

Molecular breeding of healthy vegetables

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In September 2010, a group of carrot growers in the USA announced a US\$25 million marketing campaign to promote ‘cut and peel’ carrots—also known as ‘baby carrots’—with the slogan: “Eat ‘em like junk food”. The campaign is an attempt to break into the US\$18 billion snack-food industry by “[t]aking a page out of junk food’s playbook and applying it to baby carrots” (Horovitz, 2010). It is neither the first nor only example of how food producers have tried to attract consumers with healthier meal choices including greens and fruits. Several food companies have recently introduced products that include ‘hidden’ vegetable servings in an attempt to provide vegetable-based nutrition by stealth. This approach could be beneficial for individual consumers and public health in general. A recent report from the US Centers for Disease Control indicated that only 26% of adults in the USA eat vegetables three or more times per day, which is far short of the amount recommended by the US government more than one decade ago (Severson, 2010).

Consumer interest in whole foods with enhanced nutritional qualities is at an all-time high, and more consumers are choosing foods on the basis of their healthy characteristics or direct labels that describe health benefits (Cappellano, 2009). This

development is taking place amid increasing interest in locally grown food, farmers’ markets and sustainable farming practices (Clifford, 2010). The promotion of ‘healthy’ agricultural food products has therefore coincided with a surging consumer interest in the health functionality of food, particularly fruits and vegetables. It is difficult to tell whether marketing for health functionality is spurring this interest or vice versa. Nevertheless, today’s food market offers many whole foods that are perceived and promoted as ‘health functional’ because of their phytochemical constituents: lycopene, vitamins, isothiocyanates or polyphenols, to name just a few.

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Although recognition and awareness of potentially health-functional compounds has increased during the past 15 years, much remains to be learned about the long-term effects of phytochemicals in the context of a modern human diet. Some consumers express concerns about studies—and the media coverage of results—that find evidence of the health benefits or risks of a particular food or ingredient, only for later studies to contradict these findings. Nonetheless, despite our lack of understanding, and often without substantial preliminary data, efforts have been made to enhance the concentration or quality of substances in crop plants that are thought to be health-promoting (Newell-McGloughlin, 2008). This takes place at the same time as molecular techniques and genetic modification are being applied to improve agriculture

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in the USA (Federoff *et al*, 2010). This article discusses recent attempts to characterize and modify phytochemicals in vegetable crops by using molecular approaches, focusing on those modifications that are of interest to consumers.

It should be noted at the outset that many crops were originally domesticated for both food and medicinal purposes. It is therefore not surprising that cultivated crops often have associated health benefits; in many cases the medicinal use might have preceded the food use (Rubatzky & Yamaguchi, 1997; Smartt & Simmonds, 1995). During the second half of the twentieth century, particularly in the USA, health-care practitioners and the general public moved away from food-based remedies and towards modern pharmaceuticals (Lawson, 1998). Synthetic monomolecular drugs became synonymous with the practice of modern medicine in the USA and many other parts of the developed world. However, this came at the price of traditional knowledge about the medicinal use and efficacy of many plants (Goldman, 2003; Janick, 2003). While agricultural and plant research have made strides to improve crops for several traits such as increased yield or pest resistance, researchers have only recently begun to explore molecular modifications that effect the nutritional or medicinal value of crops (Newell-McGloughlin, 2008).



Science & Society Series on Food and Science

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Increasing the health functionality of vegetable crops through breeding and/or genetic modification should create products that deliver greater health benefits than current varieties. Increasing the phytonutrient content of existing crops will have an even greater benefit for consumers who do not eat sufficient green vegetables or fruits, although from a public-health perspective it is the low level of vegetable consumption that is the real reason for concern. Nevertheless, research efforts strive to improve our understanding of crop manipulations for nutrition, and in many cases substantial progress has been made by using molecular tools.

Vegetables of the *Allium* genus such as onion, garlic, leek and chive are among the oldest crops associated with medicinal and health-related properties (Block, 2010). Some of these traits are thought to be related to the concentration and activity of organosulphur compounds in these foods (Goldman *et al*, 1996). However, simply increasing the amount of these phytonutrients to improve the medicinal benefits of these vegetables can result in greater pungency. This creates a dilemma: pungency necessitates cooking to make the foods palatable but, as several studies have demonstrated, heating can destroy other medicinal properties, such as the ability of these vegetables to inhibit platelet aggregation (Chen *et al*, 2000).

