (Basel) 12(6):E896

Zorlutuna P, Vrana NE, Khademhosseini A (2013) The expanding world of tissue engineering: the building blocks and new applications of tissue engineered constructs. IEEE Rev Biomed Eng 6:47–62

Advances in Edible Fruit Coating Materials 15

Ashitha Jose, Sunil Pareek, and E. K. Radhakrishnan

Abstract

The perishability of fresh produce during the postharvest period, before it reaches the market, is a leading cause for the food scarcity across the globe. The prevention of microbial attack and maintenance of the hydrodynamic potential as brought about through the edible coating can be an effective solution to manage the same. Although edible coatings have already been exploited over a century to expand the shelf life and freshness of the fresh produce, engineering the edible coating through the incorporation of antimicrobial agents and nanomaterials can widen its scope and application. Various bioderived substances like lipids, hydrocolloids, and their combinations have recently been used as the film forming solution to obtain tailor made properties including transparency, texture, moisture and oxygen barrier and microbial barrier properties for exploiting their applications as edible coatings. These coatings can ensure the extended shelf life, prevent the microbial load, and can also act as a nutrient fortifier depending upon the active component employed. The usage of the materials considered under the GRAS (Generally Recognized as Safe) category adds on to the safety aspect of these edible coatings. Thus, engineering edible coatings for additional properties has the potential to meet the consumer demand and can also ensure the safety of the fresh produce over a period of time.

A. Jose · E. K. Radhakrishnan (✉)
School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India
e-mail: radhakrishnanek@mgu.ac.in

S. Pareek
National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Sonipat, Haryana, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_15

Keywords

Fruit coating · Nanomaterials · Antimicrobial agents · Lipids · Wax · Cellulose · Food industry

15.1 Introduction

The world population is expected to exceed nine billion by 2050. At the same time, the food consumption pattern is affected by both urbanization and increased income. Among various edible materials, fruits and vegetables form a major dietary component. The vast majority of fruits and vegetables produced are known to be rich sources of health promoting essential elements, vitamins, minerals, phytochemicals, etc. Their ability to reduce the cholesterol content and thereby the risk of heart diseases has already been reported. The higher potassium content of the fresh produce is known to have significant effects to maintain the blood pressure and related issues. The vitamin content plays a major role in tissue repair, wound healing, and maintenance of oral health. The dietary polyphenols and carotenoids present in them provide broad spectrum of health benefits. The array of phytochemicals, the dietary fibers, and micronutrients present in these collectively maintain health and disease resistance. These have increasing potential to manage the lifestyle diseases also. The indigestible dietary fiber and the starch polysaccharides play key role to maintain healthy digestive system functions. The antioxidant capacity of the fruits and vegetables as attributed by the synergistic effect of the vitamins, polyphenols and carotenoids is well known to prevent cancer also. Especially, the fresh produces are having the higher antioxidant potential due to higher polyphenol content.

Watermelons, bananas, and apples contribute to the largest proportion of the fruits while cabbages, cucumbers, and aubergines form the major representation of vegetables. India is one of the major producers of the fresh produces. The diverse climate of India supports the growth of a wide variety of fresh fruits and vegetables. According to the national horticulture laboratory, India is a producer of 90.2 million metric tonnes of fruits and 169.1 million metric tonnes of vegetables. However, the methods used for the storage are too preliminary which generally cause severe damage to the fruits and vegetables during their storage and processing period. At the same time, the chemical methods used to prevent spoilage usually cause severe health problems and hence it can no longer be recommended.

The high demand for the exotic, tropical, and high-quality fruits has also led to the huge export across the globe. After harvesting a fruit/vegetable, it should be safe to consume, have acceptable appearance, texture, and taste till it reaches the end users. This period from field to table varies with the type of food and its processing and hence determines the acceptance in the market. This brings in the role of various food packaging including edible coatings.

As the seasonal availability and lower shelf life can affect the quality, the food processing has been introduced to ensure its availability even in the off seasons by improving the shelf life. The food coatings which are edible or those in direct contact

with the food must be safe under the conditions of the intended use. These materials must be accepted by the Food and Drug Administration (FDA) and be included under the GRAS (generally recognized safe) category after detailed analysis of the product and also the individual components. The products which are in contact with food must also be qualified for the intended conditions of consumption.

At the same time, a maximum residue limit (MRL) has also been set for the chemicals on and inside the fresh produces including pesticides, other contaminants, microbiological load, etc. The quality assurance and certification scheme introduced in the Europe during 1930s has initiated steps to maintain the quality of the fresh produces.

The current food industry is moving through a turbulent period due to the ever-increasing global demand for safe food, increasing concerns on the food insecurity and consumer demand for high quality. Proper assessment and risk mitigation form the major functions of the food industry while it also plays a major role in both health and economy of nation. Pest and pesticide control are prerequisite in the food industry along with the proper assessment of agrochemicals used. The commercial processing of the fresh produces is a relatively new industry which includes the trimming, peeling, and packaging of the food. The microbial quality and safety of the products remain as a major issue with the food industry. The produces once harvested and transported to the processing industry should be washed, dried, and analyzed for chemical contamination and microbial load followed by processing that leads to enhanced shelf life. Along with methods to enhance production of fruits and vegetables, development of innovative methods for the processing and storage of available materials is also equally important.

15.2 Food Spoilage and Edible Coatings

Even though the fruits and vegetables are rich source of vitamins, minerals, antioxidants, and other health promoting biotic and abiotic nutritional compounds, they are easily perishable. The spoilage of the fresh produce may generally be due to the attack of microorganisms, insects, or the pre- and postharvest conditions of transportation and preservations. However, this can be controlled entirely by maintaining the internal gas composition through the edible coatings which consist of antimicrobial and antioxidant agents (Park 1999).

The enrobing of the food materials either by dipping the material into the filmogenic substrate or by spraying the same over the food as an edible coating with the intention to extend its shelf life by altering the air and water permeability and maintaining the prevailing enzymatic system to prevent the deterioration of the fresh produce is generally known as the edible coatings (Fig. 15.1). They form a fortified layer over the fruit or vegetable which generally has a thickness of a few microns to millimeters. The edible polymers may be applied directly over the surface of the food to ensure better protection, preservation, and maintenance of both quality and stability. Generally, the fruits and vegetables continue to respire even after harvest. Hence the pseudo atmosphere created by the edible coatings prevents the

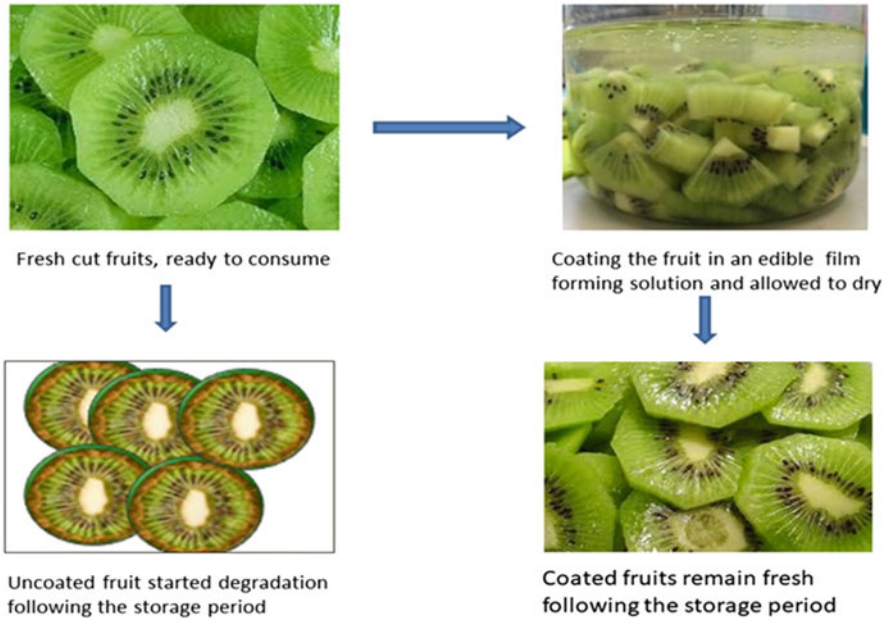

Fig. 15.1 Edible coating

water loss as well as gaseous exchange, which in turn result in low oxygen high carbon dioxide atmosphere which lets the anaerobic respiration to switch on. This low oxygen atmosphere can disrupt the ethylene production and water loss. These biochemical changes ensure the delayed ripening as evidenced by prolonged firmness, freshness, and nutritional status of the fresh produce (Cuq et al. 1998; McHugh and Senesi 2000; Dhall 2013b).

Preventing the loss of nutrients with the help of the edible polymers provides many advantages. The efficiency of the polymer used for the coating can be determined by its mass transport properties. Specifically, the edible films are formed before their application, while the coatings are formed during the application (Shit and Shah 2014). Being produced from edible and degradable materials, the edible polymers degrade faster than the synthetic packaging materials. However, the permeability and mechanical properties of the edible packaging is a challenge (Shit and Shah 2014; Sharma et al. 2019). Herbal and nanoparticle incorporated edible coating is a novel trend wherein the filler molecules incorporated in the coating are exploited to bring about their biological properties including antimicrobial, flavor enhancement, degradability, etc.

15.3 History of Edible Coatings

The history of edible coatings can be dated back to the early twelfth and thirteenth century where the wax coatings were applied to improve the shelf life of the citrus fruits such as lemon and oranges by the Chinese, who actually did not realize the function of these coatings, which actually was to slow down the exchange of respiratory gases (Park 1999). It was then replaced by hot melt paraffin wax which became commercially available in the mid 1930s.

The wax coating was known as larding in the Europe. Though the water loss and gaseous exchange was prevented in this method, the quality of the fruit remained an issue. The crude method of application of wax was to pour it in bulk over the food material. Though it was not a complete solution, the method was well accepted during the time for availing the climacteric fruits during the off seasons and to ensure the shelf life during transportation.

The realization of the deleterious effects of the common synthetic packaging has led the researchers to focus on methods which ensure the encasement of food resulting in the migration of the degradative molecule either at the natural or slower pace even after the harvest. This led to the extensive development of edible coatings from the natural polymers such as polysaccharides, lipids, and proteins either alone or in combination. The addition of surfactants, plasticizers, and other food grade additives further enhanced their innate properties (Shit and Shah 2014). Ensuring the non-toxic nature and the edibility of the developed coatings are of prime importance.

When the natural polymers were not able to meet the requirements as stand-alone molecules, the research focussed on the development of complex matrices to meet the material quality. Among the various methods employed in the development of composite materials for packaging, layer by layer (LBL) approach has been well accepted. The rational design of the composite materials in LBL approach is the alternate deposition of individual components leading to the development of final composite material. The electrostatic interaction among the various polyelectrolytes led to the development of LBL approach. The tailor-controlled approach of depositing various biopolymers in the LBL makes it to be exploited to develop various fine-tuned edible coatings (Arnon-Rips and Poverenov 2018; Decher 1997).

Modifications have also been carried out to incorporate antimicrobial components into polymer matrices. It includes crude plant extracts and other antimicrobial agents. Later on, the essential oils and nanoparticles were incorporated alone or in combination owing to its antimicrobial potency. The common essential oils used include clove oil, nutmeg oil, rosemary oil, cinnamaldehyde, etc. (Jayakumar et al. 2019). The volatile nature of these compounds is an added advantage in preventing the microbial growth. Recently, the trend has been shifted towards the exploitation of multiple essential oils to obtain enhanced activity. Alongside, the nanoparticles also gained wide attention in the food industry.

15.4 Nanoparticles in Food Industry

The nanoparticles have a larger surface area to volume ratio which defines its characteristics. They exhibit physicochemical characters that are far different from their bulk materials. The current era of nanocoatings incorporates a wide variety of nanomaterials. The surface morphology and the loading capacity of the nanoparticles can determine the efficiency of the developed nanocoatings. The photocatalytic activity of the nanoparticle requires it to be activated either through UV or visible light irradiation (Vidyalakshmi et al. 2017; Roshmi et al. 2017; Mathew et al. 2019).

Among the common nanoparticles employed, the titanium dioxide (TiO_2) nanoparticles have remarkable antimicrobial potential. It is being applied into different polymer matrices such as chitosan, and starch. A fourfold improvement in the log reduction of the *L. monocytogenes* was obtained through the use of TiO_2 nanoparticle in the poly-lactic acid (PLA) matrix upon UV illumination.

While, the silver nanoparticles possess electrocatalytic activity which is also being exposed widely in the food industry. The zinc oxide nanoparticle can act as an efficient antimicrobial barrier and permeation barrier. It has been applied to various matrices like chitosan, sago starch, fish protein, and basil seed essential oil.

Chitosan exhibits exceptional biocompatibility and film forming ability promoting its acceptance in the food industry. As per the scientific procedures for use in food (FDA/CFSAN), the Food and Drug Administration (FDA) has recognized chitosan as GRAS (generally recognized as safe) in 2005. Other commonly employed nanoparticles in the edible coating include the nano-silicon dioxide, copper oxide nanoparticles along with their combinations with essential oils. Nanocellulose is also being exploited recently in the food sector (Xing et al. 2019; Huang et al. 2017; El-Sherbiny et al. 2016; Jebel and Almasi 2016).

15.4.1 Antimicrobial Mechanism of Nanoparticles in the Food Matrix

Many mechanisms have been proposed to explain the antimicrobial potential of the nanoparticles. However, no stand-alone mechanism is effective enough, implying the inter-correlation of the proposed mechanisms.

Disruption of the microbial cell integrity through electrostatic interactions between the negatively charged bacterial surface and the cationic nanoparticles in the matrix is a common mechanism. This leads to the formation of pits and pores in the cell surface which in turn induces the cell death by cytoplasmic membrane delamination. The oxidative damage occurring in the biological macromolecules through the generation of reactive oxygen species and the hydrogen peroxide moiety through interactions with the nanoparticles when subjected to the UV or solar irradiation is another projected mechanism for the action of nanoparticles. Final proposed mechanism is the alterations in the DNA metabolism when exposed to the nanoparticles (Alizadeh-Sani et al. 2018; Kumar-Krishnan et al. 2015).

15.5 Application Methods for Edible Coating

There are various methods through which the food material can be enrobed with the developed filmogenic solution. Among them the widely utilized methods are spray coating, dip coating, brushing, extrusion, spraying, solvent casting, and overhead dip emission.

In dip coating method, the fresh produces are directly added into the prepared coating solution (either as whole fruit or processed food) for a period of time and then dried according to the developed procedure which utilizes minimum temperature not to affect the texture and quality of the fruit (Fig. 15.2a). The product will absorb the contents of the coating solutions depending on its concentration and forms a protective layer of desired thickness. The thickness of the developed coat, its uniformity, and texture depend not only on the nature of the coating solution such as viscosity and density but also on the dipping time and method employed in drying the same. The brittleness of the coating material can be tuned with the addition of plasticizers such as glycerol, mannitol, sucrose, and sorbitol. The affinity of the coating material to the rough surface of the food is a desired factor. The presence of surfactants can lead to the reduction in the surface tension of the film and thereby obtaining a uniform layer. The changes in the dilution of the dip solution owing to the surface wetness of the fruits lead to changes in the solution properties and its concentrations which might be varied with the external factors. This requires the solution to be replaced frequently and remains a major drawback of the dip coating method.

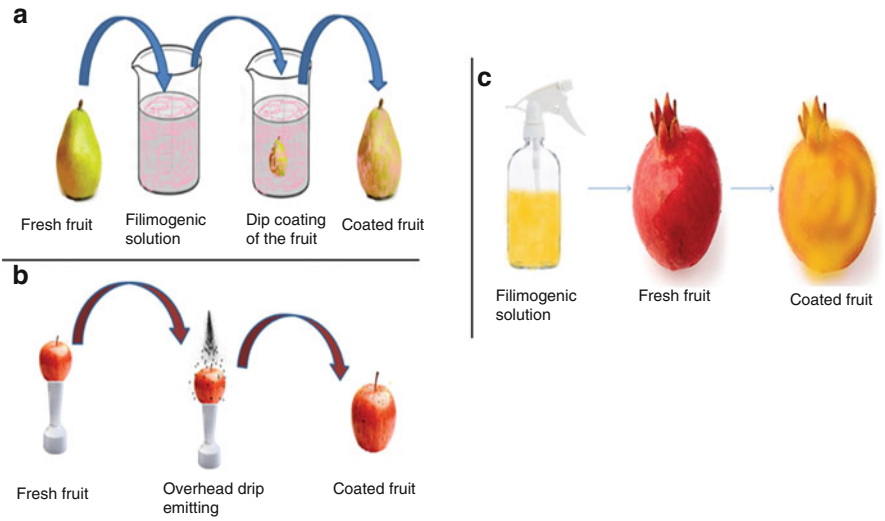

Fig. 15.2 Different methods of coating: (**a**) dip coating, (**b**) overhead drip emission, (**c**) spray coating

Requirement of the filmogenic solutions in bulk quantities so as to dip the whole fruit is another drawback. Regular replacement of the dip solution and ensuring its proper mixing are essential to ensure the even distribution of the particles and to avoid threats of contamination (Zhao 2019).

The spray coating technique (Fig. 15.2c) can ensure thin and uniform coating on the surface of the products. The development of high-pressure spray applicators and air atomizers have made the spray coating a convenient method. The solution viscosity, temperature, and surface characteristics of the solution along with the applied pressure and nozzle characteristics can alter the coating characteristics including the uniformity and thickness of the formed coat. The lower volume of the solution required and the ease of application are added advantage in this method (Zhao 2019).

Overhead drip emitters (Fig. 15.2b) of varying size can deliver variety of droplet size. The rotating brush onto which the sample is mounted ensures the development of uniform coatings.

15.6 Materials Used for Coating

The substrates utilized for the edible coating can be classified based on its chemical composition as lipids, hydrocolloids, and composites.

The hydrocolloids are the hydrophilic polymers that consist of the polysaccharides, proteins, alginates, carrageenan, carboxymethyl cellulose, and arabic gum which are used to completely dissolved in water and are used to improve the viscosity of the gelling agent. Those based on polysaccharides (chitosan, starch, cellulose, and gums) ensure a modified atmosphere in addition to the crispiness, hardness, compatibility, and thickening property (Sharma et al. 2019; Sharma et al. 2018) (Table 15.1).

15.6.1 Lipids

While the waxes, fatty acids, and aryl compounds such as carnauba wax, bees wax, paraffin wax, and mineral or vegetable oil make up the lipid coating, they are least preferred owing to their greasiness and thickness which gives a rancid flavor to the food materials in spite of its excellent moisture barrier.

