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TRANSGENIC RESEARCH OF VEGETABLE CROPS: *AN UPDATE*

Abdul Majid Ansari[1*] and Y. V. Singh[2]

[1]Junior Scientist-cum-Assistant Professor (Horticulture), Zonal Research Station (Birsa Agricultural University), Chianki, Palamau – 822102, Jharkhand, India
[2]Professor and Head, Department of Vegetable Science, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar – 263145 (U. S. Nagar), Uttarakhand, India
[*]Corresponding author E-mail: majid.gbp@gmail.com

ABSTRACT
Over the last two decades various important traits such as biotic stress resistance, product quality and storage life have been successfully engineered into crop plants including vegetable crops. Among the cultivated transgenic crop plants, herbicide tolerance has consistently been the dominant trait followed by Bacillus thuriengiensis (*Bt)* based insect resistance. Herbicide tolerant soybean and canola, and *Bt* maize and cotton constitute the four major genetically modified crops. The two commercialized transgenic vegetable crops are tomato with delayed fruit ripening and potato with insect and virus resistance. Although many more useful transgenic vegetables have been produced, the realization of their benefit at field level is slow. Molecular studies have shown that several novel traits can be introduced in vegetable crops. In recent years, considerable success has been achieved in introducing abiotic stress tolerance, quality traits and expression of various proteins and enzymes of pharmaceutical and industrial importance.

KEYWORDS: Transgenic; vegetable crops; vitamins; antioxidant; phytosterols.

INTRODUCTION

In the countries like India where the population is predominantly vegetarian, vegetables form a vital constituent of the daily diet. China is the world's largest producer of vegetables followed by India (FAO, 2011 and NHB, 2011). Total production could be greatly improved if losses due to biotic and abiotic stresses, and lack of proper storage and processing facilities could be overcome. Consequently, improvements in vegetable production, quality and prevention of post-harvest losses are a common goal in all research and development programme focused on achieving the economic, social and environmental sustainability. Over the last two decades various important traits such as biotic stress resistance, product quality and storage life have been successfully engineered into crop plants. Since the first large-scale cultivation of transgenic insect-resistant crops in 1996, a record 17.3 million farmers, in 28 countries planted 170 million hectares (420 million acres) in 2012, a sustained increase of 6% or 10.3 million hectares (25 million acres) over 2011 (James, 2012). Among the cultivated transgenic crop plants, herbicide tolerance has consistently been the dominant trait followed by *Bacillus thuriengiensis* (Bt) based insect resistance. Herbicide tolerant soybean and canola, and Bt maize and cotton constitute the four major genetically modified crops (James, 2009). The two commercialized transgenic vegetable crops are tomato with delayed fruit ripening and potato with insect and virus resistance. In India Bt brinjal hybrids have been developed by a private seed company Mahyco. The field testing and safety trials are complete but their release for large scale cultivation is still awaited. Although many more useful transgenic vegetables have been produced, the realization of their benefit at field level is slow. Molecular studies have shown that several novel traits can be introduced in vegetable crops. In recent years considerable success has been achieved in introducing abiotic stress tolerance, quality traits and expression of various proteins and enzymes of pharmaceutical and industrial importance. These recent developments made in genetic engineering of vegetable crops have been described and discussed under the above mentioned topic.

ABIOTIC STRESS TOLERANCE

Abiotic stresses such as drought, salinity and extreme temperatures cause significant losses of crop productivity and quality. In abiotic stress prone areas, vegetable production is lower, which leads to malnutrition and associated health disorders in these regions. Development of crops with an inherent capacity to withstand abiotic stresses would help stabilize the vegetable production, and significantly contribute to food and nutritional security in developing countries and semi-arid tropical regions.

