Genetic Regulation of Health Beneficial Phytonutrients in Fresh Produce

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Abstract

Compelling evidence in literature supports the hypothesis that dietary phytonutrients, especially antioxidants, maintain human health and prevent chronic diseases such as cancer, diabetes, osteoporosis, cardiovascular and age-related disorders, including dementia. Fresh fruits and vegetables are dietary sources of beneficial phytonutrients, and therefore, their consumption has increased in recent decades. However, health promoting phytonutrients in currently-used germplasm of fruits and vegetables are not at sufficient levels to achieve the threshold levels that could be beneficial. The tight, endogenous regulation of metabolic pathways for the biosynthesis of phytonutrients makes it difficult to increase their levels by conventional breeding in current fruit and vegetable germplasm. Molecular breeding and genetic engineering of crops have begun to provide novel methods to enhance levels of desirable phytonutrients by overcoming this regulation by introducing genes that are rate limiting for the production of the desirable phytonutrients. Our approach includes increasing the levels of various phytonutrients in fruits by identifying molecular regulators of biochemical pathways, and genetically engineering them to enhance metabolic activity of cells to increase the levels of desirable phytonutrients. In this chapter, we summarize various tools available to enhance levels of phytonutrients in fresh produce. A separate approach for modifying levels of phytonutrients is presented which involves engineering higher levels of biogenic amines that are molecular regulators of several cellular biochemical pathways. Along with engineering the levels of health-promoting carotenoid lycopene, we have demonstrated that spermidine and spermine also extend longevity of a ripe fruit and delay whole plant senescence. Higher endogenous levels of biogenic polyamines also activate a wide array of genes regulating transcription, translation, signal transduction, chaperone activity, stress proteins, amino acid biosynthesis, ethylene biosynthesis and action, isoprenoid pathway, and flavonoid biosynthesis.

Keywords: Fruit, phytonutrient, human health, gene regulation, metabolome

INTRODUCTION

The human health paradigm has begun to shift towards prevention rather than medical intervention to ameliorate diseases and physiological disorders. The gradual loss in physiological vigor, causing impaired homeostasis, is responsible for aging and various age related diseases and disorders. Another factor linked to aging-associated enhanced susceptibility to disease is the loss of innate immunity to disease causing organisms. Physiological factors that exhibit changes during aging include cellular senescence, mitochondrial dysfunction, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, altered inter- and intra-cellular communication, and inflammation. Genomic instability and telomere attrition have also been implicated in the mammalian aging process (López-Otín et al., 2013). Emerging evidence shows that phytonutrients, especially antioxidants, present in fruit and vegetables can alleviate some of the symptoms of disease and reduce incidence of diseases such as cancer, atherosclerosis which lead to cardiovascular diseases, hypertension, diabetes and osteoporosis

(Martin et al., 2011; Mattoo et al., 2011). Such antioxidants present in horticultural produce have been implicated in building innate immunity to fight various pathogenic organisms. Other beneficial effects of phytonutrients include counteracting harmful effects of damaging oxidants (reactive oxygen species, ROS), environmental factors including radiation and pollutants, chemicals in processed foods, consumer products, cosmetics and various drugs. Interestingly, phytonutrients present in fruits and vegetables also help alleviate the negative effects of socio-economic patterns such as sedentary life styles and lack of exercise. Unequivocal demonstration of preventive interventions/therapies of age- and lifestyle-related disorders through individually tailored diets or nutrients requires extensive time and effort. More scientific research and validation through human nutrition trials is required before phytonutrients become a 'mantra' for healthy living.

A number of bioactive compounds, with potential to overcome age- and lifestyle-related body abuse, are present in plants. However, very little is definitively known about the recommended daily allowance (RDA) for various fruit and vegetable nutrients, necessitating consumption of more than less of horticultural commodities to get a good dose of phytonutrients (Shukla and Mattoo, 2010; Fitzpatrick et al., 2012). Low levels of health promoting phytonutrients in plants are due to tight genetic and developmental controls of their metabolic pathways during plant growth and development. Conventional breeding has not been successful in boosting the levels of some phytonutrients. In recent decades, molecular breeding, in conjunction with conventional breeding, has helped overcome some of these barriers and has led to development of new cultivars with enhanced levels of some phytochemicals. However, in many instances, information about biosynthetic pathways for phytonutrients is incomplete and germplasms with higher accumulation of phytonutrients is not easily available. Modern tools of metabolomics are rapidly overcoming some of these problems (Saito and Matsuda, 2010). Molecular genetics is not only helping to overcome such problems but has also provided tools to identify and characterize genes regulating the biosynthesis of phytonutrients in plants. Notably, genetic engineering of the rate limiting steps in the biosynthesis of a compound has facilitated increased levels of phytonutrients in plant tissues/organs (Shukla and Mattoo, 2010; Fitzpatrick et al., 2012; Fatima et al., 2013; Handa et al., 2014).