Due to the hydrophobic nature, lipids act as an effective barrier to moisture alongside, it acts as an attractive gloss which can improve the visual appearance of the food stuff. However, the poor mechanical properties of the lipids remain a major hindrance in its application as an edible coating. The films made of lipid can have better moisture barrier while they hold many drawbacks such as opacity, inflexibility, waxy taste, and rancidity (Sharma et al. 2018; Quezada 2000).

Table 15.1 Coating materials used

S No	Polymer	Active compound	Substance coated	Advantage	Reference
1.	Alginate and chitosan	Nano ZnO	Guava	Shelf life extended to 20 days from 7 in normal case	Arroyo et al. (2020)
2.	Candeuba wax	Solid lipid nanoparticles	Saladette tomato	Better preservation	Miranda-Linares et al. (2018)
3.	Xanthan gum	Solid lipid nanoparticles (SLNs)	Guava	Delay the maturation—minimize the senescence	Zambrano-Zaragoza et al. (2013)
4.	PVP	Silver nanoparticles	Asparagus spears	Lower weight loss, greener color, and tender texture	An et al. (2008)
5.	Chitosan nanoparticle	Chitosan nanoparticle	Fresh-cut apples	Better antimicrobial efficiency	Pilon et al. (2015)
6.	Chitosan coatings	Ag-chitosan nanocomposites	Fresh-cut melon	Showed the highest total vitamin C, better sensory-scored	Ortiz-Duarte et al. (2019)
7.	Chitosan	Fungal chitosan nanoparticles	Postharvest table grapes	Delaying the ripening, increased moisture retention and preservation of the titratable acidity	Castelo Branco Melo et al. (2018)
8.	Gelatin	Mentha pulegium Essential oil	Strawberries	Down changes in pH, TA, weight loss, TSS, firmness, TPC, and color	Aitboulahsen et al. (2018)
9.	Chitosan	Chitosan silver nanoparticle	Melon	Reduced respiration rate, prevention of softening, improved nutritional impact	Ortiz-Duarte et al. (2019)
10.	Carboxymethyl cellulose	ZnO nanoparticles	Persimmon and tomato fruit	Delayed black spot disease	Mooktida Saekow et al. (2019)
11.	Chitosan nanoparticle	Chitosan nanoparticle	Banana	Slower ripening	Esyanti et al. (2019)

15.6.2 Polysaccharides

Polysaccharides are obtained from a wide range of sources spanning from marine to agriculture to animals even then, they do not form an effective stand-alone coating owing to its hydrophilic nature.

Polysaccharides are exploited as short term moisture barrier as they can maintain the atmosphere by acting as a sacrificial moiety (Dhall 2013a). The linear structure of some of the polysaccharides such as cellulose, amylose, and chitosan renders their respective films toughness, transparency, flexibility, and resistance to fats and oils. The lipid incorporation into these edible films can improve the water vapor barrier properties. This is a major issue with the polysaccharide packaging due to its hydrophilic nature. Figure 15.3 depicts the various polysaccharides exploited in the edible coating.

During the storage of "valencia" oranges, it was found that the incorporation of lipid components into the coating was more efficient than utilizing the polymer alone. A composite polymer matrix was chosen to optimize the hydrodynamic potential of the hydrophilic pea starch guar gum film has been reported to be improved through the incorporation of hydrophobic shellac. By the lipid incorporation into the pea starch guar gum matrix, it was found to be capable of maintaining the lower rate of fruit respiration, ethylene production, peel pitting, and weight and firmness loss which ultimately resulted in the slow degradation of the coated material. The incorporation of shellac and oleic acid resulted in the maintenance of freshness and flavor even after 4 weeks of packing (Saberi et al. 2018).

15.6.2.1 Cellulose and Derivatives

Cellulose (Fig. 15.3a) is the most abundant natural polymer with tightly packed linear structure made of anhydrous glucose with high crystallinity that protects the compound from salvation in water. The mechanical and barrier properties of the cellulose derived films maintain a direct relationship with its molecular weight.

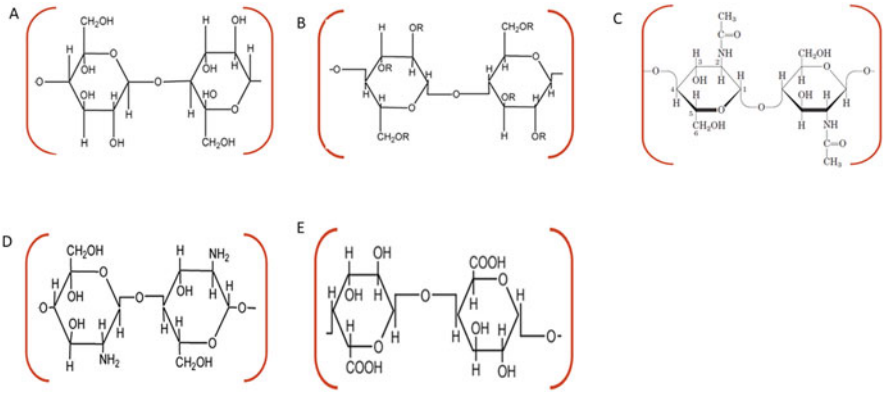

Fig. 15.3 Various polysaccharides exploited in the edible coating: (**a**) Cellulose, (**b**) Hydroxypropylmethylcellulose, (**c**) Chitin, (**d**) Chitosan, (**e**) D-galactouronic acid

The D-glucopyranose units of cellulose are linked together by β(1–4) glycosidic linkages. While the ordered regions of the linear polymer are responsible for the crystalline nature of the polymer, the structural distortions form the basis for the amorphous nature (Zhang et al. 2014).

Cellulose has mainly four polymorphs namely I, II, III, and IV. Among these, cellulose I is a crystalline form and is the most abundant form in living plants. The parallel chains of cellulose I can be converted into the antiparallel chains of cellulose II by mercerization. The active role of cellulose II in chain folding renders it a complex structure. The treatment of cellulose I and II either by ammonia or ethylene diamine generates cellulose III which upon treatment with glycerol at high temperature generates cellulose IV.

Cellulose in its natural form exhibits high mechanical property along with resistance to biological degradation and low aqueous solubility. The effective resistance to the acid hydrolysis can be attributed to the presence of strong hydrogen bonding between the fibrils. Replacement of these hydroxyl groups by acetate or methyl group can reduce the overall hydroxyl functional group and is aimed at the development of cellulose based plastics (Chivrac et al. 2009).

The poor water vapor barrier of the cellulose derived coatings can be improved by the addition of hydrophobic waxes and lipids. The bacterial cellulose however is free from impurities such as hemicellulose, pectin, and lignin, which might be seen commonly in the plant derived cellulose. It also exhibits high tensile strength, elastic modulus along with desired mechanical properties, and high water absorption.

The cellulose derivatives modified with lipid content of the bees wax or shellac have proven to be better in terms of its resistance to water in coating the mandarin oranges. *Acetobacter xylinum* mediated cellulose films (bacterial cellulose) were described to be flexible, foldable, intact, soft, opaque, and homogeneous. Its glass transition temperature of 191 °C was suitable for the minimal aging conditions and was found to be suitable for coating tablets.

Cellulose derivatives such as methyl cellulose (MC), carboxymethylcellulose (CMC), CMC sodium salt (Na-CMC), hydroxypropyl cellulose (HPC), and hydroxypropylmethylcellulose (HPMC) (Fig. 15.3b) have been used commonly in the edible coatings where they act as effective barriers (Dhall 2013a).

Jafarizadeh Malmiri et al. (Malmiri et al. 2011) were successful in developing an edible coating for the banana fingers using Na-CMC and HPMC. The effective weight loss was found to be minimal for the cellulose wrapped samples following a 10 day storage. The environmental and financial appeal along with the required properties such as flexibility and transparency have made hydroxypropyl methyl cellulose (HPMC) as edible films with a well-accepted feature in spite of its poor mechanical and barrier properties and moderate resistance to fats and oils (Quezada 2000). The nanoreinforced HPMC films have gained much attention. Reinforcement with micro-crystalline cellulose (MCC) at a nanoscale can ensure changes in tensile strength, moisture barrier, water permeability, etc. up to a 50-fold higher level (Bilbao-Sáinz et al. 2010).

15.6.2.2 Starch

The storage polysaccharides of cereals, legumes, and tubers are made of anhydrous glucose units which consists of linear amylose chains and branched amylopectin chains. Their biodegradability and low cost make them efficient replacement material for non-degradable plastic films. While the permeability of high amylose starch is far better than low amylose films, higher amylopectin content can lead to poor mechanical properties (Dhall 2013a). Another common issue with the starch is its brittleness which can be answered through the addition of biodegradable plasticizers such as glycerol and other low molecular weight poly hydroxyl compounds such as polyether and urea.

The starch derived product, glucose, when fermented produces lactic acid that can be exploited in the production of lactic acid which on polymerization produces polylactic acid and its copolymers. Another thermoplastic polyester, poly-3-hydroxybutyrate, is produced through the bacterial fermentation of glucose, acetic acid, and other feedstock. The commonly accepted food contact material, PLA, is derived from the biodegradable plant materials (corn starch and beet) and exhibits high transparency and water resistance (Tahir et al. 2020; Mallegni et al. 2018).

15.6.2.3 Chitin and Chitosan

Chitin (Fig. 15.3c) is the second most abundant natural polymer, next to cellulose. It is found in the exoskeleton of crustaceans, fungal cell wall, etc. The deacetylation of chitin by alkali yields chitosan (Fig. 15.3d). The semipermeable coatings derived from chitosan can maintain the modified atmosphere thereby decreasing the transpiration rate and thus delaying the ripening. The flexibility, strength, and barrier properties offered by chitosan make it a widely accepted choice of coating material in spite of its poor water barrier property.

Both chitin and chitosan exhibit excellent antibacterial and antifungal properties which is an added advantage in the food industry. The protonation of the amino groups converts the chitosan into a water soluble polycation enabling its application in the edible coating and packaging. Even though chitosan has excellent mechanical properties and flexibility, its sensitivity to the environmental humidity and low moisture barrier limit its application in the food industry.

Chitosan has proven antimicrobial potential wherein it reacts effectively with the negatively charged residues on fungal surface leading to the distortion of the permeability of the plasma membrane and the fungicidal activity.

Modification of the chitosan coatings with lipids is aimed at improving the water vapor barrier property of the chitosan films. The chitosan films were modified with basil and thyme oil. The addition of the essential oils enhanced the water vapor barrier (WVP) of the chitosan film owing to the negative effect of oil incorporation and cohesion forces of the matrix (Bonilla et al. 2012).

Another study which exploited the incorporation of cinnamon oil along with chitosan analyzed its effect on the postharvest storage of sweet peppers over a period of 35 days at 8 °C. It was found to be effective in minimizing the surface decay (5%) in comparison to the uncoated samples (34%). A significant change was also noticed in the sensory evaluation of the treated and untreated samples. However, the color

change was negligible for both treated and control samples as evidenced through both visual and chlorophyll analysis (Xing et al. 2015).

In another study involving both chitosan nanoparticles and *Origanum vulgare L.* essential oil, the grapes artificially contaminated with the spores of *Rhizopus stolonifer* and *Aspergillus niger* did not show any significant fungal growth when treated with the nanoparticle essential oil combination followed by storage at varying temperatures (25 and 12 °C) (Barreto et al. 2016).

15.6.2.4 Pectin

Pectin, the structural component of the plant cell wall and the intercellular cementing material is a complex polysaccharide of D-galacturonic acid (Fig. 15.3e). The gel forming nature of pectin has led to its exposure as an additive in the recent food market along with its development as an edible coating. However, it is not a stand-alone molecule in the development of edible coatings and requires the additional modification to meet the required mechanical properties, WVP, and shelf life.

A modified pectin solution, with melted beeswax, sorbitol as the plasticizer, and monoglyceride as the emulsifier was used to extend the shelf life of mangoes using dip coating approach. The weight loss was found to be significantly lower in the treated mangoes. The modification of the pectin films with α carrageenan was able to enhance the water vapor barrier and mechanical properties of the native films. The tensile properties of the complex film matrix developed were in agreement with the carrageenan concentration.

15.6.3 Proteins

The protein based edible coating can be derived from either animal or plant sources. The common coating materials include whey, zein, gluten, soy protein, etc. of the plant origin along with egg albumin and collagen of the animal origin. They exhibit good barrier property at lower relative humidity owing to the tightly packed hydrogen bonded system present in them. Even though they possess great organoleptic and mechanical properties, they are poor barrier for water (Sharma et al. 2019). The wide range of physical and mechanical properties exhibited by proteins make them a versatile agent in the food packaging/coating industry (Sharma et al. 2018).

Protein derived edible coatings are generally strong in the mechanical aspects. While they are highly permeable to gases, their moisture barrier property is not so evident. Another limiting factor in the application of protein based edible coating is the possibility of allergic reactions it may cause.

Spider silk is another edible fiber which can be exploited for the food protection. The silk solution with the film forming capability was obtained by degumming of the raw silk followed by dissolution. The silk coated fruits exhibited lesser water loss and fungal attack when compared to the control samples which demonstrated the efficacy of the natural spider silk as a coating material (Tahir et al. 2020).

15.6.3.1 Milk Protein and Derivatives

Milk proteins such as whey and casein are extensively explored in the food industry for their nutritive value. The edible films of milk origin are transparent, tasteless, flavorless, and flexible. Extended shelf life (up to 12 days) of shrimp is evident on coating the samples with the milk protein having incorporated thyme oil and trans-cinnamaldehyde. The milk protein based films were also found to be capable of reducing the lipid oxidation and SH radical production.

The predominant protein, casein, which makes up 80% of the milk proteins, when subjected to acid coagulation produces caseinate. It has a high bonding efficiency in terms of intermolecular hydrogen, electrostatic, and hydrophobic bonds which along with the random coil nature adds on to its film forming potential. While the α casein fraction contains more charged fractions and few hydrophobic residues than β casein, the latter is an excellent film forming agent owing to its lower water permeability (Lacroix and Vu 2014).

Whey protein is the soluble fraction present in the milk following caseinate coagulation during the cheese production. It makes up for around 20% of the total milk proteins and constitutes lactoglobulin, lactalbumin, bovine serum albumin, immunoglobulins, and proteose peptones.

Whey and caesinate proteins are efficient antioxidants and can be exploited in protecting the fresh produces from aging related biochemical changes. Antioxidant activities of the whey proteins are evident from the protection it offered against the fresh mushrooms and cut fruits.

15.6.3.2 Collagen and Gelatin

Collagen, commercially the most successful edible coating, present in the α and β forms makes up for about 30% mass of the body and is the main constituent of skin, tendon, and other connective tissues (Gustavson 1956). Collagen on hydrolysis yields gelatin, which is efficient in reducing the migration of moisture, oxygen, and oils.

Among the common coating materials, gelatin is a widely accepted material. The studies prove that the gelatin coating is efficient in maintaining the titratable acidity, total weight loss, total soluble solids, phenolic content, etc. of the packaged material. However, enhanced performance is ensured when the same is utilized in conjugation with the essential oils. The combination of the essential oil with the coating material can reduce the total flora, yeasts, and molds on the packaged material along with maintaining the hygienic quality (Aitboulahsen et al. 2018).

15.6.3.3 Plant Proteins

Soy protein, composed of β conglycinin and glycinin, tightly folded proteins is efficient to form film forming solution. The property enhancement can be obtained through the wide range of incorporated components including plasticizers such as glycerol and sorbitol.

Improved mechanical properties can be expected in films prepared under the alkaline pH, while the heat treatment has shown to improve the tensile strength of the

prepared films which is brought about by the exposure of the sulfhydryl groups through the unfolding of the polypeptide chains.

15.7 Extended Shelf Life of Fruits

The generation of novel products in the food industry has shifted the paradigm of the society towards ready to use food in spite of the threats they pose. The rapid development in the market trends has stimulated the research toward the generation of high-quality food with improved shelf life. The texture and appearance of the food determine its acceptability in the market. In spite of the fact that the fresh-cut fruits and vegetables are greatly influenced by the wound inflicted on them, the general process leading to the degradation and deterioration of the food is the softening, a part of the normal ripening process. However, the presence of large number of cells in the fresh-cut fruits and vegetables containing a large proportion of secondary cell wall creates lesser trouble. The release of the intracellular contents at the wounded site of these products can lead to the generation of off-flavor spillage, browning of the products, and finally the deterioration of the nutritional quality. The edible coatings can answer these issues to a large extend by enhancing the shelf life of both fresh produce and fresh-cut produces in spite of the wound inflicted on them (Porta 2013).

15.8 Microbial Growth Prevention

Another major purpose of the edible coatings is to reduce the quality deterioration of the food owing to microbial attack. The edible coatings are sufficient enough to cover up the surface micro-wounds and cracks, reducing the success rate of microbial attack. Furthermore, the incorporation of the antimicrobial components into these coatings can diminish the microbial load on the coated food material (Maherani et al. 2018). While analyzing the microbial growth on green peppers, a significant reduction was evident in the microbial load of coated samples against *Escherichia coli, Salmonella enterica*, and *Listeria innocua*.

The edible coatings applied to non-climacteric fruits such as strawberry which has a relatively high metabolic activity can not only improve the shelf life but also reduce the bacterial attack on them (Fakhouri et al. 2014). The major issue with the storage of strawberries is the yield loss in the postharvest period owing to the fungal attack. Traditionally, fungicide and colder temperature in simultaneous usage have been followed which can extend the shelf life minimally (Aitboulahsen et al. 2018). Edible coatings have found their way in enhancing the shelf life of climacteric fruits also.

15.9 Nutritional and Economical Impacts

Fruits and vegetables are fresh reservoirs of vitamins, minerals, antioxidants, and dietary fibers. Their phytochemical constituents have higher antioxidant potential which in turn can protect the consumers from stress, aging related issues, cardiac diseases, and cancer (Hassan et al. 2018). This in turn has brought about a hike in the demand of the fresh produces.

15.10 Conclusion

The addition of active ingredients into the edible coating not only enhances the safety but also improves the nutritional and sensory attributes of the food consumed. The high perishability of the fresh produce can be attributed to its water content which accounts for around 80–90% by weight. The atmosphere maintenance by the edible coating ensures the least water evaporation. The efficacy of the coating material can be improved either through the development of composite polymers or through the incorporation of herbal components/essential oils and/or nanoparticles as a filler. The paradigm shift in the research developments in edible coating showcases its efficacy as becoming the choice of the future both in terms of quality maintenance and easy handling.

