Chapter 15
Biotechnology for Nutritional and Associated Processing Quality Improvement in Potato

Som Dutt, Anshul Sharma Manjul, Mamta Chauhan, Sushil Sudhakar Changan, Pinky Raigond, Brajesh Singh and Swarup Kumar Chakrabarti

Abstract Plant products comprise majority of human food intake globally. Therefore, it is expected that better nutritional availability for human beings can be achieved by nutritional improvement of food crops. Having achieved the food sufficiency in almost all parts of the world provides us the leverage to have a paradigm shift from "quantity increase" to "quality improvement" of crops. Improvement of quality will be crucial in future; it is almost sure that there will be significant reduction in land available for agriculture as compared to that available today. This means that in the lesser agricultural land, we shall have to produce more food in terms of nutrients. Potato (*Solanum tuberosum* L.) is the world's third most important crop in terms of human consumption. It is consumed in all countries of the world whether developing or developed and has been used as a primary nutritional source in many diets and as the basis for a variety of processed products. Ability of potato to produce highest nutrition and dry matter on per unit area and time basis, among major food crops, made FAO to declare it the crop to address future global food security and poverty alleviation during 2008. Although potato is a rich source of several nutrients such as protein, vitamin C, vitamin B6, and niacin, there is ample scope for improving its nutritional quality and making it more nutritious food. Worldwide conventional breeding technologies have given the mankind a large number of varieties having improved traits as compared to their predecessors. It has led to the development of a large numbers of cultivars of various crop plants which in turn has resulted in tremendous increase in their productivity. Recent advancements in the field of agricultural biotechnology have created a new domain to complement the methods of plant breeding. These biotechnological approaches are also being used for improving the nutritional quality as well as the processing attributes of potato. Using biotechnological tools, a large number of nutrients have been improved in potato. These include phenolics, vitamins, essential amino acids, protein, carbohydrates, and minerals. Certain anti-nutritional factors have been reduced in certain potato cultivars.

S. Dutt (✉) · A. S. Manjul · M. Chauhan · S. S. Changan · P. Raigond · B. Singh · S. K. Chakrabarti
ICAR-Central Potato Research Institute (ICAR-CPRI), Shimla 171001, India
e-mail: sd_bio@yahoo.com

© Springer Nature Switzerland AG 2019

P. K. Jaiwal et al. (eds.), *Nutritional Quality Improvement in Plants*,
Concepts and Strategies in Plant Sciences,
https://doi.org/10.1007/978-3-319-95354-0_15

Processing attributes of potato have also been improved using biotechnological tools such as gene silencing and gene editing. Recent improvement in genome-editing technologies is expected to further assist plant researchers to develop nutrient-rich potatoes in more targeted manner.

Keywords Genome editing · Essential amino acid · Phenolics · Resistant starch · Cold-induced sweetening · Protein · Anti-nutritional factors

15.1 Introduction

Universally, most of the past agricultural research programs and innovations have been predominantly focused on increasing productivity of crops. In other words, "quantity" has been the center around which majority of the crop improvement and relevant resource management activities have been formulated and executed. There is no doubt that this has been the requirement of the past times where producing and making food accessible to every human being was globally the top-most priority. Having achieved the food sufficiency in almost all parts of the world provides us the leverage to have a paradigm shift from "quantity increase" to "quality improvement" of crops. Improvement of quality also becomes essential as in future, it is expected that there will be significant reduction in land available to agriculture as compared to that is available today. This suggests that in the less land availability for agriculture, we shall have to produce higher nutrients.

Food has been viewed as a source to supply amount of nutrients just sufficient for survival and normal growth. However, since evolution, human desires have always been to strive for continuous improvements in terms of physical as well as mental strengths and longevity. Fulfilling these desires definitely necessitates looking into our food components and significantly improving it in terms of their (food components) nutritional quality. Agricultural innovation has always involved new, science-based products and processes that have contributed reliable methods for increasing productivity and sustainability. Biotechnology has introduced a new dimension to such innovations, offering efficient and cost-effective means to produce a diverse array of novel, value-added products and tools.

Globally, plant products comprise the vast majority of human food intake, irrespective of location or financial status. In some cultures, either by design or default, plant-based nutrition actually comprises 100% of the diet. Therefore, it is to be expected that nutritional improvement can be achieved via modifications of staple crops. Further, it has been suggested that food components can influence physiological processes at all stages of life. For example, inverse relationships have been observed between carotenoid-rich foods and certain cancers (Botella-Pavia and Rodriguez-Conception 2006). Other nutrient-related correlations link dietary fat and fiber to the prevention of colon cancer, folate to the prevention of neural tube defects, calcium to the prevention of osteoporosis, antioxidant nutrients to the scavenging of reactive oxidant species and protection against oxidative damage of cells, etc.

Potato has a definite place in the diet and is associated with good nutrition and health. Potatoes are uniquely positioned to be a valuable source of dietary vitamins, minerals, and phytonutrients because of their high per capita consumption. In most of the developed world, potatoes are by far the most eaten vegetable. Because of this high consumption, the vitamin and phytonutrient contents of potato will have much more dietary relevance and impact than food eaten in sparse quantities. Potatoes yield more calories per acre than any other major crop, a criterion that becomes even more important in light of the planet's ever-increasing population, food shortages, price spikes, and the recent trend of utilizing farmland for other commercial purposes. Collectively, these facts emphasize the impact potatoes can have on global nutrition in the future. These facts imply that any significant improvement in nutritional quality of potato will have even more than significant impact on human health and nutrition. Here we have described the biotechnology-based approaches for improving the quality traits of potato.

Worldwide conventional breeding technologies have given the mankind a large number of varieties having improved traits as compared to their predecessors. It has led to the development of a large numbers of cultivars of various crop plants which in turn has resulted in tremendous increase in their productivity. In conventional breeding, progeny inherit genes for both desirable and undesirable traits from both parents. Desired characteristics are conserved, and undesirable ones are suppressed/eliminated by repeatedly selecting superior individuals from each generation to be the parents of the next. However, the long breeding cycles, high heterozygosities, lack of various degrees of preciseness in hybridization, low frequencies of desirable mutations, and limit of using the genetic resources of primary and secondary gene pool have made new varietal development highly resource-demanding. Recent advancements in the field of agricultural biotechnology have created a new domain to complement the methods of plant breeding. These biotechnological approaches are also being used/can be used for improving the nutritional quality as well as the processing attributes of potato.

15.2 Tuber Composition and Dietary Importance of Potato

Nutrition is the processes by which we take in and utilize food substances. Nutrition is essential for growth and development, health, and wellbeing. Essential nutrients include carbohydrate, protein, fat, vitamins, minerals, and electrolytes. Recommended dietary allowance (RDA) of these important nutrients has been defined worldwide and is revised/updated from time to time. RDA for these nutrients in India is presented in (Table 15.1). Potatoes are approximately 80% water and 20% solids, although it can vary widely from cultivar to cultivar (Fig. 15.1). Of the 20 g of solids in a 100 g tuber, about 17 g are carbohydrate and 2 g protein. In addition to carbohydrates and proteins, potatoes are a good source of many vitamins and minerals (Fig. 15.1). According to the USDA nutrient database, 100 g of potatoes contains 4% of the RDA calorie intake, 33% of the RDA of vitamin C, the most abundant vitamin

Table 15.1 Recommended dietary allowances for Indians

Nutrient	Man	Woman	Pregnant woman	Children (1–9 years)	Girls (10–17 years)	Boys (10–17 years)
Protein (g/d)	60	55	78	16.7–29.5	35–40	35–50
Calcium (mg/d)	600	600	1200	600	800	800
Iron (mg/d)	17	21	35	9–16	27	32
Vitamin A (b-carotene) (μg/d)	4800	4800	6400	3200–4800	4800	4800
Thiamin (mg/d)	1.2–1.7	1.0–1.4	1.6	0.5–0.8	1.0–1.2	1.1–1.5
Riboflavin (mg/d)	1.4–2.1	1.1–1.7	2.0	0.6–1.0	1.2–1.4	1.3–1.8
Niacin equivalent (mg/d)	16–21	12–16	18	8–13	13–14	15–17
Pyridoxin (mg/d)	2.0	2.0	2.5	0.9–1.6	1.6–2.0	1.6–2.0
Ascorbic acid (mg/d)	40	40	60	40	40	40
Dietary folate (μg/d)	200	200	500	80–120	140–200	140–200
Vit. B12 (μg/d)	1	1	1.2	0.2–1.0	0.2–1.0	0.2–1.0
Magnesium (mg/d)	340	310	310	50–100	160–235	120–195
Zinc (mg/d)	12	10	12	5–8	9–12	9–12

Adapted from National Institute of Nutrition (2011)

in potatoes and 12% of the RDA for potassium. Also, potato tubers contain an array of other small molecules, many of which are phytonutrients. These include polyphenols, flavonols, anthocyanins, phenolic compounds, carotenoids, polyamines, and tocopherols. These phytonutrients play various important roles as improving immune system, antioxidant activities, and health-promoting activities, thus are considered as important nutritional quality constituents of potato. Because of the presence of these important nutritional constituents in potato, potato is sometimes referred to as a wholesome food, though levels of these important nutrients are invariably quite low with reference to the RDA values for these nutrients. Therefore, there exists ample scope to further improve the nutritional value of potato and make it a truly wholesome food.

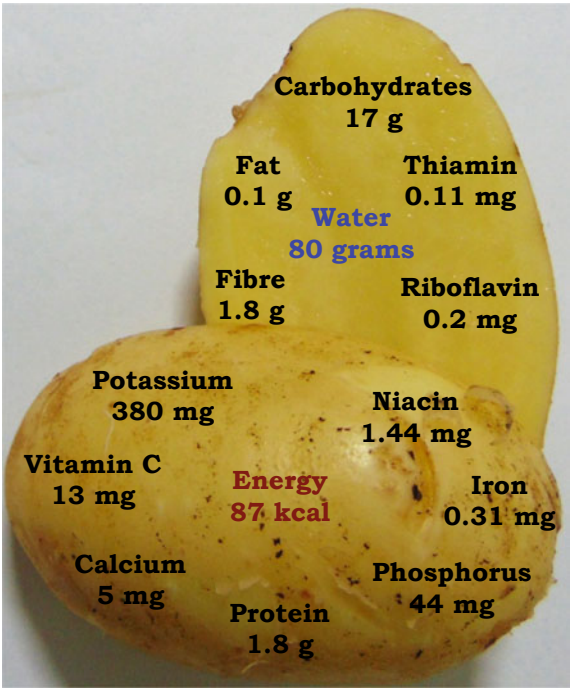

Fig. 15.1 Nutrient contents of potato (in 100 g after boiling in skin and peeling before consumption. Partially adapted from www.fao.org/potato-2008)

15.3 Biotechnological Tools for Assisting Conventional Plant Breeding

Recent advancements in the field of agricultural biotechnology have created a new domain to complement the methods of plant breeding. Genetic improvement can be achieved through conventional as well as nonconventional approaches. There are broadly three benefits to agriculture and crop improvement programs from use of biotechnology. These are: (i) reduction of the duration of the breeding programmes, (ii) to develop and multiply the healthy planting material, and (iii) genetically engineering the crop plants for trait improvement. The first two benefits hold true for all kinds of crop improvement and breeding programs. The third area, i.e., genetic engineering or recombinant DNA technology, is target trait specific.

From consumer point of view, the main quality traits in potato are nutrient content of the potato tubers, organoleptic characteristics including taste, flavor, and appearance. Therefore, for researchers improving the quality traits means improving any one, two, or all of these three characteristics of potatoes. Worldwide efforts are going on to develop the cultivars of potato with improved quality characteristics. Biotechnological tools are being continuously enriched and improved. Potatoes being one of the most important commodities for processing sector, its processing attributes are also crucial for determining their suitability for processing purposes (e.g., making

chips, French fries, and dehydrated products). This chapter is restricted to nutritional and processing trait improvement in potato using biotechnological approaches.

15.3.1 Genomic Resources for Biotechnological Applications in Potato

The generation of huge volume of the datasets of DNA sequences has gone much beyond everyone's imaginations. This has been mainly possible due to tremendous advancements in high throughputness of DNA sequencing technologies and the parallel development of sequencing storage servers and bioinformatics tools employed for DNA sequence assembly and annotations. Even in case of vegetable crops, the genome of the vegetables (including potato) belonging to more than 15 groups (tomato, potato, sweet potato, pepper, carrot, cabbage, turnip, radish, brinjal, cucumber, chenopodium, bitter gourd yam, beans, lettuce spinach, etc.) has been sequenced, and the genome sequence database is available in public domain (Table 15.2). This list of genome sequences of vegetable crops has expanded very rapidly. The assembly of these sequenced genomes is at different levels of assembly (chromosomes, scaffolds/contigs) (Table 15.2). Availability of genome sequences (including those of other vegetable crops) can be of great as a source of efficient gene isoforms for improving quality traits of potato through biotechnological approaches. Genome resequencing, single-nucleotide polymorphism (SNP) discovery through genotypic sequencing will be very useful in deciphering the genetic diversity at nucleotide sequence levels. This information in turn can be used for establishing the association between DNA/nucleotide variation and phenotypic/trait variability. The availability of genome sequences of various species within a clad/group may be very useful in performing genome-wide association mapping (GWAS) for various quality traits which will be vital for developing effective breeding strategies aiming at targeted quality trait(s) improvement. This may further help in identifying the more efficient alleles associated with desirable quality traits. However, this may need additional information about comparative kinetics of the enzymes encoded by these isoforms/alleles.

15.3.2 Transgenic-Based Tools for Quality Improvement of Potato

Genetic engineering has the application in introducing the specific traits into plants. It does not replace conventional breeding but add to the efficiency of crop improvement. It is possible due to the fact that plants are totipotent, enabling regeneration of a new plant from an isolated cell, tissue, or organ. Genetic engineering is the purposeful addition of a foreign gene or genes to the genome of an organism with the aim to

Table 15.2 Published sequenced genomes of vegetable crops

S. No.	Organism/Name	Common name	Bioproject	Size (Mb)	Scaffolds	Genes	Level of assembly
1.	*Solanum lycopersicum*	Tomato	PRJNA119	824	3224	30,336	Chromosome
2.	*Solanum pennellii*	Wild tomato	PRJEB5228	720	57,205	–	Contig
3.	*Solanum pennellii*	Wild tomato	PRJNA256426	926	12	32,519	Chromosome
4.	*Solanum arcanum*	Wild tomato	PRJEB5226	665	46,594	–	Contig
5.	*Solanum lycopersicum*	Tomato	PRJEB6302	760	13	–	Chromosome
6.	*Solanum lycopersicum, Heinz 1760*	Tomato	PRJNA41343	541	100,783	–	Scaffold
7.	*Solanum pimpinellifolium*	Wild tomato	PRJNA72351	688	309,180	–	Contig
8.	*Solanum melongena*	Egg plant	PRJDB1505	833	33,873	–	Scaffold
9.	*Capsicum annuum*	Pepper	PRJNA186921	2936	6478	41,504	Chromosome
10.	*Capsicum annuum*	Pepper	PRJNA223222	3064	35,797	35,845	Chromosome
11.	*Capsicum annuum var. glabriusculum*	Pepper	PRJNA193661	2768	16,998	–	Chromosome
12.	*Capsicum chinense*	Pepper	PRJNA331024	3071	87,978	34,974	Chromosome
13.	*Capsicum baccatum*	Hot pepper	PRJNA308879	3216	23,260	35,853	Chromosome
14.	*Solanum commersonii*	Wild potato	PRJNA269007	730	63,664	–	Scaffold
15.	*Solanum tuberosum*	Potato	PRJNA63145	706	14,854	33,410	Scaffold
16.	*Brassica oleracea var. oleracea*	Wild cabbage	PRJNA293438	489	32,886	53,670	Chromosome
17.	*Brassica oleracea var. capitata*	Cabbage	PRJNA174731	514	1816	–	Scaffold
18.	*Brassica juncea var. tumida*	Mustard	PRJNA285130	955	9746	–	Chromosome
19.	*Brassica napus*	Turnip	PRJEB5043	848	20,899	61,153	Scaffold
20.	*Spinacia oleracea*	Spinach	PRJNA396054	870	78,263	30,973	Scaffold

(continued)

Table 15.2 (continued)

S. No.	Organism/Name	Common name	Bioproject	Size (Mb)	Scaffolds	Genes	Level of assembly
21.	*Spinacia oleracea*	Spinach	PRJNA41497	494	103,502	21,539	Scaffold
22.	*Cucumis sativus*	Cucumber	PRJNA33619	196	190	20,396	Chromosome
23.	*Cucumis sativus*	Cucumber	PRJNA40333	324	13,113	–	Scaffold
24.	*Cucumis sativus*	Cucumber	PRJNA296786	343	8035	–	Contig
25.	*Chenopodium quinoa*	Chenopodium	PRJNA394242	1334	3487	58,734	Scaffold
26.	*Chenopodium suecicum*	Chenopodium	PRJNA326219	537	11,198	–	Scaffold
27.	*Chenopodium pallidicaule*	Chenopodium	PRJNA326220	337	3013	–	Scaffold
28.	*Raphanus sativus*	Radish	PRJNA344915	427	10,676	58,031	Scaffold
29.	*Raphanus sativus*	Radish	PRJNA259311	383	44,239	–	Chromosome
30.	*Raphanus sativus*	Radish	PRJDB1517	402	76,592	–	Scaffold
31.	*Raphanus sativus*	Radish	PRJDB707	383	40,123	–	Scaffold
32.	*Raphanus raphanistrum* subsp. *raphanistrum*	Wild radish	PRJNA209513	254	64,732	–	Contig
33.	*Dioscorea rotundata*	White yam	PRJDB3383	457	21	–	Chromosome
34.	*Manihot esculenta*	Cassava	PRJNA394209	582	2020	31,881	Chromosome
35.	*Manihot esculenta* subsp. *flabellifolia*	Cassava	PRJNA236442	391	54,016	–	Scaffold
36.	*Ipomoea batatas*	Sweet potato	PRJNA301667	837	28,461	–	Chromosome
37.	*Ipomoea trifida*	Wild sweet potato	PRJDB3230	513	77,400	–	Scaffold
38.	*Beta vulgaris* subsp. *vulgaris*	Sugarbeet	PRJNA41497	540	84,234	–	Scaffold
39.	*Daucus carota* subsp. *sativus*	Carrot	PRJNA326436	422	4826	36,299	Chromosome
40.	*Amaranthus hypochondriacus*	Grain amaranth	PRJNA214803	502	117,340	–	Scaffold

(continued)

Table 15.2 (continued)

S. No.	Organism/Name	Common name	Bioproject	Size (Mb)	Scaffolds	Genes	Level of assembly
41.	*Asparagus officinalis*	Garden *Asparagus*	PRJNA376608	1188	11,792	32,073	Chromosome
42.	*Vicia faba*	Faba bean	PRJEB8906	80	74,659	–	Contig
43.	*Momordica charantia, OHB3-1*	Bitter gourd	PRJDB4642	286	1052	21,623	Scaffold
44.	*Phaseolus vulgaris*	French bean	PRJNA221782	550	68,335	–	Chromosome
45.	*Lactuca sativa*	Lettuce	PRJNA68025	1134	876,110	–	Contig

Available in public domain database-NCBI; as on November 5, 2017

transfer the desired trait to the target plant. Genetic engineering physically removes the DNA from one organism and transfers the gene(s) for one or a few traits into another. Genetic engineering is mainly focused on the central dogma of biology. The components of central dogma, i.e., DNA, RNA, and proteins are manipulated to influence the targeted biological process, metabolic pathway or the trait. However, to do this we need quite a bit of information about the molecular, genetic, and biochemical basis of the target trait(s). Establishing the correlation between the gene (DNA) and the targeted trait is very crucial in achieving the success in genetic engineering. That is to identify the gene (s) which should/can be used for improving the quality trait in question. Once the genes have been identified, then the second question comes to search for the availability of the isoforms of the gene which are more efficient in improving the targeted trait. These information are very vital. Hence, the availability of genomic resources is proving to be very useful for genetic engineering.