Vitamins are vital organic compounds that are required in small amounts to maintain proteostasis in many organisms, including humans. The diverse biological roles associated with vitamins include regulation of mineral metabolism (vitamin D), antioxidant activity (vitamins A, C and E), and precursor availability of several enzyme cofactors (B complex vitamins). Many organisms either lack altogether the ability to synthesize or do not synthesize vitamins in sufficient quantities, therefore acquiring them through diet. The vitamin compounds acquired mostly through the human diet include retinol (vitamin A), thiamin diphosphate (vitamin B_1), niacin (vitamin B_3), pentothenate (vitamin B_5), pyridoxal 5'-phosphate (vitamin B_6), biotin (vitamin B_8), folate (vitamin B_9), 5'deoxyadenosyl cobalamin (vitamin B_{12}), L-ascorbate (vitamin C), cholecalciferol (Vitamin D_3), D-α-tocopherol (vitamin E), and phylloquinone (vitamin K). These organic compounds represent diverse structures and are classified by their biological activity (Fitzpatrick et al., 2012). Plant-based diets are responsible for providing to some extent many of these vitamins, including vitamins A, B_1, B_3, B_5, B_6, B_8, B_{12}, C, D_3, E and K. Deficiency in these vitamins results in a multitude of symptoms and chronic diseases. In addition to vitamins, plants provide many more reactive compounds and antioxidants that play significant roles in human health. Details of metabolic engineering of vitamins in plants have been recently summarized (Shukla and Mattoo, 2010; Fitzpatrick et al., 2012; Fatima et al., 2013). In this chapter we discuss some strategies used to increase levels of various phytonutrients such as vitamins, antioxidants and other beneficial compounds in plants.

Breeding-Based Enhancement of Phytonutrients

Plant breeding has been successfully used to enhance many agronomical attributes, including few phytonutrients in crop plants, resulting in new cultivars with greatly enhanced

crop yields. However, availability of germplasm with higher levels of phytonutrients is a prerequisite to increase levels of phytonutrients through conventional breeding. In recent years, there has been remarkable progress in analytical methods to both purify and quantify hundreds of biochemicals (Saito and Matsuda, 2010); this has helped identify, purify and quantify phytonutrients. These methods are also being used for characterization of different germplasms with enhanced levels of desirable nutrients. However, conventional breeding is a laborious process requiring quantification of the chosen phytonutrients in a large number of segregating progenies resulting from multiple crosses and backcrosses. Identification of Quantitative Trait Loci (QTLs) and availability of molecular markers associated with these QTLs has greatly accelerated the breeding processes in crop plants. The association mapping that scans the significant association between trait and genetic markers has the potential to provide new alleles for regulation of biosynthesis and cellular accumulation of a phytonutrient (Myles et al., 2009). The whole genome sequencing to map unknown mutations would likely provide new tools to discover new genes and allelic series that enhance vitamin content of food products (Austin et al., 2011). Mutagenesis and TILLING (Targeting Induced Local Lesions in Genomes) have begun to provide approaches to select against genes that negatively regulate biosynthesis or accumulation of phytonutrients (Zhang et al., 2009; Handa et al., 2011).