Water use efficiency:
Drought tolerance and water-use efficiency can be improved by precise regulation of stomatal opening. In response to drought stress, plants minimize water loss by ABA mediated stomatal closure. The signaling events that bring about stomatal closure are fairly well understood at the molecular level. The intragenic revertant of *ABA insensitive 1* mutant (*abi1-1R3*) displayed an enhanced drought tolerance compared with the wild type *Arabidopsis*. Mutations in the farnesyl transferase gene (*ERA1*) cause hypersensitivity to ABA mediated stomatal closure. Under drought stress *era1* mutants showed enhanced stomatal closure and thus reduced wilting (Pei *et al.*, 1998). Transgenic analysis of guard cell signaling components will shed light on how these genes can be utilized to improve drought tolerance of crop plants.

Compatible osmolytes:
At the cellular level osmotic stress is caused by drought, salinity and temperature extremes. Osmotic adjustment is one of the vital cellular defenses to osmotic stress. Osmotic stress may induce ion uptake (K^+), compartmentalization (Na^+ into vacuole) or synthesis of compatible solutes such as proline, betaine, polyols and soluble sugars. These organic compatible solutes protect plants from stress by (1) osmotic adjustment which helps in turgor maintenance (2) detoxification of radical oxygen species and (3) stabilization of the quaternary structure of proteins. Osmotic adjustment has been associated with an increase in crop yield under drought in many crop plants. These studies provided impetus to engineer plants that over-produce compatible solutes either constitutively or in response to stress. Several transgenic plants engineered to over-produce osmoprotectants showed enhanced abiotic stress tolerance. However, in vegetable crops only very few attempts have been made to engineer osmoprotectants. Trehalose is one of the osmoprotectants for which the role in stress tolerance has been assessed in transgenic potato. Yeast *trehalose-6-phosphate synthase* (*TPS1*) catalyzes synthesis of trehalose, which is a regulator of the carbohydrate allocation to glycolytic flux and stress survival strategies. Transgenic potato plants over-expressing the yeast *TPS1* gene showed enhanced drought tolerance. Glycinebetaine is an important compatible solute employed by many plants to combat abiotic stresses. Tomato plants do not accumulate glycinebetaine. Hence transgenic tomato plants were produced with a chloroplast-targeted *codA* gene of *Arthrobacter globiformis*, which encodes choline oxidase that catalyzes the conversion of choline to glycinebetaine. These transgenic plants exhibited enhanced chilling tolerance over various developmental phases, from seed germination to fruit production (10–30% more fruits), than their wild-type counterparts. Glycine betaine overproduction was achieved in carrot plants engineered to overexpress betaine aldehyde dehydrogenase (BADH) in plastids. These transgenic plants showed very high level of salt stress (400 mM NaCl) tolerance (Kumar *et al.*, 2004).

Ion homeostasis:
Cellular ion homeostasis under salinity is achieved by the following strategies: (1) exclusion of Na^+ from the cell by plasma membrane-bound Na^+/H^+ antiporters or by limiting the Na^+ entry, (2) utilization of Na^+ for osmotic adjustment by compartmentation of Na^+ into the vacuole through tonoplast Na^+/H^+ antiporters, and (3) Na^+ secretion. A high K^+/Na^+ ratio is essential for cellular metabolism, which is disrupted under salt stress. In plants, Na^+ competes with K^+ for uptake under saline conditions. Hence, expression of cation transport systems that specifically transport K^+ into the cell might help in maintaining ionic balance. Over-expression of yeast *HAL1*, a regulator of K^+ transport, in tomato resulted in increased K^+ accumulation and better salt tolerance under NaCl stress (Rus *et al.*, 2001), which suggests that K^+ accumulation can be genetically manipulated to improve salt tolerance of vegetable crops. The tonoplast Na^+/H^+ antiporters transport Na^+ into the vacuole by using electrochemical gradient of protons generated by the vacuolar H^+-adenosine triphosphatase (ATPase) and H^+-inorganic pyrophosphatase (PPase). This vacuolar compartmentation prevents deleterious effects of Na^+ and also helps in osmotic adjustment. Transgenic tomato plants over-expressing *A. thaliana* Na^+/H^+ antiporter *AtNHX1* gene was also able to grow and produce fruit under high salinity (200 mM NaCl). These plants accumulated high concentrations of sodium in leaves but not in fruits (Zhang and Blumwald, 2001). Thus, a significant improvement in salinity tolerance in crop plants can be achieved by engineering a single gene.