John McCallum and colleagues at the New Zealand Institute for Crop & Food Research in Christchurch have identified a quantitative trait locus (QTL) for bulb pungency and sulphur assimilation in onion, which might facilitate better selection and breeding (McCallum *et al*, 2007). Breeders have also attempted to breed onions with lower amounts of organosulphur compounds—and therefore pungency—in order to make them more palatable in raw form, thus possibly delivering greater health benefits. The irony of a breeding approach that reduces the health functionality of a vegetable crop in order to deliver health-functional compounds to the consumer is obvious. It also illustrates that simply increasing the amount of health-functional compounds in a plant need not be the ultimate goal. Instead, plant breeders must focus on the availability of these components as they actually feature in the diet. They need to work in interdisciplinary teams with nutritional and food scientists to analyse bioavailability



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and fine-tune their breeding goals accordingly. Moreover, it is important to keep in mind that increasing defensive compounds to improve pest resistance could make these crops of less interest to humans too.

When onions are cut, two compounds are formed: propanethial sulphoxide—also known as the lachrymatory factor—and 1-propanesulphenic acid. The lachrymatory factor reacts with nerve-cell membranes in the eye to produce tears, causing the familiar crying when cutting onions. In normal

conditions, levels of 1-propanesulphenic acid are low because it is rapidly converted to the lachrymatory factor. Recently, Colin Eady and colleagues (2008) at Lincoln University in Christchurch, New Zealand, silenced the gene for the lachrymatory factor enzyme by using RNA interference, to produce tearless onions: 1-propanesulphenic acid self-condenses to 1-propenyl 1-propenethiosulphinate, which then undergoes further reactions (Block, 2010). This feat of genetic engineering reduces

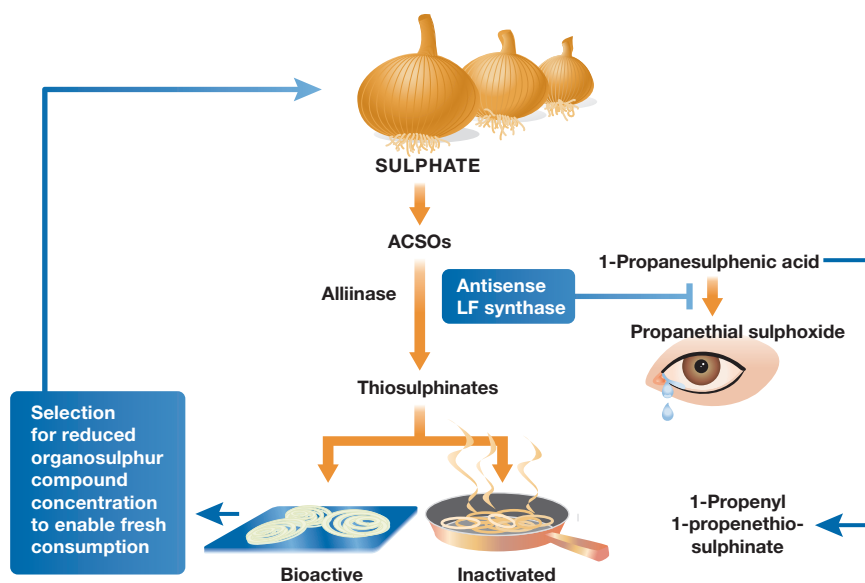


Fig 1 | Two molecular approaches for modifying the concentration of organosulphur compounds in onion. The approaches are shown in blue boxes. Antisense lachrymatory factor synthase blocks the formation of propanethial sulphoxide and eliminates tearing when chopping onions, but allows 1-propanesulphenic acid to form thiosulphinates. This, in turn, produces normal flavours and bioactivity. Heating of thiosulphinates inactivates their health-functional properties such as inhibition of platelet aggregation, but these are unaffected when onion is consumed raw. Marker-facilitated selection of low pungency onions helps in the development of new cultivars that can be consumed raw. LF, lachrymatory factor.

levels of lachrymatory factor up to 30-fold but does not diminish the overall levels of organosulphur compounds in the bulb. These ‘tearless onions’ have potential health benefits for consumers as they do not produce tears, but retain their health-promoting properties (Fig 1).