Breeding based and genetically engineering approaches to enhance levels of several vitamins and other phytonutrients, including vitamins A, C and E in a variety of edible plants have been applied successfully (Shukla and Mattoo, 2010; Fitzpatrick et al., 2012; Fatima et al., 2013). Fruits, flowers or tubers are among the best dietary sources of ascorbic acid (vitamin C) due to high accumulation of ascorbate in these tissues. However, the ascorbic acid levels vary greatly within tissues of the same species. The levels of vitamin C in tomato fruit range from 10 to >500 mg/100 g FW (Fresh Weight); in Amazonian fruit Camu camu, they can be as high as 25 mg/g FW (Fitzpatrick et al., 2012). Exploitation of this genetic variability has led to indentification of QTL location on genetic maps of several crops followed by positional cloning to identify the polymorphic locus responsible for vitamin C variation. These investigations led to identification of an allelic form of monodehydroascorbate reductase gene that co-segregates with a major QTL of vitamin C and is responsible for >80% variation in vitamin C levels in tomato fruit (Stevens et al., 2008). Breeding has been used to enhance levels of several other essential vitamins and micronutrients in staple crops (Martin et al., 2011). For example, the level of β-carotene was increased in maize from ~0 to 14 mg/g FW, in cassava from 0.4 to 1.5 mg/100 g FW, in sweet potato from 0 to 32 mg/100 g FW, total carotenes in cassava from 0.6-1.5 to 6-11.5 mg/100 g FW, iron in rice from 3 to 6-8 mg/100 g FW and in beans from 40 to 80-100 mg/100 g FW, zinc in beans from 30 to 40-50 mg/100 g FW and in rice from 13 to 35 mg/100 g FW and in wheat from 38 to 61 mg/100 g FW (Martin et al., 2011).

Metabolic Engineering-Based Enhancement of Phytonutrients

Metabolic engineering is increasingly being used to elevate the levels of biologically active phytochemicals to boost nutritional content of crops, and to meet nutritional food security for the ever increasing world population (Shukla and Mattoo, 2010; Martin et al., 2011; Fitzpatrick et al., 2012; Fatima et al., 2013). Biosynthesis and accumulation of phytochemicals are determined by the rate limiting step(s) which are regulated both at genetic and biochemical levels. Several different genetic engineering strategies have been used to enhance the levels of phytonutrients in plants (Table 1). Among them, the ectopic expression of gene(s) that encodes the rate limiting enzyme of a biochemical pathway has been used in many instances to increase levels of phytonutrients (Fatima et al., 2013). In another instance, expression of a mammalian GTP cyclohydrolase I lacking feedback regulation resulted in about 140-fold increase in pteridine and 2-fold increase in folate. Further engineering of the resulting transgenic line by crossing with another transgenic tomato expressing aminodeoxychorismate synthase resulted in about 19-fold increase in folate (Díaz de la Garza et al., 2007). Aminodeoxychorismate synthase catalyzes the first step of PABA synthesis. In yet another

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strategy, simultaneous expression of multiple genes was used to accomplish biosynthesis of a desired phytonutrient such as β-carotene (provitamin A) in rice, called 'Golden Rice'. The immature rice endosperm is capable of production of geranylgeranyl diphosphate but lacks three enzymes to synthesize β-carotene: phytoene synthase (*psy*), phytoene desaturase (*crtI*) and lycopene β-cyclase (*lcy*). Ye et al. (2000) co-transformed rice with multiple plasmids to express *psy* from daffodil (*Narcissus pseudonarcissus*), *crtI* from *Erwinia uredovora*, and *lcy* from *Narcissus pseudonarcissus*, respectively, to develop "Golden Rice" with detectable levels of β-carotene. Further investigations showed that the source of gene makes significant difference in metabolic engineering. For example, *psy* from maize instead of daffodil resulted in about 23-fold increase in total carotenoids in "Golden Rice 2" as compared to "Golden Rice" (Paine et al., 2005). Overexpression of GDP-*L*-galactose phosphorylase (*VTC2*), an enzyme that catalyzes the first committed step in the *L*-galactose ascorbate biosynthetic pathway, resulted in increasing the level of vitamin C in tomato, strawberry, and potato by 2-6 fold (Bulley et al., 2012).

  Other vitamins whose levels have been boosted by metabolic engineering include vitamin E which is made of four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ). All four differentially methylated species of tocopherols and tocotrienols are excellent lipid soluble antioxidants but α-tocopherol exhibits the highest *in vivo* vitamin E activity. By expressing α-tocopherol methyltransferase and γ-tocopherol methyltransferase, vitamin E activity was increased several-fold in soy oil and lettuce, respectively (Fatima et al., 2013). By simultaneously expressing the genes for β-carotene, ascorbate, and folate biosynthetic pathways, Naqvi et al. (2009) have demonstrated increase in β-carotene (169-fold), ascorbate (6-fold), and folate (2-fold) in corn. Expression of several genes in the flavonoid pathway have been shown to increase levels of flavonoids, carotenoids and essential fatty acids in fruits and vegetables crops (Fatima et al., 2013).