All the five steps of plant genetic engineering (i) DNA extraction, (ii) gene cloning, (iii) designing suitable gene construct, (iv) plant transformation and regeneration, and (v) backcross breeding are common to all plants except the transformation and regeneration. Some plants are easy to transform and regenerate, whereas others are recalcitrant. The transformation methods usually employed are broadly classified into two categories, viz. direct transformation methods (electroporation or PEG-mediated transformation of protoplasts, biolistics, etc.) and indirect transformation methods (i.e., requiring an intermediate biological vector, usually the bacterium *Agrobacterium tumefaciens*). Successful transformation, however, relies on various phases, being the introduction and integration of DNA into the plant genome as well as the selection and regeneration of transformed cells. Plant regeneration is generally achieved via in vitro culture systems, using a range of explants and following two alternative pathways: de novo shoot organogenesis or somatic embryogenesis. As a result of worldwide R&D interventions, transformation and regeneration methods for potatoes are available now which otherwise initially were considered recalcitrant to in vitro regeneration. Due to these efforts, it is possible to genetically engineering these vegetables crops for desired traits.

Through transgenic-based approaches, the desired traits can be manipulated by two methods. These are overexpression of the specific gene(s), and repression or inhibition of the specific gene, or both these together. Various tools and constructs have been developed in order to perform these gene overexpression-mediated or gene repression (silencing)-mediated genetic engineering for improvement of the targeted trait. These aspects of the genetic engineering have been extensively described in various literatures. The employed tools/approaches are continuously being improved for their efficiency, precision, and biosafety. The transgenic technology has achieved great success in supplementing crop breeding.

15.3.3 Genome-Editing-Based Tools for Crop Improvement

Genome-editing biotechnological approach is the latest edition to the list of biotechnological approaches for crop improvement. Application of genome editing and their principal has been reviewed by many researchers (Xiong et al. 2015). Genome-editing technologies rely on engineered endonucleases (EENs) that cleave DNA in a sequence-specific manner due to the presence of a sequence-specific DNA-binding domain or RNA sequence. Through recognition of the specific DNA sequence, these nucleases can efficiently and precisely cleave the targeted genes. The double-strand breaks (DSBs) of DNA consequently result in cellular DNA repair mechanisms, including homology-directed repair (HDR) and error-prone nonhomologous end joining breaks (NHEJ), leading to gene modification at the target sites. There are various kinds of engineered endonucleases used for genome editing and can be very useful in improving quality traits of vegetable crops. These are very briefly described as follows.

15.3.3.1 Zinc Finger Nucleases System

Zinc finger nucleases (ZFNs) are the first-generation EENs that were developed following the discovery of the functional principles of the Cys2-His2 zinc finger (ZF) domains. Each ZF protein is able to recognize three contiguous nucleotide bases within the DNA substrate. A generic ZFN monomer is fused by two functional distinct domains: an artificially prepared Cys2-His2 ZF domain at the N-terminal and a nonspecific DNA cleavage domain of the Fok I DNA restriction enzyme at the C-terminal. The dimerization of the Fok I domain is crucial for its enzymatic activity. A ZFN dimer composed of two 3- or 4-ZF domains recognizes an 18- or 24-base target sequence that, statistically, forms a unique site in the genomes of most organisms. ZFNs have been successfully applied to gene modification in model plants (*Arabidopsis*, tobacco, maize, etc.). However, obtaining functional ZFNs requires an extensive and time-consuming screening process. Further, ZFNs have other limitations, such as off-target effector even toxic to the host cells. These shortcomings limit the application of ZFNs in plant genome editing.

15.3.3.2 Transcription Activator-Like Effector Nucleases System

A newly engineered endonuclease, i.e., transcription activator-like effector nucleases (TALENs), has rapidly emerged as an alternative to ZFNs for genome editing. The broad applications of TALENs were transcription activator-like (TAL) effectors that are secreted by the plant pathogenic bacteria *Xanthomonas*. After been pumped into host cells, the TAL effectors enter the nucleus and bind to effector-specific sequences in the host gene promoters and activate transcription. The DNA recognition property of the TAL effectors is mediated by tandem amino acid repeats (34 residues in length).

Two hypervariable amino acids known as repeat-variable di-residues (RVDs) located at the 12th and 13th positions in each repeat determine the binding specificity of the TAL effectors. The TALEN monomer is fused by two independent domains: a customizable DNA-binding domain at the N-terminal and a nonspecific Fok I nuclease domain at the C-terminal. Due to easier manipulation, the genes modified by TALENs have been successfully used in rice, wheat, *Arabidopsis*, potato, tomato, etc.

15.3.3.3 Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-Associated 9 System

Recently, a new class of genome-editing technology, i.e., the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated) system, has been developed. CRISPRs were firstly identified in the *Escherichia coli* genome in 1987 as an unusual sequence element consisting of a series of 29-nucleotide repeats separated by unique 32-nucleotide "spacer" sequences. Later, repetitive sequences with a similar repeat–spacer–repeat pattern were identified in other bacterial and archaeal genomes, but the functions of these repeats remained obscure until 2005 when three independent research groups found the spacer sequence was identical to some part of the viral and plasmid sequence. Further investigations indicated that CRISPRs function through an RNA interference-like mechanism to recognize and cleave foreign DNA. The type II CRISPR/Cas from *Streptococcus pyogenes*, a short CRISPR RNA (crRNA), is able to recognize a complementary stretch of nucleotides in alien DNA and determines the sequence specificity. In addition, a transactivating crRNA (tracrRNA) is required to form a ribonucleoprotein complex with Cas9 nuclease to generate site-specific DSBs. Later, investigators found that the components of crRNA and tracrRNA could be combined into a single RNA chimera, which was termed as guide RNA (gRNA). Efficient cleavage also requires the presence of the protospacer adjacent motif (PAM) in the complementary strand following the recognition sequence. Various interventions have been carried out in CRISPR/Cas method to improve its target specificity. Presently, this technology is being applied in gene modification in various plants and holds great promise for nutritional quality improvement of potato as well.

15.4 Quality Traits of Potato Targeted Through Biotechnological Interventions

As discussed in previous sections, potato plays an important role in diet due to its nutritional content. In addition to have nutritional importance of potato as a staple food/vegetable, potatoes are also one of the most widely used food commodities for a wide range of processed products. Hence, their processing attributes may also be

considered as quality traits. Potatoes do contain some anti-nutritional factors, and thus reducing levels of these anti-nutritional factors also become improvement in quality. Improvement in these quality traits of potato employing biotechnological approaches will be described below.

15.4.1 Nutritional Quality Improvement

15.4.1.1 Phenolic Compounds

Phenolics are a diverse group of tens of thousands of different compounds. Many phenolics occur as derivatives formed by condensation or addition reactions. Chemically, a phenolic is a compound characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups. Some phenolic compounds are effective against diseases or have other health-promoting qualities including effects on longevity, mental acuity, cardiovascular disease, and eye health (Scalbert et al. 2005). Phenolics are the most abundant antioxidants in the diet. Upon consumption, phenolics are metabolized by digestive and hepatic enzymes, by the intestinal microflora and have a wide range of bioavailability (Manach and Donovan 2004).

Potatoes are an important source of dietary phenolics. Phenolic compounds belonging to various classes are present in potato. These include: (i) phenolic acids (chlorogenic acid, caffeic acid, coumaric acid, protocatechuic acid, vanillic acid, ferulic acid, cryptochlorogenic acid, neochlorogenic acid, gallic acid, p-hydroxybenzoic acid, etc.), flavonols (rutin, kaempferol rutinose, quercetin-3-o-glu-rut), flavan-3-ols (catechin, epicatechin), anthocyanidins (delphinidin, cyanidin, pelargonidin, peonidin, malvidin, anthocyanins). Variations in these phenolic compounds in potato genotypes have been reported by several studies (Table 15.3) and reviewed by various researchers (Akyol et al. 2016). As some genotypes of potato have more phenolics than other vegetables (such as tomatoes, peas, onions, French beans, cucumbers, while cabbage, carrots, lettuce), potatoes can be a substantial source of phenolics in the diet and compare very favorably to other vegetables (Chun et al. 2005). Existence of variation of several folds in phenolic content in potatoes envisions the potential to further increase its nutritional value by more fully utilizing existing germplasm. For example, a study of 74 Andean potato landraces revealed an 11-fold variation in total phenolics and a high correlation between phenolics and total antioxidant capacity (Andre et al. 2007a). Similarly, Navarre et al. (2011) screened tubers for phenolics and found over a 15-fold difference in the amount of phenolics in different potato genotypes. Although majority of phenolic compounds are found in greater concentrations in the skin, but significant quantities are also present in the flesh Silva-Beltran et al. (2017), overall the flesh typically contains more phenolics than the skin on a per tuber basis because majority of the fresh weight of a mature potato is contributed by the flesh. These main phenolic compounds found in potato have been briefly described as follows.

Table 15.3 Concentrations of the main phenolic compounds in potato

Phenolics class	Phenolic compounds	Range (mg/100 g dry extract)	References
Phenolic acids	Chlorogenic acid	27.6	Kanatt et al. (2005)
		100.0–220.0	Shakya and Navarre (2006)
		17.4–1274.6	Andre et al. (2007a)
		47.0–283.0	Leo et al. (2008)
		17.3–1468.1	Mäder et al. (2009)
		21.0–40.0	Navarre et al. (2009)
		60.0–292.0	Navarre et al. (2010)
		0.2–2193.0	Deusser et al. (2012)
	Caffeic acid	0.1–0.2	Shakya and Navarre (2006)
		5.0–50.0	Leo et al. (2008)
		1.1–172.4	Mäder et al. (2009)
		2.0–6.9	Navarre et al. (2009)
		0–41.6	Deusser et al. (2012)
	Coumaric acid	0–9.2	Leo et al. (2008)
		0–1.6	Mäder et al. (2009)
	Protocatechuic acid	0–7.6	Mäder et al. (2009)
	Vanillic acid	0–22.4	Mäder et al. (2009)
	Ferulic acid	0.6–9.0	Leo et al. (2008)
		0–3.9	Mäder et al. (2009)
		0–1.4	Deusser et al. (2012)
	Cryptochlorogenic acid	16.0–27.0	
		3.1–163.3	
		8.0–59.0	
		0.1–168.3	
	Neochlorogenic acid	2.9–9.9	
		49.2–91.2	
		0.5–1.5	
		3.0–11.0	
		0.1–87.6	
	Gallic acid	0–1.0	Mäder et al. (2009)
	p-hydroxybenzoic acid	0–7.8	Mäder et al. (2009)
Flavonols	Rutin	0.5–2.6	Shakya and Navarre (2006)

(continued)

Table 15.3 (continued)

Phenolics class	Phenolic compounds	Range (mg/100 g dry extract)	References
		0.6–1.3	Navarre et al. (2010)
		0–12.2	Deusser et al. (2012)
	Kaempferol rutinose	0.5–1.7	Navarre et al. (2010)
	Quercetin-3-o-glu-rut	2.5	Shakya and Navarre (2006)
Flavan-3-ols	Catechin	43.0–204.0	Leo et al. (2008)
		0–1.5	Mäder et al. (2009)
		0–1.4	Deusser et al. (2012)
Anthocyanidins	Anthocyanins	1.4–163.3	Andre et al. (2007b)
		87.0	Han et al. (2007)
		953.8–1630.3	Andre et al. (2007b)
		21.0–109.0	Kita et al. (2013)

Adapted from Akyol et al. (2016)

Phenolic Acids

Phenolic acids and their derivatives are a diverse class of phenolic compounds made by plants. Phenolic acids are derivatives of benzoic and cinnamic acids. The most abundant benzoic acid derivatives are p-hydroxybenzoic, vanillic, syringic, and gallic acids, while common cinnamic acid derivatives include p-coumaric, caffeic, ferulic, and sinapic acids. The derivatives differ in the degree of hydroxylation and methoxylation of the aromatic ring. Phenolic acids are produced in plants via shikimic acid through the phenylpropanoid pathway. The phenolic acids reported to be present in potato tubers are briefly being discussed below.

Chlorogenic Acid

The most abundant phenolics in tubers are caffeoyl-esters. Of the caffeoyl-esters, chlorogenic acid (CGA) comprises over 90% of a tuber's total phenolics (Malmberg and Theander 1985). CGA acid is known to provide protection against degenerative, age-related diseases, may reduce the risk of some cancers and heart disease and have anti-hypersensitive anti-viral and anti-bacterial properties (Yamaguchi et al. 2008; Nogueira and do Lago 2007). The biosynthetic pathway of CGA in plants is depicted in Fig. 15.2. This CGA biosynthetic pathway can thus be engineered for increasing the CGA content in potato. Concerns have been shown about developing high phenolic potatoes that whether they would have unacceptable levels of browning or after cooking darkening. However, studies have shown that neither the amount of total phenolics, CGA nor polyphenols oxidase correlated with the amount of browning observed in fresh-cut potatoes and that they were not rate-limiting in the development

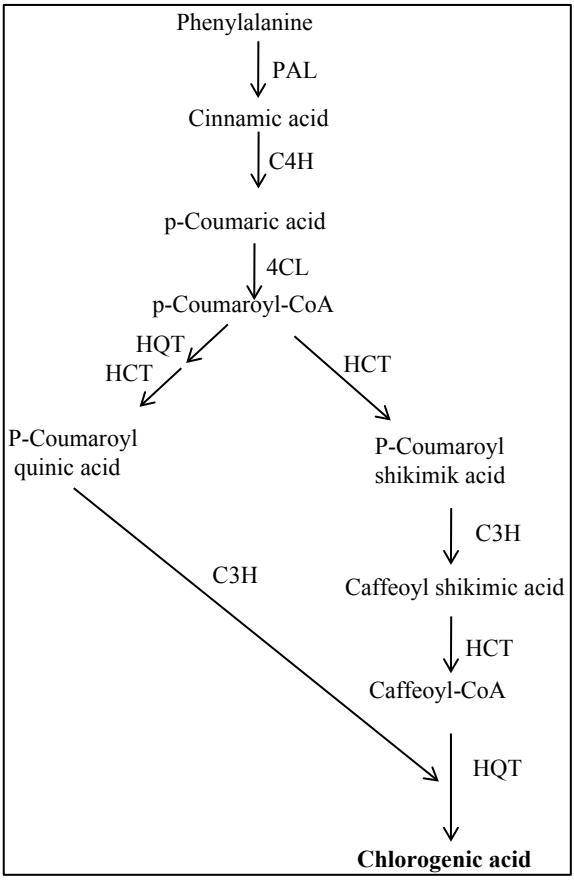

Fig. 15.2 Biosynthesis of chlorogenic acid in potato. PAL: phenylalanine ammonia-lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumaroyl:CoA-ligase; HCT: hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; C3H: *p*-coumarate 3-hydroxylase; C4H: cinnamate 4-hydroxylase; HQT: hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase

of browning (Cantos et al. 2002). Further, using QTL approach, Werij et al. (2007) found no correlation between browning and CGA.

Flavons and Flavan-3-ols

Potatoes contain flavonols such as rutin, kaempferol rutinose, and quercetin-3-o-glu-rut, but have not been thought to be important source of dietary flavonols. Numerous studies have suggested flavons having multiple health-promoting effects, including reduced risk of heart disease, lowered risk of certain respiratory diseases, such as asthma, bronchitis, and emphysema, and reduced risk of some cancers including prostate and lung cancer. One group showed that flavonols increased in fresh-cut tubers, observing concentrations up to 14 mg/100 g FW and suggested that because of the large amount of potatoes consumed, they can be valuable dietary source (Tudela et al. 2002). Various studies have reported the presence of variations in the levels of these flavons in various potato genotypes (Table 15.3). Flavan-3-ols

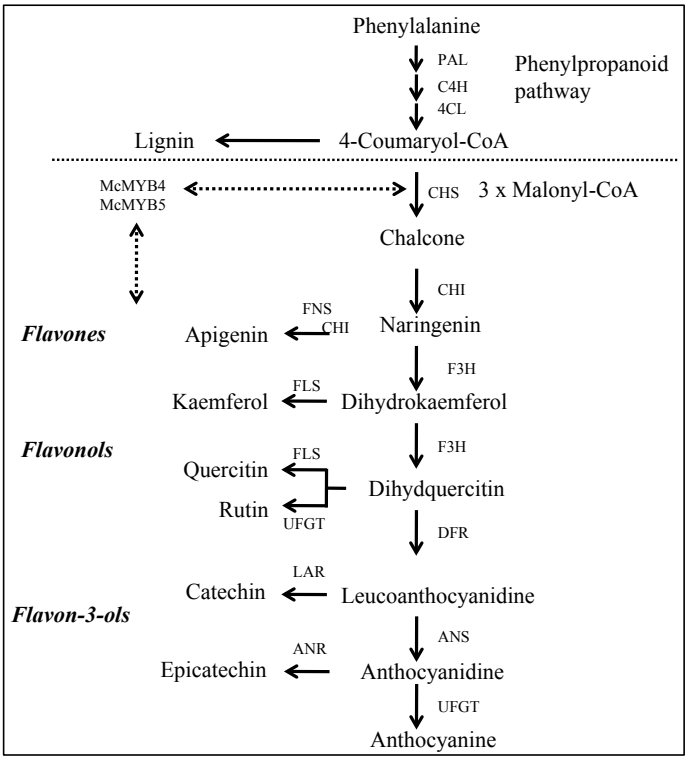

Fig. 15.3 Biosynthesis of flavons, flavan-3-ols and anthocyanins in potato. PAL: phenylalanine ammonia-lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumarate-CoA ligase; CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3 9 H: flavonoid 3 9–hydroxylase; DFR: dihydro-flavonol 4-reductase; FNS: flavone synthase; FLS: flavonol synthase; LAR: leucoanthocyanidin reductase; ANS: anthocyanidin synthase; UFGT: UDP glucose: flavonoid-3-O-glycosyltransferase

(sometimes referred to as flavanols) are derivatives of flavans and include catechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, proanthocyanidins, theaflavins, and thearubigins. Of these, some flavan-3-ols found in potato are enlisted in Table 15.3. Biosynthetic pathway of flavons and flavan-3-ols in plant is depicted as Fig. 15.3.

Anthocyanins

Potatoes, particularly colored-fleshed cultivars, can contain substantial amounts of anthocyanins, compounds that can function as antioxidants and have other health-promoting effects. Anthocyanins from potatoes have been found to have anti-cancer properties (Reddivari et al. 2007). A wide range of variations in anthocyanin content in potato have been reported (Table 15.3). Lewis et al. (1998) screened 26 colored-

fleshed cultivars for anthocyanin content and found up to 7 mg/g FW in the skin and 2 mg/g FW in the flesh. Oertel et al. (2017) screened 57 cultivars of potato for anthocyanin contents. Another study evaluated 31 colored genotypes and found a range of 0.5–3 mg/g FW in the skin and up to 1 mg/g FW in the flesh (Jansen and Flamme 2006). Brown et al. (2005) evaluated several genotypes for anthocyanins and found that whole tubers contained up to 4 mg/g FW and that anthocyanin concentration correlated with the antioxidant value. In 2005, Parr et al. reported the compounds called kukoamines in potatoes. These compounds are phenolic–polyamine conjugates and had previously only been found in a Chinese medicinal plant, in which they were being studied because they lower blood pressure. Roles of tuber polyamines include regulation of starch biosynthesis, calystegine synthesis, disease resistance, and sprouting (Tanemura and Yoshino 2006; Matsuda et al. 2005). Using high-throughput HPLC analysis, 30 putative polyamines were detected in potato tubers (Shakya and Navarre 2006).