Antioxidant defense:
Abiotic stresses cause metabolic aberrations due to their deleterious effects on rate of reaction, protein stability and membrane compartmentation. Perturbed metabolism under these abiotic stresses results in accumulation of toxic levels of reactive oxygen species (ROS) namely, superoxide radicals ($O_2{\bullet}^-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH\bullet$), which result in irreversible damage to nucleic acids, proteins and membranes. ROS detoxification through non-

enzymatic antioxidants (ascorbate, α-tocopherol, glutathione, etc.) and enzymatic antioxidants (superoxide dismutase, catalase, and enzymes of ascorbate-glutathione cycle) is an important strategy of plants to tolerate abiotic stresses. Hence, several attempts have been made to engineer stress tolerant plants by enhancing ROS scavenging capacity of plants. Inhibition of catalase by antisense RNA increases susceptibility to oxidative stress and chilling injury in transgenic tomato plants. In contrast, transgenic tomato plants ectopically expressing *Arabidopsis thaliana CBF1*, showed enhanced tolerance to oxidative stress, as *CBF1* over-expression induced a high level of expression of a catalase gene in these transgenic tomato plants (Hsieh *et al.,* 2002a and Hsieh *et al.,* 2002b).

Transcriptome engineering:
Transcriptome engineering or overexpression of a master switch gene (such as stress sensors, protein kinases or transcription factors) that regulate several target genes coding for osmolyte biosynthesis enzymes, antioxidant enzymes and stress proteins such as late embryogenesis abundant proteins, is emerging as an important tool to combat abiotic stresses. Stress-induced transcription factors such as C-repeat binding proteins (CBFs) or dehydration responsive element binding proteins (DREBs) regulate the expression of many genes for LEA-type proteins, compatible osmolytes biosynthesis and oxidative stress management. Over-expression or stress responsive (*RD29A*) promoter driven expression of *CBF3* gene in transgenic *Arabidopsis* provided protection against multiple environmental stresses such as cold, salt and drought stresses. Components of the *Arabidopsis* CBF pathway are conserved in *B. napus*, wheat, rye, and tomato. Since tomato is highly sensitive to chilling stress, efforts were made to engineer chilling tolerance in tomato by ectopic expression of *Arabidopsis CBF1* driven by a cauliflower mosaic virus 35S promoter. Transgenic tomato plants showed significantly increased chilling tolerance, as measured by survival rate, chlorophyll fluorescence, and radical elongation (Hsieh *et al.,* 2002b), as well as water deficit tolerance than that of wild-type tomato plants (Hsieh *et al.,* 2002a). These transgenic tomato plants produced more proline under drought stress and also showed oxidative stress tolerance both under chilling and drought stress (Hsieh *et al.,* 2002a and Hsieh *et al.,* 2002b).
Thus, engineering a single upstream signaling component such as transcription factors, the whole or part of the stress responsive transcriptome can be manipulated in a transgenic plant. Since several effector genes mediate abiotic stress tolerance, transcriptome engineering appears to be a viable strategy to engineer multiple stress tolerance in vegetables and other crop plants (Dalal *et al.,* 2006).

QUALITY IMPROVEMENT
Nutritional improvement:
Plants produce various compounds such as storage proteins, vitamins, flavonoids, carotenoids that perform vital functions for plants and also have nutritional importance for human beings. Vegetables are a source of minerals, proteins, micronutrients, vitamins, antioxidants, phytosterols and dietary fiber. However, some of the vegetables are deficient in essential amino acids such as methionine and lysine. The amino acid content can be modified or enhanced by expression of synthetic protein, overexpression of homologous or heterologous proteins, modifying the amino acid sequence of the protein, or through metabolic engineering. Potato is an important food crop. The nutritive value of potato protein is diminished due to deficiency in essential amino acids lysine, tyrosine, and the sulfur-containing amino acids methionine and cysteine. To improve the nutritive value of potato an *Amaranths* seed albumin gene *AmA1* has been expressed in transgenic potato tubers. This protein is non-allergenic and rich in all essential amino acids corresponding with WHO standards for human diet requirement (Chakraborty *et al.,* 2000). Similarly a 292 bp artificial gene (*asp-1*) encoding a storage protein composed of essential amino acids was introduced in sweet potato. One of the transgenic lines showed a four-fold increase in protein as compared to that of storage roots of the control plants (Egnin and Prakash, 1997). Zeh *et al*. (2001) have also reported a 30-fold increase in methionine content in transgenic potato tubers engineered with an antisense threonine synthase (TS) gene.

