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Transgenic Vegetable Breeding for Nutritional Quality and Health Benefits

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ABSTRACT

Vegetables are essential for well-balanced diets. About 3 billion people in the world are malnourished due to imbalanced diets. Vegetables can contribute to the prevention of malnutrition disorders. Genetic engineering enables vegetable breeders to incorporate desired transgenes into elite cultivars, thereby improving their value considerably. It further offers unique opportunities for improving nutritional quality and bringing other health benefits. Many vegetable crops have been genetically modified to improve traits such as higher nutritional status or better flavour, and to reduce bitterness or anti-nutritional factors. Transgenic vegetables can be also used for vaccine delivery. Consumers could benefit further from eating more nutritious transgenic vegetables, e.g. an increase of crop carotenoids by metabolic sink manipulation through genetic engineering appears feasible in some vegetables. Genetically engineering carrots containing increase Ca levels may boost Ca uptake, thereby reducing the incidence of Ca deficiencies such as osteoporosis. Fortified transgenic lettuce with zinc will overcome the deficiency of this micronutrient that severely impairs organ function. Folates deficiency, which is regarded as a global health problem, can also be overcome with transgenic tomatoes with folate levels that provide a complete adult daily requirement. Transgenic lettuce with improved tocopherol and resveratrol composition may prevent coronary disease and arteriosclerosis and can contribute to cancer chemopreventative activity. Food safety and health benefits can also be enhanced through transgenic approaches, e.g. rural African resource-poor consumers will benefit eating cyanide-free cultivars of cassava. Biotechnology-derived vegetable crops will succeed if clear advantages and safety are demonstrated to both growers and consumers.

Keywords: Antioxidants; GMOs; Horticulture; Phytochemicals; Phytonutriceuticals; Transgenic Vegetables

1. Introduction

Vegetables make up a major percentage of the human diet in many locations of the world and play a significant role in human nutrition, especially as sources of phytonutriceuticals: vitamins, minerals, dietary fiber and phytochemicals [1-3]. Some phytochemicals of vegetables are strong antioxidants and are thought to reduce the risk of chronic disease by protecting against free-radical damage, by modifying metabolic activation and detoxification of carcinogens, or even influencing processes that alter the course of tumor cells [1,2,4,5].

1.1. Addressing Malnourishment through Improving Human Diets with Vegetables

About 3 billion people in the world are manourished due to imbalanced diets [6,7]. Underconsumption of vegetables and fruits is among the top 10 risk factors leading to micronutrient malnutrition and is associated with the prevalence of chronic diseases [8,9]. More than 70% of mal-

nourished children live in Asia. At least half of the preschool children and pregnant women are affected by micronutrient deficiencies in Bangladesh, Cambodia, Nepal and the Philippines [10]. Deficiency of vitamin A and iron can result in severe anemia, impaired intellectual development, blindness, and even death.

Vegetables contain a range of macro- and micro-nutrients, including pro-vitamin A, iron and zinc, which contribute to the prevention of malnutrition disorders. Small variation in maternal diets, particularly reduction in micronutrient content, can have a significant impact on fetal growth and development. Pregnant women, in particular, benefit from good vegetable nutrition, particularly during later pregnancy, and lactation. The interplay of the different micronutrients and antioxidants found in vegetables has important health impacts, explaining for instance the higher birth weight of children in India, when mothers consume higher rates of green leafy vegetables and fruits during pregnancy [11]. Nutrition is both a quantity and a quality issue, and vegetables in all their many forms

ensure an adequate intake of most vitamins and nutrients, dietary fibers, and phytochemicals, which can bring much-needed balanced diets thereby contributing to solve many of these nutritional problems.

1.2. Biotecnology and Plant Breeding

Biotechnology is a new, and potentially powerful, tool that has been added by most of the multinational private seed sector to their vegetable breeding programs. It can augment or accelerate conventional cultivar development programs through saving time, delivering better products, and ensuring genetic uniformity, or achieving some outputs that are not possible by conventional breeding. Transgenic crops, commonly referred to as genetically modified (GM) crops enable plant breeders to bring favorable genes, often previously inaccessible, into elite cultivars, improving their value considerably and offer unique opportunities for controlling insects, viruses and other pathogens, as well as nutritional quality and health benefits. Conventional plant breeding that utilizes non-transgenic approaches will remain the backbone of vegetable genetic improvement strategies. However, transgenic crop cultivars should not be excluded as products capable of contributing to more nutritious and healthy food [12]. Many vegetable crops have been genetically modified to include resistance to pests, pathogens and herbicides, and for improved features, such as slow ripening, higher nutritional status, seedless fruit, and increased sweetness.