Biosynthetic pathway of anthocyanins in plant is depicted as Fig. 15.3. It has been demonstrated that tuber-specific expression of the native and slightly modified MYB transcription factor gene StMtf1(M) activates the phenylpropanoid biosynthetic pathway. Compared with untransformed controls, transgenic tubers contained fourfold increased levels of caffeoylquinates, including CGA (1.80 mg/g dry weight), while also accumulating various flavonols and anthocyanins. Subsequent impairment of anthocyanin biosynthesis through silencing of the flavonoid-3′,5′-hydroxylase (F3′5′h) gene resulted in the accumulation of kaempferol-rut (KAR) to levels that were approximately 100-fold higher than in controls (0.12 mg/g dry weight). The biochemical changes were associated with increased expression of both the CGA biosynthetic hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase (Hqt) gene and the upstream chorismate mutase (Cm) and prephenate dehydratase (pdh) genes. Field trials indicated that transgenic lines produced similar tuber yields to the original potato variety. Processed products of these lines retained most of their phenylpropanoids and were indistinguishable from untransformed controls in texture and taste (Rommens et al. 2008).

15.4.1.2 Carotenoids

Carotenoids are the second most abundant naturally occurring pigments on earth, with more than 750 members. Carotenoid pigments are mainly C40 lipophilic isoprenoids and synthesized in all photosynthetic organisms (bacteria, algae, and plants) and range from colorless to yellow, orange, and red, with different degree of variations. Carotenoids have numerous health-promoting properties. Some carotenoids are precursors of vitamin A and prevent human age-related macular degeneration, and some are potent antioxidant and are considered to prevent prostate cancer and cardiovascular disease. In humans, carotenoids also serve as antioxidants and reduce age-related macular degeneration of the eye, the leading cause of blindness in the elderly worldwide. An increasing interest in carotenoids as nutritional sources of

pro-vitamin A and health-promoting compounds has prompted a significant effort in metabolic engineering of carotenoid content and composition in food crops.

Potatoes also contain lipophilic compounds such as carotenoids, though in lesser amount (Table 15.4). The yellow/orange flesh color found in some potatoes is due to carotenoids. The carotenoids' content of tubers in most potato cultivars ranges between 0.2 and 36 μg/g FW (Iwanzik et al. 1983; Brown et al. 1993; Andre et al. 2007a). This variation in carotenoid concentrations has been suggested to be regulated mainly at the transcriptional levels (Morris et al. 2006). The most abundant potato carotenoids are composed mainly of the xanthophylls lutein, antheraxanthin, violaxanthin, and xanthophyll esters. Carotenoids are synthesized in plastids from isoprenoid pathway (Fig. 15.4) and are accumulated in most plant organs. Various genes of these pathways have been characterized in a range of organisms, and understanding of the regulation of the carotenoids pathway has led to devising strategies for manipulating this pathway. Numerous groups have attempted to increase potato carotenoids using transgenic strategies. The strategy commonly used in plants is to increase the biosynthetic capacity by altering the carotenogenic enzyme activities. Overexpressing a bacterial phytoene synthase in tuber of the cultivar *Desiree* increased carotenoids from 5.6 to 35 μg/g DW and changed the ratios of individual carotenoids. Beta-carotene concentrations increased from trace amounts to 11 μg/g DW and lutein levels increased 19-fold (Ducreux et al. 2005). Carotenoids have also been increased by the approaches that do not directly involve use of carotenoids biosynthesis genes, as shown by overexpression of the cauliflower *Or* gene in Desiree resulting in a sixfold increase in tuber carotenoids to about 20–25 μg/g DW (Lu et al. 2006). A twofold increase in carotenoids was observed in tubers overexpressing *Or* after six months of cold storage, but no such increase was observed in wild-type or empty-vector transformed plants (Lopez et al. 2008; Li et al. 2012). However, this is in contrast to earlier findings that potato cultivars undergo a decline in total carotenoids during cold storage (Morris et al. 2006). Cultivated diploid potatoes derived from *Solanum stenotomum* and *Solanum phureja* were found to contain up to 2000 μg/100 g FW zeaxanthin (Brown et al. 1993). A study of 24 Andean cultivars were found with almost 18 μg/g DW each of lutein and zeaxanthin and just over 2 μg/g DW of beta-carotene (Andre et al. 2007a). Overexpression of three bacterial genes in Desiree resulted in 20-fold increase in total carotenoids to 114 μg/g DW and a 360-fold increase in beta-carotene to 47 μg/g DW (Diretto et al. 2007). Bub et al. (2008) investigated whether zeaxanthin from genetically modified zeaxanthin-rich potatoes is bioavailable in humans and found that consumption of zeaxanthin-rich potatoes significantly increased chylomicron zeaxanthin concentrations suggesting that potentially such potatoes could be used as an important dietary source of zeaxanthin. Diretto et al. (2006) silenced the first dedicated step in the beta-epsilon branch of carotenoid biosynthesis, lycopene epsilon cyclase (LCY-e), by introducing, via *Agrobacterium*-mediated transformation, an antisense fragment of this gene under the control of the patatin promoter. Antisense tubers showed 2.5-fold increase in total carotenoids, with beta-carotene showing the maximum increase of up to 14-fold. The data suggested that epsilon cyclization of lycopene is a key regulatory step in potato tuber carotenogenesis.

Table 15.4 Carotenoids content reported in potato tubers (mg/kg DW* or FW**)

Carotenoid	Content	Potato cultivars	References
Total carotenoids			
	28.0*	Skin of tubers	Campbell et al. (2010)
	9.0*	Flesh of tubers	
	1.10–12.2*	Different cultivars	Hamouz et al. (2016)
	0.50–15.5*	Different cultivars	Fernandez-Orozco et al. (2013)
	0.58–1.75	Yellow cultivars	Breithaupt and Bamedi (2002)
	0.38–0.62	White cultivars	
	26.2*/5.69*	Yellow/red/purple	Brown (2005)
	8.0–20.0	Yellow–orange cvs.	
	26.0	Papa Amarilla cvs.	Brown et al. (2008)
	5.67	Inca-no-hitomi orange	Kobayashi et al. (2008)
	5.60–35.0*	Transgen. Desirée	Ducreux et al. (2005)
	3.0–36.0*	Andean landraces	Andre et al. (2007a)
	1.03–21.4	*S. phureja* accession	Bonierbale et al. (2009)
	2.57 ± 0.53*	Shetland Black	
	14.8 ± 2.22*	Red Laura	Burmeister et al. (2011)
	8.23 ± 2.98*	Boiled M. Twilight	
	1.51 ± 0.31*	Boiled Shetl. Black	Tierno et al. (2015)
	1.51 ± 0.31*	Boiled Shetl. Black	
Sum of carotenoid esters	0.41–1.31	Yellow and white	Breithaupt and Bamedi (2002)
Individual carotenoids			
All-*trans*-Lutein	1.12–17.7	Andean landraces	Andre et al. (2007b)
	0.55–1.89	*S. phureja* accession	Bonierbale et al. (2009)
	3.27–9.50*	Raw tubers	Clevidence et al. (2005)
	3.89–9.50*	Boiled tubers	
All-*trans*-Violaxanthin	trace–2.78	*S. phureja* accession	Bonierbale et al. (2009)
All-*trans*-Antheraxantin	0.03–3.54	*S. phureja* accession	Bonierbale et al. (2009)
All-*trans*-Zeaxanthin	18	Andean landraces	Andre et al. (2007b)
	12.9	*S. phureja*	Burgos et al. (2009)
	>10.0	*S. phureja*	Bonierbale et al. (2009)
	Trace–12.9	*S. phureja*	
	Trace–40*	Accession raw/boiled tubers	Clevidence et al. (2005)
All-*trans*-β-Carotene	2	Andean landraces	Andre et al. (2007b)

(continued)

Table 15.4 (continued)

Carotenoid	Content	Potato cultivars	References
	>0.1	*S. phureja* accession	Bonierbale et al. (2009)
Lutein-5,6-epoxide	Identified	Commercial, bred, old, and native cultivars	Fernandez-Orozco et al. (2013)
9-*cis*-Lutein	Identified		
13-*cis*-Lutein	Identified		
9-*cis*-Violaxanthin	+5,6-epoxide		
All-*trans*-Neoxanthin	+5,6-epoxide		
9′-*cis*-Neoxanthin	+5,6-epoxide		
Mutatoxanthin	Identified		
Luteoxanthin	+5,6 epoxide		
Neochrome	Identified		
All-*trans*-β-Cryptoxanthin	Identified		

Adapted from Lachman et al. (2016)

In 2006, Morris et al. engineered astaxanthin in potato tubers. Both *S. tuberosum* and *S. phureja* transgenic lines were produced that expressed an algal bkt1 gene, encoding a beta-ketolase, and accumulated ketocarotenoids. Two major ketocarotenoids were detected, ketolutein and astaxanthin. The level of unesterified astaxanthin reached 14 μg/g DW in some bkt1 expressing lines of *S. phureja* but was much lower in the *S. tuberosum*. Similarly, expression of *Erwinia uredovora* crtB gene encoding phytoene synthase in potato resulted in increased levels of carotenoids (Ducreux et al. 2005). The tuber of *S. tuberosum* L. cultivar Desiree normally produces tubers containing 5.6 μg carotenoid/g DW and tubers of *S. phureja* cultivar "*Mayan Gold*" contain carotenoid content of typically 20 μg carotenoid/g DW. In developing tubers of transgenic crtB Desiree lines, carotenoid levels reached 35 μg carotenoid/g DW and the balance of carotenoids changed radically compared with controls. Beta-carotene levels in the transgenic tubers reached 11 μg carotenoid/g DW, whereas control tubers contained negligible amounts and lutein accumulated to a level 19-fold higher than empty-vector transformed controls. The crtB gene was also transformed into *S. phureja* (cv. Mayan Gold), again resulting in an increase in total carotenoid content to 78 μg/g DW in the most affected transgenic line. In these tubers, the major carotenoids were violaxanthin, lutein, antheraxanthin, and beta-carotene. No increases in expression levels of the major carotenoid biosynthetic genes could be detected in the transgenic tubers, despite the large increase in carotenoid accumulation. Romer et al. (2002) genetically modified two potato varieties. By transforming with sense and antisense constructs encoding zeaxanthin epoxidase, zeaxanthin conversion to violaxanthin was inhibited. Both approaches (antisense and co-suppression) yielded potato tubers with higher levels of zeaxanthin. Depending on the transgenic lines and tuber development, zeaxanthin content was

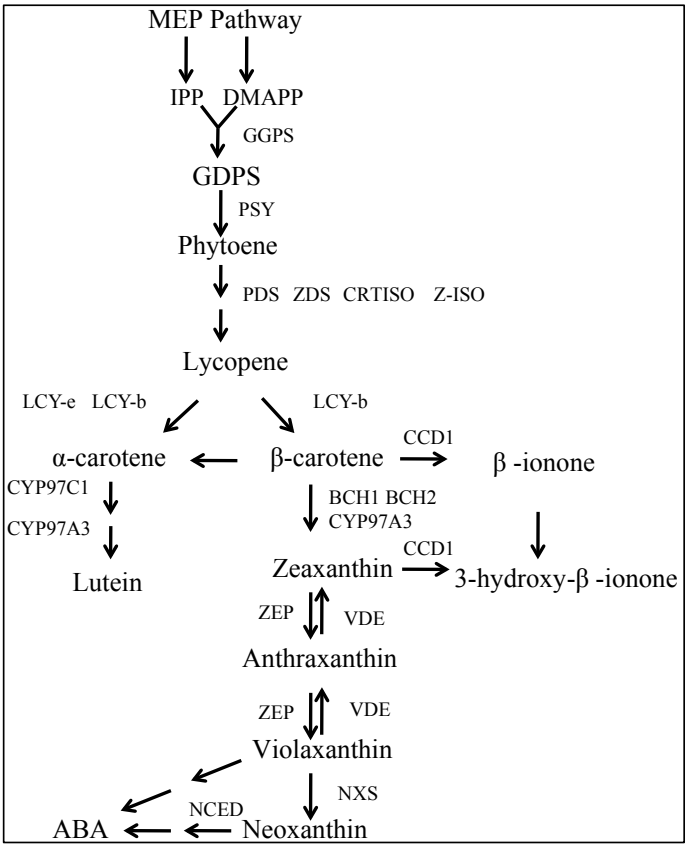

Fig. 15.4 Biosynthesis of carotenoids in potato. GGPS: geranylgeranyl pyrophosphate synthase; PSY: phytoene synthase; PDS: phytoene desaturase; ZDS: f-carotene desaturase; CRTISO: carotenoid isomerase; LCY-ε: lycopene ε-cyclase; LCY β: lycopene β-cyclase; CHY-ε: ε-ring hydroxylase; CHY-β: β-carotene hydroxylase; VDE: violaxanthin de-epoxidase; ZEP: zeaxanthin epoxidase; NXS: neoxanthin synthase; CCD: carotenoid cleavage dioxygenase; NCED: 9-cis-epoxycarotenoids dioxygenase

elevated 4–130-fold reaching values up to 40–78 μg/g DW. As a consequence of the genetic manipulation, the amount of violaxanthin was diminished dramatically and in some cases the monoepoxy intermediate antheraxanthin accumulated. In addition, most of the transformants with higher zeaxanthin levels also showed increased total carotenoid contents (up to 5.7-fold) and some of them exhibited reduced amounts of lutein. The increase in total carotenoids suggested that the genetic modification affects the regulation of the whole carotenoid biosynthetic pathway in potato tubers.

15.4.1.3 Vitamins

Vitamins are a class of organic compounds, absolutely required for the maintenance of healthy life processes. Role of vitamins in maintaining human health via regulating metabolism and supporting the biochemical process related to the energy released from food or other sources in living organisms is well established. Vitamins are also important in the synthesis of hormones, enzyme activity, red blood cells, genetic materials, and neurotransmitters (Jube and Borthakur 2006). Although vitamins are required in small amounts, their capability of sustenance and their ability to perform biochemical functions is remarkable. Based on the solubility, vitamins have been grouped into water-soluble vitamins and fat-soluble vitamins. Fat-soluble vitamins are A, D, E, and K, and the rest are water soluble. Most of the vitamins have been found to act as coenzymes; some act as growth regulators, and most of them as antioxidants. Well-known human vitamin-related disorders include blindness (Vitamin A), beriberi (Vitamin B1), pellagra (Vitamin B3), anemia (Vitamin B6), neural defects in infants (Vitamin B9), scurvy (Vitamin C), sterility-related diseases (Vitamin E), and Rickettsia (Vitamin D). In potato, predominant vitamin is vitamin C (Camire et al. 2009). Potato also contains several B vitamins (folic acid, niacin, pyridoxine, riboflavin, and thiamin), the composition of which is given in Table 15.5. Vitamin in potatoes can be increased through fortification in processed foods, conventional breeding, or through use of transgenic techniques, a process known as biofortification. The major vitamins present in potato and the research outcome to increase these vitamins level in potato are discussed below.

Vitamin C

Predominant vitamin in potatoes is vitamin C (also known as L-ascorbic acid), which ranges from 84 to 145 mg/100 g DW depending on cultivar and soil composition (Camire et al. 2009). A medium red-skinned potato (173 g) provides about 36% vitamin C of the RDA according to the USDA database (Navarre et al. 2009). Vitamin C is an important component in nutrition with the property of antioxidant, immunoprotection, cardiovascular function improvement, prevention of ailments associated with connective tissues, and help in iron metabolism. Vitamin C is a cofactor for numerous enzymes, functioning as an electron donor. The best-known symptom of vitamin C deficiency is scurvy, which in severe cases is typified by loss of teeth, liver spots, and bleeding. More than 90% of vitamin C in human diets is supplied by fruits and vegetables. It has been suggested that 100–200 mg vitamin C should be supplied by human diets, and this quantity is expected to be increasing because of increasing stress in modern life. Therefore, it is valuable to increase vitamin C content in edible products of plant. In India, the available supply of vitamin C is 43 mg/capita/day, and in the different states of India, it ranges from 27 to 66 mg/day which is far below the recommended dose of 400 mg/day by ICMR (National Institute of Nutrition 2011).