References

Aitboulahsen M, Zantar S, Laglaoui A, Chairi H, Arakrak A, Bakkali M et al (2018) Gelatin-based edible coating combined with Mentha pulegium essential oil as bioactive packaging for strawberries. J Food Qual 2018:1–7

Alizadeh-Sani M, Khezerlou A, Ehsani A (2018) Fabrication and characterization of the bionanocomposite film based on whey protein biopolymer loaded with TiO2 nanoparticles, cellulose nanofibers and rosemary essential oil. Ind Crop Prod 124:300–315

An J, Zhang M, Wang S, Tang J (2008) Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. LWT Food Sci Technol 41(6):1100–1107

Arnon-Rips H, Poverenov E (2018) Improving food products' quality and storability by using layer by layer edible coatings. Trends Food Sci Technol 75:81–92

Arroyo BJ, Bezerra AC, Oliveira LL, Arroyo SJ, Melo EA, Santos AMP (2020) Antimicrobial active edible coating of alginate and chitosan add ZnO nanoparticles applied in guavas (Psidium guajava L.). Food Chem 309:125566

Barreto TA, Andrade SC, Maciel JF, Arcanjo NM, Madruga MS, Meireles B et al (2016) A chitosan coating containing essential oil from Origanum vulgare L to control postharvest mold infections and keep the quality of cherry tomato fruit. Front Microbiol 7:1724

Bilbao-Sáinz C, Avena-Bustillos RJ, Wood DF, Williams TG, McHugh TH (2010) Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. J Agric Food Chem 58(6):3753–3760

Bonilla J, Atarés L, Vargas M, Chiralt A (2012) Effect of essential oils and homogenization conditions on properties of chitosan-based films. Food Hydrocoll 26(1):9–16

Castelo Branco Melo NF, de MendonçaSoares BL, Marques Diniz K, Ferreira Leal C, Canto D, Flores MAP et al (2018) Effects of fungal chitosan nanoparticles as eco-friendly edible coatings on the quality of postharvest table grapes. Postharvest Biol Technol 139:56–66

Chivrac F, Pollet E, Averous L (2009) Progress in nano-biocomposites based on polysaccharides and nanoclays. Mater Sci Eng R Rep 67(1):1–17

Cuq B, Gontard N, Guilbert S (1998) Proteins as agricultural polymers for packaging production. Cereal Chem 75(1):1–9

Decher G (1997) Fuzzy nanoassemblies: toward layered polymeric multicomposites. Science 277 (5330):1232–1237

Dhall R (2013a) Advances in edible coatings for fresh fruits and vegetables: a review. Crit Rev Food Sci Nutr 53(5):435–450

Dhall RK (2013b) Advances in edible coatings for fresh fruits and vegetables: a review. Crit Rev Food Sci Nutr 53(5):435–450

El-Sherbiny IM, El-Shibiny A, Salih E (2016) Photo-induced green synthesis and antimicrobial efficacy of poly (ε-caprolactone)/curcumin/grape leaf extract-silver hybrid nanoparticles. J Photochem Photobiol B Biol 160:355–363

Esyanti RR, Zaskia H, Amalia A, Nugrahapraja dH (2019) Chitosan nanoparticle-based coating as post-harvest technology in banana. J Phys Conf Ser 1204:012109

Fakhouri FM, Casari ACA, Mariano M, Yamashita F, Mei LHI, Soldi V et al (2014) Effect of a gelatin-based edible coating containing cellulose nanocrystals (CNC) on the quality and nutrient retention of fresh strawberries during storage. IOP Conf Ser Mater Sci Eng 64:012024

Gustavson KH (1956) Chemistry and reactivity of collagen. Academic Press, New York

Hassan B, Chatha SAS, Hussain AI, Zia KM, Akhtar N (2018) Recent advances on polysaccharides, lipids and protein based edible films and coatings: a review. Int J Biol Macromol 109:1095–1107

Huang S, Guild B, Neethirajan S, Therrien P, Lim LT, Warriner K (2017) Antimicrobial coatings for controlling Listeria monocytogenes based on polylactide modified with titanium dioxide and illuminated with UV-A. Food Control 73:421–425

Jayakumar A, Heera KV, Sumi TS, Joseph M, Mathew S, Praveen G et al (2019) Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application. Int J Biol Macromol 136:395–403

Jebel FS, Almasi H (2016) Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films. Carbohydr Polym 149:8–19

Kumar-Krishnan S, Prokhorov E, Hernández-Iturriaga M, Mota-Morales JD, Vázquez-Lepe M, Kovalenko Y et al (2015) Chitosan/silver nanocomposites: synergistic antibacterial action of silver nanoparticles and silver ions. Eur Polym J 67:242–251

Lacroix M, Vu KD (2014) Edible coating and film materials: proteins. In: Innovations in food packaging. Elsevier, Amsterdam, pp 277–304

Maherani B, Harich M, Salmieri S, Lacroix M (2018) Antibacterial properties of combined non-thermal treatments based on bioactive edible coating, ozonation, and gamma irradiation on ready-to-eat frozen green peppers: evaluation of their freshness and sensory qualities. Eur Food Res Technol 245(5):1095–1111

Mallegni N, Phuong T, Coltelli M-B, Cinelli P, Lazzeri A (2018) Poly (lactic acid)(PLA) based tear resistant and biodegradable flexible films by blown film extrusion. Materials 11(1):148

Malmiri JH, Osman A, Tan C, Rahman AR (2011) Evaluation of effectiveness of three cellulose derivative-based edible coatings on changes of physico-chemical characteristics of 'Berangan' banana (Musa sapientum cv. Berangan) during storage at ambient conditions. Int Food Res J 18 (4):1381

Mathew S, Raveendran A, Mathew J, Radhakrishnan EK (2019) Antibacterial effectiveness of rice water (starch)-capped silver nanoparticles fabricated rapidly in the presence of sunlight. Photochem Photobiol 95(2):627–634

McHugh T, Senesi E (2000) Apple wraps: a novel method to improve the quality and extend the shelf life of fresh-cut apples. J Food Sci 65(3):480–485

Miranda-Linares V, Escamilla-Rendón P, Del Real-López A, González-Reza RM, Zambrano-Zaragoza ML (2018) Solid lipid nanoparticles based edible coating for saladette tomato preservation. Acta Hortic 1194:305–312

Mooktida Saekow MN, Tongdeesoontorn W, Hamauzu Y (2019) Effect of carboxymethyl cellulose coating containing ZnO-nanoparticles for prolonging shelf life of persimmon and tomato fruit. J Food Sci Agric Technol 5:41–48

Ortiz-Duarte G, Pérez-Cabrera LE, Artés-Hernández F, Martínez-Hernández GB (2019) Ag-chitosan nanocomposites in edible coatings affect the quality of fresh-cut melon. Postharvest Biol Technol 147:174–184

Park HJ (1999) Development of advanced edible coatings for fruits. Trends Food Sci Technol 10 (8):254–260

Pilon L, Spricigo PC, Miranda M, de Moura MR, Assis OBG, Mattoso LHC et al (2015) Chitosan nanoparticle coatings reduce microbial growth on fresh-cut apples while not affecting quality attributes. Int J Food Sci Technol 50(2):440–448

Porta R (2013) Edible coating as packaging strategy to extend the shelf-life of fresh-cut fruits and vegetables. J Biotechnol Biomater 03(04):1–3

Quezada GJ (2000) Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion-based edible films. J Membr Sci 180(1):37–46

Roshmi T, Jishma P, Radhakrishnan E (2017) Photocatalytic and antibacterial effects of silver nanoparticles fabricated by Bacillus subtilis SJ 15. Inorg Nano-Met Chem 47(6):901–908

Saberi B, Golding JB, Marques JR, Pristijono P, Chockchaisawasdee S, Scarlett CJ et al (2018) Application of biocomposite edible coatings based on pea starch and guar gum on quality, storability and shelf life of 'Valencia' oranges. Postharvest Biol Technol 137:9–20

Sharma P, Shehin VP, Kaur N, Vyas P (2018) Application of edible coatings on fresh and minimally processed vegetables: a review. Int J Veg Sci 25(3):295–314

Sharma P, Shehin V, Kaur N, Vyas P (2019) Application of edible coatings on fresh and minimally processed vegetables: a review. Int J Veg Sci 25(3):295–314

Shit SC, Shah PM (2014) Edible polymers: challenges and opportunities. J Polym 2014:1–13

Tahir H, Pervez N, Nadeem J, Khan A, Hassan Z (2020) Esculent coating of spider silk enhanced the preservation and shelf life of apricot. Braz J Biol. https://doi.org/10.1590/1519-6984.193974

Vidyalakshmi N, Thomas R, Aswani R, Gayatri G, Radhakrishnan E, Remakanthan A (2017) Comparative analysis of the effect of silver nanoparticle and silver nitrate on morphological and anatomical parameters of banana under in vitro conditions. Inorg Nano-Met Chem 47 (11):1530–1536

Xing Y, Lin H, Cao D, Xu Q, Han W, Wang R et al (2015) Effect of chitosan coating with cinnamon oil on the quality and physiological attributes of China jujube fruits. Biomed Res Int 2015:1

Xing Y, Li W, Wang Q, Li X, Xu Q, Guo X et al (2019) Antimicrobial nanoparticles incorporated in edible coatings and films for the preservation of fruits and vegetables. Molecules 24(9):1695

Zambrano-Zaragoza ML, Mercado-Silva E, Ramirez-Zamorano P, Cornejo-Villegas MA, Gutiérrez-Cortez E, Quintanar-Guerrero D (2013) Use of solid lipid nanoparticles (SLNs) in edible coatings to increase guava (Psidium guajava L.) shelf-life. Food Res Int 51(2):946–953

Zhang Y, Rempel C, Mclaren D (2014) Edible coating and film materials: carbohydrates. In: Innovations in food packaging. Elsevier, Amsterdam, pp 305–323

Zhao Y (2019) Edible coatings for extending shelf-life of fresh produce during postharvest storage. Elsevier, Amsterdam

Systems Biology Approaches for Food and Health

16

Rajesh Kumar Pathak and Dev Bukhsh Singh

Abstract

In recent years, systems biology has emerged as a powerful tool to understand crop plant systems for augmenting food production and how their dietary components promote our health and prevent from diseases as well as to investigate the bioactive molecules involved in such effects. Systems-based approaches hold immense potential to understand the molecular mechanism of important and complex traits linked with food productivity in crop plants via combining several "omics" genomics, transcriptomics, proteomics, and metabolomics, and other omics approaches. Computational approaches and predictive models allow the broad analysis of the particular response of key genes at the molecular level and its role in improving the nutritional quality of plant products, and their effects on human health. This has resulted in the identification of some important genes, and proteins engaged in plant growth, development, and nutritional, as well as the discovery of bioactive molecules required for human health. This chapter specifically focus on the use of systems biology approaches to breaking yield barriers for food productivity and improving the nutritional values of food for our health through integration of multi-omics data for modeling, simulation, and network analysis, which leads to molecular breeding and genetic manipulation of key candidate genes in crop plants and predict the response of food/diets in different conditions of body for designing of functional food and nutraceuticals for benefits of the society.

R. K. Pathak
School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India

D. B. Singh (✉)
Department of Biotechnology, Institute of Biosciences & Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur, Uttar Pradesh, India

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_16

Keywords

Systems biology · Omics · Network · Crop plants · Food · Health

16.1 Introduction

Several complex traits, manifested by genetic and epigenetic interaction, govern plant productivity. Two main methods are generally used to study such significant agricultural features: phenotypic to genotypic and genotypic to phenotypic (Kumar et al. 2015; Pathak et al. 2018a). However, there is a wide gap in these methods, so systems biology is the most promising discipline to studying the molecular mechanism of such characteristics regulated by complicated gene regulatory networks and pathways that will allow us to produce smart, high yielding crops with sufficient amount of health benefiting nutrients (Kumar et al. 2018). Developing smart crops are a component of innovation, producing more food with enough nutrients in a shorter period, reducing our need for agrochemicals such as pesticides and fungicides, and adding sustainable agriculture to the environment (Pathak et al. 2018b). This has become essential for ensuring food and nutritional security.

Expected changes in weather and its variability, generally extreme temperatures and changes in rainfall, will make crop plant improvement even more important for food production (Varshney et al. 2011; Kumar et al. 2015). Since there is also interest in producing health-promoting food, the future has big possibilities for crop improvement with the goal of functional food and nutraceuticals development. Advances in our understanding of multi-omics data, their integration and analysis, and newer systems-based approaches to using this knowledge for enhancing the quality of components have the ability to add value to the plant products, which will definitely contribute to human health benefits (Panagiotou and Nielsen 2009; Kumar et al. 2015).

In recent years, the term "systems biology" has emerged to illustrate the frontier of cross-disciplinary research in biotechnology and natural sciences; besides, it is established as a language of modern biology (Kitano 2002). Nearly two decades have passed since the beginning of the modern form of systems biology (Kitano 2015). Recently it is known to recognize and decode complete biological systems through data integration, modeling, and simulation analysis and predicting the behavior of each component and their interactions. It is an attempt to study living systems by identifying all elements and connections between these elements from a system-wide view.

The first step in a system-based strategy is to define each element involved in system functionality (genomics, transcripts, proteomes, and metabolites), followed by perturbation analysis of system and determine its response under specified circumstances. The identified behavior of systems can then be integrated into system function models. New hypotheses produced by these models can be experimentally evaluated, leading to updated models and new hypotheses that can be tested. The

system-oriented study is the mainstream strategy today and has been implemented effectively in the field of agricultural and plant sciences for producing more food with well nutritional quality. This chapter offers the latest developments and advances in the practical uses of systems biology for food and health.

16.2 Networks in Systems Biology

The word "network" is a keyword for systems biology. It organizes biological system complexity as components (nodes) and relationships (edges) among them. Biological processes are often depicted in the form of networks such as gene regulatory, protein–protein interactions network (PPIs), signal transduction, and metabolic networks (Pathak et al. 2017a).

16.2.1 Gene Regulatory Network

It is a common word for transcription-level cellular control of protein synthesis. Gene regulation can also be viewed as a cell's reaction to an inner stimulus. Often one gene is controlled by another gene through the respective protein called a transcription factor, so gene regulation is coordinated in a network of gene regulators.

The gene regulatory network explains precisely how genomic sequence codes and regulates the expression of gene sets that progressively produce developmental patterns and build different differentiation states (Hasty et al. 2001; Sullivan et al. 2014).

16.2.2 Signal Transduction Network

The network of signal transduction connects intracellular processes to extracellular environments and regulates cellular functions in response to multiple external stimuli. It is initiated by receptor binding of extracellular ligands, leading in one or more particular responses. Different extracellular ligands, such as hormones, growth factors, and cytokines react on the outer surface of a cell and are responsible for activation of gene or energy production as per the need of the systems (Junker and Schreiber 2008; Yan et al. 2014).

16.2.3 Protein-Protein Interaction Network

The studying protein-protein interactions (PPIs) of all proteins found in an organism remain one of the challenging tasks in modern biology (Snider et al. 2015). Such information is essential for understanding cellular pathways and designing an efficient approach for increasing food productivity. A protein may interact with

others to form a protein–protein complex or trigger other proteins. PPIs form a big network, their visualization helps biologists to investigate the role of proteins and fetched novel insights into mechanisms within and across cellular structures and compartments to formulate and test individual gene function hypotheses experimentally.

16.2.4 Metabolic Network

Plant metabolite biosynthesis and function have been researched for centuries (Stitt et al. 2010). Metabolic networks reveal the relationship among tiny biomolecules (metabolites) and enzymes (proteins) that communicate with them to catalyze a biochemical reaction (Castrillo et al. 2013). Metabolic network modeling, metabolic flux analysis are a key area in systems biology, which opens a roadmap for large scale production of health benefiting molecules in crop plants through metabolic engineering.

16.3 Integrative Systems Biology: Top-Down Approach

It starts with collecting samples and performing general wet lab experimentations such as isolation of DNA, RNA, Protein, etc, and high-throughput experiments using several omics platforms like Illumina, PacBio, Microarray, Mass Spectrometry, etc. followed by data integration, bioinformatics analysis, and results in interpretation for novel insight. It attempts to construct integrative models based on data analysis at the molecular level with emphasis on precise queries at a particular scale. This approach is also referred to as a top-down approach for decoding the intricacy of any biological system or system of systems (Pathak et al. 2017a; Kumar et al. 2018).

16.3.1 Integration of Big Data Generated Through Several Omics Platforms

High-throughput omics technologies such as genomics, transcriptomics, proteomics, metabolomics, and others have been used to generate big data. Without the integration of such data, no useful information is produced. In past years, scientists are focusing on single gene and protein but due to advances in the field of molecular biology and biotechnology, scientists are continuously working for the development of novel algorithm and tools for integration of such big data for investigating the function of thousands of genes/proteins holistically in a single task. Systems biology tools have enormous applicability to the integration of big data generated through several omics platforms for visualization of whole plant systems, and this information can be utilized for the discovery of important traits (Kumar et al. 2015; Pathak et al. 2017a).

16.4 Predictive Systems Biology: Bottom-Up Approach

Predictive systems biology deals with the function and molecular mechanism of various components found in biological systems via the building of the mathematical model and hypothesis formulation through detailed information obtained from integrative systems biology and is referred to as a bottom-up approach. The key steps in this approach are pathway modeling, network building, and analysis of perturbation through simulation with the help of systems biology tools. It visualizes the whole picture of biological systems concerning particular response and time. This will lead to wetlab experimentation in a precise and cost-effective way (Pathak et al. 2013, 2017a; Gupta and Misra 2016).

16.4.1 Modeling and Simulation of Biological Pathway

Biological pathway modeling can be done using two kinds of modeling techniques: mathematical modeling and network analysis (Gupta 2018). In mathematical modeling, the system learns and examines the network by changing the reactions and entities in the form of a matrix. Several mathematical formulation and methods were intended and created to study and evaluate the diverse biological pathway with inter as well as intra interactions (Tagore et al. 2008). Boolean network, stoichiometric methods, and ordinary differential equations are used to model large scale signaling pathways, metabolic pathways, gene regulatory networks, and genome-scale networks and simulate to predict its dynamics behaviors' to time and particular response.

The network-based method is associated with graph theory, which finds and link connections among nodes by edges in the pathway. Where all biological entity, i.e. gene, protein, or metabolites indicate a node and edges are shown as interaction type between the pairs of a node. Generally, it is represented as a directed or undirected graph (Gupta et al. 2013; Gupta 2018). In probabilistic graph model, Bayesian networks are introduced to learn from gene expression data, besides other networks model used in biology are Gaussian network, Helmholtz machine, latent variable models, density estimation, maximum likelihood, hidden Markov model, and generative topographic mapping (Tegnér et al. 2009; Wang et al. 2015; Gupta 2018).