Recently, Dias and Ortiz [13] did an analysis of the status (until 2010) of transgenic tomato, eggplant, potato, cucurbits, brassicas, lettuce, alliums, sweet corn, cowpea, cassava, sweet potato, and carrots. From this analysis we noticed several benefits of transgenic vegetables to nutritional quality and health benefits, which are very important for consumers. This article discusses recent attempts to characterize and modify phytonutriceuticals in vegetable crops by using transgenic approaches, focusing on those modifications that are of interest for improving human diets and health.

2. Transgenic Breeding and Nutritional Health Benefits

2.1. Vitamins and Carotenoids

Vitamin A is a major public health problem in much of the developing world. It is estimated that in developed countries, almost 70% of vitamin A comes from animal sources while 30% is derived from plant-based foods. In contrast, people in developing countries derive about 70% to 80% of vitamin A from plant-based foods. Vegetarians and populations with limited access to animal products depend on provitamin A carotenoids. Vitamin A deficiency affects approximately 25% of the developing world's preschoolers. It is associated with blindness, susceptibility to

disease and higher mortality rates. It leads to the death of approximately 1 to 3 million children each year [14,15]. The most famous attempt to combat vitamin A deficiency is the development of GM "Golden Rice". Ye, *et al*. [16] genetically transformed rice genotypes with carotenoid biosynthetic genes to deliver more vitamin-A precursors in the diet. Although "Golden Rice" has not yet been commercialized [17], it has shown the potential for genetic manipulation of carotenoid biosynthesis in crops [18]. One of the most obvious benefits of enhancing carotenoid levels is the 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. There are a range of other approaches to enhance the carotenoid levels in potatoes and other root vegetables. Diretto, *et al*. [19], have silenced the first step in the beta-epsilon branch of carotenoid biosynthesis, lycopene epsilon cyclase (*LCY-e*) in potato—a tuber crop that contains low levels of carotenoids. This antisense tuber-specific silencing of the gene results in significant increases in carotenoid levels, with up to 14-fold more β-carotene. Cervantes-Flores, *et al*. [20] have also recently reported in sweet potato the identification of quantitative trait loci (QTL) for dry matter, starch content and β-carotene content, opening up the possibility of genetic manipulation and further enhancement of this root crop.

Fruit-derived carotenoids might have greater health potential than root carotenoids because they have better bioavailability. Modification of carotenoid-containing fruit crops such as tomato and pepper might therefore have better outcomes than modification of root and tuber crops. Tomato fruit and its processed products are the principal dietary sources of carotenoids such as lycopene and β-carotene. Therefore, there is considerable interest in elevating the levels of carotenoids in tomato fruit by genetic manipulation and thereby improving the nutritional quality of the crop. To enhance the carotenoid content and profile of tomato fruit, Romer, *et al*. [21] produced transgenic lines containing a bacterial carotenoid gene (*crtI*) encoding the enzyme phytoene desaturase, which converts phytoene into lycopene. Expression of this gene in transgenic tomato plants of the cultivar "Ailsa Cray" did not elevate total carotenoid levels. However, the β-carotene content increased about threefold, up to 45% of the total carotenoid content. The alteration in carotenoid content of these transgenic tomatoes did not affect growth and development. Levels of noncarotenoid isoprenoids were unchanged in the transformants. The phenotype has been found to be stable and reproducible over at least four generations. Fraser, *et al*. [22] reported also an increase in tomato fruit carotenoids phytoene, lycopene, β-carotene and lutein in cultivar "Ailsa Craig". Phytoene synthase from the bacterium *Erwinia uredovora* (crtB) has been