Plants may have multiple vitamin C biosynthetic pathways; with all of the enzymes of the L-galactose pathway have been characterized (Laing et al. 2007; Wolucka and

Table 15.5 Nutrient composition of potato (*Solanum tuberosum*), white, flesh, and skin, raw per 100 g

Nutrient		Units	Value per 100 g
Proximates	Water	g	81.6
	Energy	kJ	288
	Protein	g	1.68
	Total lipid (Fat)	g	0.1
	Ash	g	0.94
	Carbohydrate, by diff.	g	15.7
	Fiber, total dietary	g	2.4
	Sugar, total	g	1.15
	Sucrose	g	0.28
	Glucose (destrose)	g	0.53
	Fructose	g	0.34
	Lactose	g	0
	Maltose	g	0
	Galactose	g	0
	Starch	g	13.5
	Available carbohydrate[2]	g	14.65
Minerals	Calcium, Ca	mg	9
	Iron, Fe	mg	0.52
	Magnesium, Mg	mg	21
	Phosphorous, p	mg	62
	Potassium, K	mg	407
	Sodium, Na	mg	6
	Zinc, Zn	mg	0.29
	Copper, Cu	mg	0.116
	Manganese, Mn	mg	0.145
	Selenium, Se	mg	0.3
Vitamins	Vitamin C	mg	19.7
	Thiamin	mg	0.071
	Riboflavin	mg	0.034
	Niacin	mg	1.066
	Pantothenic acid	mg	0.281
	Vitamin B-6	mg	0.203
	Folate, total	mcg	18
	Folic acid	mcg	0
	Folate, food	mcg	18

(continued)

Table 15.5 (continued)

Nutrient		Units	Value per 100 g
	Folate DFE	Mcg_DFE	18
	Choline, total	mg	11
	Betaine	mg	0.2
	Vitamin B12	mcg	0
	Vitamin A IU	IU	8
	Vitamin A RAE	Mcg_RAE	0

USDA National Nutrient Database, No. 11365

Van Montagu 2007). One study examined tuber vitamin C content in 75 genotypes and found concentrations ranging from 11.5 to 29.8 mg/100 g FW (Love et al. 2004). This study also reported that some genotypes had more consistent concentrations of vitamin C than others across multiple years or when grown in different locations and suggests that the year may have a bigger effect than location. Dale et al. (2003) measured vitamin A in 33 cultivars grown in three locations around Europe and found vitamin C in a range of 13–30.8 mg/100 g FW. Extensive research work has been undertaken at molecular levels of vitamin C biosynthetic pathway in plants. An outline of plant vitamin C biosynthesis pathway is represented in Fig. 15.5. This knowledge has made it possible to manipulate vitamin C content in several crops (including potato) using various approaches including genetic engineering based. Transfer of L-gulone-γ-lactone oxidase gene from rat to potato resulted in 40% increase in vitamin C (Jain and Nessler 2000). Overexpression of gene encoding enzyme D-galacturonic acid reductase (catalyzes reduction of D-galacturonic acid or L-galactonic acid in the pathway for ascorbic acid biosynthesis via uronic acids) from strawberry in potato gave rise to twofold increase in tuber ascorbate content with respect to wild-type plants (Hemavathi et al. 2010; Vathi et al. 2009, 2011). Qin et al. (2011) transformed potato with its native cytosolic- and chloroplastic-targeted *DHAR* cDNAs, each under the control of the *CaMV 35S* promoter. Overexpression of cytosol-targeted *DHAR* led to increased ascorbate content in both tubers and leaves while overexpressing the chloroplastic enzyme also affected leaf ascorbate content. Bulley et al. (2012) reported an up to threefold increase in ascorbate through the overexpression of a single potato gene, GDP-L-galactose phosphorylase. In another report, the potato transgenic lines were developed by overexpressing DHAR gene, driven by the CaMV35S constitutive promoter and a tuber-specific patatin promoter. The AsA level in tubers of patatin: DHAR transgenic lines showed an enhanced level (up to 1.3-folds) as compared to that of control plants (Young et al. 2008). In another report, two independent transgenic potato lines were developed by overexpression of cytosolic DHAR (Cyt DHAR) gene and chloroplast DHAR (Chl DHAR) gene (Qin et al. 2011). The Cyt DHAR gene considerably augmented DHAR activities and AsA contents in potato tubers and leaves, because overexpression of Chl DHAR gene could only increase DHAR activities and AsA contents in leaves, not in tubers. These results indicated that AsA level of potato is enhanced by increasing recycling ascorbate via

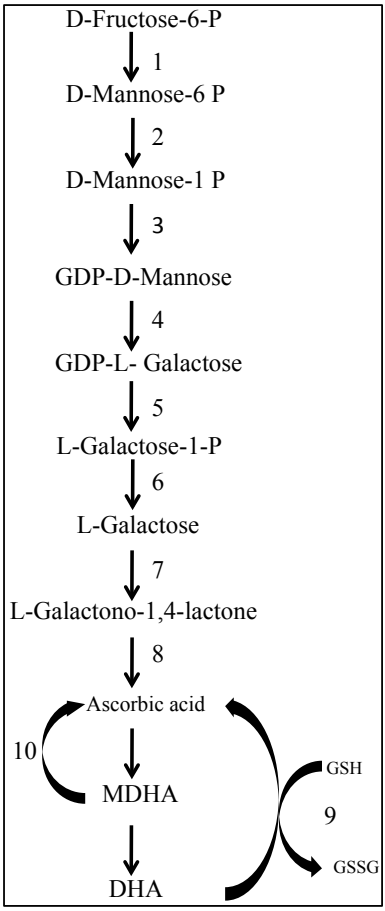

Fig. 15.5 Ascorbic acid biosynthesis and recycling pathways in plants. 1: mannose-6-phosphate isomerase; 2: phosphomannomutase; 3: GDP-mannose pyrophosphorylase (mannose-1-phosphate guanylyltransferase); 4: GDP-mannose-3′,5′-epimerase; 5: phosphodiesterase; 6: sugar phosphatase; 7: L-galactose dehydrogenase; 8: L-galactono-1,4-lactone dehydrogenase; 9: dehydroascorbate reductase; 10: mono-dehydro-ascorbate reductase

DHAR overexpression. Similarly, the potato transformation was done using the gene construct with potato isolate GGP (GDP-L-galactose phosphorylase) gene under the control of polyubiquitin promoter (tubers only). The molecular and biochemical study revealed that transgenic potato showed an increase in tuber ascorbate of up to threefold (Bulley et al. 2012).

Vitamin A

Vitamin A deficiency is one of the most prevalent nutrient deficiencies in many underdeveloped regions of the world, where it affects an approximately 250 million children under 5 years of age. Beta-carotene is the primary substrate for synthesis of vitamin A in humans. Plant pro-vitamin A carotenoids are the primary dietary precursors of vitamin A. While many fruits and vegetables have high levels of pro-

vitamin A carotenoids, staple crops contain low levels of these compounds, which contributes to the global prevalence of vitamin A deficiency. Vitamin A deficiency (VAD) is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections. To help combat vitamin A deficiency, a global effort is underway to increase pro-vitamin A content in major food crops including potato. Cultivated potato is extremely poor in pro-vitamin, i.e., β-carotene. However, metabolic engineering efforts to accumulate high levels of β-carotene in potato tubers proved successful. Ducreux et al. (2005) worked on two potato cultivars to increase the carotenoid content of potato tubers. *S. tuberosum* cv Desiree, which typically accumulates 5.6 μg/g DW carotenoids with negligible β-carotene content and *S. phureja* cv. Mayan Gold which typically accumulates 20 μg/g DW carotenoids. Both cultivars were transformed with the phytoene synthase gene (crtB) (for place of this enzyme in carotenoid biosynthetic pathway kindly see Fig. 15.4) from *E. uredovora*. Transgenic potato showed an accumulation of 35 total carotenoids and 11 μg/g DW β-carotene in developing tubers of Desiree and 78 μg/g DW in Mayan Gold tubers. In another study, the gene encoding lycopene ε-cyclase (Lcy-ε) (for place of this enzyme in carotenoid biosynthetic pathway kindly see Fig. 15.4) was targeted with a tuber-specific antisense construct in order to suppress epsilon cyclization of lycopene and direct the flux toward β-β-carotenoid branch (Diretto et al. 2006). Results showed a tuber-specific increase in the accumulation of β-carotene (up to 14-fold) and β-β-carotenoids (up to 25-fold) with a decrease in accumulation of lutein. When the β-carotene hydroxylation step of the β-β-carotenoid branch was targeted by tuber-specific antisense silencing of the beta-carotene hydroxylase (*chy1* and *chy2*) (for place of this enzyme in carotenoid biosynthetic pathway kindly see Fig. 15.4), a 38-fold increase in tuber β-carotene content was achieved (Diretto et al. 2007). Similarly, by silencing the β-carotene hydroxylase gene in potato using RNAi, Van Eck et al. (2010) were able to significantly increase beta-carotene content of tubers, even in lines that normally accumulate only low levels of zeaxanthin.

Vitamin E

Vitamin E (also known as tocopherols) is another essential nutrient for human health, but is consumed at suboptimal levels. The importance of vitamin E for reproductive health was recognized as early as 1922. Humans and other animals are not capable of synthesizing tocopherol (vitamin E) autonomously and must be obtained from their diet. The vitamin E (α-tocopherol) is only synthesized by photosynthetic organisms which show potent antioxidant activity and vital for human health, however, consumed at the suboptimal level. The metabolic pathways involved in tocopherol biosynthesis in plants have been deciphered to a greater extent. A generalized pathway of the vitamin E metabolic biosynthesis in plants is represented in (Fig. 15.6). In 2008, Crowell et al. reported the development of transgenic tuber over accumulating vitamin E where the transgenic potato lines developed via *Agrobacterium*-mediated transformation using two vitamin E biosynthetic genes, p-hydroxyphenylpyruvate dioxygenase (At-HPPD) and homogentisate phytyl transferase (At-HPT), isolated

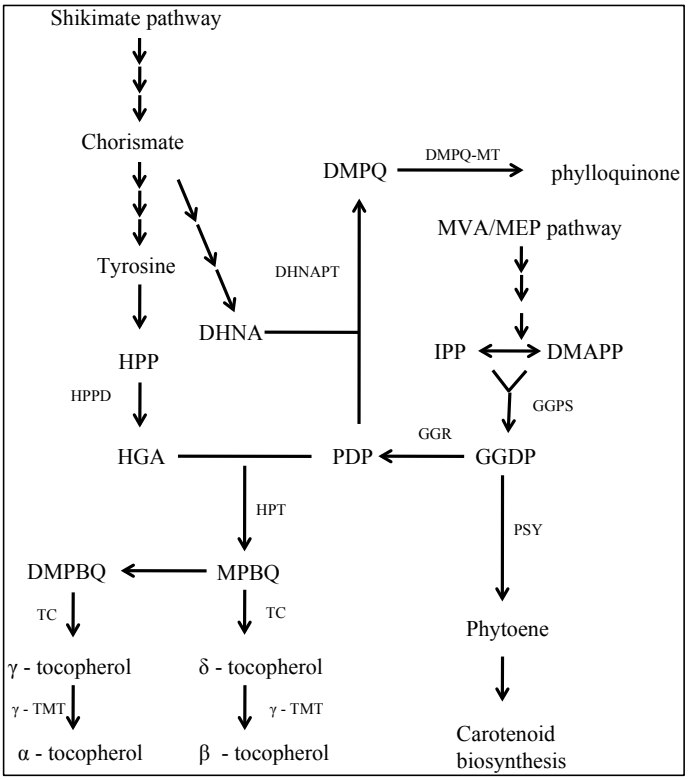

Fig. 15.6 Biosynthesis of tocopherols in plants. HPPD: p-hydroxyphenyl-pyruvate dioxygenase; HPT: homogentisate phytyltransferase; MPBQ: methylphytylbenzoquinone; MT: methyltransferase; γ-TMT: γ-tocopherol methyltransferase

from *Arabidopsis thaliana*. Biochemical and molecular analysis revealed that the overexpression of At-HPPD and At-HPT resulted in a maximum 266 and 106% increase in alpha-tocopherol, respectively, still lesser alpha-tocopherol than leaves or seeds. This might be limiting factors for tocopherol accumulation in potato tubers due to physiological and biochemical regulatory constraints. Overexpression of *Arabidopsis At-HPPD* and homogentisate phytyl transferase (*At-HPT*) genes in potato transgenics was carried in an attempt to increase vitamin E content of potato. *At-HPPD* resulted in maximum 266% increase in α-tocopherol, and overexpression of *At-HPT* yielded a 106% increase in potato.

Vitamin B9

Vitamin B_9, also known folates, is used as generic name to designate tetrahydrofolate (THF) and its on-carbon (C1) unit derivatives. Folates are essential micronutrients in the human diet. Folates are important cofactors involved in C1 unit transfer reac-

tions. Folates exist in various forms. All-native reduced folate derivatives are very sensitive to oxidative cleavage at the C9 and N10 bond; however, there are marked differences in stability of those species, 5-formyl-THF being the most stable natural folate, THF the least, and 5-methyl-THF intermediate. Folate deficiency is associated with the increase risk of neural tube defects, cardiovascular diseases, megaloblastic anemia, and some cancers (Bailey et al. 2003; Scott 1999). Unfortunately, folate intake is suboptimal in most of the world's populations, even in developed countries (Scott et al. 2000). Therefore, there is an urgent need to increase folate content and bioavailability in staple foods. Because of its large consumption worldwide, potato is an appealing target for enrichment.

Importance of folates in human diets urges to increase the folate levels in potato. Humans are not capable of synthesizing folates and thus require dietary supply. Plants represent the major source of folate in the diet. As such potato is in the lower range of folate contents among plant foods, even then potato is a well-known significant source of folates in the diet due to its high level of consumption more so that for its endogenous content. Several studies reported folate concentrations in potatoes of usually unspecified genotypes, and the reported values can vary substantially depending on the analytical method used. Values for folate concentrations in mature raw potato vary between 12 and 37 μg/100 g FW (Konings et al. 2001; Vahteristo et al. 1997) except a study by McKillop et al. (2002) who reported an exceptionally high folate concentration (125 μg/100 g FW). The USDA National nutrient Database for Standard Reference (SR20) gives values of 14 and 18 μg/100 g FW for raw potatoes. Goyer and Navarre (2007) determined total folate concentration of potato tubers from >70 cultivars, advanced breeding lines, and wild species and found showed an approximately threefold difference in folate values ranging from 0.46 to 1.37 μg/g DW or 11 to 35 μg/100 g FW. Vahteristo et al. (1997) determined that raw potatoes contain 21 μg/100 g FW of 5-methyl-THF, 3 μg/100 g FW of THF, and traces of 10-formyl folic acid, an oxidation product of 10-formyl-THF. Konings et al. (2001) showed that >95% of folates were present as a 5-methyl-THF derivative in potato tubers, the rest comprising 10-formyl folic acid and folic acid, and that total folate derivatives were >90% polyglutamylated. Therefore, polyglutamated forms of 5-methyl-THF seem to constitute most of the folate pool in potato tuber as is the case in most fruits and vegetables. This variation in folic acid content in various potato genotypes can be utilized through transgenic approaches for improving the folic acid content in popular commercial cultivars of potato. Nevertheless, improving folate contents using genetic engineering has been thought to be possible. As folate biosynthesis has been fairly delineated in recent years, metabolic engineering of the pathway is feasible. Recently, De Lepeleire et al. (2018) provided a proof of concept that additional introduction of HPPK/DHPS and/or FPGS, downstream genes in mitochondrial folate biosynthesis, enable augmentation of folates to satisfactory levels (12-fold) and observed folate stability upon long-term storage of tubers. This engineering strategy can serve as a model in the creation of folate-accumulating potato cultivars, readily applicable in potato-consuming populations suffering from folate deficiency.

Vitamin B6

Vitamin B_6 (chemically know as pyridoxine) is water soluble and like folate has several vitamins. Vitamin B_6 may be involved in more bodily functions than any other nutrient, is a cofactor for many enzymes, especially those involved in protein metabolism, and is also a cofactor for folate metabolism. Vitamin B_6 has anti-cancer activity, is a strong antioxidant, and is involved in hemoglobin biosynthesis, lipid and glucose metabolism, and immune and nervous system function (Tambasco-Studart et al. 2005; Theodoratou et al. 2008; Denslow et al. 2005). Possible consequences of deficiency include anemia, impaired immune function, depression, confusion, and dermatitis (Spinneker et al. 2007).

The most significant sources of Vitamin B_6 are animal proteins, starchy vegetables (potatoes), bananas, avocados, walnuts, peanuts, and legumes. Potatoes are an important source of dietary vitamin B_6 (Kant and Block 1990) with a medium-baked potato (173 g) providing about 26% of the RDA (USDA National Nutrient Database SR20). Very little research has been conducted on this vitamin in potato; thus, little is known about how much its concentration vary among genotypes. Rogan et al. (2000) had reported the content of Vitamin B_6 in potato in the range of 0.26–0.82 mg/200 g FW have been reported. Vitamin B_6 content varies substantially among the potato genotypes. There is thus great potential for improving potato further through increasing the content of this specific phytonutrient, by either breeding or genetic manipulation to fortify the B_6 vitamin as a healthy food resource for human nutrition. Work on elucidation of metabolism of vitamin B_6 in plants is in progress which may be of vital importance for improving the vitamin B_6 content in potatoes. Recently, Bagri et al. (2018) developed the of transgenic potato cv. Kufri Chipsona overexpressing key vitamin B_6 pathway gene, the PDXII from *A. thaliana* under the control of CaMV 35S constitutive promoter. Transgenic tubers exhibited 107–150% increase in vitamin B_6 accumulation in comparison to the untransformed controls potato.

15.4.2 Protein and Essential Amino Acids

Origin of name "Protein" (derived from the Greek word "*proteios*" means primary) itself justifies it as one of the primary components of the living cells and is the most important nutrient for humans. Lack of sufficient proteins in diet leads to deleterious effects on growth and development in human beings. The deleterious effects of diets that are sufficient in protein quantity but deficient in protein quality are well documented: poor growth, tissue wasting, and in severe cases, death. Lack of sufficient protein in diet is known as protein energy malnutrition (PEM), and this is the most lethal form of malnutrition and affects every fourth child worldwide. Building blocks of proteins are twenty common amino acids. Humans like other animals can only produce about half of the 20 common amino acids needed for life, the rest amino acids must be obtained via diet, and these amino acids are referred to as essential amino acids. Plant proteins contribute about 65% of the per capita supply of protein on

worldwide basis. Among plants, cereal grains, tubers, and food legumes are the most important suppliers of proteins. As the world's population increases (and with it the load on our agricultural resources), the need to make good-quality protein available efficiently and economically becomes increasingly important. The importance and urgency of providing humans with quality proteins are reflected in the growing scientific and industrial interest in augmenting the nutritive value of the world's protein sources. Major efforts have been made to enhance the overall protein content and/or to improve the essential amino acid composition of plant protein. The latter may be considered as improving the quality of the targeted protein(s). Most plant proteins are incomplete sources of amino acids. Among essential amino acids, methionine (Met), lysine (Lys), and tryptophan (Trp) are present in very low quantity as compared to other food sources (Table 15.6). This clearly shows that there is urgent need and ample scope for improving these essential amino acids in potato. Because of the importance of dietary protein and the fact that plants are its major source, development of strategies to increase protein levels and the concentration of essential amino acids in food crops is of primary importance in a crop improvement program. In potato, protein content ranges from 1 to 1.5% of tuber fresh weight (Ortiz-Medina and Donnelly 2003). Compared with other, it is negligible a source, potatoes are not typically considered to be good dietary protein sources due to their low overall protein content although it has excellent biological value of 90–100 (Camire et al. 2009). Keeping these facts in view, genetic engineering-based strategies and the efforts to enhance the protein quality/quantity and essential amino acids (specifically methionine, lysine and tryptophan) in various crop plants including potato have been targeted worldwide. Here, efforts been made in potato are described.

Table 15.6 Lys, Met, and Trp contents (mg/100 g food) in the major protein sources worldwide

Food	Lysine	Methionine	Tryptophan
Potatoes	*130*	*30*	*30*
Beans	1870	260	230
Peas	610	100	100
Soybean	1900	580	450
Maize	290	190	70
Barley	380	180	150
Rice	290	170	90
Wheat	380	220	150
Nuts	750	330	450
Pig meat	2200	750	310
Freshwater fish	2020	700	240
Marine fish, other	2050	600	240

Adapted from Le et al. (2016)

15.4.2.1 Role of Methionine, Lysine, and Tryptophan in Humans

Roles of these three essential amino acids, viz. lysine, methionine, and tryptophan have been described in several literatures. Methionine acts as a precursor for the synthesis of S-adenosylmethionine (SAM). SAM is a substrate involved in epigenetics and in fatty acid oxidation. Methionine also acts as an important methyl donor in human metabolism. Lack of methionine in diet leads to methylation-related disorders such as fatty liver, tumorigenesis, neurological disorders, and atherosclerosis. The limited availability of methionine leads to DNA strand breakage and fragmentation, which may be significant to the carcinogenic process (Garcia et al. 2011; Forges et al. 2007; Guthikonda and Haynes 2006; Scott and Weir 1998; Fowler 2005). Lysine plays several important roles in defense mechanism of humans. Lysine deficiency decreases defense ability of mammalian cells to viruses. Lysine deficiency is also the major cause of the osteoporosis in humans. Defects of lysine metabolism may result in familial hyperlysinemia due to genetic disorder (Gaby 2006; Sacksteder et al. 2000; Civitelli et al. 1992; Galvez et al. 2008). A diet deficient in methionine and lysine intake reduces biological value of plant-based nutrition to 50–70%, compared to a balanced diet with high abundance of essential amino acids. Unlike other amino acids, tryptophan acts as a precursor to several neurochemicals, such as serotonins and melatonin. Tryptophan deficiency in daily diet leads to several symptoms. Stresses caused by the loss of sleep were reportedly caused by the lack of tryptophan in daily food (Badawy 2013). Loss of lysine, methionine, and tryptophan in diet caused several symptoms include weight loss, decrease in muscle mass, and stress caused by losing sleep.