Carotenoids, such as β-carotene and lycopene, give the fruit its characteristic color. Carotenoids are good antioxidants and are precursors of vitamin A. These are synthesized through the isoprenoid biosynthetic pathway. Hence genetic manipulation of this pathway may contribute to the organoleptic and nutritional qualities of vegetables. An effort to increase the pro-vitamin A content in tomato was made by engineering a bacterial gene encoding for the phytoene-desaturase enzyme that converts phytoene to lycopene into transgenic tomato. These transgenic plants produced three-fold more β-carotene content than that of control plants. Similarly, a six-fold increase in carotenoid content and two- to three-fold increase in α-tocopherol content was achieved in transgenic potato plants by antisense or co-suppression of the zeaxanthin epoxidase gene that results in the inhibition of zeaxanthin conversion to violaxanthin. Recently an

Erwinia uredovora crtB gene encoding phytoene synthase was introduced in potatoes. This resulted in four- to seven-fold increase in carotenoid content in the transgenic tubers with β-carotene and lutein accumulation reaching up to 11 and 19-fold higher than control tubers (Ducreux *et al.,* 2005).

Another group of metabolites exploited for its antioxidant property are the flavonoids. These are a diverse group of polyphenolic secondary metabolites, which impart color to the fruit. Flavonoids have been correlated with a decreased risk of cardiovascular disease in humans. Since natural variation for flavonoid content of tomato fruit is limited and most of it is present in the peel, a transgenic approach has been used to increase the flavonoid content by over-expression of either the enzymes involved in flavonoid biosynthesis or transcription factors that regulate the genes of this pathway. Transgenic tomato plants expressing petunia *CHI-A* gene encoding chalcone isomerase showed significant increase in flavonoid content. Similarly, a 10-fold increase in flavonoid content has been achieved by ectopic expression of the maize transcription factors LC and C1 in transgenic tomato (Le Gall *et al.,* 2003).

Improvement of aroma:
Flavor and aroma have an important influence on people's choice of foods. The aromas of fruits, vegetables, and flowers are mixtures of volatile metabolites such as alcohols, phenols, ethers, aldehydes, ketones, etc. Some of the short-chain aldehydes and alcohols, which contribute to flavor, are derived from lipid components by the action of lipases, hydro peroxide lyases, and alcohol dehydrogenases. These transgenic tomato plants showed changes in certain flavor compounds such as *cis*-3-hexenol, 1-hexanol, hexanal, and *cis*-3-hexenal. In another study, overexpressing a nonspecific tomato alcohol dehydrogenase gene in tomato fruits altered the levels of aroma-determining short chain aldehydes and alcohols. This change in aroma volatiles resulted in intense 'ripe fruit' flavor in tomato fruit. One of the important volatile compounds that influence the quality of the flavor of tomatoes and their products is the acyclic monoterpene alcohol, linalool. Linalool, imparts a sweet, floral alcoholic note to fresh tomatoes. Hence linalool levels were altered by engineering the *S*-linalool synthase (LIS) gene from *Clarkia brewery* in tomato plants. The expression of *S*-linalool synthase enzyme, which catalyzes the formation of linalool, resulted in elevated levels of linalool and linalool derivatives in the transgenic fruit (Lewinsohn *et al.,* 2001).