Several epidemiological studies in Asia, the USA and Europe have suggested that the consumption of vegetables from the Brassicaceae family, notably broccoli, reduce the risk of lung, breast, colon, and prostate cancer (Juge *et al*, 2007). The phytonutrients thought to be responsible for these health benefits are the isothiocyanates iberin and sulphoraphane. Sulphoraphane was initially thought to induce phase II enzymes in humans, which act against potentially carcinogenic compounds entering the body through the digestive system. However, it is not clear to what degree the anti-carcinogenic activity of sulphoraphane is achieved by phase II enzyme induction; it also seems that sulphoraphane can induce apoptosis and cell-cycle arrest in a variety of cell types.

While research continues into the health-promoting mechanisms of *Brassica*

isothiocyanates, others have developed high-glucosinolate broccoli germplasm that results in plants that produce mainly isothiocyanates, compared with standard broccoli cultivars that also produce nitriles (Sarikamis *et al*, 2006). Scientists and breeders introgressed chromosome segments from a wild ancestor, *Brassica villosa*, to enhance glucosinolate levels. They also found *B. villosa* alleles that determine whether hydrolysis generates iberin or sulphoraphane. These findings suggest that high-glucosinolate broccoli might be suitable for increasing the amount of sulphoraphane in the diet.

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The extent to which vegetable brassicas protect against cancer probably depends on the genotype of the consumer, in particular the allele present at the *GSTM1* locus. This gene codes for the enzyme glutathione transferase, which catalyses the conjugation of glutathione with isothiocyanates. Approximately 50% of humans carry a

deletion of the *GSTM1* gene (Juge *et al*, 2007), which reduces their ability to conjugate, process and excrete isothiocyanates. Individuals with two null alleles for *GSTM1* might gain less protection from these varieties of vegetable.

The most commonly consumed *Brassica* vegetable in Asia is *Brassica rapa*. *B. rapa* contains different isothiocyanates to *Brassica oleracea* and recent evidence suggests that individuals who are null for *GSTM1* can gain a protective benefit from *B. rapa* (Gasper *et al*, 2005). This example illustrates another aspect of complexity in breeding for health functionality in vegetable crops: human genetic variability has not generally been considered in the context of breeding programmes, but it might have important implications. Thus, when establishing breeding targets, it is important to explore the extent to which human variability affects the bioavailability and processing of health-functional compounds and influences health outcomes for a particular commodity.

In many regions of the developing world, vitamin-A deficiency, which causes childhood blindness and other maladies, is common. The most famous attempt to combat this problem is the development of ‘golden rice’ by Ingo Potrykus, Peter Beyer and their colleagues. They genetically transformed rice genotypes with carotenoid biosynthetic genes (Ye *et al*, 2000) to deliver more vitamin-A precursors in the diet. Although golden rice has not yet been translated to a commercial product (Enserink, 2008), it has demonstrated the potential for genetic manipulation of carotenoid biosynthesis in other crops (DellaPenna & Pogson, 2006). One of the most obvious benefits of enhancing carotenoid levels is the serendipitous increase in pigmentation, which can lead to more deeply coloured vegetables that are often preferred by consumers. Thus, increasing levels of carotenoid is doubly beneficial, both in terms of nutrition and aesthetics.

