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Biotechnological improvement of nutritional and therapeutic value of cultivated potato

Deepak Singh Bagri[1], Devanshi Chandel Upadhyay[1], Subodh Kumar Jain[1], Chandrama Prakash Upadhyay[1]

[1]Department of Biotechnology, Dr Harisingh Gour Central University, Sagar-470003, Madhya Pradesh, India

TABLE OF CONTENTS

1. ABSTRACT

Genetic engineering is recognized as a powerful tool for altering the genetic characteristic of crop plants. Genetic engineering has tremendous potential in developing improved potato varieties with desired agronomic traits and has been utilized for improvement of several crop plants including potato to enhance essential amino acid, protein and lipids/ carbohydrates contents as well to improve stress tolerance. The pathway engineering of amino acid revealed dramatic changes in essential amino acid content and protein quality. Similarly, the vitamin pathway engineering of potato has been proved to enhance the vitamin content with increased cellular antioxidant activities. Secondary metabolites such as flavonoids have also been altered through the genetic engineering of potato. This review provides detailed reports on the advances made in genetic transformation of potato for enrichment in its nutritional and therapeutic value by an increase in functional secondary metabolites, carbohydrate, essential amino acids, proteins, lipids, vitamins and edible vaccines.

2. INTRODUCTION

Potato (*Solanum tuberosum* L., L stands for "Linnaeus" system of nomenclature) is a worldwide significant crop grown as a nutritionally valuable food in the form of tubers. Potato has been extensively studied because of its usage as a stable food crop and potentially valuable source of compounds with health benefits. The potato tubers develop by the morphological change of the underground stem into stolon which finally converts into tubers containing axillary dormant buds. Potato is grown from the botanical seeds or propagated vegetatively by planting pieces of tubers containing eyes or dormant buds which grow into new shoots (sprouts) when grown under suitable conditions. In addition, the natural property of potato is conserved due to the vegetative mode of propagation (1). Today the potato is cultivated in about 125 countries and about a billion people worldwide consume them daily. The annual world production of potato exceeded 376 million metric tonnes with China being the top producer. Higher yield per unit area and nutritional value have led to an increase in potato

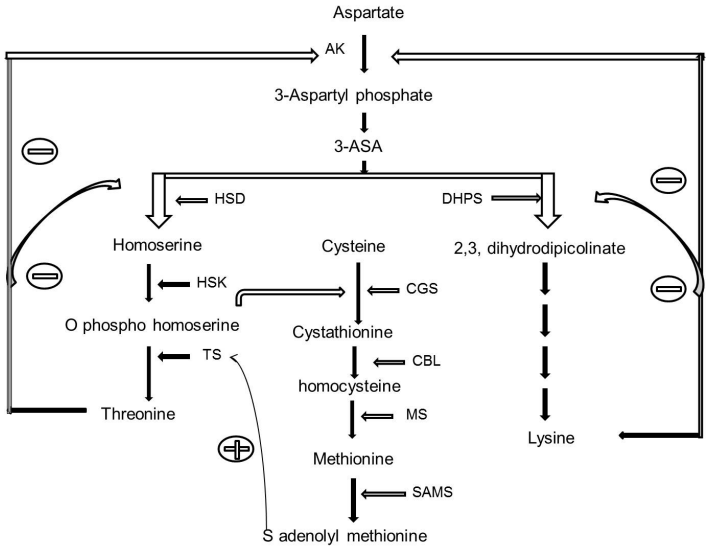

Figure 1. Schematic diagram of essential amino acid synthesis pathway. The aspartate family biosynthetic pathway of the essential amino acids lysine, threonine, and methionine. Curved arrows with (−) sign represents the major feedback inhibition by the end-product amino acids. The arrow with a (+) sign represents enzyme activation.

production over past years compared with other tuber crops (2). Harvesting of potato in developing countries is better adapted than the developed countries probably due to its increased per-capita consumption; however, the demand of potato in developed countries is also increased due to consumption as fast food in the form of fries and chips (3). The nutritional quality of potato is compromised due to the cultivation of varieties with higher yield and lesser nutrients such as vitamins and protein (4). New cultivars of potatoes with better yield, disease resistance, and other desirable traits are being developed with the help of breeding techniques; however, nutritional enhancement of crop plants like the potato, rice is crucial as the worldwide vegetarian population is devoid of the basic nutrition to maintain health (5). Following the rational development of genetic engineering, many genetically modified potatoes with higher proteins, vitamins, amylose/amylopectin content, antioxidant levels, and tuber yield have also been developed (6).

Several strategies have been adopted for bio-fortification of potatoes such as farming practices, breeding techniques, and genetic engineering. The simplest approach relies on farming practices where suitable minerals such as inorganic compounds added to the soil to increase the mineral content of the tubers. In addition, the conventional breeding technique assisted with mutagenesis has also been used as an alternative approach for developing the nutrient rich crops, however, these approaches are time-consuming to identify the useful traits and breed them into the selective cultivars. The modern transgenic approach uses the selective genetic information to introduce the functional gene directly into the plant genome through easily available natural or artificial techniques. Genetic engineering technique is well adapted to introduce a desirable character into elite transgenic potato varieties to generate superior quality of potato. However, these transgenic varieties of potatoes are not permitted for food use in many countries because of the concerns related to consumer health and the environment. Until these genetically modified potatoes have been given proper clearance by the food authorities and acceptance by the consumers, they may have a good scope for their use in nonfood or other industrial applications.