overexpressed in tomato cultivar "Ailsa Craig". Fruit-specific expression was achieved by using the tomato polygalacturonase promoter, and the CRTB protein was targeted to the chromoplast by the tomato phytoene synthase-1 transit sequence. Total fruit carotenoids of primary transformants (T(0)) were 2-4-fold higher than the controls, whereas phytoene, lycopene, β-carotene, and lutein levels were increased 2.4-, 1.8-, and 2.2-fold, respectively. The biosynthetically related isoprenoids, tocopherols plastoquinone and ubiquinone, were unaffected by changes in carotenoid levels. The progeny (T(1) and T(2) generations) inherited both the transgene and phenotype. Ripe tomato fruits accumulate large amounts of lycopene and small amounts of β-carotene (pro-vitamin A). Lycopene is transformed into β-carotene by the action of lycopene beta-cyclase (beta-Lcy). Rosati, et al. [23] introduced, via Agrobacterium-mediated transformation, DNA constructs aimed at up-regulating (OE construct) or down-regulating (AS construct) the expression of the beta-Lcy gene in a fruit-specific fashion. Three tomato transformants containing the OE construct show a significant increase in tomato fruit β-carotene content. The tomato fruits from these plants display different colour phenotypes, from orange to orange-red, depending on the lycopene/beta-carotene ratio. Fruits from AS transformants show up to 50% inhibition of beta-Lcy expression, accompanied by a slight increase in lycopene content. Leaf carotenoid composition is unaltered in all transformants. In most transformants, an increase in total carotenoid content is observed with respect to the parental line. This increase occurs in the absence of major variations in the expression of endogenous carotenoid genes.

Current advances in genetic engineering of brassicas have enable also the production of plants with alterations in a range of vitamins or amino acids for improved human nutrition. Lu, et al. [24] developed transgenic cauliflower with high levels of β-carotene accumulation, and Wahlroos, et al. [25] produced oilseed Brassica rapa with increased histidine content. It is likely in the future that more transgenic vegetable brassicas with altered vitamin or amino acid content will be also developed.

Vitamin E, which includes tocopherols, is a lipid soluble antioxidant. There are α, β, γ, and δ isoforms of tocopherol with relative vitamin E potencies of 100%, 50%, 10%, and 3%, respectively. Conversion of γ-tocopherol to α-tocopherol in vegetable crops could increase their value and importance in human health because vitamin E reduces the risk of several serious disorders (e.g. cardiovascular diseases and cancer), slows ageing and enhances the function of the immune system. Cho et al. [26] developed transgenic lettuce plants of the cultivar "Chungchima" expressing a cDNA encoding γ-tocopherol methyltransferase from Arabidopsis thaliana to improve tocopherol composition. Transgene inheritance and expression

in transformed plants increased enzyme activity and conversion of γ-tocopherol to the more potent α form.

2.2. Folates

Folate deficiency, regarded as a global health problem, causes neural tube defects and other human diseases. Folates are synthesized from pteridine, p-aminobenzoate (PABA), and glutamate precursos. Díaz de la Garza, et al. [27,28] developed trangenic tomatoes by engineering fruit-specific overexpression of GTP cyclohydrolase I that catalyzes the first step of pteridine synthesis, and aminodeoxychorismate synthase that catalyzes the first step of PABA synthesis. Vine-ripened fruits contained on average 25-fold more folate than controls by combining PABA- and pteridine overproduction traits through crossbreeding of transgenic tomato plants. The achieved folate level provides a complete adult daily requirement with less than one standard serving. This strategy could have substantial implications for the biosynthesis of folate in vegetables that already produce this vitamin and for the general fortification of vegetables.

2.3. Calcium and Zinc

Vegetables offer consumers a diverse mixture of nutrients that promote human health more beneficially than dietary supplements. However, essential nutrients such as calcium (Ca) could be limiting in plant-based diets. Consequently, genetically engineering vegetables containing increased Ca levels may boost Ca uptake, thereby reducing the incidence of Ca deficiencies such as osteoporosis. In this regard, Park, et al. [29] modified carrots to express increased levels of the plant Ca transporter sCAX1. These carrot lines were fertile and displayed no adverse phenotypes. Further mice and human feeding trials demonstrated increased Ca absorption from sCAX1-expressing transgenic carrots compared to controls [30]. This research supports alternative means of biofortifying vegetables with bioavailable Ca. Zinc is also an essential element in human nutrition, as its deficiency severely impairs organ function. It is also important for development of intellectual capacity. In experiments to fortify lettuce with this element, Zuo, et al. [31] used Agrobacterium-mediated gene delivery of a mouse metallothionein mutant β-cDNA in the lettuce cultivar "Salinas 88". The concentration of zinc in the lettuce transgenic plants increased to 400 μg/g dry weight, which is considerably higher than in wild-type plants.