Browning and loss of the flavor compound methional during processing are two problems associated with quality of potato. Transgenic potatoes have been generated in which browning is overcome by antisense inhibition of polyphenol oxidase. One obvious way to retrieve the flavor in potato is by addition of methional in processed potato, which would be uneconomical. The other approach can be to increase the level of its precursor, methionine. Cystathionine gamma-synthase (CGS) is a key enzyme regulating methionine biosynthesis in plants. To increase the level of soluble methionine in potato, *Arabidopsis thaliana* CGS cDNA was introduced under transcriptional control of the cauliflower mosaic virus 35S promoter into Russet Burbank potato (Di *et al.,* 2003). This strategy led to a 2.4- to 4.4-fold increase in methional level in baked tubers of field-grown transgenic potato lines. The increase observed in methional levels also correlated with the soluble methionine level in the tubers from the same lines measured before processing (Di *et al.,* 2003).

Seedless vegetables:
The development of the fruit in the absence of pollination and/or fertilization is known as parthenocarpy. Since pollination and fertilization are adversely affected by environmental stresses such as low or high temperature, parthenocarpy gives advantage for stability in productivity under these stress conditions. Moreover, the seedless nature of parthenocarpic fruits increases consumer acceptance, makes processing of vegetables easier and also improves the quality of the vegetables like brinjal (eggplant), where seeds are associated with bitter substances. The absence of pollen will also alleviate environmental concerns regarding the transfer of transgenes to non-transgenic by cross-pollination. Parthenocarpy has been shown to be regulated by auxins. Hence, efforts have been made to engineer parthenocarpy either by increasing the auxin production or sensitivity of the ovary to auxins. Expression of *iaaM* gene driven by the ovule specific promoter *DefH9* has been shown to confer parthenocarpy to transgenic tomato (Ficcadenti *et al.,* 1999) and eggplant (Rotino *et al.,* 1997), and also resulted in high yield and fruit quality in eggplant (Donzella *et al.,* 2000). Recently, the *Agrobacterium rhizogenes*-derived gene *rolB* has been used as an alternative approach for the induction of parthenocarpy in tomato. Transgenic tomato plants transformed with the *rolB* gene under the control of ovary- and young-fruit-specific promoter *TPRP-F1* developed parthenocarpic fruits. The fruit size and morphology,

including jelly fill in the locules of the seedless fruits, were comparable to those of seeded fruits of the parental line (Carmi *et al.*, 2003).

PHARMACEUTICAL AND INDUSTRIAL USE

The ability to transfer genes across different plant species and kingdoms through genetic engineering is being exploited in terms of Bio-farming. Bio-farming refers to production of proteins and biomolecules in transgenic plants at agricultural scale. The proteins mainly include antigens, antibodies, enzymes that are of immense importance in therapeutics, pharmaceutical and industrial applications. Though many of these proteins are being made in bacterial, fungal or animal systems, plants are now being preferred for manufacturing these proteins. The use of plants as bio-factories is attributed to many factors, such as plants offer cost effective and environmentally safe production of proteins as they use low-cost inputs such as light, water and minerals, plants allow mass production, suitable for production of eukaryotic proteins which may require post-translational modification, oligomerization etc. and naturally do not contain human pathogens. Among vegetables, potato and tomato are the most commonly used host systems for bio-farming. The feasibility of vegetables as plant factories is very well illustrated in the form of edible vaccine, plantibodies (plant derived antibodies) and plant derived recombinant enzymes (Dalal *et al.,* 2006).

Edible vaccines:

The advancement in medical sciences has succeeded in the fight against infectious diseases of humans by producing vaccines. However, widespread vaccination remains elusive for the majority of the world population due to constraints on vaccine production in animals, transport and delivery. Plants offer an effective way to circumvent these problems as virtually any protein can be expressed in a plant cell, antigens can be produced on a large scale in the crops, and the crops can be grown locally which will reduce transport requirements. Antigens can be expressed in plant storage organs, which are viable at room temperature, thus eliminating the need for refrigeration of antigens during transport and storage. Other advantages of vaccine production in plant storage organs include elimination of the need for purification of antigen and possible source of contamination with pathogenic viruses that occasionally occurs when vaccine production is done in animals (Dalal *et al.,* 2006). The vaccines can be produced in edible parts of plants thus oral administration of vaccine will also abolish the need for needles, a possible source of contamination, and would not require trained personnel.