15.4.2.2 Genetic Engineering for Improving Methionine, Lysine, and Tryptophan in Potato

Genetic engineering exploits the metabolic pathway genes or the associated transcription factors. Hence, information at the genes levels of the metabolic pathways aimed for manipulation is prerequisite. As far as lysine, methionine, and tryptophan biosynthesis is concerned, their metabolic pathways in plants are fairly known. The success of the genetic approach has been mostly restricted to improving protein quality in model plants with enriched lysine, methionine, and tryptophan production. These are briefly described as follows. Lysine and methionine are synthesized by aspartate pathway within the chloroplast (Fig. 15.7). They share the initial three steps of this biosynthetic pathway. The first enzymatic step of the aspartate family is catalyzed by aspartate kinase which has multiple isoenzymic forms. Using ATP and Mg^{2+}, phosphorylation of aspartate leads to the formation of aspartyl phosphate which is subsequently oxidized to aspartate semialdehyde by aspartate semialdehyde dehydrogenase (ASDH). In the last step of the common pathway, ASD forms either dihydrodipicolinate (DHDP), a precursor of diaminopimelic acid and lysine, or O-phosphohomoserine (OPH). OPH may be channeled to threonine or methionine (Azevedo et al. 2006). Methionine is converted from OPH in three enzymatic steps:

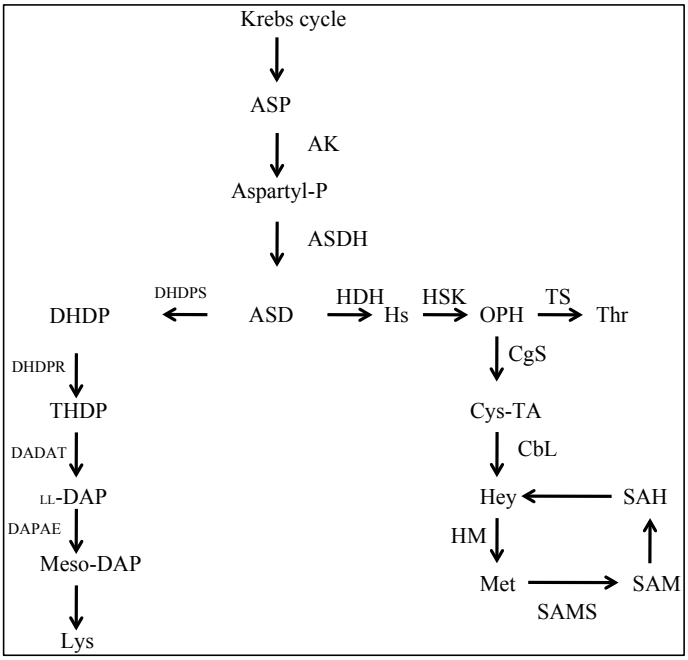

Fig. 15.7 Aspartate pathway leading to the biosynthesis of Met and Lys. Asp: Aspartate; AK: Aspartate kinase; ASD: aspartate semialdehyde; ASDH: aspartic semialdehyde dehydrogenase; DHDP: dihydrodipicolinate; DHDPS: dihydrodipicolinate synthase; HS: homoserine; HDH: homoserine dehydrogenase; OPH: O-phosphohomoserine; HSK: homoserine kinase; Thr: threonine; TS: Thr synthase; CysTA: cystathionine; CgS: cystathionine-synthase; HcY: homocysteine; CbL: cystathionine-lyase; SAH: Sadenosylhomocysteine; HM: homocysteine methyltransferase; SAM: S-adenosyl-methionine; SAMS: S-adenosyl-methionine synthetase; THDP: tetrahydrodipicolinate; DAPAE: DAP epimerase; DAP: diaminopimelate; DAPAT: DAP-aminotransferase

CgS catalyzes the formation of the thioether cystathionine from substrates of cysteine, the sulfur atom donor, and OPH by *trans*-sulfuration. The next step converts the intermediate to homocysteine and subsequently to methionine. In this mechanism, reactions are catalyzed by the enzymes CgS, cystathionine-lyase (CbL), and methionine synthase (MS), in that order. Almost 80% of methionine is converted into SAM, and the remainder takes part as a protein constituent (Hesse and Hoefgen 2003). Tryptophan biosynthesis too takes place in chloroplasts and is synthesized from chorismate (Fig. 15.8). Anthranilate synthase (AS) catalyzes the first reaction of the tryptophan biosynthesis which converts chorismate and an amine donor (usually glutamine) to form anthranilate; its activity is subject to feedback inhibition by Trp. In subsequent step, anthranilate phosphoribosylanthranilate transferase catalyzes a conversion of anthranilate and phosphoribosylpyrophosphate to phosphoribosylanthranilate and inorganic pyrophosphate. The third enzyme in the biosynthesis of tryptophan is phosphoribosylanthranilate isomerase (PAI) activity converting phosphoribosylanthranilate to L-(O-carboxyphenylamino)-L-deoxyribulose-5-phosphate

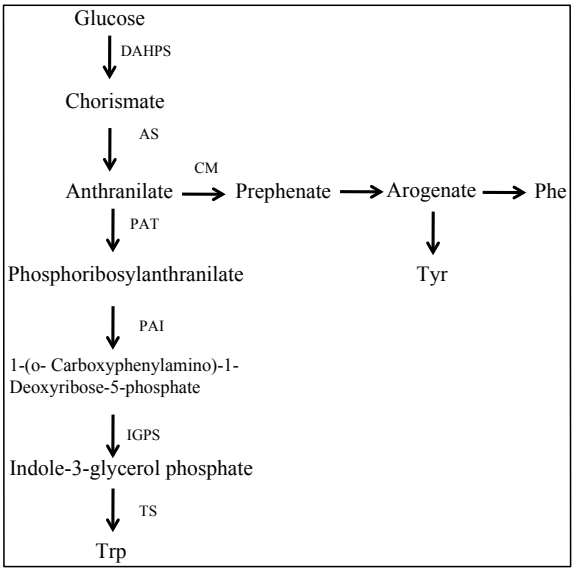

Fig. 15.8 Tryptophan biosynthesis pathway. AS: Anthranilate synthase; PAT: phosphoribosylanthranilate transferase; PAI: phosphoribosyl anthranilate isomerase; IGPS: indole-3-glycerol phosphate synthase; Trp: tryptophan; TS: Trp synthase; Tyr: tyrosine; Ser: serine; IAA: indole-3-acetic acid; Phe: phenylalanine; AH: arogenate dehydro; DAHPS: DAHP synthase

(CDRP). Then, indole-3-glycerol phosphate synthase (IGPS) accepts CDRP as the substrate which is transferred to indole-3-glycerol phosphate (Tzin and Galili 2010).

The major genetic engineering-based strategies for improving protein quantity/quality can be broadly grouped into three categories. These three groups are: (1) genetic engineering of essential amino acids. In potato tubers, in addition to amino acids present in proteins, some amino acids are "free" in the cytosol of seed cells and available to be digested within the tuber. These "free" amino acids also represent the pool available to the plant cell for protein synthesis and, to some extent, limit the amount and type of protein synthesized by the cell. Thus, genetic engineering to increase the level of amino acid synthesis has the potential to both remove some of the limitations to protein synthesis and enrich the "free amino acid content" of the plant. (2) Genetic engineering to enhance the levels of natural high-quality proteins within the plant tissue. In this approach, the gene copy number and transcription rate for specific genes are increased, or genes with appropriated essential amino acid profile from different organism can be imported for heterologous expression in the desired tissue (in case of potato, it is off-course tubers). (3) Improving the nutritional quality of protein plant synthesise, through protein engineering and/or design. Under this approach, the amino acid content of proteins expressed in potato tubers can be tailored, or entirely new, modified proteins with more desirable complement of amino acids can be designed and expressed. Although these three approaches can be followed separately, however, application of any one of these three strategies invariably results into more than one outcome in terms of improvement in protein quantity/protein quality/free amino acid levels. Therefore, various research works pertaining to improvement of proteins/amino acids in potato are discussed together under one section.

Efforts are being on for increasing content of various essential amino acids (methionine, lysine, tryptophan, threonine, etc.) in potato. Advances in biotechnology allowed the use of transgenic approach to increase the content of specific essential amino acids in a target plant. It was first demonstrated by the significant enhancement of methionine content in tobacco seed proteins through expressing transgene encoding a methionine-rich protein from Brazil nut (Altenbach et al. 1989). Beauregard et al. (1995) created an 11-kD synthetic protein, MBI, with 16% Met and 12% Lys, and transformed soybean using vectors targeted to seed protein storage bodies using appropriate leader sequences and seed-specific promoters. This was also achieved in a nonseed food crop, sweet potato (*Ipomoea batatas*), modified with an artificial storage protein gene (Egnin and Prakash 1997). These transgenic plants exhibited two- and fivefold increases in the total protein content in leaves and roots, respectively, over that of control plants. A significant increase in the level of essential amino acids, such as Met, Thr, Trp, Ile, and Lys, was also observed. In potato, higher methionine levels increase the nutritional quality and promote the typically pleasant aroma associated with baked and fried potatoes. Several attempts have been made to elevate tuber methionine levels by genetic engineering of methionine biosynthesis and catabolism. Chakraborty et al. (2000) developed transgenic potato overexpressing the sunflower albumin or an amaranth seed albumin (*AmA1*), driven under the constitutive promoters, which resulted in five- to sevenfold increase in total methionine level in tubers. Further analysis of transgenic potato lines with enhanced methionine amino acid via tuber-specific expression of a seed protein, *AmA1* (*Amaranth albumin* 1), revealed an increase in total protein contents up to 60% in comparison to the transformed potato (Chakraborty et al. 2010). Similarly, the methionine was also enhanced in transgenic potato by overexpression of gene encoding *PrLeg* polypeptide (isolated from *Perilla*), driven under the tuber-specific *patatin* promoter. This resulted in an increase in ~3.5-fold methionine in transgenic potato without changes in other amino acids or growth, development, and yield of the potato (Goo et al. 2013). It was also reported that higher isoleucine accumulation in transgenic tubers enhanced the methionine accumulation via methionine gamma-lyase (MGL) catabolism pathway (Huang et al. 2014). Recently, Kumar and Jander (2017) reported that overexpression of *A. thaliana* cystathionine γ-synthase gene in *S. tuberosum* increased methionine levels in tubers. Also, silencing *S. tuberosum* methionine γ-lyase gene, a gene encoding protein which causes degradation of methionine into 2-ketobutyrate, resulted in increase in methionine levels in tubers. Further, they reported that *S. tuberosum* cv. Désirée plants with *A. thaliana* cystathionine γ-synthase gene overexpression and *S. tuberosum* methionine γ-lyase gene silenced by RNA interference accumulated higher free methionine levels than either single-transgenic line. The paradise nut 2S seed protein is abundant Met residues (16 mol%). To explore the feasibility of further increasing Met content of this protein, modifications were made in the sequence region between the Cys-6 and Cys-7 codons of *PN2S* cDNA to contain 19, 21, and 23 mol% Met, respectively. All the three modified Met-rich *PN2S* were expressed, processed, and accumulated in transgenic tobacco seeds. The same modifications were also made in the Brazil nut 2S protein, and the chimeric genes were used to transform potato. Results revealed that the mutated Met-enriched BN2S proteins

were expressed and accumulated as well as normal 2S protein in the leaves and tubers of transgenic potato. The accumulation of the methionine-rich protein could make a significant enhancement in methionine levels in seed protein of transgenic potato (Tu et al. 1998). In another study, attempts were made to increase the Met content in potato tubers through heterologous overexpression of *Arabidopsis* cystathionine γ-synthase (*CgSΔ90*), which is not regulated by Met in potato plants and a storage Met-rich 15-kD zein in Desiree cultivar. There was sixfold increase in free Met content and in the Met content of the zein containing protein fraction of the transgenic tubers. In addition, in line with higher Met content, the amounts of soluble isoleucine and serine were also increased. However, all the lines with higher Met content *CgCΔ90* expressions were phenotypically abnormal showing severe growth retardation, changes in leaf architecture, and 40–60% reduction in tuber yield. Furthermore, the color of the transgenic tubers was altered due to reduced amounts of anthocyanin pigments.

In 1989, Yang et al. inserted the high essential amino acid encoding DNA (*HEAAEDNA*) into the chloramphenicol acetyltransferase (*CAT*) coding sequence to generate a CAT-HEAAE fusion protein. Transgenic study indicated that CAT-HEAAE protein was accumulated at 0.02–0.35% of total tuber protein in transgenic potato. Based on the structurally well-studied maize zeins, the group later designed and synthesized another artificial storage protein (ASP1) composed of 78.9% essential amino acids and estimated to possess a more stable storage protein like structure in plants (Kim et al. 1992). The 284-bp *asp1* gene, under the control of *CaMV 35S* promoter, was normally expressed in transgenic tobacco leaves resulting in the accumulation of relatively high levels of ASP1 proteins. Surprisingly, the overall levels of total amino acid and protein were found to be increased remarkably in transgenic potato. Gene silencing by RNAi technology also has been tried in potato to increase the essential amino acid content. The threonine synthase (*TS*) involved in synthesis of threonine in potato was targeted for silencing so as to divert the cycle and increase the Met content (Fig. 15.7). A reduction of 6% TS activity levels in transgenic potato which increased the methionine levels up to 30-fold developing on the transgenic line and environmental conditions and had no reduction in threonine (Zeh et al. 2001). Most enzymes in biosynthesis pathways leading to amino acids are inhibited by their end-products (allosteric regulation). 2S-sunflower seed protein has been characterized for its IgE-binding capacity; the protein possesses a significant amount of sulfur-containing amino acids (Hudson et al. 2005). This could be used to improve protein quality of other crops through genetic engineering. A chimeric gene encoding a methionine-rich Brazil nut (*Bertholletia excelsa*) protein contains over 18% methionine, whereas most proteins contain only a few percent of methionine. To increase methionine levels in plants, several transgenic approaches have been used. Cystathionine γ-synthase (CGS), the first committed enzyme in the methionine biosynthesis pathway, was overexpressed in transgenic potato plants. The transformation of PrLeg gene into potato, which contains low amounts of sulfur-containing amino acids, was found to enhance Met content in the tubers (Goo et al. 2013).

15.4.2.3 Minerals

Humans require various minerals to maintain health and for proper growth, and plants are essential source of such minerals (Welch 2002). Minerals can generally be classified as (a) major minerals [such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), cobalt (Co), manganese (Mn), nitrogen (N), and chlorine (Cl)] and (b) minor/trace minerals [such as iron (Fe), copper (Cu), selenium (Se), nickel (Ni), lead (Pb), sulfur (S), boron (B), iodine (I), silicon (Si), and bromine (Br)]. Importance of optimal intake of these minerals to maintain good health has been universally recognized (Avioli 1988). Potatoes are an important source of different dietary minerals. However, there are significant differences in major and trace mineral contents among different genotypes of potato (Randhawa et al. 1984; True et al. 1979). The minerals present in significant concentrations in potato are given in (Table 15.6). In addition to genetic factors, many other factors affect the mineral composition of potatoes; these include: location, stage of development, soil type, soil pH, soil organic matter, fertilization, irrigation, and weather. Therefore, the same genotype grown in different locations may have different mineral concentration due to these environmental factors (Burgos et al. 2007). The available information pertaining to the mineral content of potatoes and their improvement is described below.

In terms of mineral content, potato is best known as an important source of dietary potassium. Potassium plays a fundamental role in acid–base regulation, fluid balance, required for optimal functioning of the heart, kidneys, muscles, nerves, and digestive systems. Health benefits of sufficient potassium intake include reduced risk of hypokalemia, osteoporosis, high blood pressure, stroke, inflammatory bowl disease, kidney stones, and asthma. Potato is listed as providing 18% of the RDA of potassium. Potato qualifies for a health claim approved by the U.S. Food and Drug Administration, which states: "Diets containing foods that are good source of potassium and that are low in sodium may reduce the risk of high blood pressure and stroke." Potassium varies from 3550 to 8234 μg/gFW (Casanas et al. 2002; Rivero et al. 2003). The dietary reference intake of potassium for adult men and women is 3000–6000 mg per day. The US National academy of Sciences has recently increased the recommended intake for potassium from 3500 mg to at least 4700 mg per day. Besides potassium, phosphorus is the main mineral in potato tubers. It has many roles in the human body and is a key player for healthy cells, teeth, and bones. Inadequate phosphorus intake results in abnormally low serum phosphate levels, which affect loss of appetite, anemia, muscle weakness, bone pain, rickets/osteomalacia, susceptibility to infection, numbness and tingling of the extremities, and difficulty in walking. In potatoes, phosphorus ranges from ~1300 to 6000 μg/g DW (Lisinka and Leszczynski 1989). Daily requirement of phosphorus is 800–1000 mg. Potato is listed as providing 6% of the RDA of phosphorus. Calcium is important for bone and tooth structure, blood clotting, and nerve transmission. Deficiencies are associated with skeletal malformation and blood pressure abnormalities. The RDA for calcium is 600–1200 mg (Table 15.1). Potatoes are a significant source of calcium and have been shown to provide 2% of the RDA of calcium. Among 74 Andean landraces

screened, calcium ranged from 271 to 1093 μg/g DW (Andre et al. 2007b). Magnesium is required for normal functioning of muscles, heart, and immune system. Magnesium also helps maintain normal blood sugar levels and blood pressure. Potato magnesium levels range from 142 to 359 μg/g FW (Casanas et al. 2002; Rivero et al. 2003) and provides 6% of the RDA of magnesium. Manganese has a role in blood sugar regulation, metabolism, and thyroid hormone function. RDA for manganese is 2–10 mg. The range of potato manganese content has been reported from 0.73 to 3.62 μg/gFW (Rivero et al. 2003) to 9–13 μg/g DW (Orphanos 1980). Copper is needed for synthesis of hemoglobin, proper ion metabolism, and maintenance of blood vessels. The RDA for copper is 1.5–3.0 mg. Copper in potatoes varies from 0.23 to 11.9 mg/kg FW (Randhawa et al. 1984; Rivero et al. 2003). Like zinc, copper is also high in yellow-fleshed potatoes (Dugo et al. 2004).

Iron deficiency affects more than 1.7 billion people worldwide and has been called the most widespread health problem in the world by the World Health Organization. Due to severe iron deficiency, more than 60,000 women die in pregnancy and childbirth each year, and almost 500 million women of childbearing age suffer from anemia. Dietary iron requirements depend on numerous factors, for example, age, sex, and diet composition. Potato is a modest source of iron. Potato is listed as providing 6% of the RDA of iron. Iron content in cultivated potato tubers has been found in the range of 0.3–2.3 mg 100 g FW or 6–158 μg/g DW (True et al. 1979; Andre et al. 2007b). Potato iron has been suggested to be quite bioavailable because it has very low levels of phytic acid unlike the cereals. Zinc is needed for body's immune system to properly work and is involved in cell division, cell growth, and wound healing. Iron and zinc deficiencies result in decreased immune function and can interfere with growth and development (Zimmermann and Hurrell 2002). The RDA for zinc is 15–20 mg, and potato is listed as providing 2% of the RDA of zinc. The zinc content ranges from 1.8 to 10.2 μg/g FW (Andre et al. 2007b; Randhawa et al. 1984; Rivero et al. 2003). Yellow-fleshed potatoes from different cultivars contain zinc in 0.5–4.6 μg/g FW (Dugo et al. 2004).