16.5 Integrated Computational and Experimentation Based Analysis in Systems Biology for Food and Health

Systems biology aims to provide a clear image of complicated biological systems through interconnected biochemical reaction networks information's obtained from both wet laboratory experiments and computational investigation. It is a twenty-first-century science that is renewing the reductionist strategy to a worldwide perspective and adding food and health study to a new dimension (Kumar et al. 2018). Food and

health systems biology includes incorporating biochemical reaction networks through experimental and computational methods to provide an understanding of complex traits in crop plants linked to dietary values. It offers a clear knowledge of the dynamics of systems in distinct physiological and environmental circumstances, so it requires computational software's and databases for curation, modeling pathways, analyzing, and visualizing complete systems at molecular to the cellular level (Kumar et al. 2015).

16.5.1 Development of Tools and software's for Data Integration, Modeling and Simulation

Development of tools and software's for integration of omics data, modeling, and simulation, for biological system analysis is a big and challenging task because it requires a team of scientists from interdisciplinary fields such as life sciences, mathematics, statistics, physics, chemistry, and computer science to design efficient algorithms and develop better tools. Advances in several omics platforms and systems biology tools provide a better understanding of the intricacies of plant reactions to abiotic and biotic stresses and environmental conditions. This will guide the scientist to develop functional food for health in the future. Here, we highlighted some highly cited tools of systems biology and their applications (Table 16.1).

16.5.2 Development of Databases for Deposition of Omics Data, Modeled Pathway, and Bioactive Compounds

An exciting job for bioinformaticians and systems biologists is to document the accessible information from literature, omics data generated through high-throughput technology, isolated and characterized bioactive molecules, and modeled pathway created by computational tools. The primary goal is to gather enormous amounts of biological information and develop databases in a well-organized form. These databases can be further utilized by the scientific community for the prediction and discovery of new information related to food and health (Kumar et al. 2018). Systems biology resources available for research and analysis are listed in Table 16.2.

16.6 Important Traits in Crop Plants Linked with Food Productivity and Maintenance of Human Health

Plants on planet earth are vital facilitators of human life. It is playing an essential role in mediating air quality, food availability, and agricultural resources sustainability. However, crop plants are constantly interacting with their surroundings, and are often hampered by different kinds of stress such as biotic and abiotic (Shameer et al.

Table 16.1 List of some important systems biology software's/tools for improving food and health status

S. no.	Software's/tools	Application	Availability
1	MATLAB	MATLAB is biological systems modeling and simulation software for predicting the nature of systems	https://www.mathworks.com/products/matlab.html
2	R/Bioconductor	Bioconductor offers tools for high-throughput omics data analysis and understanding. It utilizes the R programming language for data analysis	https://www.bioconductor.org/
3	MapMan	MapMan is a tool that shows big data sets on the metabolic pathway or other process diagrams for novel insight	https://mapman.gabipd.org/home
4	Cytoscape	Cytoscape is used for visualization and integration of molecular interaction networks and pathways for the identification of key components involved in different biological processes	https://cytoscape.org/
5	CellDesigner	CellDesigner is a pathway modeling and simulation tool with a graphical user interface (GUI) and promotes the format of systems biology markup language (SBML) for storing of biological data	http://www.celldesigner.org/
6	JDesigner	JDesigner is a GUI based modeling and simulation environment for biochemical networks	http://jdesigner.sourceforge.net/Site/JDesigner.html
7	SBMLsqueezer	It produces kinetic rate equations for biochemical networks as per the context of each reaction. It can be utilized for the generation of the kinetics equation using CellDesigner	http://www.ra.cs.uni-tuebingen.de/software/SBMLsqueezer/
8	COPASI	COPASI is used for biochemical network simulation and its dynamics assessment	http://copasi.org/
9	NetworkAnalyzer	It helps to computes many topological parameters of directed and undirected biological networks loaded into Cytoscape; and assists in the identification key molecular targets for further investigation	http://apps.cytoscape.org/apps/networkanalyzer
10	AutoDock	It is a software platform used for identification of bioactive molecules for plant and human health via targeting key molecular targets obtained from network modeling through molecular docking	http://autodock.scripps.edu/

Table 16.2 List of some important databases used in systems biology research for food and health

S. no.	Database	Utility for systems biology	Availability
1	Sequence read archive (SRA)	SRA provides biological sequence information and data generated through several high-throughput sequencing platforms to the scientific community for further investigation via comparing data sets and their integration to other data for novel insight	https://www.ncbi.nlm.nih.gov/sra
2	Gene expression omnibus (GEO)	GEO is a functional genomics database contains microarray, next-generation sequencing, and other experimental data for network construction and systems-based analysis	https://www.ncbi.nlm.nih.gov/geo/
3	BioModels	BioModels is a repository of biological system mathematical models. It can be utilized for editing and simulation of the model for predicting the dynamic behavior of systems in appropriate conditions	https://www.ebi.ac.uk/biomodels/
4	KEGG	Kyoto encyclopedia of genes and genomes (KEGG) is a database for knowing the biological system's high-level features and their utility for further research and development	https://www.genome.jp/kegg/
5	PANTHER	The PANTHER is a comprehensive database resource that incorporates genomes, classifications of gene function, pathways and computational tools for statistical analysis, and allows biologists to evaluate large scale experimental data	http://www.pantherdb.org/
6	STRING	STRING is a protein–protein interaction (PPIs) database. It uses a number of functional classification schemes such as gene ontology, Pfam, and KEGG to highlight functional enrichments in user-provided lists of protein. This also provides PPIs network that can be utilized for the identification of key protein(s) as molecular targets	https://string-db.org/
7	PlantCyc	PlantCyc contains information about metabolic pathways in plants for metabolic modeling and flux analysis	https://www.plantcyc.org/databases/plantcyc
8	BRENDA	BRENDA is one of the most extensive enzyme repositories in the world. It is utilized for metabolic engineering and other enzymes related studies	https://www.brenda-enzymes.org/index.php
9	MINT	MINT is a protein–protein interaction database derived from the scientific literature and by expert curators. It is utilized for PPIs network analysis	https://mint.bio.uniroma2.it/

(continued)

Table 16.2 (continued)

S. no.	Database	Utility for systems biology	Availability
10	PubChem	PubChem is a database of chemistry. It contains information about bioactive molecules, their structure, chemical properties, etc. it is utilized in the identification of novel molecules for plants and human health	https://pubchem.ncbi.nlm.nih.gov/

2019). These are the main reason for the declining nutritional contents of seed and food productivity. Besides, many diseases and disorders are linked to nutrition due to imbalance of micro and macronutrients, or presence of some toxic materials in seed because of applications of synthetic agrochemicals in crop field (Van Ommen and Stierum 2002; Pathak et al. 2017b; Kumar et al. 2018). Sustainable farming depends on conserved intervention to manage soil and water for agricultural and wild biodiversity, minimizing the use of external inputs. Systems biology has the potential for designing of crop plants as per need of the time via decoding the intricate nature of different traits such as growth and development, nitrogen use efficiency, water use efficiency, photosynthetic efficiency, plant architecture, abiotic and biotic stress tolerance and, macronutrient and micronutrient improvement for filling the gaps between genotype and phenotype to producing smart crop plants for sustainable agriculture, and boosting their yield potential with enough nutrients for health (Kumar et al. 2015).

16.7 Systems Biology for Food Productivity

Physiology of plants is an assembly of distinct biological phenomena ranging from intracellular molecular communications to the entire phenotypic response. Systems-based approaches can decode these multi-scale communication networks and bridge the connection between genotype and phenotype (Muers 2011; Kumar et al. 2015). The organization and dynamics of these communication networks are accountable for the management of a cell's phenotypic condition. Various cells and different tissues co-ordinate to generate an organ level response that further controls plant physiological states concerning a particular condition, such a strategy would be very helpful in understanding and handling issues related to food and health (Mochida and Shinozaki 2011). In recent years, systems-based approaches have got tremendous demand in food and agricultural research for augmenting productivity and quality of food (Fig. 16.1).

16.7.1 Breaking Yield Barriers

The key objective of scientists working in the field of crop improvement is to maximize yield. The world's population is increasing rapidly, and large quantities

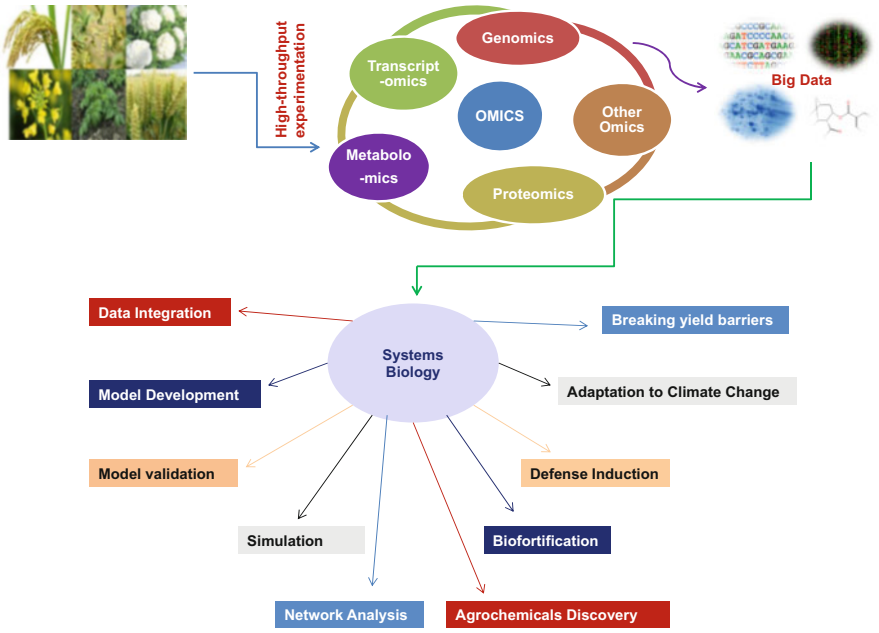

Fig. 16.1 Systems biology approach for understanding big data about plants and their application for improving food productivity

of food products are needed to save people's lives. Recent developments in system biology can decode the complexity of the multiple traits associated with yield via integration, and analysis of big molecular data (Sheehy et al. 2008; Kumar et al. 2015). This will lead to enhance photosynthesis, water use efficiency, nitrogen use efficiency and modify other physiological mechanisms through biotechnological approaches for the development of crop plants with higher yield potential.

16.7.2 Adaptation to Climate Change

Crop plants are highly affected by high or low temperatures, shortages and excess of water during growing, flowering, fruiting and ripening stages of their life, and these are directly linked to our food and health. Recently published data have depicted the opportunity of the design of adaptation to climate change strategies (1) a better understanding of driving procedures under future climate change, and (2) a combination of genetic and crop development models may be at the expenditure of a number of the genes/traits examined. Significantly, the latter may involve extra complexity in modeling research of crop systems. System biology methods will, therefore, be helpful for modularity in crop models as well as testing of the individual component against observational data that would be one of the key elements in an attempt to simulate crop breeding strategies under future climate situations

(Ramirez-Villegas et al. 2015; Kumar et al. 2015). The information obtained from the simulation will definitely help in the development of climate-friendly crop plants, which may easily adopt in any climate condition.

16.7.3 Defense Induction and Management of Crop Plant Diseases

Resistance development through defense induction and management of diseases has always been the primary goal of any crop improvement program. Plant–pathogen interaction is a well-described mechanism that includes activating different signaling pathways during the manifestation of defense response through pathogen attack. This type of response makes it easier for the host plant to prevent further infections. The use of holistic systems-based approaches for disease resistance and management could not only help us to understand plant defense signals but could also reveal novel insights into the networks of molecular interactions associated with de novo resistance development and food productivity (Pathak et al. 2017a).

16.7.4 Biofortification and Development of Nutraceutical

Biofortification and development of nutraceuticals are a primary goal to enrich our plant products with vital micronutrients and proteins. Biofortification of staple food crops has been discovered to fix the issue of malnutrition associated with poor people found in rural areas. They also play an essential role in designing and development of functional foods (nutraceuticals) for nutrition, health, and well-being through the implementation of systems biology approaches (Pathak et al. 2018a). Recent studies have shown that much research is needed to develop novel crop plant varieties with improved dietary quality using interdisciplinary methods to accomplish the objective of offering additional health advantages to the society (Ciccolini et al. 2017; Yadava et al. 2018).

16.7.5 Identification of Agrochemicals

Crop protection chemistry plays a crucial role in ensuring enough food supply to a rapidly growing world population. In the view of ever more stringent demands about efficacy, potency, and environmental safety, the finding of new agrochemicals has become a complex and resource-intensive undertaking (Lamberth et al. 2013). Remarkable increase in information on genome sequences, PPIs are helping in identification of molecular targets for agrochemical discovery through systems biology, in combination with advances in bioinformatics and computational chemistry opens up exciting opportunities for application of molecular modeling in prediction of 3D structure of targets and their interaction studies with small molecules, driven by rapidly improving algorithms assist to tackle today's challenges of pest and pathogen resistance for sustainable agriculture with cost-

effectiveness (Lamberth et al. 2013; Pathak et al. 2016; Verma et al. 2017; Mamgain et al. 2018).

16.8 Systems Biology Approaches for Health: Personalized Nutrition Based on Diet

Personalized nutrition is rapidly becoming a reality owing to several technological, science, and social innovations complementing and extending current suggestions on nutrition for public health. It provides nutritional suggestions based on a person's health status and particular biological requirements (Fig. 16.2). The biology that strengthens these suggestions is complicated, and therefore any suggestions must take into consideration for various biological processes and subprocesses that occur in different tissues, and how dietary nutrients interact with these processes and environmental variables. However, after the publication of the Human Genome Project (HGP), researchers are continuously focusing and working in the area of nutrigenomics. It is an emerging field of nutritional science that utilizes omics science and technology to search, access and decipher the various responses obtained through an administration of a certain diet among people or any population

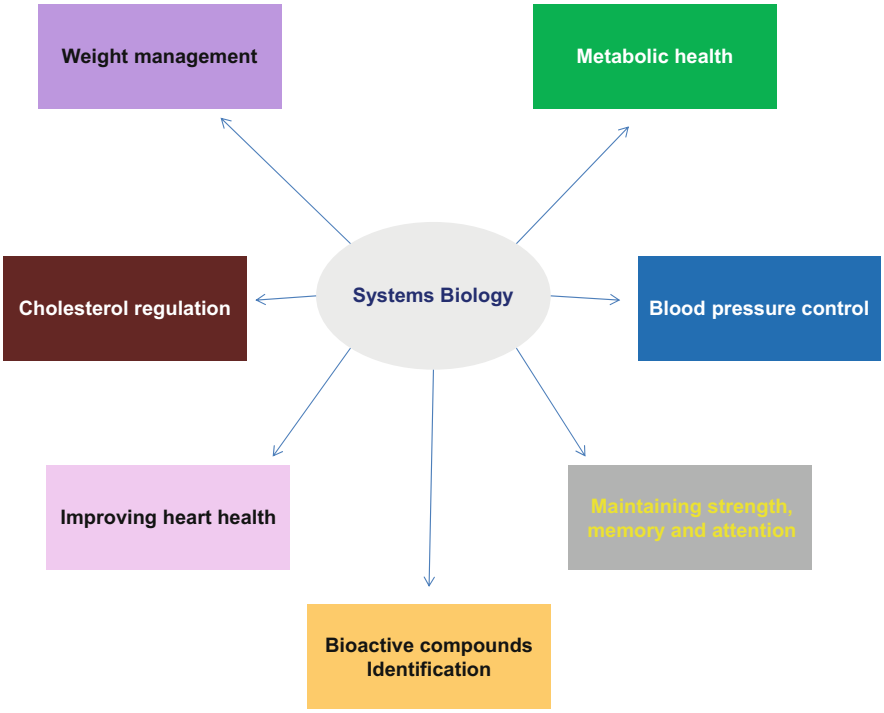

Fig. 16.2 Systems biology for understanding and improving health status

(Sales et al. 2014). Therefore, a system-based approach that considers the most appropriate interacting biological mechanisms is necessary to formulate the best suggestions via integration and modeling of nutri-genomics and other omics data to help people for achieving their wellness objectives (van Ommen et al. 2017).

16.8.1 Weight Management

Overweight can boost the risk of many issues with health, including diabetes, heart disease, and many other illnesses. Keeping an optimal body weight and or body shape is necessary for a healthy life but it is directly linked with our food lifestyle (van Ommen et al. 2017; Ramos-Lopez et al. 2017). Therefore, due to advances in systems biology, it will be possible to recommend a diet based on the genome organization of people for weight management.

16.8.2 Metabolic Health

People can use energy from all three sources of macronutrients (carbohydrate, protein, and lipid) and can deal with extreme ratio modifications between the three. However, macronutrient abundance and decrease have their advantages and disadvantages depending on the state and/or objectives of a people health; however, nutritional suggestions are often widespread, which can lead individuals to receive health advice or change their diet in ways that are counterproductive to personal and overall public health objectives (van Ommen et al. 2017; Ramos-Lopez et al. 2017).

16.8.3 Cholesterol Regulation

Dysregulation in cholesterol metabolism is a serious problem because it is linked to many diseases. Recently, a whole-body cholesterol metabolism mathematical model was developed to predict its dynamics and dysregulation concerning age. Such types of models can explore possible nutritional and lifestyle schemes that can mitigate the impacts of aging on the metabolism of cholesterol (Mc Auley et al. 2012; Ramos-Lopez et al. 2017). Therefore, reduce and optimize the equilibrium between high-density lipoprotein and low-density lipoprotein cholesterol in people who are affected is a major objective of systems biology for the investigation of appropriate diet composition.

16.8.4 Blood Pressure Control

A high protein diet can control the level of blood pressure and decrease the risk of hypertension (Rebholz et al. 2012; Buendia et al. 2014). Evidence is contradictory in terms of total fat consumption. The Dietary approaches to stop hypertension

(DASH) diet, designed to reduce hypertension, limits total fat to around 27E%. Nevertheless, evidence suggests that not only the total quantity, type, and source of fat are important (Houston 2014). Maintaining the level of blood pressure is a challenging task, and the application of systems biology can solve such type of intricate problems (van Ommen et al. 2017).

16.8.5 Improving Heart Health

Several processes can lead to hypertension, which including endothelial dysfunction, renin-angiotensin-aldosterone dysfunction, and or disruption of folate/homocysteine pathways (Pilic et al. 2016; van Ommen et al. 2017). Vitamin C has been suggested to improve endothelial function and thus health status (Houston 2014). Supplementation with vitamin C in patients with diabetes, atherosclerosis, and heart failure has been shown to enhance endothelial function, but no effect has been observed in healthy volunteers (Ashor et al. 2015). Diet having low salt, and more potassium is found to be beneficial in the control and prevention of hypertension (Aaron and Sanders 2013; Aburto et al. 2013; van Ommen et al. 2017). Therefore, more research is needed for predicting and validating the response of particular diets and recommending it for the functioning of the heart.