2.4. Phosphorous

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)—*i.e.*; the storage form of phosphorus—which cannot be digested by non-ruminants such as humans. Humans therefore excrete this excess phosphorus in their waste. Furthermore, phytic acid can act as metal chelator of important minerals such as calcium, magnesium, iron, zinc and can contribute to mineral deficiencies in people. 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 [32] and common bean [33], which extends this potential benefit to vegetable legumes. In addition to improving the phosphorus cycle, low-phytic-acid mutants provide further nutritional benefits, increasing the availability of minerals such as calcium, magnesium, iron, and zinc 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 [34]. Several interesting biotechnological approaches have been suggested to remedy this problem, including embryo-specific silencing of an ABC transporter responsible for phytic acid accumulation [35] and the engineering of high-phytase seeds [36]. 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 [37].

2.5. Flavonoids

Flavonoids are polyphenols whose dietary intake has the potential to prevent chronic diseases. Schijlen, *et al.* [38] introduced heterologous, flavonoid pathway genes—stilbene synthase, chalcone synthase, chalcone reductase, chalcone isomerase, and flavone synthase—to produce novel flavonoids in tomato fruit. These novel flavonoids-flavones and flavonols-increased threefold, mostly in the Q12 peel, which had higher total antioxidant capacity. These findings add further support to the potential of engineering tomato fruit for accumulation of high levels of beneficial nutrients. Similarly, the poliphenol resveratrol—a stilbenoid—shows cancer chemo-preventative activity and may prevent coronary heart disease and arteriosclerosis. Liu, *et al.* [39] showed, in a quantitative analysis, that resveratrol in transgenic lettuce plants reached 56.0 ± 5.52 μ/g leaf fresh weight, which is comparable to that in the skin of grape fruit (*Citrus × paridisi* Macfad.). Flavonoids such as anthocyanins are known as antioxidants *in vitro* and can reduce the risk of many diseases related to aging. However, some vegetable brassicas, such as cauliflower, are low in anthocyanins. In an attempt to

manipulate pigment biosynthesis to increase the health benefits of brassica vegetables, the effect of a regulatory locus of flavonoid content was assessed. *Agrobacterium tumefaciens*—mediated transformation of a *B. oleracea* line, selected for high transformation ability by Sparrow, *et al.* [40], was used to produce plants transgenic for the maize Lc (leaf color) locus. Lc is a regulatory gene in the anthocyanin pathway, and it is expected that its presence will increase the flavonoid content. Seedling explants were cocultivated with *Agrobacterium tumefaciens* strain LBA4404 containing a binary vector Q27 with a neomycin phosphotransferase II (NPTII) gene. Under tissue culture conditions, Lc-containing plants were green with no visible increase in anthocyanin production. However, after transfer to the greenhouse, the exposure to high light intensity led to visible signs of pigmentation within one week. Increased pigmentation was apparent in stems, petioles, main leaf veins, and sepals. Lc-containing lines had 10 to 20 times higher levels of total anthocyanins than controls. In addition, antioxidant activity of Lc-containing lines was 1.5 times higher than that of controls [41]. Potato does not normally produce anthocyanin, but germplasm expressing anthocyanin pigment has been developed and is attracting interest from consumers. Stushnoff, *et al.* [42] in potato 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, who can also benefit from native Andean potatoes that do not always show desired tuber shapes for both table and processing industry.

2.6. Organosulphur Compounds: Glucosinolates and Thiosulfides

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 [43]. The phytochemicals thought to be responsible for these health benefits are the isothiocyanates sulphoraphane and indole-3-carbinol. 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 [44]. 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 [45]. Chromosome segments from a wild ancestor, *Brassica villosa*, have been introgressed to enhance glucosinolate levels. *B. villosa* alleles determine whether hydrolysis generates indole-3-carbinol or sulphoraphane. Hence, highglucosinolate broccoli might be suitable for increasing the amount of sulphoraphane in the diet. 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 [43], which reduces their ability to conjugate, process and excrete isothiocyanates. Individuals with two null alleles for *GSTM1* might gain less protection from these cultivars of vegetable. The most commonly consumed *Brassica* vegetable in Asia is *Brassica rapa*. *B. rapa* contains different isothiocyanates to *B. oleracea* and recent evidence suggests that individuals who are null for *GSTM1* can gain a protective benefit from *B. rapa* [46]. 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 plant breeding programmes, but it might have important implications. Thus, when establishing vegetable 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.