Improving Mineral Content in Potatoes Using Genetic Engineering

Very few reports are there about research attempts improving mineral content in potato through biofortification. Because plants cannot synthesize these minerals, they must be acquired from soil. As a result, engineering of plant mineral content is quite different from modifications of improving other nutrition associated constituents such as proteins and vitamins that the plant itself synthesizes. There are four main strategies which can be employed for improving the mineral contents in potatoes (Fig. 15.9); these are: (i) improving minerals uptake from soil, (ii) increasing transport to storage organ, (iii) increasing storage capacity of sink, and (iv) decreasing anti-nutrient (phytic acid, phytase, etc.) components which reduce availability of the minerals. Research to improve the mineral composition of crop plants has mostly focused on iron content. Several reports exist in this particular area, most of which describe research that was performed on iron biofortification in rice crop. However,

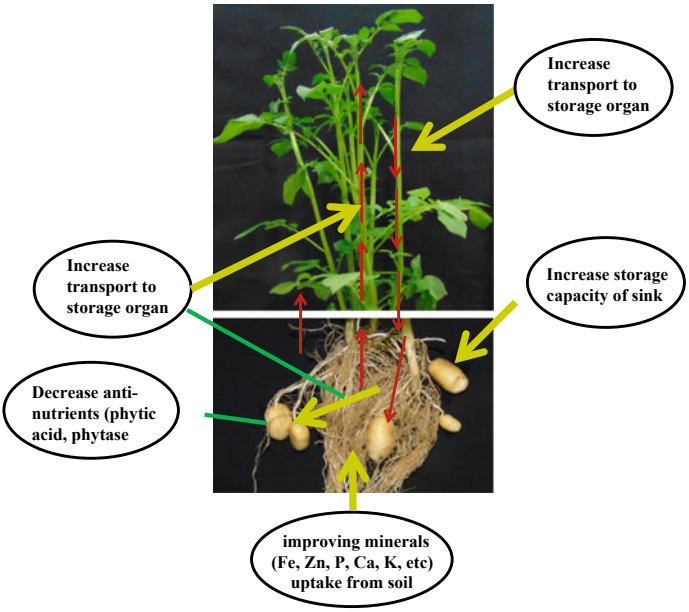

Fig. 15.9 Approaches for improving mineral content in potato. The four main strategies include: (i) improving minerals uptake from soil, (ii) increasing transport to storage organ, (iii) increasing storage capacity of sink, (iv) decreasing anti-nutrient (phytic acid, phytase, etc.) components which reduce availability of the minerals

very less research in this regard in potato has been carried out worldwide. An attempt was made to over express *Arabidopsis sCAX* (Cationic Exchanger 1) and H^+/Ca^{2+} transporter genes in potato. Transgenic tubers expressing *sCAX1* displayed up to threefold more calcium content compared to wild type without significant alteration in growth and development. The trait was also found to be stably inherited when monitored over three generations (Park et al. 2005). In other work, a chimeric, N-terminus-truncated *Arabidopsis* cation transporter (*CAX2B*) that contains a domain from *CAX1* for increased substrate specificity was over expressed in potato to improve calcium accumulation. The transgenic plants had 50–65% improved tuber calcium content relative to wild type, with stable inheritance and no deleterious effects on plant growth or development (Kim et al. 2006).

15.4.3 Reducing Anti-nutritional Factors

15.4.3.1 Glycoalkaloids

Steroidal alkaloids (SAs) and their glycosylated forms, i.e., steroidal glycosylated alkaloids (SGAs) are toxic compounds mainly produced by members of the Solanaceae and Liliaceae plant families. In humans and animals, steroidal alkaloids

are considered anti-nutritional factors because they affect the digestion and absorption of nutrients from food and might even cause poisoning. In spite of the first report on steroidal alkaloids nearly 200 years ago, much of the molecular basis of their biosynthesis and regulation remains unknown. It has been perceived that elaborating the knowledge regarding the steroidal alkaloids biosynthetic pathway, the subcellular transport of these molecules, as well as the identification of regulatory and signaling factors associated with steroidal alkaloids metabolism, will also provide the means to develop, through classical breeding or genetic engineering, crops with modified levels of anti-nutritional SAs (Cardenas et al. 2015). Recently, co-expression analysis and metabolic profiling revealed metabolic gene clusters in tomato and potato that contain core genes required for production of the prominent SGAs.

The presence of SGAs in potatoes has been of a particular concern due to their toxicity to humans (Friedman et al. 1997). In potatoes, SGAs are found in every plant organs (roots, tubers, stolons, stems, foliage, flowers, and fruits) with fresh weight concentrations ranging from 10 mg per kg (fresh weight) in tubers to 5000 mg per kg (fresh weight) in the flowers (Smith et al. 1996). Solanine and chaconine, derived from the aglycone solanidine, are the most prevalent glycoalkaloids found in cultivated potato (Dale et al. 1993). Solanine and solasonine have a common sugar moiety (solatriose) while chaconine and solamargine have chacotriose in common. The alkamine steroidal skeletons (aglycones) of the glycoalkaloids are classified into two groups, the spirosolanes and solanidanes, of which solasodine and solanidine are representatives, respectively. These compounds are derived from mevalonic acid. The use of wild germplasm in potato breeding is extensive and the main source of transmission of unusual SGAs (Väänänen et al. 2006).

Elimination of solanidine glycosylation has been found to decrease toxicity of edible tuber. Antisense DNA constructs of *SGT1* coding for solanidine galactosyl transferase involved in α-solanine biosynthesis, *SGT2* coding for solanidine glucosyltransferase involved in α-chaconine biosynthesis, or *SGT3* coding for sterol rhamnosyltransferase, the last step in the triose formation of α-chaconine and α-solanine (McCue et al. 2005, 2006, 2007), reduced the corresponding glycoalkaloids in transgenic potato plants. Antisense silencing of a potato gene encoding a sterol alkaloid glycosyl transferase (*sgt1*) resulted in complete inhibition of α-solanine accumulation. But this decrease was compensated by elevated levels of α-chaconine and resulted in wild-type total steroidal glycoalkaloids (SGA) levels in transgenic lines (McCue et al. 2005). Arnqvist et al. (2003) overexpressed soybean (*Glycine max*) type 1 sterol methyl transferase (*GmSTM1*) in potato (cv. Desiree) in an attempt to reduce glycoalkaloids. The transgenic potato showed decreased glycoalkaloid levels in leaves and tubers, down to 41 and 63% of wild-type levels, respectively. In 2002, Esposito et al. estimated the glycoalkaloid content in potatoes improved with nonconventional breeding approaches. They performed chemical analyses on two distinct groups of new potato genotypes. The first group contained clones transformed with the gene ech42 encoding for an endochitinase. The second included interspecific hybrids between the cultivated potato *S. tuberosum* and the wild species *Solanum commersonii*, obtained either by somatic fusion or by sexual hybridization. The results suggested that chitinase gene insertion did not alter other metabolic

pathways of potato tubers and did not cause unintentional pleiotropic effects. In interspecific hybrids, wide variability for all of the parameters analyzed was found. In a number of genotypes, glycoalkaloid levels were close to or lower than those of the control varieties, suggesting that selection for low glycoalkaloid content is possible. The results also indicated that glycoalkaloids from *S. commersonii* may be lost rapidly. Recently, co-expression analysis and metabolic profiling revealed metabolic gene clusters in tomato and potato that contain core genes required for production of the prominent SGAs (Cardenas et al. 2015).

15.4.3.2 Acrylamide and Allergens

Acrylamide has been classified as probable carcinogen in humans and has neurological and reproductive effects. It is formed from free asparagine and reducing sugars during high-temperature cooking and processing of common foods. Potato and cereal products are major contributors to dietary exposure to acrylamide. One of the promising approaches to reduce the acrylamide formation in plant-based processed products is to develop crop varieties with lower concentrations of free asparagine and/or reducing sugars, and of best agronomic practice to ensure that concentrations are kept as low as possible (Halford et al. 2012). Chawla et al. (2012) reported that simultaneous silencing of asparagine synthetase (Ast)-1 and -2 reduced asparagine formation and, consequently, reduces the acrylamide-forming potential of tubers. However, phenotypic analysis revealed that the phenotype of silenced lines appears normal in the greenhouse, but field-grown tubers were small and cracked. Assessing the effects of silencing StAst1 and StAst2 individually, they found that yield drag was mainly linked to down-regulation of StAst2. Interestingly, tubers from untransformed scions grafted onto intragenic StAst1/2-silenced rootstock contained almost the same low ASN levels as those in the original silenced lines, indicating that ASN is mainly formed in tubers rather than being transported from leaves. Further, field studies demonstrated that the reduced acrylamide-forming potential achieved by tuber-specific StAst1 silencing did not affect the yield or quality of field-harvested tubers.

Allergies to potatoes appear to be relatively uncommon. Patatin, the primary storage protein in potato unfortunately, has also been suggested to be major allergen in potato. Patatin may be cross-reactive for persons with allergy to latex, and children with atopic dermatitis appear to have increased sensitivity to this potato protein (Schmidt et al. 2002). However, boiling of potatoes reduce or nullify the allergic reaction. No significant work has been carried out to remove or minimize the allergic potential of potatoes. The biotechnological way to overcome this patatin-associated allergy may be developing potato cultivar engineered for the patatin protein at specific site(s) which is/are responsible for inducing the allergic response upon consumption.

15.4.4 Improving Carbohydrates Quality

After cellulose, starch is the second most abundant compound produced in higher plants. Starch represents the most important carbohydrate used for food and feed purposes. While cellulose is a structural component of plants, starch mainly serves as a compound to temporarily store energy that can be accessed at a later time point. Chemically, starch is an alpha-glucan (α-glucan) and composed of two types of polysaccharides: amylose and amylopectin (Fig. 15.10). Amylopectin is highly branched, leaving more surface area available for digestion. It is broken down quickly and thus produces a larger rise in blood glucose. On the other hand, amylose is a straight chain, which limits the amount of surface area exposed for digestion. Therefore, digestion of amylose is slow than that of amylopectin and hence is responsible for resistant nature of starch. Thus, improving the resistant starch content refers to increasing the amylose content of the target crop. Resistant starch provides health benefits such as glycemic control, control of fasting plasma triglyceride and cholesterol levels and absorption of minerals. In view of the industrial application and the nutritional benefits of resistant starch, researchers around the globe have been working to increase the RS content of the plants. The approaches for increasing the RS content in plants include natural selection, conventional breading as well as transgenic. All these approaches are based on biosynthetic pathways of starch metabolism. The key enzymes for starch biosynthesis are AGPase, starch synthases, and branching enzymes. Generation of the sugar nucleotide ADP glucose is catalyzed by AGPase. Starch synthases catalyze the polymerization of glucose residues resulting in formation of α-1,4 glucans. Branching enzymes cleave α-1,4 glucans and reattach the cleaved chain to an α-1,4 glucan chain by an α-1,6 glycosidic linkage, thereby forming a branch (Fig. 15.10).

As potato contains high starch, they have been genetically modified for increasing the resistant starch content (i.e., amylose content). In 2000, Schwall et al. developed very-high-amylose potato starch by manipulating starch branching enzymes through genetic engineering. They simultaneously inhibited two isoforms of starch branching enzyme to below 1% of the wild-type activities which resulted in altered starch granule morphology and composition. In these, potato amylopectin was found to be absent, whereas the amylose content increased to levels comparable to the highest commercially available maize starches. Expression of amylosucrase in potato resulted in larger starch granules with rough surfaces and novel physicochemical properties, including improved freeze–thaw stability, higher end viscosity, and better enzymatic digestibility. In 2005, Blennow et al. reported genetic engineering of potato tuber starch by simultaneous antisense suppression of the starch branching enzyme (SBE) I and II isoforms. Starch prepared from 12 independent lines, and three control lines were characterized with respect to structural and physical properties. The lengths of the amylopectin unit chains and the concentrations of amylose and monoesterified phosphate were significantly increased in the transgenically engineered starches. With the aim of increasing starch content, Regierer et al. (2002) modulated the adenylate pool by changing the activity of the plastidial adenylate

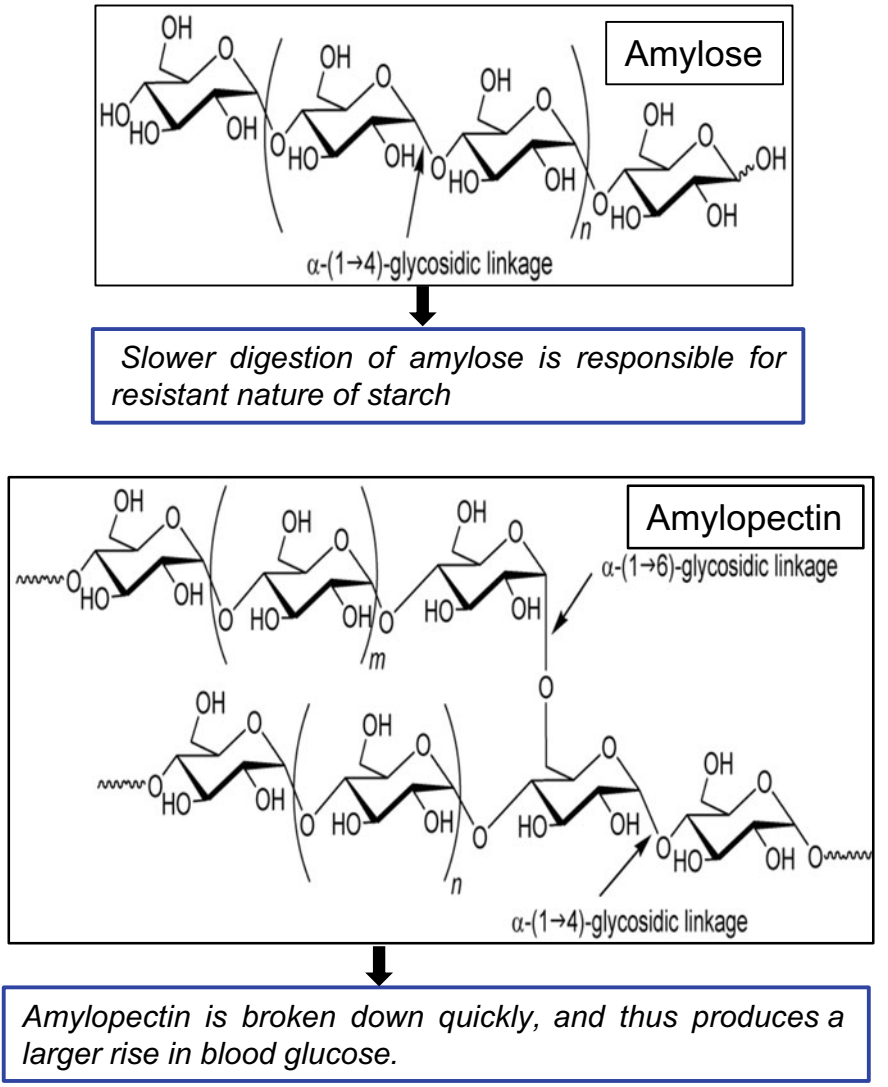

Fig. 15.10 Starch components in potato. Starch has two components, viz. amylose and amylopectin. Amylose is considered as resistant starch

kinase in transgenic potato plants. A substantial increase in the level of adenylates and, most importantly, an increase in the level of starch to 60% above that found in wild-type plants were observed.

Beaujean et al. (2000) demonstrated that it is possible to replace starch degradation using microbial enzymes via a system where the enzymes are produced directly in the plants, but active only at high temperature, thus offering novel and viable strategies for starch-processing industries. They reported for the first time that starch was degraded and glucose and fructose were produced directly when crushed potato tubers expressing a starch degrading bifunctional gene were heated for 45 min at 65 °C. To achieve this, we have constructed a fusion gene encoding the thermostable enzymes: alpha-amylase (*Bacillus stearothermophilus*) and glucose isomerase (*Thermus thermophilus*) under the control of the granule-bound starch synthase promoter. This enzymatic complex produced in transgenic tubers was only active at high temperature (65 °C). More than 100 independent transgenic potato plants were regenerated. The biochemical analyses performed on young and old tubers after high-temperature treatment (65 °C) revealed an increase in the formation rate of fructose and glucose by a factor of 16.4 and 5.7, respectively, in the transgenic tubers as compared to untransformed control tubers. Potato tuber pectin is rich in galactan (oligomer of beta-1,4-linked galactosyl residues). Oxenboll Sorensen et al. (2000) expressed a fungal endo-galactanase cDNA in potato under control of the granule-bound starch synthase promoter to obtain expression of the enzyme in tubers during growth. Analyses of transgenics revealed alterations in pectin composition. Monosaccharide composition of total cell walls and isolated rhamnogalacturonan I fragments showed a reduction in galactosyl content to 30% in the transformants compared with the wild type.

15.4.5 Improving Processing Attributes

Accumulation of reducing sugars (primarily glucose and fructose) in cold-stored potato tubers is referred to as "cold-induced sweetening" (CIS). CIS makes the cold-stored potatoes unfit for processing purposes such as chips and French fries making (Fig. 15.11). Two separate metabolic events are critical in determining a potato tuber's ability to produce sugars in the cold storage: the ability to form sucrose and the ability to hydrolyze sucrose to the reducing sugars glucose and fructose. The control of sucrose synthesis is controlled by several related enzymes while reducing sugar formation is more specifically related to level of vacuolar acid invertase activity. Role of vacuolar acid invertase in cold-induced sweetening has been demonstrated by various researchers (Fig. 15.12). Bhaskar et al. (2010) demonstrated that silencing the potato vacuolar acid invertase gene (VInv) prevented reducing sugar accumulation in cold-stored tubers. Potato chips processed from VInv silencing lines were light in color even when tubers were stored at 4 °C. Comparable, low levels of VInv gene expression were observed in cold-stored tubers from wild potato germplasm stocks that are resistant to cold-induced sweetening. Wiberley-Bradford et al. (2014) have

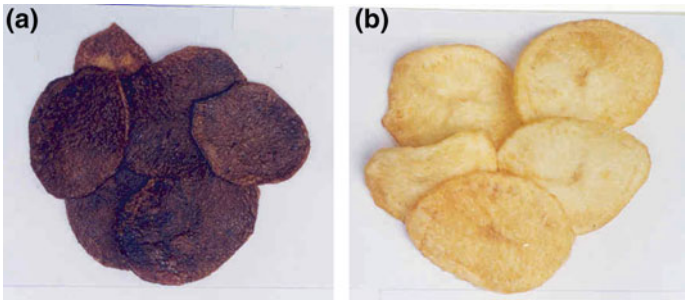

Fig. 15.11 Chips prepared from potatoes having high glucose content due to cold-induced sweetening (**a**); and from potatoes having low glucose content (**b**)

shown that vacuolar invertase (VInv) silencing significantly reduced cold-induced sweetening in stored potato tubers, likely by means of differential VInv expression early in storage. Zhu et al. (2014) suppressed the expression of Vacuolar Invertase gene (VInv) in cultivars Russet Burbank and Ranger Russet using RNA interference to determine if this approach could control sugar-end defect formation. Acid invertase activity and reducing sugar content decreased at both ends of tubers. Clasen et al. (2016) used transcription activator-like effector nucleases (TALENs) to knockout vacuolar invertase gene (VInv, which encodes a protein that breaks down sucrose to glucose and fructose) within the commercial potato variety, Ranger Russet. They isolated 18 plants containing mutations in at least one VInv allele, and five of these plants had mutations in all VInv alleles. Tubers from full VInv-knockout plants had undetectable levels of reducing sugars, and processed chips contained reduced levels of acrylamide and were lightly colored. These results provide a framework for using TALENs to quickly improve traits in commercially relevant autotetraploid potato lines.