Vaccines produced in edible parts of plants, e.g. grain, tuber or fruit, are known as edible vaccines. The development of edible vaccines is primarily focused on pathogens, which invade the host via the mucosal surfaces lining the digestive, respiratory and urino-reproductive tracts of the body. The concept of plant derived vaccine was realized when Hepatitis B surface antigen was expressed in tobacco by Mason *et al*. (1992). Subsequently several attempts were made to express antigens related to various diseases in edible parts of the plants (James, 2009). The vaccination of Hepatitis B virus is based on highly immunogenic surface antigen (HBsAg), which is secreted in infected patient's serum in the form of virus like particles (VLPs). The HBsAg has been expressed in tobacco and potato (Mason *et al.,* 1992 and Richter *et al.,* 2000). The HbsAg extracted from transgenic tobacco leaves invoked immune response when administered parentally (Mason *et al.*, 1992). To make HBsAg vaccine suitable for oral delivery, Richter *et al.* (2000) developed transgenic potatoes expressing HBsAg under the transcriptional control of a tuber specific patatin promoter. Potato tubers accumulated 1.1 μg of HBsAg g^{-1} fresh tuber. Mice fed with transgenic tuber (5.5 μg $dose^{-1}$) developed primary immune response, which could be boosted by single sub-immunogenic dose of commercial HBsAg vaccine indicating that plants expressing HBsAg vaccine in edible tissue may be used for oral immunization of Hepatitis B (Richter *et al.,* 2000).

The edible vaccines were initially targeted against enteric diseases, which are the most common cause of child mortality worldwide, especially in developing countries. Enteric disease may result from bacterial and viral infections. One of the bacterial pathogens is *Vibrio cholerae*, which is the causative agent of cholera. A non-toxic immunogenic B subunit (CTB) of enterotoxin that helps the toxin to bind to gut cells was expressed in potato. Mice administered with transgenic potato tubers expressing CTB fused with ER retention signal showed induction of both serum and intestinal CTB specific antibodies. Similar to cholera, enteroxygenic *Escherichia coli* also colonizes the small intestine and produces enterotoxin leading to diarrhea. The enterotoxin (LT) from *E. coli* consists of one A subunit and pentameric B subunits. The non-toxic immunogenic B subunit has been engineered to express in tobacco and potato. For expression in potato tubers synthetic *LT-B* was expressed under a class I patatin promoter. The extracted protein from transgenic potato was used for immunization of mice. The recombinant protein was immunogenic and it could elicit local and systemic IgA response in parenteral primed mice. Antigens for some of the enteric viral pathogens such as Norwalk virus and rotaviruses were expressed in potato. The mice immunized orally by feeding the potato tubers expressing recombinant Norwalk virus-like particle (rNV) were shown to develop serum IgG specific for rNV produced transgenic potatoes expressing capsid structural protein VP6 of Murine rotavirus. Oral immunization of mice with these transformed potato tuber tissues generated detectable antibody response against the rotavirus capsid protein. The success in mouse trials was followed by human trials. Human volunteers orally immunized with either bacterial (LT-B) or viral (Norwalk virus capsid protein) protein developed specific serum and immune responses against these antigens (Tacket *et al.,* 1998 and Tacket *et al.,* 2000).

Since enteric diseases are caused by multiple pathogens it would be desirable to generate multicomponent vaccines that can provide protection against such pathogens simultaneously. Hence a strategic epitope fusion construct consisting of subunits B and A2 of the cholera toxin (CT) linked with rotavirus enterotoxin and enterotoxigenic *E. coli* fimbrial antigen genes was made and transferred into potato. This trivalent edible vaccine generated significant immune response against these antigens in orally immunized mice (Dalal *et al.,* 2006).