This has been achieved through breeding programmes to enhance orange-fleshed sweet potatoes, for example, which have a particular potential for improving the nutritional status of millions of people in the developing world. This crop delivers dietary carotenoids, which can be converted to vitamin A by the body. Such potatoes have already been developed and adapted for local conditions in Kenya to provide a new source of β -carotene (Hagenimana

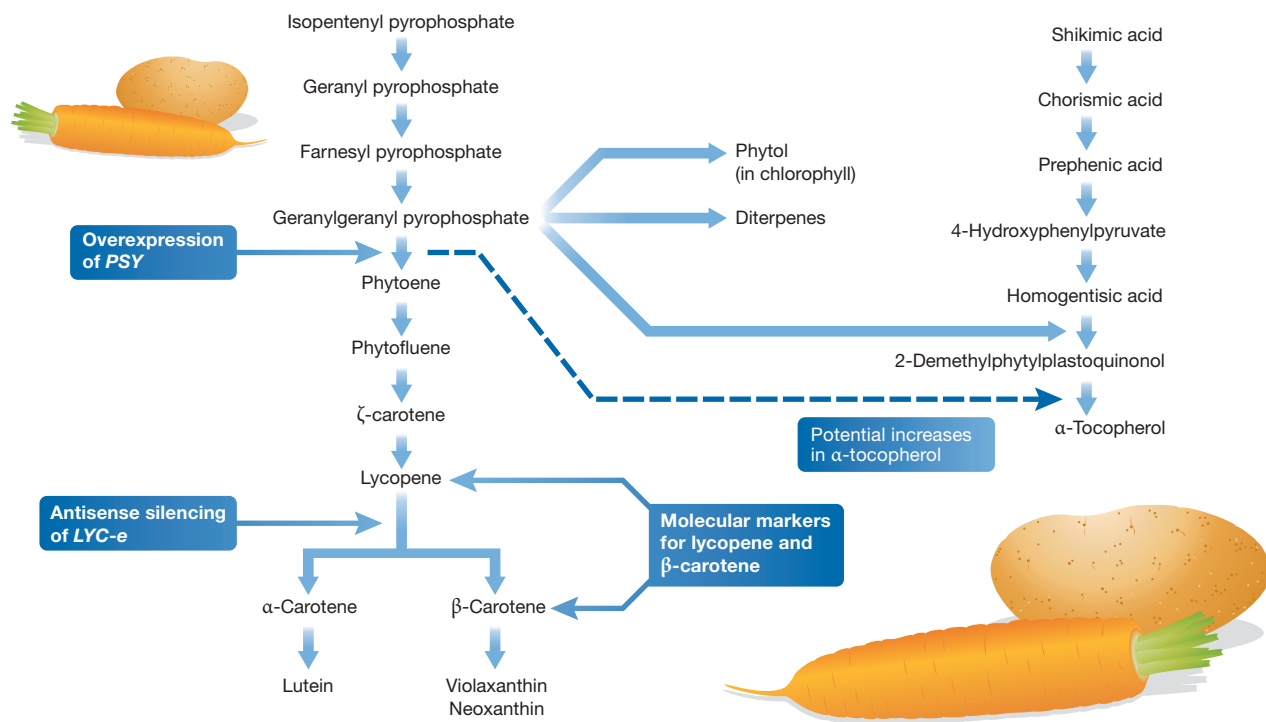


Fig 2 | Three molecular approaches for modifying carotenoid levels in vegetable crops. The approaches are shown in blue boxes. Overexpression of the *PSY* allele, which converts geranylgeranyl pyrophosphate into phytoene, has shown promise as a mechanism by which to enhance native carotenoid levels (Fraser *et al*, 2009). This approach has also demonstrated corresponding increases in tocopherols, which are responsible for pro-vitamin E activity. Antisense silencing of the lycopene cyclase enzyme *LYC-e* has similarly increased β -carotene levels. Molecular markers linked to key quantitative trait loci in the carotenoid pathway are being used to enhance carotenoid levels in vegetable crops.

et al, 1999). Cervantes-Flores *et al* (2010) have recently reported the identification of a QTL for dry matter, starch content and β -carotene content, opening up the possibility of genetic manipulation and further enhancement of this crop.

There are a range of other approaches to enhance the carotenoid levels in potatoes and other root vegetables. The screening of metabolites by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, for example, might be useful for evaluating the potential of germplasm for producing phytochemicals such as carotenoids (Fraser *et al*, 2007).