Rommens et al. (2006) improved potato storage and processing characteristics through all-native DNA transformation. They simultaneously lowered the expression of Ranger Russet's tuber-expressed polyphenol oxidase, starch-associated R1, and phosphorylase-L genes. This genetic modification was accomplished without inserting any foreign DNA into the plant genome. French fries from the intragenic potatoes also contained reduced amounts of the anti-nutritional compound acrylamide while, unexpectedly, displaying enhanced sensory characteristics. Processed potato tuber texture is an important trait that influences consumer preference, a detailed understanding of tuber textural properties at the molecular level is lacking. Tuber pectin methyl esterase activity is a potential factor impacting on textural properties. Expression of a gene encoding an isoform of pectin methyl esterase (PEST1) was associated with cooked tuber textural properties. Ross et al. (2011) changed the texture of potato by engineering pectin and thus changed the texture of potato. They used transgenic approach to investigate the impact of the PEST1 gene. Antisense and overexpressing potato lines were generated. In overexpressing lines, tuber pectin methyl esterase activity was enhanced by up to 2.3-fold, whereas in antisense lines, pectin methyl

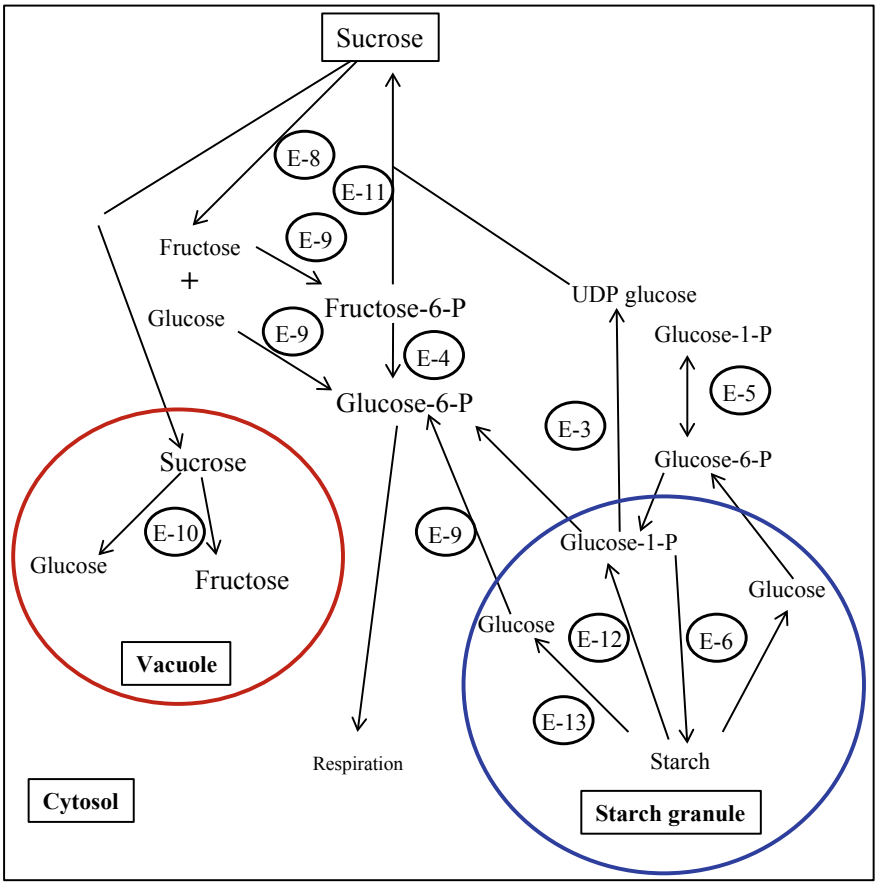

Fig. 15.12 Carbohydrate metabolism in stored potato tubers. Enzymes: (E1) sucrose synthase; (E2) fructokinase; (E3) UDP glucose pyrophosphorylase; (E4) phosphohexose isomerase; (E5) phosphoglucomutase; (E6) ADP glucose pyrophosphorylase; (E7) starch synthases, branching enzymes; (E8) neutral invertase; (E9) hexokinase; (E10) acid invertase; (E11) sucrose phosphate synthase; (E12) starch phosphorylase; (E13) amylases, debranching enzymes. Partially adapted from Dale and Bradshaw (2003)

esterase activity was decreased by up to 62%. Pectin methyl esterase isoform analysis indicated that the PEST1 gene encoded one isoform of pectin methyl esterase. Analysis of cell walls from tubers from the overexpressing lines indicated that the changes in pectin methyl esterase activity resulted in a decrease in pectin methylation. Analysis of processed tuber texture demonstrated that the reduced level of pectin methylation in the overexpressing transgenic lines was associated with a firmer processed texture. Thus, there was a clear link between pectin methyl esterase activity, pectin methylation, and processed tuber textural properties (Ross et al. 2011). Potato polyphenol oxidases are the enzymes responsible for enzymatic browning reaction observed in impacted, damaged, or sliced tubers. These oxidative deterioration reac-

tions alter the organoleptic properties of food and greatly affect potato tuber quality. Llorente et al. (2011) silenced the *PPO* gene in transgenic potato which reduced the enzymatic browning and enhanced the shelf life of potato.

15.5 Future Prospects

Malnutrition is the most chronic and pressing agricultural and human health problem of the twenty-first century. Potato being an important constituent of our diets is expected to play vital role in tackling this serious malnutrition problem. Therefore, improvements in the nutritional quality of potato would have most visible positive impact. For achieving this, full potential of biotechnological tools must be put in use in association with conventional plant breeding programs with the sole aim of bio-fortifying potatoes with superior nutritional levels. The genomic resources need to be continuously enriched to have deeper insights for identifying key molecular regulators which can be utilized through biotechnological approaches in potato with the aim of developing nutritionally superior cultivars. Present mechanisms put in place for evaluation and recommendation for commercial application of the genetically engineered superior potato, and other crops need to be streamlined so that the real benefit of the biofortified nutritionally superior food can reach the consumers.

References

Akyol H, Riciputi Y, Capanoglu E, Caboni MF, Verardo V (2016) Phenolic compounds in the potato and its byproducts: an overview. Int J Mol Sci 17:E835

Altenbach SB, Pearson KW, Meeker G, Staraci LC, Sun SM (1989) Enhancement of the methionine content of seed proteins by the expression of a chimeric gene encoding a methionine-rich protein in transgenic plants. Plant Mol Biol 13:513–522

Andre C, Oufir M, Guignard C, Hoffmann L, Hausman J, Evers D, Larondelle Y (2007a) Antioxidant profiling of native Andean potato tubers (*Solanum tuberosum* L.) reveals cultivars with high levels of β-carotene, α-tocopherol, chlorogenic acid, and petanin. J Agric Food Chem 55:10839–10849

Andre CM, Ghislain M, Bertin P, Oufir M, Herrera Mdel R, Hoffmann L, Hausman JF, Larondelle Y, Evers D (2007b) Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. J Agric Food Chem 55:366–378

Arnqvist L, Dutta PC, Jonsson L, Sitbon F (2003) Reduction of cholesterol and glycoalkaloid levels in transgenic potato plants by overexpression of a type 1 sterol methyltransferase cDNA. Plant Physiol 131:1792–1799

Avioli LV (1988) Calcium and phosphorus. In: Shils ME, Young E (eds) Modern nutrition in health and disease, 7th edn. Lea & Febiger, Philadelphia, pp 142–158

Azevedo RA, Lancien M, Lea PJ (2006) The aspartic acid metabolic pathway, an exciting and essential pathway in plants. Amino Acids 30:143–162

Badawy AA (2013) Tryptophan: the key to boosting brain serotonin synthesis in depressive illness. J Psychopharmacol (Oxford) 27:878–893

Bagri DS, Upadhyaya DC, Kumar A, Upadhyaya CP (2018) Overexpression of PDX-II gene in potato (*Solanum tuberosum* L.) leads to the enhanced accumulation of vitamin B6 in tuber tissues and tolerance to abiotic stresses. Plant Sci 272:267–275

Bailey LB, Rampersaud GC, Kauwell GPA (2003) Folic acid supplements and fortification affect the risk for neural tube defects, vascular disease and cancer: evolving science. J Nutr 133:1961S–1968S

Beaujean A, Ducrocq-Assaf C, Sangwan RS, Lilius G, Bülow L, Sangwan-Norreel BS (2000) Engineering direct fructose production in processed potato tubers by expressing a bifunctional alpha-amylase/glucose isomerase gene complex. Biotechnol Bioeng 70:9–16

Beauregard M, Dupont C, Hefford MA (1995) Design, expression and initial characterization of MB1, a de novo protein enriched in essential amino acids. Biotechnology 13:974–981

Bhaskar PB, Wu L, Busse JS, Whitty BR, Hamernik AJ, Jansky SH, Buell CR, Bethke PC, Jiang J (2010) Suppression of the vacuolar invertase gene prevents cold-induced sweetening in potato. Plant Physiol 154:939–948

Blennow A, Wischmann B, Houborg K, Ahmt T, Jorgensen K, Engelsen SB, Bandsholm O, Poulsen P (2005) Structure function relationships of transgenic starches with engineered phosphate substitution and starch branching. Int J Biol Macromol 36:159–168

Bonierbale M, Grüneberg W, Amoros W, Burgos G, Salas E et al (2009) Total and individual carotenoid profiles in *Solanum phureja* cultivated potatoes: II development and application of near-infrared reflectance spectroscopy (NIRS) calibrations for germplasm characterization. J Food Compos Anal 22:509–516

Botella-Pavia P, Rodriguez-Conceptıon M (2006) Carotenoid biotechnology in plants for nutritionally improved foods. Physiol Plant 126:369–381

Breithaupt DE, Bamedi A (2002) Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): new insights into an ancient vegetable. J Agric Food Chem 50:7175–7181

Brown CR (2005) Antioxidants in potato. Am J Potato Res 82:163–172

Brown CR, Edwards CG, Yang CP, Dean BB (1993) Orange flesh trait in potato: inheritance and carotenoid content. J Am Soc Hortic Sci 118:145–150

Brown CR, Culley D, Yang C, Durst R, Wrolstad R (2005) Variation of anthocyanins and carotenoid contents and associated antioxidant values in potato breeding lines. J Am Soc Hortic Sci 130:174–180

Brown CR, Durst RW, Wrolstad R, De Jong W (2008) Variability of phytonutrient content of potato in relation to growing location and cooking method. Potato Res 51:259–270

Bub A, Möseneder J, Wenzel G, Rechkemmer G, Briviba K (2008) Zeaxanthin is bioavailable from genetically modified zeaxanthin-rich potatoes. Eur J Nutr 47:99–103

Bulley S, Wright M, Rommens C, Yan H, Rassam M, Lin-Wang K, Andre C, Brewster D, Karunairetnam S, Allan AC, Laing WA (2012) Enhancing ascorbate in fruits and tubers through overexpression of the L-galactose pathway gene GDP-L-galactose phosphorylase. Plant Biotechnol J 10:390–397

Burgos G, Amoros W, Marote M, Stangoulis J, Bonierbale M (2007) Iron and zinc concentration of native potato cultivars from a human nutrition perspective. J Sci Food Agric 87:668–675

Burgos G, Salas E, Amoros W, Auqui M, Muñoa L, Kimura M, Bonierbale M (2009) Total and individual carotenoid profiles in *Solanum phureja* of cultivated potatoes: I concentrations and relationships as determined by spectrophotometry and HPLC. J Food Compos Anal 22:503–508

Burmeister A, Bondiek S, Apel, L Kühne C, Hillebrand S et al (2011) Comparison of carotenoid and anthocyanin profiles of raw and boiled *Solanum tuberosum* and *Solanum phureja* tubers. J. Food Compos Anal 24:865–872

Camire ME, Kubow S, Donnelly DJ (2009) Potatoes and human health. Crit Rev Food Sci Nutr 49:823–840

Campbell R, Ducreux LJM, Morris WL, Morris JA, Suttle JC et al (2010) The metabolic and developmental roles of carotenoid cleavage dioxygenase 4 from potato (*Solanum tuberosum* L). Plant Physiol 154:656–664

Cantos E, Tudela JA, Gil MI, Espin JC (2002) Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. J Agric Food Chem 50:3015–3023
Cardenas PD, Sonawane PD, Heinig U, Bocobza SE, Burdman S, Aharoni A (2015) The bitter side of the nightshades: genomics drives discovery in *Solanaceae* steroidal alkaloid metabolism. Phytochemistry 113:24–32
Casanas R, Gonzalez M, Rodriguez E, Morrero A, Diaz C (2002) Chemometric studies of chemical compounds in five cultivars of potatoes from Tenerife. J Agric Food Chem 50:2076–2082
Chakraborty S, Chakraborty N, Datta A (2000) Increased nutritive value of transgenic potato by expressing a non-allergenic seed albumin gene from *Amaranthus hypochondriacus*. Proc Natl Acad Sci USA 97:3724–3729
Chakraborty S, Chakraborty N, Agrawal L, Ghosh S, Narula K, Shekhar S, Naik SP, Pande PC, Chakrborti SK, Datta A (2010) Next generation protein-rich potato expressing the seed protein gene Am A1 is a result of proteome rebalancing in transgenic tuber. Proc Natl Acad Sci USA 107:17533–17538
Chawla R, Shakya R, Rommens CM (2012) Tuber-specific silencing of asparagines synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. Plant Biotechnol J 10:913–924
Chun OK, Kim DO, Smith N, Schroeder D, Han JT, Lee CY (2005) Daily consumption of phenolics and total antioxidant capacity from fruits and vegetables in the American diet. J Sci Food Agric 85:1715–1724
Civitelli R, Villareal DT, Agnusdei D, Nardi P, Avioli LV, Gennari C (1992) Dietary L-lysine and calcium metabolism in humans. Nutrition 8:400–405
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 14:169–176
Clevidence B, Haynes K, Rao D, Novotny J (2005) Effect of cooking method on xanthophyll content of yellow-fleshed potato. US Japan Nat Resour Protein Panel 34:280–284
Crowell EF, McGrath JM, Douches DS (2008) Accumulation of vitamin E in potato (*Solanum tuberosum*) tubers. Transgenic Res 17:205–217
Dale MF, Bradshaw JE (2003) Progress in improving processing attributes in potato. Trends Plant Sci 8:310–312
Dale MFB, Grfiffiths DW, Bain H, Todd D (1993) Glycoalkaloid increase in *Solanum tuberosum* on exposure to light. Ann Appl Biol 123:411–418
Dale MF, Griffiths DW, Todd DT (2003) Effects of genotype, environment, and postharvest storage on the total ascorbate content of potato (*Solanum tuberosum*) tubers. J Agric Food Chem 51:244–248
De Lepeleire J, Strobbe S, Verstraete J, Blancquaert D, Ambach L, Visser RGF, Stove C, Van Der Straeten D (2018) Folate biofortification of potato by tuber-specific expression of four folate biosynthesis genes. Mol Plant 11:175–188
Denslow SA, Walls AA, Daub ME (2005) Regulation of biosynthetic genes and antioxidant properties of vitamin B_6 vitamers during plant defense responses. Physiol Mol Plant Pathol 66:244–255
Deusser H, Guignard C, Hoffmann L, Evers D (2012) Polyphenol and glycoalkaloid contents in potato cultivars grown in Luxembourg. Food Chem 135:2814–2824
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 26(6):13
Diretto G, Welsch R, Tavazza R, Mourgues F, Pizzichini D, Beyer P, Giuliano G (2007) Silencing of beta-carotene hydroxylase increases total carotenoid and beta-carotene levels in potato tubers. BMC Plant Biol 2:7–11
Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA (2005) Metabolic engineering of high carotenoid potato tubers containing enhanced levels of beta-carotene and lutein. J Exp Bot 56:81–89

Dugo G, La Pera L, Lo Turco V, Giuffrida D, Restuccia S (2004) Determination of copper, zinc, selenium, lead and cadmium in potatoes (*Solanum tubersom* L.) using potentiometric stripping methods. Food Addit Contam 2:649–657

Egnin M, Prakash CS (1997) Transgenic sweetpotato expressing a synthetic storage protein gene exhibits high level of total protein and essential amino acids. In Vitro Cell Dev Biol 33:52A

Esposito F, Fogliano V, Cardi T, Carputo D, Filippone E (2002) Glycoalkaloid content and chemical composition of potatoes improved with nonconventional breeding approaches. J Agric Food Chem 50:1553–1561

FAO (2008) International year of the potato. http://www.fao.org/potato-2008/en/index.html

Fernandez-Orozco R, Gallardo-Guerrero L, Hoirnero-Méndez D (2013) Carotenoid profiling in tubers of different potato (Solanum sp) cultivars: accumulation of carotenoids mediated by xanthophyll esterification. Food Chem 141:2864–2872

Forges T, Monnier-Barbarino P, Alberto JM, Gueant-Rodriguez RM, Daval JL, Gueant JL (2007) Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update 13:225–238

Fowler B (2005) Homocysteine: overview of biochemistry, molecular biology, and role in disease processes. Semin Vasc Med 5:77–86

Friedman M, McDonald GM, Keszi MF (1997) Potato glycoalkaloids: chemistry, analysis, safety and plant physiology. Crit Rev Plant Sci 16:55–132

Gaby AR (2006) Natural remedies for Herpes simplex. Altern Med Rev 11:93–101

Galvez AF, Revilleza MJ, de Lumen BO, Krenz DC (2008) Food for health in the pacific rim. In: Enhancing the biosynthesis of endogenous methionine-rich proteins (MRP) to improve the protein quality of legumes via genetic engineering. Food & Nutrition Press, Inc., pp 540–552

Garcia MM, Gueant-Rodriguez RM, Pooya S, Brachet P, Alberto JM, Jeannesson E, Maskali F, Gueguen N, Marie PY, Lacolley P, Herrmann M, Juilliere Y, Malthiery Y, Gueant JL (2011) Methyl donor deficiency induces cardiomyopathy through altered methylation/acetylation of PGC-1α by PRMT1 and SIRT1. J Pathol 225:324–335

Goo YM, Kim TW, Lee MK, Lee SW (2013) Accumulation of PrLeg, a *Perilla legumin* protein in potato tuber results in enhanced level of sulphur-containing amino acids. C R Biol 336:433–439

Goyer A, Navarre DA (2007) Determination of folate concentrations in diverse potato germplasm using a trienzyme extraction and a microbiological assay. J Agric Food Chem 55:3523–3528

Guthikonda S, Haynes WG (2006) Homocysteine: role and implications in atherosclerosis. Curr Atheroscler Rep 8:100–106

Halford NG, Curtis TY, Muttucumaru N, Postles J, Elmore JS, Mottram DS (2012) The acrylamide problem: a plant and agronomic science issue. J Exp Bot 63:2841–2851

Han KH, Matsumoto A, Shimada K, Sekikawa M, Fukushima M (2007) Effects of anthocyanin-rich purple potato flakes on antioxidant status in F344 rats fed a cholesterol-rich diet. Br J Nutr 98:914–921

Hemavathi UCP, Upadhyaya CP, Akula N, Young KE, Chun SC, Kim DH, Park SW (2010) Enhanced ascorbic acid accumulation in transgenic potato confers tolerance to various abiotic stresses. Biotechnol Lett 32:321–330

Hamouz K, Pazderu K, Lachman J, Cepl J, Kotíkova Z (2016) Effect of cultivar, flesh colour, locality and year on carotenoid content in potato tubers. Plant Soil Environ 62:86–91