The concept of edible vaccine has been extended for various other diseases such as anthrax, cancer, bronchiolitis, etc. (James, 2009). Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. Protective antigen (PA), the potent molecule for vaccination against anthrax has been expressed in tobacco and tomato (Kumar *et al*. unpublished). Human papillomavirus (HPV) infection has been associated with cervical cancer. An attempt was made to produce edible vaccine by engineering the HPV type 16 major capsid protein (L1) in transgenic tobacco and potato plants. The immunogenicity test of this recombinant protein by feeding transgenic potato tubers to mice revealed an anti-L1 response in about 50% of the animals. The edible vaccines can be of tremendous help in treating autoimmune diseases such as diabetes mellitus, which require relatively large quantities of antigen for inducing immune tolerance. Antigens for diabetes mellitus, human GAD65 and human insulin have been engineered in transgenic carrot and potato respectively. Oral administration of transgenic potatoes to diabetic mice resulted in a substantial reduction in insulitis and a delay in the progression of clinical diabetes. These results indicate the feasibility of oral delivery of plant derived antigens for imparting immuno tolerance against this T cell-mediated autoimmune disease. There are various groups involved in making edible vaccines for animals as well. In two independent studies, vaccine antigens for hemorrhagic disease of rabbits and infectious bronchitis (IB) of chickens have been expressed in transgenic potatoes and have also been successfully tested on rabbits and chickens respectively. Recently generated an edible vaccine, which is effective against simian immunodeficiency virus (SIV) which infects African green monkeys and various primates. A fusion gene comprising of the cholera toxin B subunit (CTB) linked with the simian immunodeficiency virus (SIVmac) Gag p27 capsid gene (CTB–Gag) was transferred into potato. The fusion gene CTB–Gag was biologically active and made up approx. 0.016–0.022% of the total soluble tuber protein. Although the pre-clinical trials for plant based vaccines have been performed (Tacket *et al.,* 1998 and Tacket *et al.,* 2000), many challenges including optimization of expression level to suit the dosage requirements and stabilization during post-harvest storage need to be addressed. Vegetables, which are used as salads are good candidates for oral immunization.

Enzymes and plantibodies:

Vegetables have also been used for bio production of therapeutically valuable proteins (Dalal *et al.,* 2006). Treatment of organophosphate poisoning requires large amounts of cholinesterases. Transgenic tomato plants expressing recombinant isoform of human acetyl cholinesterase have been generated which produced active and stable acetyl cholinesterase, with the kinetic characteristics similar to that of the human enzyme (Mor *et al.,* 2001). Another human protein, α-interferon has also been successfully engineered in potato plants. Plant-derived antibodies, also known as plantibodies, have extensive medical uses such as passive immunization, diagnostics and targeted drug delivery. However, the potential of antibodies as therapeutics gets constrained due to the limited amount and high cost of production. Plants provide suitable machinery for production of antibodies in large amounts. Antibody production has been successfully demonstrated in tobacco, soybean and potato. These antibodies can be produced and stored in leaves, seeds or storage organs. Various experiments have shown that antibodies can be expressed in potato tubers. The antibodies constituted up to 2% of the total soluble protein and could be stored in tubers in cold storage for about 18 months with 50% of the antibodies retaining their function.

Industrial applications:
Use of plants as bio-factories for producing industrial enzymes, chemicals and raw materials can be carried out in two ways. One way is to introduce a foreign protein gene and produce that in high amounts, the other way is to modify the plants metabolic pathways to generate sufficient end product, by product or a novel biomolecule. The first category involves production of enzymes such as cellulases, amylases, which are required in large quantities and foreign proteins such as silk protein, or sweet protein 'monellin'. Cellulases find wide application in food ingredients, beverage and textile industries. Dai *et al.* (2000) generated transgenic potatoes expressing endoglucanase (E1), a cellulase gene from *Acidothermus cellulolyticus*. The gene expression driven by leaf specific promoter and RbcS-2A signal peptide, resulted in E1 protein accumulation that was up to 2.6% of total leaf soluble protein. Human milk proteins such as lactoferrin and casein have also been expressed in transgenic potatoes. These can be used as supplement in human infant formulas and baby foods to enhance nutrition, digestibility and antimicrobial properties. The remarkable mechanical property of spider silk finds several industrial applications. However, obtaining spider silk in large quantities from a natural source is very difficult. Hence efforts have been made to obtain large quantities of spider dragline silk by over-expressing *Nephila clavipes* dragline protein gene in tobacco and potato. Dragline protein accumulation of up to 2% of the soluble protein has been achieved through this gene in transgenic potato lines.