Such techniques enable a very fine-scale analysis of carotenoid profiles in plant tissues and have shown that potato tubers contain low levels of carotenoids that are composed mainly of the xanthophylls lutein, antheraxanthin, violaxanthin and xanthophyll esters, none of which can be converted into vitamin A. Gianfranco Diretto and colleagues (2006) at the Casaccia Research Centre in Rome, Italy, have therefore silenced the first

step in the beta-epsilon branch of carotenoid biosynthesis, lycopene epsilon cyclase (*LYC-e*). This antisense tuber-specific silencing of the gene results in significant increases in carotenoid levels, with up to 14-fold more β -carotene.

Brian Just, Phil Simon and colleagues (2007) at the University of Wisconsin in the USA have identified several molecular markers for genes that are related to carotenoid content in carrot. By using these markers, they have identified carrot lines with higher levels of carotenoids, which are now being used by commercial seed companies. Overall, carotenoid levels in carrot have increased dramatically in the past four decades through traditional breeding to reach levels of 1,000 p.p.m. carotenoids, on a fresh weight basis (Simon & Goldman, 2007).

Importantly, fruit-derived carotenoids might have greater health potential than root- or leaf-derived carotenoids because they have better bioavailability. Modification of carotenoid-containing fruit crops such as

pepper and tomato might therefore have better outcomes than modification of root crops such as carrot.

Similarly, potato—which usually accumulates lutein and violaxanthin—has been genetically modified to accumulate zeaxanthin (Römer *et al*, 2002). Serendipitously, this resulted in elevated transcript levels of phytoene synthase and a concomitant two- to three-fold increase in levels of α -tocopherol (vitamin E; Römer *et al*, 2002). The carotenoid and tocopherol pathways are biochemically linked and, therefore, modifications aimed at one or the other might have corresponding effects (Fig 2).

Another approach to improving the health functionality of vegetable crops is to reduce the concentration of anti-nutritional factors. These are naturally occurring compounds with inhibitory effects on the nutritive potential of plants. In many cases, anti-nutritional factors are produced *in planta* for pest control, but have secondary effects on human

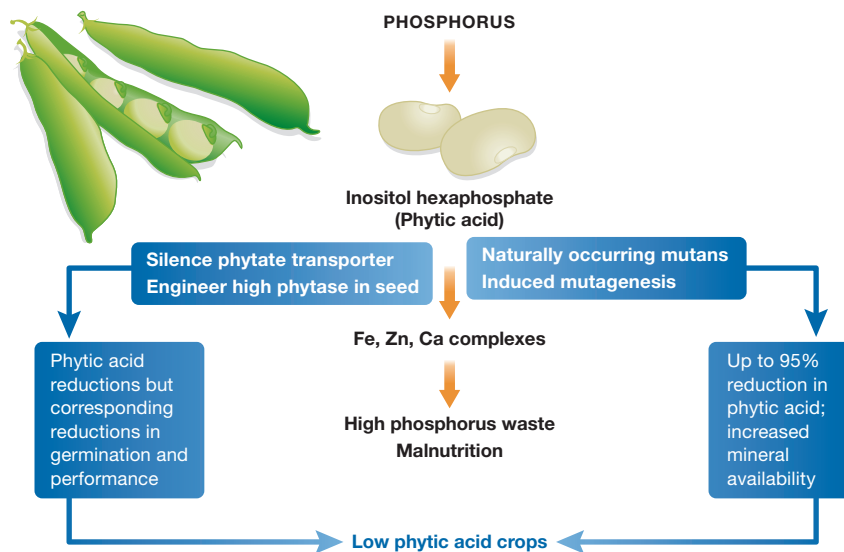


Fig 3 | Four molecular approaches for modifying phytic acid accumulation in seeds. The approaches are shown in blue boxes. Silencing of a key phytate transporter and engineering of increased phytase activity in seeds will reduce phytic acid levels, but it compromises germination and plant performance. Identification of naturally occurring low-phytic-acid mutants and development of low-phytic-acid genotypes through induced mutagenesis have resulted in very low phytic acid levels in seeds. All four of these methods have the potential to lead to low phytic acid crops.

nutrition. Faba bean (*Vicia faba* L.), for instance, contains condensed tannins that reduce the value of the inherently high protein levels of the crop. Tannins can be removed by the activity of two genes, *zt-1* and *zt-2*, which are pleiotropic for white-flowered plants. Gutierrez *et al* (2008) have identified a sequence characterized amplified repeat (SCAR) marker linked to the *zt-2* gene that is associated with increased protein levels and reduced fibre content of bean seeds, which should facilitate the development of tannin-free faba varieties.