Vegetables of the *Allium* genus such as onion, garlic, leek and chive are among the oldest crops associated with health-related properties [47]. Some of these traits appear to be related to the concentration and activity of organosulphur compounds in these vegetables [48]. The unique flavor and odor of alliums is derived from the hydrolysis of organosulfur compounds, which produces pyruvate, ammonia, and volatile sulfur compounds [49]. This reaction is catalyzed by the enzyme alliinase, which is contained in vacuoles within cells and released upon disruption of the tissue [50]. Variations in the ratios of these volatile sulfur compounds are responsible for the difference in flavors and odors between *Allium* species [49]. Along with health and nutritional benefits associated with these compounds, these thiosulfides are also major contributors to the bitter taste of some onions [49,50]. Three sets of transgenic onion plants containing antisense alliinase gene constructs (a CaMV 35S-driven antisense root alliinase gene, a CaMV 35S-driven antisense bulb alliinase, and a bulb alliinase promoter-driven antisense bulb alliinase) have been recently produced [51]. Results from the antisense bulb alliinase lines have been much more encouraging, and three lines were produced with barely

detectable bulb alliinase levels and activity. Progress has been confounded by the poor survival of transgenic plants. Transgenic hybrid onion seed from these transgenic lines has been developed by crossing a nontransgenic open-pollinated parental line with a transgenic parental plant carrying a single transgene in the hemizygous state. Some resulting seed produced by the nontransgenic parents will be hemizygous for the transgene and can be selected to obtain F_1 heterozygous individuals containing the transgene. Self-fertilization of these individuals produces homozygous, hemizygous, and null F_2 progeny for the transgene locus. These homozygous individuals can then be used to generate the bulk seed required for the production of commercial transgenic onion lines with less bitter taste.

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, Eady, *et al*. [52] silenced the gene for the lachrymatory factor enzyme by using RNAinterference, to produce tearless onions: 1-propanesulphenic acid self-condenses to 1-propenyl 1-propenethiosulphinate, which then undergoes further reactions [47]. This feat of genetic engineering reduces 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.

2.7. Sweet Taste

In attempts to reduce bitterness in lettuce, Sun, *et al*. [53] cloned the gene for the sweet and taste modifying protein miraculin from the pulp of berries of *Richadella dulcifica*, which is a West African shrub. This gene, with the CaMV 35S promoter, was introduced into the lettuce cultivar "Kaiser" using *A. tumefaciens* GV2260. Expression of this gene in transgenic plants led to the accumulation of significant concentrations of the sweet enhancing protein. People suffering diabetes may use miraculin, which is active at extremely low concentrations, as a food sweetener. The first successful study conducted to engineer genetically the taste of tomato fruit involved transformation of tomato with the thaumatin gene from the African plant katemfe (*Thaumatococcus daniellii*) [54]. Thaumatin is a sweet-tasting protein. Fruit from T_2 transgenic plants tasted sweeter than the control plants, leaving a unique and sweet-specific after taste.

Fructans and fructose polymers, sometimes known as inulin, might also have health-functional properties be-

cause they promote the growth of beneficial microbes in the gut, add sweetness without adding calories, and contribute to the fibre content of foods. Hellwege, *et al*. [55], 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.

2.8. Anti-Nutritional Factors

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 nutrition. The first transgenic cassava plants became available in the mid-1990s [56-58] as plants with reduced cyanogenic content [59-61], which can benefit resource-poor people in rural Africa where this starchy root crop is the base of their diet. Faba bean (*Vicia faba*) 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*. [62] 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 faba bean seeds, which should facilitate the development of tannin-free faba cultivars. 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 [63]. 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 and McConn [64] 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 genetically 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.

2.9. Vaccine Delivery

Some vegetables, mainly tomato, have also been genetically modified to be used as vaccine delivery. Plant delivery of oral vaccines has attracted much attention because this strategy offers several advantages over vaccine delivery by injection [65-67]. Oral vaccines also offer the