A sweet protein Monellin from the *Dioscoreophyllum cumminsii* plant has been expressed in lettuce and tomato (Penarrubia *et al.*, 1992). Monellin is 100,000 times sweeter than sucrose. Thus, monellin can replace sucrose, which is extensively used in confectionery, jams, cakes, and biscuits. Due to high calorific value and dental caries-inducing properties, sucrose had been replaced by artificial sweeteners. Artificial sweeteners have low calorific value and hence are advantageous for persons on a restrictive diet. However, these artificial sweeteners also have limitations in terms of taste and are suspected to be carcinogenic. Hence an alternate has been presented in the form of palatinose. Palatinose is a low calorific structural isomer of sucrose with physio-chemical properties similar to that of sucrose. For converting sucrose into palatinose, an enzyme sucrose isomerase from *Erwinia rhapontici* was cloned under the control of tuber specific promoter and transferred to potato. The transgenic potato plants accumulated 1.7–16 μmol g^{-1} of palatinose in tubers. Thus, production of sweeteners with low calorific value in transgenic fruits and vegetables not only fulfills the requirements of a low calorie sweetener but also represents an alternative strategy to enhance the flavor and quality of the fruit.

FUTURE PROSPECTS
Significant progress has been made in engineering crop plants for introduction of novel traits of agricultural, pharmaceutical and industrial value (Dalal *et al.*, 2006). The rate of increase in the area of cultivation of transgenic crops clearly indicates that agriculture in future will be dominated by transgenic crop varieties. Although transgenic tomato "Flavr Savr" was the first transgenic crop to be commercialized, presently soybean, maize, cotton and canola are the four major transgenic crops in cultivation. Progress in commercialization of transgenic vegetable crops is still slow although a variety of transgenic vegetables have been field-tested. Among vegetable crops, the commercialization has been limited to insect- and virus-resistant potato, and tomato with improved shelf life. One major limitation is that the public acceptance of genetically modified food is not wide spread even in developed countries. It is very important to spread the information about the safety and importance of transgenic crops. Transgenic crops are more relevant for developing countries already burdened with enormous population. In addition to meeting the demand for more food, there is an urgent need to reduce the chemical inputs in agriculture so as to preserve the environmental quality and protect biodiversity. This would also lead towards the improvement of economic security of the resource-poor and small farmers. Transgenic vegetables will be of great benefit to alleviate human suffering in the third world. The battle against life threatening diseases such as cholera, hepatitis and diarrhea will be easier to win by using engineered vegetables. Malnutrition can be overcome by vegetables improved for these specific traits. There is a need for technology and product transfer to developing countries mediated by individuals and philanthropic organizations as has been done in the case of "Golden rice". In addition, public research systems in developing countries need to intensify efforts to develop technologies of their own.

Some of the limitations in transgenic applications need to be resolved for wider application and acceptance of transgenic technology. Environmental risks such as cross-pollination with closely related wild relatives of the crop plants and effect of transgene products on human health need to be assessed carefully on a case-by-case basis (Dalal *et al.*, 2006). Moreover, there are wide spread concerns about the use of antibiotic and herbicide resistance genes as selectable markers from the point of view of ecological and human safety. Use of alternate methods to obtain marker free transgenic plants may enhance the public acceptance of transgenic crops. Development of binary vectors or mini-chromosomes for multiple gene transfer and improvement in transformation systems for vegetable crops may further increase our capability to introduce traits with long lasting value.

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