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Calcium oxalate is another common anti-nutritional factor in plants. It is most commonly found as deposits in the vacuole of specialized cells called idioblasts (Nakata, 2003). The specific function of calcium oxalate accumulation in plants is not known; it might have a role in calcium regulation, ion balance, plant protection, detoxification or light gathering. There have

been several attempts to reduce the amount of calcium oxalate in plant tissues by using molecular approaches. Nakata & McConn (2003) identified mutants of barrel clover (*Medicago truncatula*) that are deficient in calcium oxalate and not compromised in growth. This suggests that it might be possible to engineer plants with low or very low calcium oxalate levels; however, if calcium oxalate has a role in plant protection, low-calcium oxalate crops would require other protection strategies.

In addition to phytonutrients, plants also contain a wide range of non-organic micronutrients that are essential for a healthy diet. These have also become the target of plant modifications to develop health-functional vegetables. Jay Morris and colleagues (2008) at Texas A&M University in the USA, for example, have developed a genetically modified carrot that expresses increased levels of a plant calcium transporter (*sCAX1*); these plants have approximately two-fold higher levels of calcium in the edible portions of the carrots. The researchers labelled these modified carrots with isotopic calcium and fed them to mice and humans. They found increased calcium absorption from *sCAX1*-expressing carrots compared with controls in both cases.

Humans and animals also need to consume sufficient dietary phosphorus. Plant seeds usually contain an adequate amount of phosphorus, but most of it is in the form of phytic acid (inositol hexaphosphate), which cannot be digested by non-ruminants such as humans, pigs, fish and chickens. Humans and domestic animals therefore excrete this excess phosphorus in their waste, which is a significant cause of water pollution and eutrophication. To overcome this problem, animal feed can be supplemented with phytase to degrade phytate or with another form of phosphorus from rock phosphate.

A more elegant solution is the development of low-phytic-acid crops. About 20 years ago, researchers identified the first mutants in plants including corn, rice, wheat, soybean, barley and *Arabidopsis* that have lower amounts of phytic acid in their seeds and an increased amount of phosphorus. Low-phytic-acid germplasm accessions have been identified in lentil (Thavarajah *et al*, 2009) and common bean (Fileppi *et al*, 2010), which extends this potential benefit to vegetable crops. In addition to improving the phosphorus cycle, low-phytic-acid mutants provide further nutritional benefits, increasing the availability of minerals such as iron, zinc and calcium, as these are no longer chelated by phytic acid.

However, reducing phytic acid has resulted in a concomitant reduction in seed and plant performance; it compromises germination, emergence, stress tolerance and yield (Raboy, 2007). Several interesting biotechnological approaches have been suggested to remedy this problem, including embryo-specific silencing of an ABC transporter responsible for phytic acid accumulation (Shi *et al*, 2007) and the engineering of high-phytase seeds (Raboy, 2009). Biotechnological solutions might ultimately help to avoid phytic acid accumulation in seeds, but scientists have already demonstrated that mutagenesis was sufficient to develop a low-phytic acid mutant with yield performance comparable with its wild counterpart (Campion *et al*, 2009; Fig 3).

Fructans and fructose polymers, sometimes known as inulin, might also have health-functional properties because they promote the growth of beneficial microbes in the gut, add sweetness without adding calories, and contribute to the fibre content of foods. Elke Hellwege and co-workers (2000) at the Max Planck Institute for Molecular Plant Physiology in Golm,

Germany, developed transgenic potato plants that produce inulin by the expression of the 1-*SST* (sucrose:sucrose 1-fructosyltransferase) and 1-*FFT* (fructan:fructan 1-fructosyltransferase) genes from globe artichoke. The results suggested that these enzymes might be sufficient to produce inulin molecules of various lengths in plants.