16.8.6 Maintaining Strength, Memory and Attention

Feeling powerful, avoiding muscle tiredness, maintaining and achieving optimum short-term and or working memory, and maintaining and achieving optimum attention is a personal goal (van Ommen et al. 2017). However, this will completely depend on our diets and lifestyle, but generally, we can see that the strength, memory, and attention are reduced rapidly in many people's specially children due to malnutrition (Kar et al. 2008). Besides, it will also reduce in old peoples. Therefore, we can design functional food and nutraceutical to overcome such problems. Systems biology can help to predict the response of diets in such conditions to achieve personal goals. This is a prerequisite for the professional and reliable development and supply of personalized nutrition in a healthy community.

16.8.7 Identification of Bioactive Compounds for Treatment of Human Diseases

Food bioactive compounds are constituents of extra nutrition that typically found in food but in a small quantity. Many bioactive compounds seem to have positive impacts on health. A lot of scientific research is required before we can begin to build science-based recommendations of diet. Nevertheless, there is adequate evidence that food rich in bioactive compounds are recommended (Chinchole et al. 2017; Kokane et al. 2018; Teodoro 2019). Identification of such bioactive compounds from

food can be screened against a particular target(s) followed by analysing its pharmacokinetics, i.e. absorption, distribution, metabolism, and excretion (ADME), and pharmacodynamics, i.e. toxicity (T) study and prediction of response against particular disease condition using systems biology. This leads to the identification of natural drug-like molecule(s), which can be utilized for treatment and prevention of diseases (Sagar et al. 2014; Pathak et al. 2018b; Rana et al. 2019).

16.9 Future Scope

The growing demands for healthy food and major challenges in production are the key factors that force the scientific community to adopt innovative approaches. Systems biology-based approaches have tremendous potential to decode the big experimental data associated with plant growth and their development, plant–pathogen interactions, and its physiological processes, and other pathway and network associated with crop plant yields and nutritional quality of seeds for improving food productivity, and food with a rich amount of nutrients. Besides, it also can recommend diets by analyzing and simulating nutrigenomics data for our healthy life. Therefore, the availability of big omics data about crop plants in public domain and continuous sequencing of crop plant genomes and their improved assembly data along with generation of other data, i.e. transcriptome, proteome and metabolome developing a road map for producing sufficient amount of foods. The data of nutrigenomics will also help in understanding food-gene interaction at molecular level through systems biology. Such information will be beneficial for precise nutrition that recommending personalized diets for resolving several health-related problems and ensuring a good life.

16.10 Conclusion

Good nutrition is essential for better health and prevention, therapy and management of illness, and is directly linked to food. The world's population is growing rapidly, requiring more and more food to satisfy people's needs. The bottom-up and top-down approaches of systems biology enable us to offer new molecular perspectives via visualizing the whole crop plants systems through breaking the yield barrier and dissecting the complexity of nutrients pathway for increasing crop production with good quality seed and its nutritional content. Progress in system biology continues, and accessible databases are updated on time with new data as well as software and an algorithms development program are also running in many research institutions and software R&D divisions of the worlds with aim of systems analysis and formulation of mathematical models describing the structure of the systems for novel discovery. The molecular sketch thus obtained from such analysis will be useful for fulfilling the demand of food and health in the future.

References

Aaron KJ, Sanders PW (2013) Role of dietary salt and potassium intake in cardiovascular health and disease: a review of the evidence. In Mayo clinic proceedings Amsterdam, Elsevier 88:9, pp. 987-995

Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP (2013) Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ 346:f1378

Ashor AW, Siervo M, Lara J, Oggioni C, Afshar S, Mathers JC (2015) Effect of vitamin C and vitamin E supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials. Br J Nutr 113(8):1182–1194

Buendia JR, Bradlee ML, Singer MR, Moore LL (2014) Diets higher in protein predict lower high blood pressure risk in Framingham offspring study adults. Am J Hypertens 28(3):372–379

Castrillo JI, Pir P, Oliver SG (2013) Yeast systems biology: towards a systems understanding of regulation of eukaryotic networks in complex diseases and biotechnology. In: Handbook of systems biology. Academic Press, San Diego, pp 343–365

Chinchole M, Pathak RK, Singh UM, Kumar A (2017) Molecular characterization of EcCIPK24 gene of finger millet (Eleusine coracana) for investigating its regulatory role in calcium transport. 3 Biotech 7(4):267

Ciccolini V, Pellegrino E, Coccina A, Fiaschi AI, Cerretani D, Sgherri C, Quartacci MF, Ercoli L (2017) Biofortification with iron and zinc improves nutritional and nutraceutical properties of common wheat flour and bread. J Agric Food Chem 65(27):5443–5452

Gupta PKP (2018) Biological systems and pathway modeling approaches. Austin J Biotechnol Bioeng 5(3):1099

Gupta MK, Misra K (2016) A holistic approach for integration of biological systems and usage in drug discovery. Network modeling analysis in health informatics and bioinformatics 5(1):4 Vienna Springer

Gupta MK, Singh DB, Shukla R, Misra K (2013) A comprehensive metabolic modeling of thyroid pathway in relation to thyroid pathophysiology and therapeutics. OMICS 17(11):584–593

Hasty J, McMillen D, Isaacs F, Collins JJ (2001) Computational studies of gene regulatory networks: in numero molecular biology. Nat Rev Genet 2(4):268

Houston M (2014) The role of nutrition and nutraceutical supplements in the treatment of hypertension. World J Cardiol 6(2):38

Junker BH, Schreiber F (2008) Analysis of biological networks. Wiley Interscience, Somerset. ISBN: 978-0-470-04144-4

Kar BR, Rao SL, Chandramouli BA (2008) Cognitive development in children with chronic protein energy malnutrition. Behav Brain Funct 4(1):31

Kitano H (2002) Computational systems biology. Nature 420(6912):206

Kitano H (2015) Accelerating systems biology research and its real world deployment. NPJ Syst Biol Appl 1:15009

Kokane SB, Pathak RK, Singh M, Kumar A (2018) The role of tripartite interaction of calcium sensors and transporters in the accumulation of calcium in finger millet grain. Biol Plant 62 (2):325–334

Kumar A, Pathak RK, Gupta SM, Gaur VS, Pandey D (2015) Systems biology for smart crops and agricultural innovation: filling the gaps between genotype and phenotype for complex traits linked with robust agricultural productivity and sustainability. OMICS 19(10):581–601

Kumar A, Pathak RK, Gayen A, Gupta S, Singh M, Lata C, Sharma H, Roy JK, Gupta SM (2018) Systems biology of seeds: decoding the secret of biochemical seed factories for nutritional security. 3 Biotech 8(11):460

Lamberth C, Jeanmart S, Luksch T, Plant A (2013) Current challenges and trends in the discovery of agrochemicals. Science 341(6147):742–746

Mamgain S, Dhiman S, Pathak RK, Baunthiyal M (2018) In'silico'identification of agriculturally important molecule (s) for defense induction against bacterial blight disease in soybean (Glycine max). Plant Omics 11(2):98

Mc Auley MT, Wilkinson DJ, Jones JJ, Kirkwood TB (2012) A whole-body mathematical model of cholesterol metabolism and its age-associated dysregulation. BMC Syst Biol 6(1):130

Mochida K, Shinozaki K (2011) Advances in omics and bioinformatics tools for systems analyses of plant functions. Plant Cell Physiol 52(12):2017–2038

Muers M (2011) Systems biology: plant networks. Nat Rev Genet 12(9):586

Panagiotou G, Nielsen J (2009) Nutritional systems biology: definitions and approaches. Annu Rev Nutr 29:329–339

Pathak RK, Taj G, Pandey D, Arora S, Kumar A (2013) Modeling of the MAPK machinery activation in response to various abiotic and biotic stresses in plants by a system biology approach. Bioinformation 9(9):443

Pathak RK, Taj G, Pandey D, Kasana VK, Baunthiyal M, Kumar A (2016) Molecular modeling and docking studies of phytoalexin (s) with pathogenic protein (s) as molecular targets for designing the derivatives with anti-fungal action on'Alternaria'spp. of'Brassica'. Plant Omics 9(3):172

Pathak RK, Baunthiyal M, Pandey N, Pandey D, Kumar A (2017a) Modeling of the jasmonate signaling pathway in Arabidopsis thaliana with respect to pathophysiology of Alternaria blight in Brassica. Sci Rep 7(1):16790

Pathak RK, Baunthiyal M, Shukla R, Pandey D, Taj G, Kumar A (2017b) In silico identification of mimicking molecules as defense inducers triggering jasmonic acid mediated immunity against alternaria blight disease in brassica species. Front Plant Sci 8:609

Pathak RK, Baunthiyal M, Pandey D, Kumar A (2018a) Augmentation of crop productivity through interventions of omics technologies in India: challenges and opportunities. 3 Biotech 8(11):454

Pathak RK, Gupta A, Shukla R, Baunthiyal M (2018b) Identification of new drug-like compounds from millets as xanthine oxidoreductase inhibitors for treatment of Hyperuricemia: a molecular docking and simulation study. Comput Biol Chem 76:32–41

Pilic L, Pedlar CR, Mavrommatis Y (2016) Salt-sensitive hypertension: mechanisms and effects of dietary and other lifestyle factors. Nutr Rev 74(10):645–658

Ramirez-Villegas J, Watson J, Challinor AJ (2015) Identifying traits for genotypic adaptation using crop models. J Exp Bot 66(12):3451–3462

Ramos-Lopez O, Milagro FI, Allayee H, Chmurzynska A, Choi MS, Curi R, De Caterina R, Ferguson LR, Goni L, Kang JX, Kohlmeier M (2017) Guide for current nutrigenetic, nutrigenomic, and nutriepigenetic approaches for precision nutrition involving the prevention and management of chronic diseases associated with obesity. J Nutrigenet Nutrigenomics 10 (1-2):43–62

Rana G, Pathak RK, Shukla R, Baunthiyal M (2019) In silico identification of mimicking molecule (s) triggering von Willebrand factor in human: a molecular drug target for regulating coagulation pathway. J Biomol Struct Dyn 14:1–3

Rebholz CM, Friedman EE, Powers LJ, Arroyave WD, He J, Kelly TN (2012) Dietary protein intake and blood pressure: a meta-analysis of randomized controlled trials. Am J Epidemiol 176 (suppl_7):S27–S43

Sagar M, Pathak RK, Pandey RK, Singh DB, Pandey N, Gupta MK (2014) Binding affinity analysis and ADMET prediction of epigallocatechine gallate (EGCG) derivatives for AP-1 protein: a drug target for liver cancer. Network modeling analysis in health informatics and bioinformatics 3(1):66 Vienna Springer

Sales NM, Pelegrini PB, Goersch MC (2014) Nutrigenomics: definitions and advances of this new science. J Nutr Metab 2014:1

Shameer K, Naika MB, Shafi KM, Sowdhamini R (2019) Decoding systems biology of plant stress for sustainable agriculture development and optimized food production. Prog Biophys Mol Biol 145:19

Sheehy JE, Gunawardana D, Ferrer AB, Danila F, Tan KG, Mitchell PL (2008) Systems biology or the biology of systems: routes to reducing hunger. New Phytol 179(3):579–582

Snider J, Kotlyar M, Saraon P, Yao Z, Jurisica I, Stagljar I (2015) Fundamentals of protein interaction network mapping. Mol Syst Biol 11(12):848

Stitt M, Sulpice R, Keurentjes J (2010) Metabolic networks: how to identify key components in the regulation of metabolism and growth. Plant Physiol 152(2):428–444

Sullivan AM, Arsovski AA, Lempe J, Bubb KL, Weirauch MT, Sabo PJ, Sandstrom R, Thurman RE, Neph S, Reynolds AP, Stergachis AB (2014) Mapping and dynamics of regulatory DNA and transcription factor networks in A. thaliana. Cell Rep 8(6):2015–2030

Tagore S, Gomase VS, De RK (2008) Pathway modeling: new face of graphical probabilistic analysis. J Proteomics Bioinformatics 01:281–286

Tegnér JN, Compte A, Auffray C, An G, Cedersund G, Clermont G et al (2009) Computational disease modeling - fact or fiction? BMC Syst Biol 3:56

Teodoro AJ (2019) Bioactive compounds of food: their role in the prevention and treatment of diseases. Oxidative Med Cell Longev 2019:3765986

Van Ommen B, Stierum R (2002) Nutrigenomics: exploiting systems biology in the nutrition and health arena. Curr Opin Biotechnol 13(5):517–521

van Ommen B, van den Broek T, de Hoogh I, van Erk M, van Someren E, Rouhani-Rankouhi T, Anthony JC, Hogenelst K, Pasman W, Boorsma A, Wopereis S (2017) Systems biology of personalized nutrition. Nutr Rev 75(8):579–599

Varshney RK, Bansal KC, Aggarwal PK, Datta SK, Craufurd PQ (2011) Agricultural biotechnology for crop improvement in a variable climate: hope or hype? Trends Plant Sci 16(7):363–371

Verma S, Pathak RK, Kasana V, Kumar A (2017) Binding affinity analysis of cinnamanilide and α-aminophosphonic acid derivatives for acetohydroxyacid synthase through molecular docking. Int J Agri Environ Biotechnol 10(3):271

Wang RS, Saadatpour A, Albert R (2015) Boolean modeling in systems biology: an overview of methodology and applications. Phys Biol 9:055001

Yadava DK, Hossain F, Mohapatra T (2018) Nutritional security through crop biofortification in India: status & future prospects. Indian J Med Res 148(5):621

Yan H, Yoo MJ, Koh J, Liu L, Chen Y, Acikgoz D, Wang Q, Chen S (2014) Molecular reprogramming of Arabidopsis in response to perturbation of jasmonate signaling. J Proteome Res 13(12):5751–5766

Advances of Next-Generation Sequencing (NGS) Technologies to Enhance the Biofortifications in Crops

17

Nisha Singh, Vacha Bhatt, Nitika Rana, and S. M. Shivaraj

Abstract

The modern-day agriculture is feeding the global population but not providing adequate essential nutrients which are known as "*Hidden Hunger*." Deficiency of essential nutrients in daily diet is the cause of extensive health problems in developing countries, biofortification is the process intended to improve the nutrition level in all crops. The improvement of crop cultivars with increased level of nutrients in their edible parts is necessary to cope with nutrient deficiencies. Biofortification is the process of crafting nutritionally dense crops containing vitamins, minerals, and crucial macro-micronutrients through conventional plant-breeding methods, agronomic practices, genetic engineering, and next-generation sequencing (NGS) technologies. Rapid advancements in omics-NGS technologies have contributed immensely to the improvement of crop plants. Here, we summarized recent advances in NGS technologies that have contributed toward the progress in crop biofortification. As the cost of sequencing has declined, breeders started employing cutting-edge NGS technologies to sequence large plant populations to identify the genetic basis of agriculturally important traits and to predict the breeding value of individuals. Modern

N. Singh (✉)
Institute for Genomic Diversity, Cornell University, Ithaca, New York, USA

ICAR-National Institute for Plant Biotechnology, New Delhi, India
e-mail: ns868@cornell.edu

V. Bhatt
Savitribai Phule Pune University, Pune, Maharashtra, India

N. Rana
National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India

S. M. Shivaraj
National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India

University Laval, Quebec City, QC, Canada

T. R. Sharma et al. (eds.), *Advances in Agri-Food Biotechnology*,
https://doi.org/10.1007/978-981-15-2874-3_17

molecular breeding techniques like genome-wide association study (GWAS) and genomic selection (GS) are increasing the efficiency of breeding to enhance the rate of nutritionally rich cultivars of food crops in the most cost-efficient way. The biofortification of crops opens a new arena to address the malnutrition challenge that the world is facing.

Keywords

Biofortification · Nutrient deficiency · Micronutrients · NGS technologies · Nutrition

17.1 Introduction

The global population is increasing rapidly, and it is estimated that by 2050 it will reach 10 billion, which would require an increased cereal production (Ray et al. 2012). Thus, to suffice the food requirement in the future, scientists need a "second green revolution" to fulfill the needs of a growing population by increasing the yield potential of crop varieties. However, the narrow genetic base of the cultivated crop varieties is causing problems to achieve this. To break the yield plateau, it is critical to expand the genetic base of the varieties by introgression of novel genes. Apart from enhancing crop yield, it is necessary to provide attention to grain quality and nutritional aspects since half of the world's population is suffering from vitamin and mineral deficiency (World Food Program 2015). More than three-billion people are suffering from micronutrient deficiency and 3.1 million children die each year due to malnutrition (Gearing 2015). In developed countries, fortification programs are adopted to manage nutrient deficiency, but a similar strategy is not affordable in developing countries. Hence, an alternative and cost-effective strategy are required to improve the nutritional and nutraceutical quality of the food grains consumed by the people. To improve the nutritional quality of important cereals and pulses, research should be focused on the development of high-yielding cultivars with enhanced nutrients through selective breeding or genetic modification (Garg et al. 2018; Rana et al. 2019). The cereals such as rice, wheat, maize, cassava are the major part of the diet of the population and they contain an insufficient amount of vitamin A, iron, zinc, calcium, iodine, manganese, copper, or selenium compared to recommended daily requirements. Among the cereals, rice is consumed by more than half of the world's population and the rice grain is relatively low in essential micronutrients such as iron (Fe), zinc (Zn), and calcium (Ca) as compared to other cereals, pulses, and tubers (Adeyeye et al. 2000). However, the rice bran, the outer layer of the grain is an important source of protein, vitamins, minerals, phytosterols, and antioxidants (Iqbal et al. 2005; Schramm et al. 2007). Rice bran protein is having unique nutraceutical and anti-cancer properties, hence used potentially by industries (Saunders 1990; Shoji et al. 2001). It is also used as a hypoallergenic food ingredient in the infant (Helm and Burks 1996). Rice bran will be removed from the grains during processing hence improvement in these components within the rice grains will help to reduce malnutrition. Several conventional plant-breeding approaches

have been employed to produce new varieties with improved nutrient content besides increasing yield potential. Traditional plant breeding involves identification and development of parental lines with increased nutrient content and crossing them with elite lines and selecting the segregants for several generations to produce plant genotypes with the desired nutrient and agronomic traits (Pfeiffer and McClafferty 2007). The recent advances in crop genomics, specifically the application of high-throughput NGS technologies empower the decoding sequence of whole genome and identification of complete gene set of a species. Similarly, the transcriptomics allows the study of the expression pattern of an entire genes catalogs. Furthermore, the sequence data provide information about the basis of genetic variation in the form of single-nucleotide polymorphisms (SNPs), InDels, and expression level which further provide a relationship between genotype and phenotype. Evaluation of the existing genetic diversity in different germplasm resources is essential for the detection of novel genes or quantitative trait locus (QTL) for nutrition and important quality traits. Mining of these useful alleles from wild relatives and landraces has relevance in crop improvement. To improve agronomic and quality traits of crops the in-depth analysis of population structure, their characterization and evolutionary relationships among cultivar groups are required for the efficient breeding program. The germplasm utilization strategies involve the collection and their characterization to comprehend the basic morphological and molecular variations of nutrient contents available in wild crop relatives and landraces.