Hesse H, Hoefgen R (2003) Molecular aspects of methionine biosynthesis. Trends Plant Sci 8:259–262

Huang T, Joshi V, Jander G (2014) The catabolic enzyme methionine gamma-lyase limits methionine accumulation in potato tubers. Plant Biotechnol J 12:883–893

Hudson AO, Bless C, Macedo P, Chatterjee SP, Singh BK, Gilvarg C, Leustek T (2005) Biosynthesis of lysine in plants: evidence for a variant of the known bacterial pathways. Biochim Biophys Acta 1721:27–36

Iwanzik W, Tevini M, Stute R, Hilbert R (1983) Carotinoidgehalt undzusammensetzung verschiedener deutscher Kartoffelsorten und deren Bedeutung für die Fleischfarbe der Knolle. Potato Res 26:149–162

Jain AK, Nessler CL (2000) Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants. Mol Breed 6:73–78

Jansen G, Flamme W (2006) Coloured potatoes (*Solanum tuberosum* L.)-anthocyanin content and tuber quality. Genet Resour Crop Evol 53:1321–1331

Jube S, Borthakur D (2006) Recent advances in food biotechnology research. In: Hui YH, Nip W-K, Nollet LML, Paliyath G, Sahlstrom S, Simpson BK (eds) Food biochemistry and food processing. Blackwell Publishing, Oxford, UK, pp 35–70

Kant AK, Block G (1990) Dietary vitamin B-6 intake and food sources in the US population: NHANES II, 1976-1980. Am J Clin Nutr 52:707–716

Kanatt S, Chander R, Radhakrishna P, Sharma A (2005) Potato peel extract: a natural antioxidant for retarding lipid peroxidation in radiation processed lamb meat. J Agric Food Chem 53:1499–1504

Kim JH, Cetiner S, Jaynes JM (1992) Enhancing the nutritional quality of crop plants: design, construction and expression of an artificial plant storage protein gene. In: Bhatnagar D, Cleveland TE (eds) Molecular approaches to improving food quality and safety. An avi book, New York, pp 1–36

Kim CK, Han JS, Lee HS, Oh JY, Shigaki T, Park SH, Hirschi K (2006) Expression of an Arabidopsis CAX2 variant in potato tubers increases calcium levels with no accumulation of manganese. Plant Cell Rep 25:1226–1232

Kita A, Bąkowska-Barczak A, Hamouz K, Kułakowska K, Lisińska G (2013) The effect of frying on anthocyanin stability and antioxidant activity of crisps from red- and purple-fleshed potatoes (*Solanum tuberosum* L.). J Food Comp Anal 32:169–175

Kobayashi A, Ohara-Takada A, Tsuda S, Matsuura-Endo Ch, Takada N et al (2008) Breeding of potato variety 'Inca-no-hitomi' with a very high carotenoid content. Breed Sci 58:77–82

Konings EJ, Roomans HH, Dorant E, Goldbohm RA, SarisWH, van den Brandt PA (2001) Folate intake of the Dutch population according to newly established liquid chromatography data for foods. Am J Clin Nutr 73:765–776

Kumar P, Jander G (2017) Concurrent overexpression of *Arabidopsis thaliana* cystathionine γ-synthase and silencing of endogenous methionine γ-lyase enhance tuber methionine content in *Solanum tuberosum*. J Agric Food Chem 65:2737–2742

Laing WA, Wright MA, Cooney J, Bulley SM (2007) The missing step of the L-galactose pathway of ascorbate biosynthesis in plants, an L-galactose guanyltransferase, increases leaf ascorbate content. Proc Natl Acad Sci USA 104:9534–9539

Lachman J, Hamouz M, Orsak M, Kotikova Z (2016) Carotenoids in potatoes—a short overview. Plant Soil Environ 62:474–481

Le DT, Chu HD, Le NQ (2016) Improving nutritional quality of plant proteins through genetic engineering. Curr Genomics 17:220–229

Leo L, Leone A, Longo C, Lombardi D, Raimo F, Zacheo G et al (2008) Antioxidant compounds and antioxidant activity in "early potatoes". J Agric Food Chem 56:4154–4163

Lewis CE, Walker JRL, Lancaster JE, Sutton KH (1998) Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: coloured cultivars of *Solanum tuberosum* L. J Sci Food Agric 77:45–57

Li L, Yang Y, Xu Q, Owsiany K, Welsch R, Chitchumroonchokchai C, Lu S, Van Eck J, Deng XX, Failla M, Thannhauser TW (2012) The *Or* gene enhances carotenoid accumulation and stability during post-harvest storage of potato tubers. Mol Plant 5:339–352

Lisinka G, Leszczynski W (1989) Potato science and technology. Elsevier, London and New York, pp 41–42

Llorente B, Alonso GD, Bravo-Almonacid F, Rodríguez V, López MG, Carrari F, Torres HN, Flawia MM (2011) Safety assessment of nonbrowning potatoes: opening the discussion about the relevance of substantial equivalence on next generation biotech crops. Plant Biotechnol J 9:136–150

Lopez AB, Van Eck J, Conlin BJ, Paolillo DJ, O'Neill J, Li L (2008) Effect of the cauliflower *Or* transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. J Exp Bot 59:213–223

Love SL, Salaiz T, Shafii B, Price WJ, Mosley AR Thornton RE (2004) Stability of expression and concentration of ascorbic acid in North American potato germplasm. HortScience 39:156–160

Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran DM et al (2006) The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. Plant Cell 18:3594–3605

Mäder J, Rawel H, Kroh L (2009) Composition of phenolic compounds and glycoalkaloids-solanine and chaconine during commercial potato processing. J Agric Food Chem 57:6292–6297

Malmberg, AG Theander O (1985) Determination of chlorogenic acid in potato tubers. J Agric Food Chem 33:549–551

Manach C, Donovan JL (2004) Pharmacokinetics and metabolism of dietary flavonoids in humans. Free Radic Res 38:771–785

Matsuda F, Morino K, Ano R, Kuzawa M, Wakasa K, Miyagawa H (2005) Metabolicflux analysis of the phenylpropanoid pathway in elicitor-treated potato tuber tissue. Plant Cell Physiol 46:454–466

McCue KF, Shepherd LVT, Allen PV, Maccree MM, Rockhold DR, Corsiru DL, Davies HV, Belknap WR (2005) Metabolic compensation of steroidal glycoalkaloid biosynthesis in potato tubers—using reverse genetics to confirm the *in vivo* enzyme function of asteroidal alkaloid galactosyl transferase. Plant Sci 168:267–273

McCue KF, Allen PV, Shepherd LVT, Blake A, Whitworth J, Maccree MM, Rockhold DR, Stewart D, Davies HV, Belknap WR (2006) The primary in vivo steroidal alkaloid glucosyltransferase from potato. Phytochemistry 67:1590–1597

McCue KF, Allen PV, Shepherd LVT, Blake A, Maccree MM, Rockhold DR, Novy RG, Stewart D, Davies HV, Belknap WR (2007) Potato glycosterol rhamnosyltransferase, the terminal step in triose side-chain biosynthesis. Phytochemistry 68:327–334

McKillop DJ, Pentieva K, Daly D, McPartlin JM, Hughes J, Strain JJ, Scott JM, McNulty H (2002) The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. Br J Nutr 88:681–688

Morris WL, Ducreux LJ, Fraser PD, Millam S, Taylor MA (2006) Engineering ketocarotenoid biosynthesis in potato tubers. Metab Eng 8:253–263

National Institute of Nutrition, Indian Council of Medical Research, Hyderabad (2011) Dietary guidelines for Indians—a manual, 2nd edn

Naverre DA, Goyer A, Shakya R (2009) Nutritional value of potatoes: vitamin, phytonutrient, and mineral content. In: Singh J, Kaur L (eds) Advances in potato chemistry and technology, pp 395–424

Navarre D, Pillai S, Shakya R, Holden M (2011) HPLC profiling of phenolics in diverse potato genotypes. Food Chem 127:34–41

Navarre DA, Shakya R, Holden J, Kumar S (2010) The effect of different cooking methods on phenolics and vitamin C in developmentally young potato tubers. Am J Potato Res 87(4):350–359

Nogueira T, do Lago CL (2007) Determination of caffeine in coffee products by dynamic complexation with 3,4-dimethoxycinnamate and separation by CZE. Electrophoresis 28:3570–3574

Oertel A, Matros A, Hartmann A, Arapitsas P, Dehmer KJ, Martens S, Mock HP (2017) Metabolite profiling of red and blue potatoes revealed cultivar and tissue specific patterns for anthocyanins and other polyphenols. Planta 246:281–297

Orphanos PT (1980) Dry matter content and mineral composition of potatoes grown in Cyprus. Potato Res 23:371–374

Ortiz-Medina E, Donnelly DJ (2003) Concentration and distribution of total soluble protein in fresh and stored potato tubers. Acta Hortic 619:323–328

Oxenboll Sorensen S, Pauly M, Bush M, Skjot M, McCann MC, Borkhardt B, Ulvskov P (2000) Pectin engineering: modification of potato pectin by in vivo expression of an endo-1,4-beta-D-galactanase. Proc Natl Acad Sci USA 97:7639–7644

Park S, Cheng NH, Pittman JK, Yoo KS, Park J, Smith RH, Hirschi KD (2005) Increased calcium levels and prolonged shelf life in tomatoes expressing Arabidopsis H^+/Ca^{2+} transporter. Plant Physiol 139:1194–1206

Parr AJ, Mellon FA, Colquhoun IJ, Davies HV (2005) Dihydrocaffeoyl polyamines (kukoamine and allies) in potato (*Solanum tuberosum*) tubers detected during metabolite profiling. J Agric Food Chem 53:5461–5466

Qin AG, Shi QH, Yu XC (2011) Ascorbic acid contents in transgenic potato plants overexpressing two dehydroascorbate reductase genes. Mol Biol Rep 38:1557–1566

Randhawa KS, Sandhu KS, Kaur G, Singh D (1984) Studies of the evaluation of different genotypes of potato (*Solanum tuberosum* L) for yield and mineral contents. Plant Foods Hum Nutr 34:239–242

Reddivari L, Vanamala J, Chintharlapalli S, Safe SH, Miller JC Jr (2007) Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through activation of caspase-dependent and caspase-independent pathways. Carcinogenesis 28:2227–2735

Regierer B, Fernie AR, Springer F, Perez-Melis A, Leisse A, Koehl K, Willmitzer L, Geigenberger P, Kossmann J (2002) Starch content and yield increase as a result of altering adenylate pools in transgenic plants. Nat Biotechnol 20:1256–1260

Rivero RC, Hernandez PS, Rodriguez EMR, Martin JD, Romero CD (2003) Mineral concentrations in cultivars of potatoes. Food Chem 83:247–253

Rogan GJ, Bookout JT, Duncan DR, Fuchs RL, Lavrik PB, Love SL, Mueth M, Olson T, Owens ED, Raymond PJ, Zalewski J (2000) Compositional analysis of tubers from insect and virus resistant potato plants. J Agric Food Chem 48:5936–5945

Romer S, Lubeck J, Kauder F, Steiger S, Adomat C, Sandmann G (2002) Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. Metab Eng 4:263–272

Rommens CM, Ye J, Richael C, Swords K (2006) Improving potato storage and processing characteristics through all-native DNA transformation. J Agric Food Chem 54:9882–9887

Rommens CM, Richael CM, Yan H, Navarre DA, Ye J, Krucker M, Swords K (2008) Engineered native pathways for high kaempferol and caffeoylquinate production in potato. Plant Biotechnol J 6:870–886

Ross HA, Morris WL, Ducreux LJ, Hancock RD, Verrall SR, Morris JA, Tucker GA, Stewart D, Hedley PE, McDougall GJ, Taylor MA (2011) Pectin engineering to modify product quality in potato. Plant Biotechnol J 9:848–856

Sacksteder KA, Biery BJ, Morrell JC, Goodman BK, Geisbrecht BV, Cox RP, Gould SJ, Geraghty MT (2000) Identification of the alpha-aminoadipic semialdehyde synthase gene, which is defective in familial hyperlysinemia. Am J Hum Genet 66:1736–1743

Scalbert A, Manach C, Morand C, Remesy C, Jimenez L (2005) Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 45:287–306

Schmidt MHH, Raulf-Heimsoth M, Posch A (2002) Evaluation of patatin as a major cross-reactive allergen in latex-induced potato allergy. Ann Allergy Asthma Immunol 89:613–618

Schwall GP, Safford R, Westcott RJ, Jeffcoat R, Tayal A, Shi YC, Gidley MJ, Jobling SA (2000) Production of very-high-amylose potato starch by inhibition of SBE A and B. Nat Biotechnol 18:551–554

Scott J, Rebeille F, Fletcher J (2000) Folic acid and folates: the feasibility for nutritional enhancement in plant foods. J Sci Food Agric 80:795–824

Scott JM (1999) Folate and vitamin B12. Proc Nutr Soc 58:441–448

Scott JM, Weir DG (1998) Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. J Cardiovasc Risk 5:223–227

Shakya R, Navarre D (2006) Rapid screening of ascorbic acid, glycoalkaloids, and phenolics in potato using high-performance liquid chromatography. J Agric Food Chem 54:5253–5260

Silva-Beltran NP, Chaidez-Quiroz C, Lopez-Cuevas O, Ruiz-Cruz S, Lopez-Mata MA, Del-Toro-Sanchez CL, Marquez-Rios E, Ornelas-Paz JJ (2017) Phenolic compounds of potato peel extracts: their antioxidant activity and protection against human enteric viruses. J Microbiol Biotechnol 27:234–241

Smith DB, Roddick JG, Jones JL (1996) Potato glycoalkaloids: some unanswered questions. Trends Food Sci Technol 7:126–131

Spinneker A, Sola R, Lemmen V, Castillo MJ, Pietrzik K, González-Gross M (2007) Vitamin B6 status, deficiency and its consequences—an overview. Nutr Hosp 22:7–24

Tambasco-Studart M, Titiz O, Raschle T, Forster G, Amrhein N, Fitzpatrick TB (2005) Vitamin B6 biosynthesis in higher plants. Proc Natl Acad Sci USA 102:13687–13692

Tanemura Y, Yoshino M (2006) Regulatory role of polyamine in the acid phosphatase from potato tubers. Plant Physiol Biochem 44:43–48

Theodoratou E, Farrington SM, Tenesa A, McNeill G, Cetnarskyj R, Barnetson RA, Porteous ME, Dunlop MG, Campbell H (2008) Dietary vitamin B6 intake and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 17:171–182

Tierno R, Hornero-Méndez D, Gallardo-Guerrero L, López-Pardo R, Ruiz de Gallareta JI (2015) Effect of boiling on the total phenolic, anthocyanin and carotenoid concentrations of potato tubers from selected cultivars and introgressed breeding lines from native potato species. J Food Compos Anal 41:58–65

True RH, Hogan JM, Augustin J, Johnson SJ, Teitzel C, Toma RB, Orr P (1979) Mineral composition of freshly harvested potatoes. Am Potato J 56:339–350

Tu HM, Godfrey LW, Sun SS (1998) Expression of the Brazil nut methionine-rich protein and mutants with increased methionine in transgenic potato. Plant Mol Biol 37:829–838

Tudela JA, Cantos E, Espin JC, Tomas-Barberan FA, Gil MI (2002) Induction of antioxidant flavonol biosynthesis in fresh-cut potatoes. Effect of domestic cooking. J Agric Food Chem 50:5925–5931

Tzin V, Galili G (2010) The biosynthetic pathways for shikimate and aromatic amino acids in *Arabidopsis thaliana*. Arabidopsis Book 8:e0132

Väänänen T, Ikonen T, Rokka V-M, Kuronen P, Serimaa R, Ollilainen V (2006) Correction. Influence of incorporated wild *Solanum* genomes on potato properties in terms of starch nanostructure and glycoalkaloid content. J Agric Food Chem 54:4496–4497

Vahteristo L, Lehikoinen K, Ollilainen V, Varo P (1997) Application of an HPLC assay for the determination of folate derivatives in some vegetables, fruits and berries consumed in Finland. Food Chem 59:589–591

Van Eck J, Zhou X, Lu S, Li L (2010) Modulation of carotenoid accumulation in transgenic potato by inducing chromoplast formation with enhanced sink strength. Methods Mol Biol 643:77–93

Vathi H, Upadhyaya CP, Young KE, Nookaraju A, Kim HS, Heung JJ, Oh MH, Aswath CR, Chun SC, Kim DH, Park SW (2009) Over-expression of strawberry D-galacturonic acid reductase in potato leads to accumulation of vitamin C with enhanced abiotic stress tolerance. Plant Sci 177:659–667

Vathi H, Upadhyaya CP, Nookaraju A, Kim HS, Heung JJ, Oh MH, Chun SC, Kim DH, Park SW (2011) Biochemical analysis of enhanced tolerance in transgenic potato plants overexpressing D-galacturonic acid reductase gene in response to various abiotic stresses. Mol Breed 28:105–115

Welch RM (2002) The impact of mineral nutrients in food crops on global human health. Plant Soil 247(1):83–90. Progress in plant nutrition: plenary lectures of the XIV international plant nutrition colloquium (November 2002)

Werij JS, Kloosterman B, Celis-Gamboa C, De Vos CR, America T, Visser RG, Bachem CW (2007) Unravelling enzymatic discoloration in potato through a combined approach of candidate genes, QTL, and expression analysis. Theor Appl Genet 115(2):245–252

Wiberley-Bradford AE, Busse JS, Jiang J, Bethke PC (2014) Sugar metabolism, chip color, invertase activity, and gene expression during long-term cold storage of potato (*Solanum tuberosum*) tubers from wild-type and vacuolar invertase silencing lines of Katahdin. BMC Res Notes 7:801

Wolucka BA, Van Montagu M (2007) The VTC2 cycle and the *de novo* biosynthesis pathways for vitamin C in plants: an opinion. Phytochemistry 68:2602–2613

Xiong JS, Ding J, Li Y (2015) Genome-editing technologies and their potential application in horticultural crop breeding. Hortic Res 2:15019

Yamaguchi T, Chikama A, Mori K, Watanabe T, Shioya Y, Katsuragi Y, Tokimitsu I (2008) Hydroxyquinone-free coffee: a double-blind, randomized controlled dose-response study of blood pressur. Nutr Metab Cardiovasc Dis 18:408–414

Yang MS, Espinoza NO, Dodds JH, Jaunes JM (1989) Expression of a synthetic gene for improved protein quality in transformed potato plants. Plant Sci 64:99–111

Young MG, Hyun JC, Tae WK, Cheol HL, Mi-Jeonghn SCB, Kang JC, Jae A, Chung-han C, Shin-Woo L (2008) Expressional characterization of dehydroascorbate reductase C-DNA in transgenic potato plants. J Plant Biol 51:35–41

Zeh M, Casazza AP, Kreft O, Roessner U, Bieberich K, Willmitzer L, Hoefgen R, Hesse H (2001) Antisense inhibition of threonine synthase leads to high methionine content in transgenic potato plants. Plant Physiol 127:792–802

Zhu X, Richael C, Chamberlain P, Busse JS, Bussan AJ, Jiang J, Bethke PC (2014) Vacuolar invertase gene silencing in potato (*Solanum tuberosum* L.) improves processing quality by decreasing the frequency of sugar-end defects. PLoS One 9:e93381

Zimmermann MB, Hurrell RF (2002) Improving iron, zinc and vitamin A nutrition through plant biotechnology. Curr Opin Biotechnol 13:142–145