hope of more convenient immunization strategies and a more practical means of implementing universal vaccination programs worldwide. Tomato has been tested for expression of vaccines that can address human health issues of the developing world. Transgenic tomato plants potentially can bring several positive effects and improve human health. McGarvey, *et al*. [68] engineered tomato plants of cultivar "UC82b" to express a gene encoding a glycoprotein (G-protein), which coats the outer surface of the rabies virus. The recombinant constructs contained the G-protein gene from the environmental risk assessment strain of rabies virus. The G-protein was expressed in leaves and fruit of the transgenic plants, and it was found localized in Golgi bodies, vesicles, lasmalemma, and cell walls of vascular parenchyma cells. Ma, *et al*. [69] overexpressed hepatitis E virus (HEV) open reading frame 2 partial gene in tomato plants, to investigate its expression in transformants, the immunoactivity of expressed products, and explore the feasibility of developing a new type of plant-derived HEV oral vaccine. The recombinant protein was produced at 61.22 ng/g fresh weight in tomato fruits and 6.37 to 47.9 ng/g fresh weight in the leaves of the transformants. It was concluded that the *HEV-E*2 gene was correctly expressed in transgenic tomatoes and that the recombinant antigen derived had normal immunoactivity. These transgenic tomato plants are valuable tools for the development of edible oral vaccines. Chen, *et al*. [70] developed an effective antiviral agent against enterovirus 71 (EV71), which causes seasonal epidemics of hand, foot, and mouth disease associated with fatal neurological complications in young children, by transforming the gene for VP1 protein—a previously defined epitope and also a coat protein of EV71—in tomato plant. VP1 protein was first fused with sorting signals to enable it to be retained in the endoplasmic reticulum of tomato plant, and its expression level increased to 27 mg/g in fresh tomato fruit. Transgenic tomato fruit expressing VP1 protein was then used as an oral vaccine, and the development of VP1-specific fecal IgA and serum IgG were observed in BALB/c mice. Additionally, serum from mice fed transgenic tomato could neutralize the infection of EV71 to rhabdomyosarcoma cells, indicating that tomato fruit expressing VP1was successful in orally immunizing mice. Moreover, the proliferation of spleen cells from orally immunized mice was stimulated by VP1 protein and provided further evidence of both humoral and cellular immunity. Results of this study not only demonstrated the feasibility of using transgenic tomato as an oral vaccine to generate protective immunity in mice against EV71 but also the probability of enterovirus vaccine development. The Gram-negative bacterium *Yersinia pestis* causes plague, which has affected human health since ancient times. It is still endemic in Africa, Asia, and the American continent. There is the urgent need for a safe

and cheap vaccine due to the increasing reports of the incidence of antibiotic-resistant strains and concern with the use of *Y. pestis* as an agent of biological warfare. Out of all the *Y. pestis* antigens tested, only F_1 and Vinduce a good protective immune response against a challenge with the bacterium [71]. Alvarez, *et al*. [72] reported the expression in tomato of the *Y. pestis* F_1-Vantigen fusion protein. The immunogenicity of the F_1-V transgenic tomatoes was confirmed in mice that were injected subcutaneously with bacterially produced F_1-V fusion protein and boosted orally with transgenic tomato fruit. Expression of the plague antigens in the tomato fruit allowed producing an oral vaccine candidate without protein purification and with minimal processing technology, offering a good system for a largescale vaccination programs in developing countries. The future of edible plant-based vaccines through transgenic approaches will depend on producing them safely on sufficient amounts.

3. Constraints for Transgenic Vegetable Breeding

As described consumers could benefit from improved access to more nutritious transgenic vegetables and enhanced food safety through transgenic approaches. However, transgenic crop technology for horticulture remains still in its infancy for several reasons. Vegetables are considered minor crops. Consequently, fewer resources are allocated to transgenic research of horticultural crops compared to field crops, especially by the multinational private seed corporations. While it is becoming less expensive to create transgenic crops, developing a marketable product and responding to the regulatory requirements remains very costly. Development and regulatory costs can be recouped more readily if the product is grown on an extensive area, which is generally not the case for individual vegetable crops. For this reason, most large multinational seed corporations have abandoned the development of transgenic vegetable crops.