17.1.1 Need for Biofortification in Crops

The continued consumption of nutrient deficient food results in poor health, disability, impaired development, stunted mental and physical growth, diminished livelihoods, increased morbidity (Caballero 2002). Biofortification is one of the most environmentally sustainable, long-term solution and economically viable strategy to combat malnutrition. Biofortification uses plant breeding, and advanced molecular breeding and genetic engineering techniques to produce crop plants with increased micronutrient levels, reduced antinutrient substances, and increased levels of promoters which enhance the nutrient absorption/bioavailability (Bouis et al. 2013). Now, it is important to eradicate malnutrition in the human population by feeding micronutrient-rich food grains derived by combining available genetic resources. Biofortification is essentially aided by mining crop genomes to obtain genes of interest. In past decades, several biofortified varities have been released especially in staple crops, for those reference genomes are present in the public domain (Table 17.1). To meet the specific dietary requirements of rural populations, it is necessary to biofortify crops to enhance micronutrient levels. For example, iron-biofortification in rice, beans, sweet potato, cassava, and legumes. Zinc-biofortification of wheat, rice, beans, sweet potato, and maize. Provitamin A carotenoid profuse biofortification of sweet potato, maize, and cassava. Protein and amino acid biofortification of sorghum and cassava. The NGS technologies will drive the

Table 17.1 List of biofortified crops released

Crop	Nutrient	Countries of first release	Release year
Sweet potato	Provitamin A	Mozambique, Uganda	2007
Cowpea	Iron	India	2008
Beans	Iron, zinc	Rwanda, DRC	2012
Pea	Iron, zinc	USA	2012
Pearl millet	Iron, zinc	India	2013
Cassava	Provitamin A	Nigeria	2013
Maize	Provitamin A	Zambia, Nigeria	2012
Rice	Iron, zinc	Bangladesh, India	2013
Wheat	Iron, zinc	India, Pakistan	2013
Mung Bean	Iron, zinc	India	2013
Lentils	Iron, zinc	India, Nepal, Bangladesh	2012, 2013, 2013

way forward for the discovery and transfer of genes and QTL associated with an these improved nutritional profile from the diverse crop genetic resources.

17.1.2 Methods to Develop Biofortified Crops

There are different approaches adopted to implement biofortification in crop plants, and the approach varies from crop to crop (Fig. 17.1). With the recent advancement of genomic technologies and their employment for GS, GWAS, marker-assisted selection (MAS), agronomic intervention, genetic transformation along with the conventional breeding methods and agronomic intervention will boost the biofortification in crops. However, conventional breeding methods were based on rigorous phenotypic selection, thus it consumes more time to develop nutrition-dense elite crop varieties. In this context, combined approach with use of both the NGS technologies and conventional breeding methods will accelerate crop improvement. To develop a biofortified crop the primary step is to identify representative targeted populations. The selection should be made for the prevalent micronutrient deficiencies, the primary crop which is highly produced and consumed, and the importance should be given to self or locally produced plants (Ortiz-Monasterio et al. 2007). Therefore, germplasm collection is a good initiative for biofortification of crops. Screening germplasm for the genotypes, and sorting them which can accumulate different level of nutrients in grains to construct a Germplasm Association Panel (GAP) for that particular crop. Subsequently, the identified genotypes are used for mapping of genotypic differences. Crossing promising genotypes obtained from GAP and selecting the progenies with desirable agronomic traits for several generations to develop new biofortified varieties. The novel biofortified varieties are then tested at multiple locations, to assess genetic and environment (G $\times$ E) interactions. It is reported that the variability of mineral content in the germplasm is determined by the genotype, the environment, and their interaction, but the impact of these factors differs for different minerals and crops. Once the variety is developed, the most important aspect is to assess consumer acceptance by

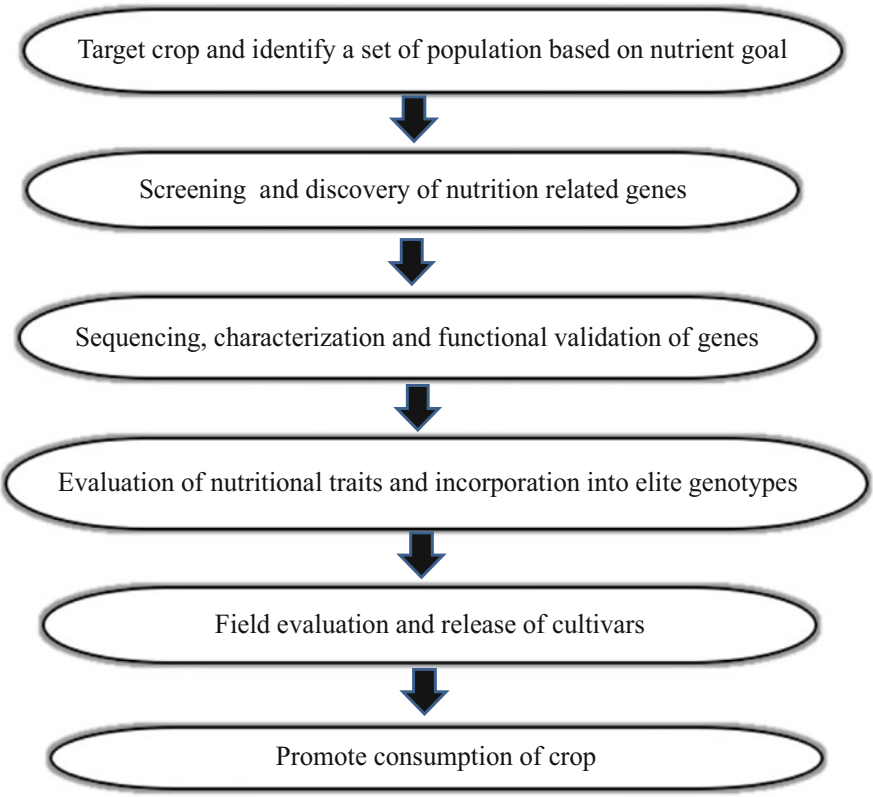

Fig. 17.1 Generalized strategy being used to develop biofortified crop varieties

evaluating appearence, taste, and cooking quality (Khoshgoftarmanesh et al. 2010). Finally, the functioning of the newly released variety was analyzed in terms of micronutrient retention, micronutrient bioavailability and its impact on human health.

17.2 Role of NGS Technologies to Enhance the Biofortification in Crops

During the last decade, research has shown that biofortification is an effective complement for those approaches undertaken to address issues related to micronutrient deficiency and human health problems (Saltzman et al. 2013). Biofortification involves increasing the micronutrient content or its bioavailability in targeted crop plants, widely consumed by low-income families globally. NGS assisted the high-throughput decoding of several crop genomes has a way to revolutionize the functional genomics, genetics, and breeding applications. Several resequencing projects in diverse crops have contributed enormously to the information on the genome-wide structural variations and genetic diversity. Currently, DNA markers

are extensively used for genetic diversity analysis, association mapping, evolutionary studies, fingerprinting, and breeding applications. They are immensely useful in developing improved nutritional rich crop varieties and have reduced time in releasing new crop varieties. In this context, NGS technologies generate huge amounts of sequence data, providing cost-effective genome-wide sequence reads leading to the discovery of several SNPs. The most powerful advantages of NGS technologies is SNP genotyping, a rapid and cost-effective tool to genotype breeding populations, which assists breeders to implement molecular marker discovery, genomic diversity study, genetic linkage analysis, haplotype-based gene analysis, GWAS, and genomic selection (He et al. 2014).

The recent development in sequencing technologies includes first generation sequencing technology (FGS) like, Sanger sequencing. Second-generation technologies (SGT), such as GS FLX Titanium/GS Junior, Roche/454 FLX Pyrosequencer, Solid Sequencer (Applied Biosystems), Genome Analyzer (Solexa/Illumina), and third-generation sequencing (TGS) technologies, such as HiSeq/MiSeq from Illumina, PacBio and Oxford Nanopore Technology, Ion Torrent PGM/Proton (Life Technologies). Among these the third-generation NGS technologies have gained immense popularity in recent years because of their throughput, read lengths, and low sequencing cost. Comparison of different NGS platforms and their applications is listed in Table 17.2. NGS technologies are commonly used for quantitative trait mapping (QTL mapping), epiQTL analysis, TILLING study, HapMap, mutational map (MutMap), GWAS, genotyping by sequencing (GBS), genomic selection (GS),whole-genome sequencing (WGS), whole-genome resequencing (WGRS), de novo sequencing, whole-genome bisulfite sequencing (WGBS), exome sequencing, transcriptomics, differential gene expression and epigenetic (MeDIP; ChIP)/analysis, restriction-site-associated DNA sequencing (RAD-seq), small RNA profiling, SHORE map (Zargar et al. 2015; Bhat et al. 2018; Chaudhary et al. 2019; Kumawat et al. 2019). In this perspective, NGS technologies assist us to examine the nutrition traits, pathways, and accumulation mechanisms. Using collective genomics, population genetics, quantitative genetics, transcriptomics, proteomics, metabolomics, ionomics, and nanotechnology approaches enhanced our understanding of the precise selection of particular trait of interest (Figs. 17.2 and 17.3).

17.2.1 Single-Nucleotide Polymorphism (SNP) and Its Applications in Crops

SNP markers could play a significant role to enhance the qualitative and quantitative traits in crop plants in a short time frame. Earlier, the plant geneticist and breeders were using traditional molecular markers, such as restriction fragment length polymorphism (RFLP) (Botstein et al. 1980), random amplified polymorphic DNA (RAPD) (Williams et al. 1990), simple sequence repeats (SSR) (Zietkiewicz et al. 1994). Amplified fragment length polymorphism (AFLP) (Mueller and Wolfenbarger 1999) was used to identify the genetic basis of complex traits in

Table 17.2 Comparison of different NGS platforms and their applications

NGS platforms	Applications	Features and costs	Bioinformatics challenges
MiniSeq and MiSeq	Small whole genome, targeted gene sequencing, targeted gene expression profiling, small RNA and miRNA, DNA–protein interaction, 16S metagenomic sequencing	Low to mid sample throughput. 1.7–15 gb. Compatible with almost all types of applications. User-friendly workflow with no need for automation. Affordable instrument price, and reasonable per sample cost ($120 per 5 MB genome). Accuracy 99.9% for >75 to 90% of bases	Run and short-read length, synchronization problems
HiSeq	Throughput length 10–1000 gb. Whole-genome sequencing, population-scale studies, exome sequencing, targeted gene sequencing, complete transcriptome, gene expression profiling, small RNA and miRNA, methylation, shotgun metagenomics. DNA–protein interaction	The high initial investment, run length, read accuracy, throughput, low per sample cost. Accuracy 99.9% for >75 to 85% of bases	Phasing and synchronization problems
NovaSeq	Read accuracy, throughput 2000–6000 gb. Whole-genome sequencing, population-scale studies, exome sequencing, targeted gene sequencing, gene expression profiling, whole transcriptome, small RNA and miRNA, methylation, shotgun metagenomics, DNA–protein interaction	The high initial investment, run, and read length. Low per sample cost. Accuracy 99.9% for >75 to 85% of bases	Coverage, alignment, and phasing issues
Ion Torrent	Fast run time, a broad range of applications. Targeted DNA–RNA seq, exome sequencing, de novo sequencing, gene expression profiling, transcriptome profiling, small RNA sequencing, ChIP-seq	The library preparation process is lengthier read length, semi-conductor technology, no requirement for optical scanning and fluorescent nucleotides	High error rates in homopolymers

(continued)

Table 17.2 (continued)

NGS platforms	Applications	Features and costs	Bioinformatics challenges
10× Genomics Chromium	De novo genome assembly and scaffolding, phasing, identification of large structural variation (more than 10 kb), and single-cell based gene expression	Low cost and input DNA requirement linked reads (~100 kb) obtained from a collection of short-read sequences; moderately costlier than short reads	Sparse sequencing rather than true long reads; more difficult to align, with poorer resolution due to locally repetitive sequences. The paired-end library can be sequenced and later assembled into big scaffolds by one assembly program: SUPERNOVA
Oxford Nanopore Sequencing	De novo genome assembly, detection of structural variant, resolution of gene isoform epigenetic modifications, and metagenomics	Single-molecule long reads averaging ~10 kb with some >1 Mb; high read depth, accuracy, several times more costly than short reads. Fast run times and compact nature	Raw reads with high error rates containing false deletions and homopolymer errors; algorithms for new alignment and error correction are necessary
PacBio SMRT Sequencing	De novo genome assembly, detection of structural variant, resolution of gene isoform analysis of transcriptomes, structural variants, epigenetic modifications. Targeted transcript	Single-molecule long reads averaging ~10 kb and few approaching 100 kb; several times more costly than short reads	Raw reads with high error rates dominated by false insertions; algorithms for new alignment and error correction are required
Bionano Genomics Optical Mapping	Genome scaffolding and identification of large structural variants (more than 10 kb), de novo sequencing hybrid scaffolding, targeted sequencing, metagenomics, epigenetics	Optical mapping of long DNA fragments (~250 kb or longer) labeled with fluorescent probes; less costly than short reads	Fewer algorithms required to discover high-confidence alignment between sequence assembly and an optical map
Hi-C-based analysis	Construction of high-quality genome-wide chromatin maps with a very low amount of input material, sparse sequencing with the highly variable genomic distance between pairs (1 kb to >1 Mb), useful for high-quality draft assembly	Genome scaffolding and phasing, genomic distance is more predictable. Pairs of short reads (<100 bp) obtained from cross-linking chromatin interactions; moderately more costly than short reads	Sparse sequencing with the highly variable genomic distance between pairs (1 kb to 1 Mb or longer)

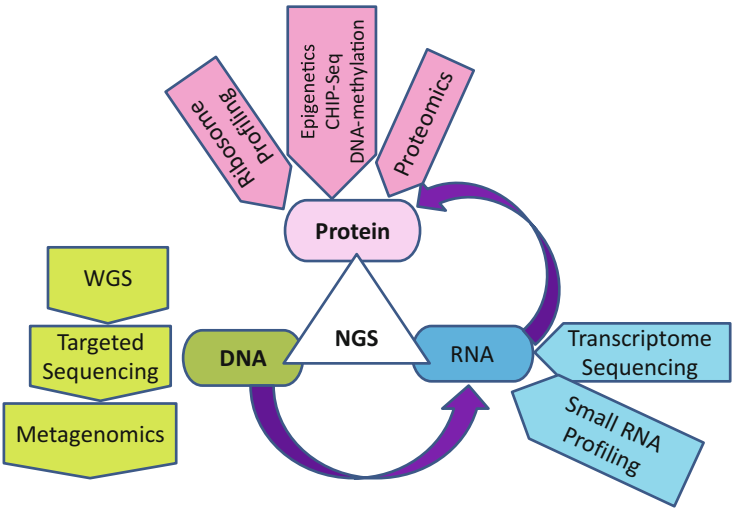

Fig. 17.2 Listed some of the different applications of NGS technologies that span across the central dogma of molecular biology

plants. But these markers have some limitations such as limited chromosome coverage, a low resolution, labor-intensiveness, and cost-effectiveness which resulted in a low-density linkage map. For fast-track development of superior nutrition-dense varieties, there is a need to map and tag agronomically important genes and understand the basis of allelic variation at these loci. The high-throughput SNP genotyping provided better altrnative to the traditional markers used in many breeding programs. However, it also imposes a series of organizational, economic, and technical hurdles. To resolve these problems, SNP based resources are being developed and made available to the public for application-based crop research (Singh et al. 2015). The preface of new generation sequencing technologies has dramatically changed the scenario for the detection and utilization of genome-wide polymorphisms (Feng et al. 2009). SNPs are known as new generation's most abundant molecular markers. They are found in both coding and non-coding regions of nuclear as well as plastid DNA (Kwok et al. 1996). Currently, various methods for SNP genotyping are available, although all of them may not be equally useful. The SNP genotyping assays can be broadly classified into two types as (1) gel-based and (2) non-gel based. Gel-based assay comprises of cleaved amplified polymorphic sequence (CAPS) markers (Thiel et al. 2004; Komori and Nitta 2005) or allele-specific amplification methods (Drenkard et al. 2000). Presently, the latter one is widely preferred by the scientific community because of its assay accuracy, high density, speed, robustness, simple data analysis, and cost-effectiveness (Gupta and Sankararamakrishnan 2009). A range of high-throughput SNP genotyping technologies are available to run flexible sets of SNP assays. In this context, the Illumina BeadArray Technology employs beads with specific oligos that fit into

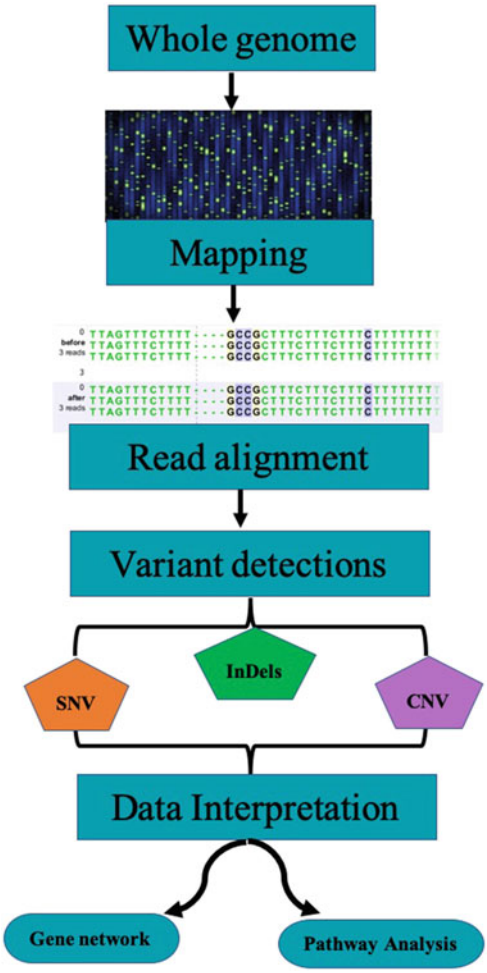

Fig. 17.3 The pipeline of NGS data analysis and applications

patterned microwells assisting highly multiplexed SNP detection. The GoldenGate assays employ allele-specific oligos for hybridization, subsequently allele-specific extension and fluorescent scanning (Shen et al. 2005). The BeadArray technology was further extended to high-density arrays using Infinium assays, on a two-color single base extension from a single hybridization probe per SNP marker. GoldenGate assays are using VeraCode microbeads with the BeadXpress Reader to genotype up to 384 SNPs with the fluidic system instead of printed arrays (Lin et al. 2009). Recently the Affymetrix Axiom technology, based on a two-color, ligation-based assay with 30-mer probes, allows simultaneous genotyping of 384 samples with 50 K SNPs or 96 samples × 650 K SNPs (Hoffmann et al. 2011). SNPs are widely used in breeding applications which include marker-assisted and genomic selection, association and QTL mapping, haplotype and pedigree

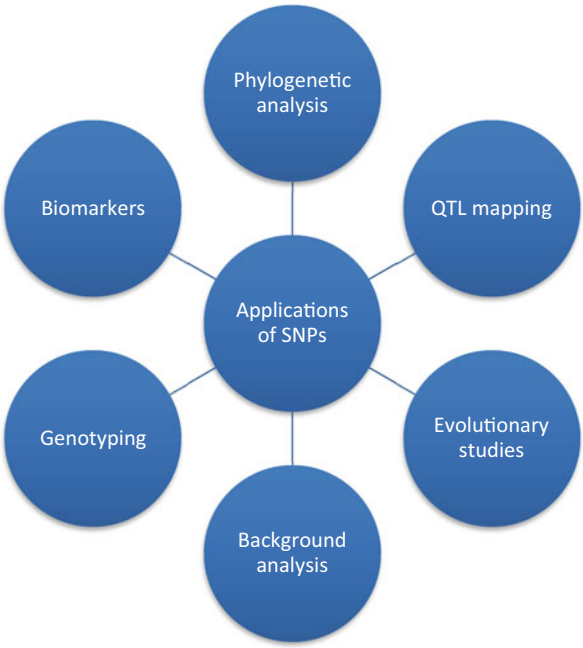

Fig. 17.4 Elucidation of several applications of SNPs for crop improvement

analysis, positional cloning, variety identification, and seed purity testing (Jannink et al. 2010). They assist breeders to analyze the array of alleles present in different germplasm resources and also to identify the combinations of alleles that perform better in target environments (Collard and Mackill 2007; Heffner et al. 2009; Jannink et al. 2010).