Molecular Engineering of Transcription Factors to Enhance Phytonutrient Production and Accumulation

  In addition to genes encoding the metabolic pathways, transcription factors from various plant sources have been used to enhance phytonutrients. Overexpression of *Rosea1* and *Delila* increased flavonoid (anthocyanin) content in tomato pericarp to levels similar to that present in anthocyanin-rich blackberries and blueberries (Butelli et al., 2008): ectopic expression of flavonoid-related *R2R3-MYB* resulted in increase in the levels of anthocyanidins including petunidin, malvidin, and delphinidin in transgenic tomatoes. Overexpression of *TOMATO AGAMOUS-LIKE 1 (TAGL1)*, a *MADS*-box transcription factor, resulted in higher accumulation of lycopene and naringenin chalcone (Itkin et al., 2009); RNAi suppression of the *DE-ETIOLATED1 (DET1)* gene (a photomorphogenesis regulatory gene) caused several-fold increase in carotenoid, tocopherol, phenylpropanoids and flavonoids with no detrimental effects on yield (Enfissi et al., 2010).

Molecular Engineering of Molecular Regulators to Enhance Phytonutrient Production and Accumulation

  Tomato engineered to enhance levels of spermidine (Spd) and spermine (Spm) exhibited 2 to 3-fold increase in lycopene (120-175 mg/g FW) and choline; in addition, this transgenic event also led to enhancing other attributes such as fruit vine-life and higher processing quality (Mehta et al., 2002; Mattoo et al., 2006). Further metabolomic analyses using NMR profiling revealed higher levels of glutamine, asparagine, citrate, fumarate, malate, and an unidentified compound A, and lower levels of valine, aspartic acid, sucrose, and glucose in high Spd/Spm fruits (Mattoo et al., 2006). The nitrogen sensing/signaling and carbon metabolism were preferentially activated in the high Spd/Spm transgenics suggesting that Spd and Spm are perceived as nitrogenous metabolites that stimulate carbon sequestration in the fruit cells (Mattoo et al., 2006). Similar results were reported later by Neily et al. (2011). The pathways involved in the accumulation of several of these phytochemicals exhibited positive

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correlation with Spd and Spm levels and negative correlation with putrescine (Put) levels (Mattoo et al., 2010). Transcriptome analyses suggested that Spd and Spm act as anabolic regulators that enhance accumulation of many metabolites whereas Put acts as a catabolic regulator (Handa and Mattoo, 2010). Enhancing metabolic profile by regulating the metabolic pathway, as shown in the case of polyamine biosynthesis, provides another novel approach to increase levels of desirable phytonutrients.

CONCLUSIONS

Transgenically produced crops via a precision-based tool of genetic engineering add to the genetic resources for obtaining insight about genetic, biochemical and physiological regulation of various metabolic pathways and the production of bio-functional molecules. Such knowledge is useful for paving a path to produce highly nutritious, 'speciality crops' for the public good. Deciphering transcriptome, proteome and metabolome of the novel transgenics is providing much needed knowledge-base to ease the concerns of the society and help open the market for genetically engineered horticulture crops. A case in point is the acceptability of and subsequent higher sales of Hawaii-grown transgenic papaya in the United States. Moreover, as we progress further and generate more understanding of the processes involved, this knowledge will catalyze further efforts to develop precision-based newer strategies for redesigning metabolic pathways in a suitable manner such that desired levels of a particular phytonutrient (antioxidant) in crops are achieved. Moreover, in order to derive maximum health benefits and meet the demands for public good, we need unambiguous data from precise nutrition studies to determine what dose of an antioxidant is sufficient to provide prevention or cure against a disease, and what form makes a value-added fruit bioavailable.

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Table

Table 1. Genetic engineering of tomato fruit to enhance phytonutrients.

Metabolite	Fold-increase	Metabolite	Fold-increase
Flavonoids		**Flavor volatile**	
Flavonoids (total)	3.5 to10	1-nitro-2-phenylethane	10
Flavonols (peel)	10 to 78	methyl salicylate	123
Rutin flavonol	16 to 20	**Carotenoids**	
Chlorogenic acid	27	Phytosterol (Total)	2.4
Dicaffeoyl quinic acid	26	Carotenoids	1.2-2
Tricaffeoyl quinic acid	42	Phytoene	2.3 to 2.4
Quercetin rutinoside	67	β-carotene	2.2 -31.7
Kaempferol rutinoside	593	Lycopene	1.3-3.0
Anthocyanins	500	Phytofluene	1.7
		Lutein	1.5 to 1.7
		Xanthophyll (Total)	10

Redrawn from Handa et al. (2014).