Generally there are many cultivars of the same vegetable species on the market and the life span of an individual cultivar can be quite short. Introducing a transgene into a breeding program can be complicated and cost prohibitive, especially in crops with difficulty for using backcrossing (e.g. cassava, potato or sweet potato). Deregulation of a transgenic trait is event specific in many countries. For many vegetable species it is not possible to develop a single transgenic event that can be converted into many different cultivars of a single or closely related group of vegetable species through conventional breeding. For example, *Brassica* contains about 40 closely related commercialized crops, including cabbage (*B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), Brussels sprouts (*B. oleracea* var. *gemmifera*), turnip (*B. rapa* var.

rapa), broccoletto (*B. rapa* ssp. *utilis*), Chinese cabbage (*B. rapa* ssp. *pekinensis*), pak-choy (*B. rapa* ssp. *chinensis*), choysum (*B. rapa* ssp. *parachinensis*), swede or rutabaga (*B. napus* ssp. *napobrassica*), vegetable rape (*B. napus* ssp. *napus*), and various mustards (*B. juncea*, *B. carinata* and *B. nigra*) [73]. No single parent exists that can be used to backcross the transgene into the many different types of *Brassica* botanical varieties and subspecies. Individual events would have to be developed for many of the crop types, and deregulation of more than one event for a single protein is problematic for most business models. Because of the regulatory costs currently involved with GM vegetable crops, it is difficult for either the public or private sector to develop novel products specifically for small vegetable markets, including specialty vegetable crops in the developed and developing world and almost any crop in countries with relatively small agricultural sectors. For the few transgenic vegetable crops that are being developed, novel or unconventional strategies have been employed to bring the crops to markets, e.g. private-public partnerships in which the private sector would focus on selling hybrids to higher-end growers while the public sector would focus on low-resource farmers.

4. Safety of Transgenic Crops

Although transgenic cultivars have proven to be a powerful tool for nutritional health benefits, many countries are still engaged in discussions about their potential food safety. Consumer antagonism has precluded many farmers and other end users from sharing the benefits that these crops provide. Transgenic crops must pass a rigorous assessment for potential risks based on scientific data. The objective of this appraisal is to determine whether the transgenic crop is as safe as its conventional counterpart without transgenic modification. For this purpose, scientific data have to be produced to demonstrate that transgenic plants are safe for the environment and do not impose any health hazard for the consumers. In the USA, the process of deregulation is an interactive process between the industry, government agencies and any other stakeholder that feels concerned, where the industry has to provide scientific evidence as requested by the government agency to prove that there is no reasonable doubt on the safety of the transgenic crop.

The World Health Organization [74], the Food and Agriculture Organization of the United Nations [75], the Royal Society of London, the US National Academy of Sciences, the Brazilian Academy of Sciences, the Chinese Academy of Sciences, the Indian National Science Academy, the Mexican Academy of Sciences and the Third World Academy of Sciences [76], the American College of Nutrition [77], the Society of Toxicology [78], the British Medical Association [79], and the Union of

German Academies of Sciences and Humanities [80], among others, have stated that GM crops approved for commercialization, do not pose more risk to human health than conventional crops, and they should be considered as safe as conventional ones.

The world has witnessed a steady increase of transgenic crop area in the last 1.5 decades. Extensive research has produced no evidence that transgenic crops approved by the authorities impose a greater risk to human and animal health than conventional crops. The Federal Office of Consumer Protection and Food Safety of Germany and partners [81] issued the report "Biological and Ecological Evaluation towards Long-term Effects" (also known as the BEETLE report) with the aim of providing scientific data to the European Commission. The BEETLE report reviewed in excess of 100 publications and consulted 52 experts in health issues to assess the possible long-term effect of GM crops on the health of consumers and the environment. This report concluded that so far no adverse effects to human health from eating GM food have been found. The report further stated that although unexpected negative effects are known in conventional crops, none has yet been detected in GM crops. The report concludes that there is a negligible probability for adverse effects to consumers' health in the long term.

5. Conclusion

Transgenic cultivars can improve nutritional quality and health benefits and make important contributions to sustainable vegetable production by overcoming limiting factors in production, mainly virus diseases and pests, which are not easily addressed through conventional vegetable breeding alone. The variety of transgenic and conventional breeding techniques being used to enhance the nutritional quality and health benefits of vegetable crops is increasing. Molecular approaches hold great promise for future modifications. To be successful, however, more interdisciplinary work is required and besides molecular biologists and plant breeders is necessary to involve nutritional and food scientists as well as others from biomedical fields to ascertain the true function of specific plant compounds. A barrier to the successful use of transgenic 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 two transgenic vegetable species—squash and sweet corn—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.

Transgenic vegetable crops are not a silver bullet for achieving nutritional quality and health benefits but coupled with conventional breeding can be a powerful tool for making available better vegetables. Biotechnology-derived vegetable crops will succeed if clear advantages and safety are demonstrated to both growers and consumers.

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