SNP resources also help breeders to more efficiently evaluate and utilize the wealth of natural genetic variations that exist in both wild and cultivated germplasm with the objective of improving the crop productivity. It is also employed in identifying recombination breakpoints in fine mapping of the genes within the identified QTLs in bi-parental crosses. Genewise SNP haplotype-based phylogenetic and network analysis in domestication genes was proven to trace the origin and domestication history of crops (Fig. 17.4). Agronomically important traits like hybrid vigor, grain yield, and quality are affected by many genes/QTLs each with a small effect on the trait. RICE6K showed that the array is suitable for rice germplasm fingerprinting, genotyping bulked segregant analysis (BSA), seed authenticity check, and genetic background selection (Yu et al. 2014). Similarly, Affymetrix 44 K array has been designed for rice SNP genotyping for association studies in rice (McCouch et al. 2010; Zhao et al. 2011). RiceSNP50 using Illumina Infinium platform was successfully used for variety verification and trait introgression and GWAS (Chen et al. 2014). Recently, a genome-wide high-density 700K SNP Affymetrix chip was designed for GWAS in rice to map grain length trait and for salt stress tolerance in rice (McCouch et al. 2016). Although all the

abovementioned SNP arrays are genome-wide representation except the gene-based 6K SNP Infinium array of stress-responsive genes in rice for genome-wide association mapping of salinity tolerance in rice (Kumar et al. 2015). Therefore, SNP markers have become extremely popular in plants to create supersaturated genetic maps, enabled genome-wide tracking, fine mapping of target regions, rapid association of markers with a trait, and accelerated cloning of gene/QTL of interest.

Hence, to date, more than 50 SNP arrays and 15 different types of genotyping by sequencing(GBS) platforms have been developed in more than 25 crop species including perennial trees, for instance, soybean (SoySNP50K) (Song et al. 2013), Cotton SNP63K (Hulse-Kemp et al. 2015), maize SNP600K (Unterseer et al. 2014), sunflower, Apple480K SNP (Bianco et al. 2016), groundnut *Arachis*58K SNP (Pandey et al. 2017), oat (Tinker et al. 2014), and wheat (Wang et al. 2014; Winfield et al. 2016) have accelerated the pace and gains of plant breeding. The GBS approach has been widely used in applications like GWAS, GS, and QTL mapping mostly due to the reduced cost and high-throughput level (Bastien et al. 2014; Sonah et al. 2015). The crop breeding programs are well versed with the NGS tools and the integrated omics approaches are being routinely employed for the development of high yielding, nutritive, and climate-smart novel varieties (Deshmukh et al. 2014; Chaudhary et al. 2015, 2019; Shivaraj et al. 2019).

SNP markers are highly abundant and biallelic nature which makes it more efficient and reliable for a variety of functions in crop improvement, including linkage map construction, genetic diversity analysis, marker-trait association, and MAS. The importance of having a large pool of available SNPs in a sub-set containing the most informative and useful SNPs can be chosen for varied applications. For some applications, it is important to select a sub-set of evenly distributed SNP markers. A key step is validating SNPs for their performance with specific marker assays, since not every SNP will be converted into a reliable marker across different genotyping systems. At present several genotyping technologies are available that can genotype thousands of markers simultaneously and can be applied in different species at the same time (Gupta et al. 2008). Two major types of high-throughput genotyping platforms are available in the market that can be used for genomic breeding, DNA sequencing (Davey et al. 2011), and DNA array. The Illumina offers two different types of genotyping platforms. GoldenGate array for medium-density genotyping contains 96-1536 SNPs per array, and the Infinium array for high-density genotyping contains up to 1 M SNPs per array (Fan et al. 2006). In this situation, GoldenGate assays based on VeraCode technology using Illumina BeadXpress seem to be the most cost-effective platform. The current high-throughput genotyping platforms, whether based on the Affymetrix GeneChip or Illumina's Beadchip, are able to deliver genome-wide coverage and robust performance that allows laboratories worldwide to successfully complete GWA studies (Table 17.3). The low-assay throughput technologies are robust and deliver high-sample throughput and play a key role in replication studies. SNP markers have gained considerable importance in plant genetics and breeding because of their excellent genetic attributes and suitability for genetic diversity analysis, evolutionary relationships, and understanding of population structure.

Table 17.3 Examples of high-throughput SNP genotyping technologies

Genotyping platforms	Technology	SNP × sample combinations	Capital investment	Cost per sample	Advantages
Illumina Infinium iSelect HD	Fixed array	3072–700 K SNPs × 24 samples	High (iScan)	Moderate to high	Highly multiplexed
Affymetrix Axiom	Fixed array	50 K SNPs × 384 samples; 650 K SNPs × 96 samples	High (GeneTitan)	Moderate to high	Highly multiplexed
Douglas Array Tape	Flexible, PCR-based	1 SNP/sample × 76,800 reactions/reel	Very high (Nexar, Soellex, Araya)	Very low	Ultra high throughput
Fluidigm Dynamic Arrays	Flexible, PCR-based	96 SNPs × 96 samples; 24 SNPs × 192 samples	Moderate (IFC Controller, FC1, EP1)	Low	High throughput
RE-based GBS	GBS	~10 K to 100 K SNPs × 96 or 384 samples	Low to moderate	Low to moderate	Lots of data relative to the cost
Amplicon sequencing	GBS	Variable (e.g., 20–500 SNPs × 48–384 samples)	Low to moderate	Low to moderate	Multiple targeted loci at once

17.2.2 Advantages and Applications of SNP Genotyping in Crop Breeding

Development and applications of molecular markers in plant breeding have received remarkable attention. It started with low throughput and reached up to high throughput as a major landmark was reached with the breakthrough of SNP markers. Earlier, SSR markers were widely used for genotyping but due to some limitations in practical plant breeding, it has been replaced with SNP markers. The limitations of SSR markers are that multiple alleles per locus, data from different platforms or populations can be difficult to compile and compare because it varies. SSR motifs are finite in a genome, unevenly distributed, cost-ineffective, laborious, and time-consuming (Xu and Crouch 2008). Therefore, SNP genotyping techniques are an ideal platform for studies ranging from single-gene markers to whole-genome profiling. The most powerful advantages of SNP genotyping technique is that it is a rapid and cost-effective tool to genotype breeding populations, allowing plant breeders to implement GWAS, genomic diversity study, genetic linkage analysis, molecular marker discovery, and genomic selection for breeding programs (He et al. 2014). Usually, SNPs are biallelic, although very rarely tri- or tetra-allelic forms can also be found. Therefore, ease of data management along with their flexibility, speed, and cost-effectiveness is the range of genotyping platforms available. Due to its biallelic nature different genotyping platforms will provide the same allele calls once proper data QC has been performed (Thomson 2014).

These range from high-density marker-throughput technologies, such as highly multiplexed fixed arrays providing over one million SNP loci per run, to high-sample-throughput technologies that enable running of hundreds of samples per day with low-cost SNP assays. Therefore, these genotyping platforms have been highly optimized for speed, efficiency, robustness, and cost-effectiveness. Although many of these systems require a large initial capital investment, the end result is that the cost per sample has decreased to the point where it is significantly cheaper to genotype a breeding population than to phenotype it. The application of NGS technologies has led to remarkable advances in whole-genome sequencing, which provides ultra-throughput sequences to revolutionize plant genotyping and breeding. SNP markers are extremely useful in plant genetics and breeding applications. For instance, gene mapping and tagging, bulk segregation analysis, foreground selections, background selections, genetic diagnosis, forensic examination, phylogenetic analysis, and evolutionary studies (McCouch et al. 2010; Chen et al. 2014; Singh et al. 2015).

17.3 Application of Transcriptomics in Biofortification

Development in transcriptome sequencing has made it easy to engineer crops with enhanced key nutrients. Transcriptome provides the complete expression profile of a particular plant in the respective condition. We can utilize this characteristic feature to study the genes responsible for nutrient uptake in plants. We can even design

experiments by comparing transcriptome data of different cultivars showing varying nutrient contents. Moreover, the transcriptome of samples under different treatments can also be studied to check the expression of nutrient-related genes. With the help of these overexpressed genes, transporters, transcription factors, etc. can be known which will further transferred into other plants to achieve biofortification. Table 17.4 shows the use of transcriptome profiling for biofortification. There are various techniques available for transcriptome. There is huge data available that can be used for omics-assisted breeding approaches for crop improvement. Figure 17.5 presents a schematic representation of various techniques of transcriptome analysis which can be utilized for biofortification. Likewise, in Fig. 17.6 describes the workflow of transcriptome profiling for biofortification.

Table 17.4 Applications of the transcriptome to achieve biofortification

Biofortified plant	Micronutrient	Observation	References
Lettuce	Iodine	Transcriptome profiling of Caco-2 cancer cell line showed that after treatment with iodine biofortified lettuce extract Suppression of proliferation of Caco-2 achieved more effectively than the extract from non-fortified lettuce	Koronowicz et al. (2016)
Medicago truncatula	Iron	Transcription profiling revealed that the phosphatase gene is responsible for iron deficiency in M. truncatula	Santos et al. (2013)
Phaseolus vulgaris	Iron	Defensin gene identified in P. vulgaris for iron deficiency	Santos et al. (2013)
Glycine max	Iron	Overexpression of zinc ion binding gene in G. max responsible for iron deficiency	Santos et al. (2013)
Rice	Zinc	Transporters for higher zinc uptake, phosphate transporter-exporter (PHO), proton-coupled peptide transporters (POT) and vacuolar iron transporter (VIT) in different rice cultivar has been identified via transcriptome sequencing	Neeraja et al. (2018)
Rice	Iron	Genes related to iron toxicity identified	Quinet et al. (2012)
Arabidopsis thaliana	Magnesium	Identification of genes related to Mg uptake and Mg deficiency via transcriptome sequencing	Yardley (2009)
Finger millet	Calcium	Revealed genes responsible for calcium uptake and transport	Vinoth and Ravindhran (2017)

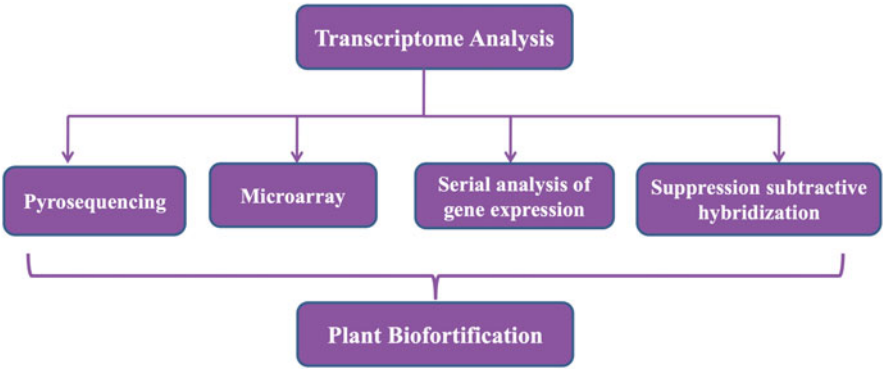

Fig. 17.5 Schematic representation of various techniques of transcriptomics applied in biofortification

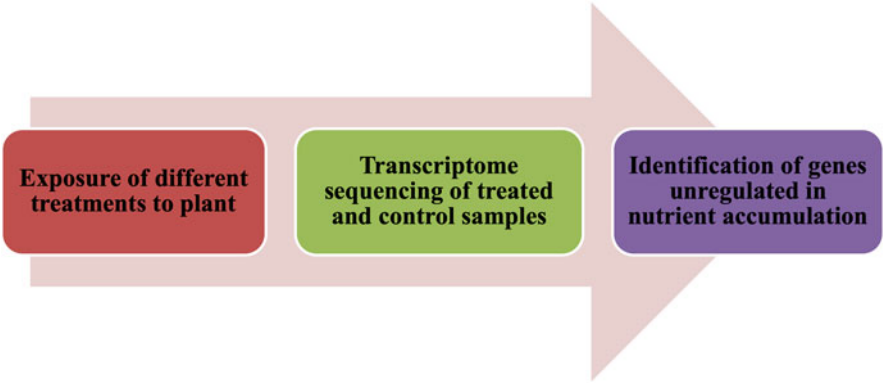

Fig. 17.6 The workflow of transcriptome profiling for biofortification

17.3.1 Small RNA Profiling and Biofortification

Small RNA is 21–24 nucleotide long RNA that plays an important role in gene expression in both plants and animals (Pant et al. 2009; Zhao et al. 2012). In plants, two major types of small RNA have been identified, namely small interfering RNAs (siRNAs) and microRNAs (miRNAs) which function as negative regulators in gene expression (Hsieh et al. 2009; Zhao et al. 2012). miRNAs are generally 21 nucleotides long and are generated from ssRNA hairpin-shaped precursors, whereas siRNAs are generally 24 nucleotides in length and are produced from long dsRNA precursor (Zhao et al. 2012). miRNA is formed with the help of RNase III enzyme DICER-LIKE1 (DCL1) or DCL, whereas siRNA requires RNA-dependent RNA polymerase activity (Hsieh et al. 2009). In recent years,

high-throughput sequencing (HTS) has arisen as a direct method for small RNA profiling. Both recognized and unrecognized small RNA semi-open-ended analysis is allowed in HTS compared to microarray or quantitative RT-PCR (qRT-PCR). However, there are some limitations regarding this method, such as it lacks a statistical method for analysis, there are problems due to the large dataset during data processing and mapping it on a reference genome (Fahlgren et al. 2009). Some miRNAs have been identified to play a prominent role in nutrient deficiency. Hsieh et al. (2009) showed the role of miRNA during nutrient deficiency. In Arabidopsis thaliana, through deep sequencing of small RNA, they analyze differentially expressed miRNA or other small RNA in response to phosphate (Pi) deficiency. They identified that during Pi deficiency, a significant amount of miR399 gets accumulated. Their findings also revealed a miRNA/ta-siRNA (microRNAs/trans-acting small interfering RNAs) regulatory network and its target gene. By suppressing the expression of such miRNA nutrient deficiency can be overcome.

17.3.2 Utilization of Whole-Genome Sequence Data for Biofortification

Recent advancement in sequencing technology has made it possible to sequence the whole genome of plants in less time and reduced cost, due to which a lot of crop plants have been sequenced and more are being sequenced now. The complete genome sequence data of many crops like soybean, rice, wheat, maize, groundnut, pearl millet, mustard, etc. are available now. The exploitation of these data for biofortification provides a direct benefit to researchers. The whole-genome sequence not only gives complete information about the location of a particular gene but also provides information about the presence of genes related to nutrients. Through these data SNPs present in nutrient related genes can be studied. This may provide a good lead when working on specific nutrient enrichment. Another approach for utilizing sequencing technology is germplasm screening in which genetic variations within natural populations can be studied. It is a useful tool to discover the gene responsible for elevated micronutrient content in particular cultivar (Karley and White 2009). Whole-genome sequencing and resequencing can be used to develop GWAS, phylogeny, tagging, mutant mapping, and QTL which can be further utilized to achieve biofortification. A combined use of whole-genome sequence data and functional genomics can provide better chances of developing crops with higher nutrient value. Figure 17.7 explains a flow chart of the use of whole-genome sequencing technology in the development of biofortified crops.