Another approach to improving the health functionality of vegetable crops is to reduce the concentration of anti-nutritional factors

Polyphenolic compounds such as anthocyanins have also attracted attention in recent years for their potential antioxidant activity and related health benefits. Potato does not normally produce anthocyanin, but germplasm expressing anthocyanin pigment has been developed and is attracting interest from consumers. Cecil Stushnoff and co-workers (2010) at Colorado State University (Fort Collins, USA) identified 27 genes that are differentially expressed in purple and white tuber tissues. One of these genes—which encodes a novel single-domain MYB transcription factor—has the potential to influence anthocyanin-pigment production in potato. The resulting purple potato might offer both novelty and health functionality to consumers.

Similarly to the production of phenolic compounds in crops or plant organs, fortification of foods by the addition of specific micronutrients has the potential to provide health benefits (DellaPenna, 2007). Indeed, fortification has been a tremendous success for public health in many areas of the world. Recent attempts to biofortify foods include the addition of folic acid, one of the key B vitamins in the human diet. Rocio Diaz de la Garza *et al* (2007) at the University of Florida, USA, have described the genetic engineering of a key component of folic-acid synthesis in tomato through overexpression of the enzyme aminodeoxychorismate synthase (ADCS). Crosses between this and other transgenic lines—for another key enzyme in the folic acid pathway—have generated plants with folate levels up to 25-fold higher than controls. This strategy could have substantial implications for the biosynthesis of folate in crops that already produce this vitamin and for the general fortification of crops.

Feverfew (*Tanacetum parthenium* Schulz-Bip) has been used as a medicinal plant for millennia to treat headaches and fever. The active compound is a sesquiterpene lactone known as parthenolide produced in the aerial portion of the plant. By using colchicine to double the chromosomes of diploid feverfew, it was possible to substantially increase the concentration of parthenolides in the tetraploid derivatives (Majdi *et al*, 2010). Indeed, ploidy manipulation, which has been used successfully in potato and other crops, might be an interesting route by which to modify health-functional compounds. Increases in ploidy often result in larger cells and more biomass—which could be an advantage for secondary compounds—however, autopolyploids might cause difficulties for fertility and seed production.

The variety of molecular and breeding techniques being used to enhance the health functionality of vegetable crops is increasing, although there are no clear and standard protocols for such modifications. Molecular-marker-facilitated selection, transformation, chromosome manipulations and the creation and identification of useful mutant alleles have all been successfully used in these strategies. However, several concerns underlie future approaches to improving vegetable health functionality: bio-availability of the target compounds, their biosynthesis in species or plant tissues where these compounds are not naturally produced, overexpression of transgenes, removal of anti-nutritional factors or defensive compounds that have other purposes in the plant, and the genetic variability in humans who consume these crops. Plant breeders must therefore tailor strategies to species and compounds.

Molecular approaches hold great promise for future modifications. To be successful, however, more interdisciplinary work is required that involves nutritional and food scientists as well as others from biomedical fields to ascertain the true function of specific plant compounds.

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A barrier to the successful use of molecular techniques might be the acceptance—or lack thereof—of transgenic vegetable crops by the public. Strategies for improving the health functionality of vegetables that rely on transgenic approaches offer great scientific promise, but have so far been met with public scepticism, and even fear. Thus far, only one transgenic vegetable species—squash—has been approved and sold commercially for any length of time in the USA, and, despite the brief appearance and quick disappearance of transgenic tomatoes and potatoes in the past twenty years, there is widespread doubt whether more genetically modified vegetable crops will be approved in the near term. It remains to be seen what the marketplace will bring in the next decade. Fortunately for both scientists and consumers, increasing interest in this area has fuelled research, which bodes well for improving our understanding of the health functionality of vegetables and the potential for developing transgenic vegetable crops.

Research and its application to improve crops are therefore only one side of the coin; the willingness of the public to choose a healthier diet and eat these fruits and vegetables is the other

The last challenge, however, is perhaps the hardest. For as long as many consumers prefer a diet rich in meat and carbohydrates and shun fruits and vegetables, even the most sophisticated methods to increase health functionality of vegetables will not have much impact on public health. Research and its application to improve crops are therefore only one side of the coin; the willingness of the public to choose a healthier diet and eat these fruits and vegetables is the other.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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