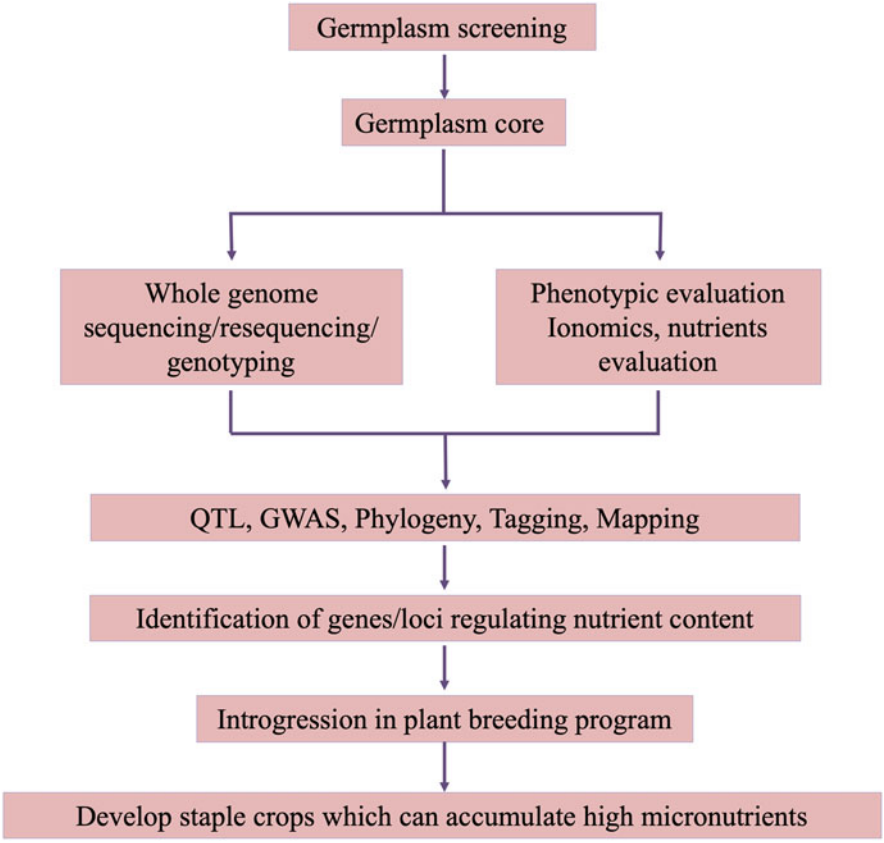

Fig. 17.7 Flow chart for development of biofortified crop using whole-genome sequencing technology

17.4 Outlook

Biofortification is a reasonable and cost-effective means of delivering micronutrients to populations to overcome "unseen hunger." A multi-tier synchronized strategy will help in eradicating problems associated with hidden hunger. Presently, sequencing of the whole genome is becoming cheaper, major hindrance is bioinformatics analysis of sequence for better understanding of basics in metabolic pathway and identification of true positive traits. Exploiting the genetic resources using conventional breeding and further adapting NSG has occupied a central key site in a breeding pipeline to accelerate the precision of trait mapping and trait transfer. These novel genomic approaches to engineer designer crops with enhanced key micronutrient levels in the background of elite variety will be the future goal to ensure nutritional security, especially in a developing country. Biofortified crops will remarkably help the entire community depending on plant-based food for essential

nutrients to overcome the nutritional challenges in order to achieve malnutrition-free society. Recently attempts have been made toward biofortification in legumes, however some orphan crops like pigeon pea, urd bean, mung bean, and millets also need more attention as they are the chief source of protein, vitamins, micronutrients, and minerals. There is immense opportunity to utilize pulses for biofortification of micronutrients with a proper understanding of micronutrient distribution and its accumlation in each of the tissues. Our immediate goal is to attain a malnutrition-free human population by feeding micronutrient enriched food sources. In the area of nutrition genomics, we have to work in collaboration with developed and developing countries to design the smart and quick nutritional strategies that the biofortified varieties can actually reduce iron deficiencies, zinc deficiencies, and vitamin A deficiencies under controlled conditions. Furthermore, we need to put our efforts on combining nutritional character with climate-smart traits while breeding new varities. However critical selection of the nutrient which need to be improved depending on the crop is necessary.

17.5 Conclusions

It is well reported that there are severe health issues related to micronutrition deficiencies, prevention of these deficiencies is a worldwide goal. The lack of nutritious food has enforced many people to depend on food fortification, supplements, and agronomic practices as interventions. Biofortification of staple crops such as rice, wheat, maize, pearl millet, and pulses is considered to be the most sustainable method of intervention and is largely facilitated by employing modern genetics and functional genomic approaches. In this regard, the use of available genetic resources and diverse germplasm collections will accelerate this research. Biofortification is not only a silver bullet that can completely eliminate all nutrition-related problems. Biofortification of different crop varieties provides a sustainable way to produce micronutrient-rich crops. From an economic viewpoint, biofortification is actually a one-time investment and offers a cost-effective, long-term, and sustainable approach in fighting against hidden hunger because once the biofortified crops are developed, there are no costs of buying the fortificants and adding them to the food supply during processing. In this vision, organizations such as the World Health Organization (WHO) and the Consultative Group on International Agricultural Research (CGIAR) have included the development of nutritionally enhanced high-yielding biofortified crops as one of their main objectives to eradicate this problem from the world.

Several NGS techniques have driven research to enhance the productivity, sustainability, and nutritional quality of food production and it is now possible to identify QTLs and candidate genes to determine valuable traits. There are a variety of platforms that aid the development of the nutrient-rich crop, including the use of genetic maps, GWAS, GS, synteny studies, QTL fine mapping for targeting candidate genes, and genetic engineering technology. The synteny based identification of genes governing nutritional traits in other important crops can be achieved using existing genomic resources from model plant species. The common genes associated

with nutrition biosynthesis pathways can be identified and targeted through omics techniques, transgene-based, gene editing (CRISPR) and breeding methods. Advances in crop genomics research in recent years thus offer the opportunities to enhance the prediction of phenotypes from genotypes for cereal breeding. NGS techniques contributed to the identification and tagging of some agronomically important genes and QTLs for agricultural applications. It will further improve the tagging of nutrition-related important genes and QTLs. As the understanding of genetic variations for micronutrients in the genome increases, this will facilitate the discovery and validation of genes associated with high micronutrient uptake and lead to improved varieties that can be accessed by smallholders and the wider population. Biofortified crops have a promising future as these have the potential to eradicate micronutrient malnutrition among billions of poor people, especially in developing countries.

References

Adeyeye E, Arogundade L, Akintayo E, Aisida O, Alao P (2000) Calcium, zinc and phytate interrelationships in some foods of major consumption in Nigeria. Food Chem 71(4):435–441

Bastien M, Sonah H, Belzile F (2014) Genome wide association mapping of Sclerotinia sclerotiorum resistance in soybean with a genotyping-by-sequencing approach. Plant Genome 7(1):plantgenome2013.10.0030

Bhat JA, Shivaraj S, Ali S, Mir ZA, Islam A, Deshmukh R (2018) Genomic resources and omics-assisted breeding approaches for pulse crop improvement. In: Pulse improvement. Springer, Cham, pp 13–55

Bianco L, Cestaro A, Linsmith G, Muranty H, Denancé C, Théron A, Poncet C, Micheletti D, Kerschbamer E, Di Pierro EA (2016) Development and validation of the Axiom® Apple480K SNP genotyping array. Plant J 86(1):62–74

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32(3):314

Bouis, H., Low, J., McEwan, M., Tanumihardjo S., (2013) Bio henolic compounds: structure, c ion

Caballero B (2002) Global patterns of child health: the role of nutrition. Ann Nutr Metab 46(Suppl. 1):3–7

Chaudhary J, Patil GB, Sonah H, Deshmukh RK, Vuong TD, Valliyodan B, Nguyen HT (2015) Expanding omics resources for improvement of soybean seed composition traits. Front Plant Sci 6:1021

Chaudhary J, Alisha A, Bhatt V, Chandanshive S, Kumar N, Mir Z, Kumar A, Yadav SK, Shivaraj S, Sonah H (2019) Mutation breeding in tomato: advances, applicability and challenges. Plan Theory 8(5):128

Chen C, Wu W, Sun X, Li B, Hu G, Zhang Q, Li J, Zhang H, Li Z (2014) Fine-mapping and candidate gene analysis of BLACK HULL1 in rice (Oryza sativa L.). Plant Omics 7(1):12

Collard BC, Mackill DJ (2007) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc., B 363(1491):557–572

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12 (7):499

Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R, Vuong T, Valliyodan B, Nguyen HT (2014) Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci 5:244

Drenkard E, Richter BG, Rozen S, Stutius LM, Angell NA, Mindrinos M, Cho RJ, Oefner PJ, Davis RW, Ausubel FM (2000) A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. Plant Physiol 124(4):1483–1492

Fahlgren N, Sullivan CM, Kasschau KD, Chapman EJ, Cumbie JS, Montgomery TA, Gilbert SD, Dasenko M, Backman TW, Givan SA (2009) Computational and analytical framework for small RNA profiling by high-throughput sequencing. RNA 15(5):992–1002

Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112(6):1164–1171

Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Front Nutr 5:12

Gearing M (2015). Good as gold: can golden rice and other biofortified crops prevent malnutrition. Science in the News, Harvard University. http://sitn.hms.harvard.edu

Gupta AB, Sankararamakrishnan R (2009) Genome-wide analysis of major intrinsic proteins in the tree plant Populus trichocarpa: characterization of XIP subfamily of aquaporins from evolutionary perspective. BMC Plant Biol 9(1):134

Gupta P, Rustgi S, Mir R (2008) Array-based high-throughput DNA markers for crop improvement. Heredity 101(1):5

He J, Zhao X, Laroche A, Lu Z-X, Liu H, Li Z (2014) Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Plant Sci 5:484

Heffner EL, Sorrells ME, Jannink J-L (2009) Genomic selection for crop improvement. Crop Sci 49 (1):1–12

Helm R, Burks A (1996) Hypoallergenicity of rice protein. Cereal Foods World 41(11):839–843

Hoffmann TJ, Kvale MN, Hesselson SE, Zhan Y, Aquino C, Cao Y, Cawley S, Chung E, Connell S, Eshragh J (2011) Next generation genome-wide association tool: design and coverage of a high-throughput European-optimized SNP array. Genomics 98(2):79–89

Hsieh L-C, Lin S-I, Shih AC-C, Chen J-W, Lin W-Y, Tseng C-Y, Li W-H, Chiou T-J (2009) Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. Plant Physiol 151(4):2120–2132

Huang X, Feng Q, Qian Q, Zhao Q, Wang L, Wang A, Guan J, Fan D, Weng Q, Huang T (2009) High-throughput genotyping by whole-genome resequencing. Genome Res 19(6):1068–1076

Hulse-Kemp AM, Lemm J, Plieske J, Ashrafi H, Buyyarapu R, Fang DD, Frelichowski J, Giband M, Hague S, Hinze LL (2015) Development of a 63K SNP array for cotton and high-density mapping of intraspecific and interspecific populations of Gossypium spp. G3 Genes Genomes Genetics 5(6):1187–1209

Iqbal S, Bhanger M, Anwar F (2005) Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem 93(2):265–272

Jannink J-L, Lorenz AJ, Iwata H (2010) Genomic selection in plant breeding: from theory to practice. Brief Funct Genomics 9(2):166–177

Karley AJ, White PJ (2009) Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Curr Opin Plant Biol 12(3):291–298

Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M (2010) Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A rev Agron Sustain Dev 30(1):83–107

Komori T, Nitta N (2005) Utilization of the CAPS/dCAPS method to convert rice SNPs into PCR-based markers. Breed Sci 55(1):93–98

Koronowicz AA, Kopeć A, Master A, Smoleń S, Piątkowska E, Bieżanowska-Kopeć R, Ledwożyw-Smoleń I, Skoczylas Ł, Rakoczy R, Leszczyńska T (2016) Transcriptome profiling of Caco-2 cancer cell line following treatment with extracts from iodine-biofortified lettuce (Lactuca sativa L.). PLoS One 11(1):e0147336

Kumar V, Singh A, Mithra SA, Krishnamurthy S, Parida SK, Jain S, Tiwari KK, Kumar P, Rao AR, Sharma S (2015) Genome-wide association mapping of salinity tolerance in rice (Oryza sativa). DNA Res 22(2):133–145

Kumawat S, Rana N, Bansal R, Vishwakarma G, Mehetre ST, Das BK, Kumar M, Kumar Yadav S, Sonah H, Sharma TR (2019) Expanding avenue of fast neutron mediated mutagenesis for crop improvement. Plan Theory 8(6):164

Kwok P-Y, Deng Q, Zakeri H, Taylor SL, Nickerson DA (1996) Increasing the information content of STS-based genome maps: identifying polymorphisms in mapped STSs. Genomics 31 (1):123–126

Lin CH, Yeakley JM, McDaniel TK, Shen R (2009) Medium-to high-throughput SNP genotyping using VeraCode microbeads. In: DNA and RNA profiling in Human Blood. Springer, Cham, pp 129–142

McCouch SR, Wright MH, Tung C-W, Maron LG, McNally KL, Fitzgerald M, Singh N, DeClerck G, Perez FA, Korniliev P, Greenberg AJ, Naredo MEB, Mercado SMQ, Harrington SE, Shi Y, Branchini DA, Kuser-Falcão PR, Leung H, Ebana K, Yano M, Eizenga G, McClung A, Mezey J (2016) Erratum: Corrigendum: Open access resources for genome-wide association mapping in rice. Nat Commun 7(1)

McCouch SR, Zhao K, Wright M, Tung C-W, Ebana K, Thomson M, Reynolds A, Wang D, DeClerck G, Ali ML (2010) Development of genome-wide SNP assays for rice. Breed Sci 60 (5):524–535

Mueller UG, Wolfenbarger LL (1999) AFLP genotyping and fingerprinting. Trends Ecol Evol 14 (10):389–394

Neeraja C, Kulkarni KS, Babu PM, Rao DS, Surekha K, Babu VR (2018) Transporter genes identified in landraces associated with high zinc in polished rice through panicle transcriptome for biofortification. PLoS One 13(2):e0192362

Ortiz-Monasterio JI, Palacios-Rojas N, Meng E, Pixley K, Trethowan R, Peña RJ (2007) Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J Cereal Sci 46 (3):293–307

Pandey MK, Agarwal G, Kale SM, Clevenger J, Nayak SN, Sriswathi M, Chitikineni A, Chavarro C, Chen X, Upadhyaya HD (2017) Development and evaluation of a high density genotyping 'Axiom_Arachis' array with 58 K SNPs for accelerating genetics and breeding in groundnut. Sci Rep 7:40577

Pant BD, Musialak-Lange M, Nuc P, May P, Buhtz A, Kehr J, Walther D, Scheible W-R (2009) Identification of nutrient-responsive Arabidopsis and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. Plant Physiol 150 (3):1541–1555

Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47 (Supplement_3):S-88–S-105

Quinet M, Vromman D, Clippe A, Bertin P, Lequeux H, Dufey I, Lutts S, Lefevre I (2012) Combined transcriptomic and physiological approaches reveal strong differences between short-and long-term response of rice (Oryza sativa) to iron toxicity. Plant Cell Environ 35 (10):1837–1859

Rana N, Rahim MS, Kaur G, Bansal R, Kumawat S, Roy J, Deshmukh R, Sonah H, Sharma TR (2019) Applications and challenges for efficient exploration of omics interventions for the enhancement of nutritional quality in rice (Oryza sativa L.). Crit Rev Food Sci Nutr:1–17. https://doi.org/10.1080/10408398.2019.1685454

Ray DK, Ramankutty N, Mueller ND, West PC, Foley JA (2012) Recent patterns of crop yield growth and stagnation. Nat Commun 3:1293

Santos CS, Silva AI, Serrão I, Carvalho AL, Vasconcelos MW (2013) Transcriptomic analysis of iron deficiency related genes in the legumes. Food Res Int 54(1):1162–1171

Saltzman A, Birol E, Bouis H et al (2013) Biofortification: progress toward a more nourishing future Glob. Food Secur 2(1):9–17

Saunders R (1990) The properties of rice bran as a foodstuff. Cereal Foods World 35(7):632–636

Schramm R, Abadie A, Hua N, Xu Z, Lima M (2007) Fractionation of the rice bran layer and quantification of vitamin E, oryzanol, protein, and rice bran saccharide. J Biol Eng 1(1):9

Shen R, Fan J-B, Campbell D, Chang W, Chen J, Doucet D, Yeakley J, Bibikova M, Garcia EW, McBride C (2005) High-throughput SNP genotyping on universal bead arrays. Mutat Res 573 (1-2):70–82

Shivaraj S, Dhakate P, Sonah H, Vuong T, Nguyen HT, Deshmukh R (2019) Progress toward development of climate-smart flax: a perspective on omics-assisted breeding. In: Genomic designing of climate-smart oilseed crops. Springer, Cham, pp 239–274

Shoji Y, Mita T, Isemura M, Mega T, Hase S, Isemura S, Aoyagi Y (2001) A fibronectin-binding protein from rice bran with cell adhesion activity for animal tumor cells. Biosci Biotechnol Biochem 65(5):1181–1186

Singh N, Jayaswal PK, Panda K, Mandal P, Kumar V, Singh B, Mishra S, Singh Y, Singh R, Rai V (2015) Single-copy gene based 50 K SNP chip for genetic studies and molecular breeding in rice. Sci Rep 5:11600

Sonah H, O'Donoughue L, Cober E, Rajcan I, Belzile F (2015) Identification of loci governing eight agronomic traits using a GBS-GWAS approach and validation by QTL mapping in soya bean. Plant Biotechnol J 13(2):211–221

Song Q, Hyten DL, Jia G, Quigley CV, Fickus EW, Nelson RL, Cregan PB (2013) Development and evaluation of SoySNP50K, a high-density genotyping array for soybean. PLoS One 8(1): e54985

Thiel T, Kota R, Grosse I, Stein N, Graner A (2004) SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development. Nucleic Acids Res 32(1):e5–e5

Thomson MJ (2014) High-throughput SNP genotyping to accelerate crop improvement. Plant Breed Biotechnol 2(3):195–212

Tinker NA, Chao S, Lazo GR, Oliver RE, Huang Y-F, Poland JA, Jellen EN, Maughan PJ, Kilian A, Jackson EW (2014) A SNP genotyping array for hexaploid oat. Plant Genome 7(3): plantgenome2014.03.0010

Unterseer S, Bauer E, Haberer G, Seidel M, Knaak C, Ouzunova M, Meitinger T, Strom TM, Fries R, Pausch H (2014) A powerful tool for genome analysis in maize: development and evaluation of the high density 600 k SNP genotyping array. BMC Genomics 15(1):823

Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security. Front Plant Sci 8:29

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol J 12(6):787–796

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18 (22):6531–6535

Winfield MO, Allen AM, Burridge AJ, Barker GL, Benbow HR, Wilkinson PA, Coghill J, Waterfall C, Davassi A, Scopes G (2016) High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. Plant Biotechnol J 14(5):1195–1206

World Food Programme (2015) Types of malnutrition. http://www.wfp.org/hunger/malnutrition/types

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Sci 48(2):391–407

Yardley AW (2009) Dietary magnesium: new research. Nova Science Publishers, New York

Yu H, Xie W, Li J, Zhou F, Zhang Q (2014) A whole-genome SNP array (RICE 6 K) for genomic breeding in rice. Plant Biotechnol J 12(1):28–37

Zargar SM, Raatz B, Sonah H, Bhat JA, Dar ZA, Agrawal GK, Rakwal R (2015) Recent advances in molecular marker techniques: insight into QTL mapping, GWAS and genomic selection in plants. J Crop Sci Biotechnol 18(5):293–308

Zhao K, Tung C-W, Eizenga GC, Wright MH, Liakat Ali M, Price AH, Norton GJ, Rafiqul Islam M, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR (2011)

Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat Commun 2(1)

Zhao Y-T, Wang M, Fu S-X, Yang W-C, Qi C-K, Wang X-J (2012) Small RNA profiling in two Brassica napus cultivars identifies microRNAs with oil production-and development-correlated expression and new small RNA classes. Plant Physiol 158(2):813–823

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20(2):176–183