Following sections discuss diverse transgenic approaches used to improve the nutrient content in cultivated potato

3. PATHWAY ENGINEERING FOR ENHANCED AMINO ACID/PROTEIN

Plant growth and development is due to interactions among several environmental factors including the source-sink phenomenon. The photosynthetic assimilate move from source to sinks in the form of sucrose. A relation between sucrose content and amino acid biosynthesis in plants established using the functional genomics approaches. Functional genomics analysis of major enzymes involved in amino acid biosynthesis strongly suggested that increase in amino acids is directly dependent on the sucrose supply at the cellular level and tightly controlled at the transcription level, thus, regulating the amino acid biosynthesis of potato (7). A generalized pathway of essential amino acid biosynthesis in the plant is represented in Figure 1

3.1. Transgenic potato with enhanced amino acid content

Functional analysis the genes involved in methionine and cysteine metabolism in potato revealed that cystathionine-beta-lyase (CBL) is the rate-limiting enzyme for essential amino acid biosynthesis in potato (8). Scientists at NIPGR, New Delhi, India developed the transgenic potato overexpressing the sunflower albumin or an amaranth seed albumin *(AmA1)* driven under the constitutive promoters, which resulted in five to seven folds increase in total methionine level in tubers (9). Since increased methionine content of plant is of commercial interest, the emerging knowledge has also been exploited by molecular breeders; however, the success is still awaited. Analysis of transgenic potato lines with enhanced methionine amino acid via tuber-specific expression of a seed protein, *AmA1* (Amaranth Albumin 1) revealed an increase in total protein contents up to 60% in comparison to the transformed potato (15). In addition, this increase in total protein content corroborated with higher concentrations of other essential amino acids in transgenic tubers which are otherwise inadequate in potato. Similarly, the methionine amino acid were also enhanced in transgenic potato by overexpression of the gene encoding *PrLeg* polypeptide (isolated from *Perilla*) driven under the tuber-specific *patatin* promoter. This resulted in an increase in *Ca*. 3.5-folds methionine in transgenic potato without changes in other amino acids or growth, development and yield of the potato (10). It was also reported that higher isoleucine accumulation in transgenic tubers enhanced the methionine accumulation via methionine gamma-lyase (MGL) catabolism pathway (11).

3.2. Transgenic potato for quality proteins

There are few reports on enhancing the total protein content in potato via transgenic approaches. The transgenic potato lines were developed by insertion and overexpression of the gene involved in lactic-β-casein biosynthesis. Biochemical analysis of these transgenic tubers showed relatively fewer increase casein in tubers; however, the experiment provided a proof of concept of expression of the casein protein in the edible crops. Later, the successful expression of lactoferrin or casein (human milk like proteins) in potato tuber tissues opens the novel path for developing hypoallergenic human milk-like-proteins in the formulation of baby or infant formula foods (13). The transgenic potato over expressing the casein might be the most important in future particularly to produce human milk-like-proteins. The potato flours are also used in food industry which is an important ingredient for several products. To prepare better quality potato flour, the transgenic potato was developed by over expression of low-weight gluten known as LMW-GS-MB1 gene via *Agrobacterium* mediated transformation method. The expression of this gene induced the polymerization of gluten via interconnecting them either mutually or with other constituents present in tubers, resulting in enhanced potato flour matrix and viscosity (14).

4. PATHWAY ENGINEERING FOR THE HIGH VITAMIN CONTENT

Vitamins play a major role in maintaining human health via regulating metabolism and supporting the biochemical process related to the energy released from food or other sources in living organisms. Vitamins are also important in the synthesis of hormones, enzyme activity, red blood cells, genetic materials and neurotransmitters (16). There are several reports of development of transgenic potato tuber with enhanced vitamin content.

4.1. Transgenic potato with enhanced carotenoids

Vitamin A and is an important factor for developing visual pigments of “rod and cone” cells in the retina which is synthesized in cells via its precursor ß-carotene. The oxidative cleavage of the ß-carotene in intestine yields two molecules of retinal which ultimately converts into retinol or vitamin-A by a reduction reaction. Major carotenoids present in potato are antheraxanthin, violaxanthin, xanthophylls lutein, and xanthophyll esters, without provitamin-A activity (17). The generalized impression of tocopherol and carotenoid biosynthesis pathway in plants is represented in Figure 2**.** Researchers have attempted to enhance carotenoids content in potato via transgenic strategies. As a first report, transgenic potato lines were developed by overexpression of a bacterial *phytoene synthase* gene and an increase in carotenoids was observed up to seven folds as compared to wild type potato with a change in overall ratios of individual cellular carotenoids (18). Metabolic engineering in potato by overexpression of the *lycopene epsilon cyclase* gene revealed the enhancement of the carotenoid in comparisons to the untransformed control plants (19). In another study, the commercially important keto-carotenoids including astaxanthin were overexpressed in potato via transgenic approaches. Additionally, a transgenic potato line accumulating zeaxanthin was co-transformed with the *crtO b-carotene ketolase gene,* (isolated from the cyanobacterium *Synechocys tis* and driven by constitutive promoter *CaMV35S*) in order to support the formation of 3-hydroxylated and 4-ketolated-b-carotene (20). In a recent report, the transient overexpression of two different *di-hydroxyphenylalanine* (*DOPA*) *dioxygenases* (*DOD*s) genes, with the feeding of DOD substrate (L-DOPA) was enough to induce betalain production in cell cultures of potato (21). However, the development of stable transgenic potato by over-expressing the *CrtRb2* gene (*β-Carotene Hydroxalase 2)* and *PSY2* gene *(Phyto*

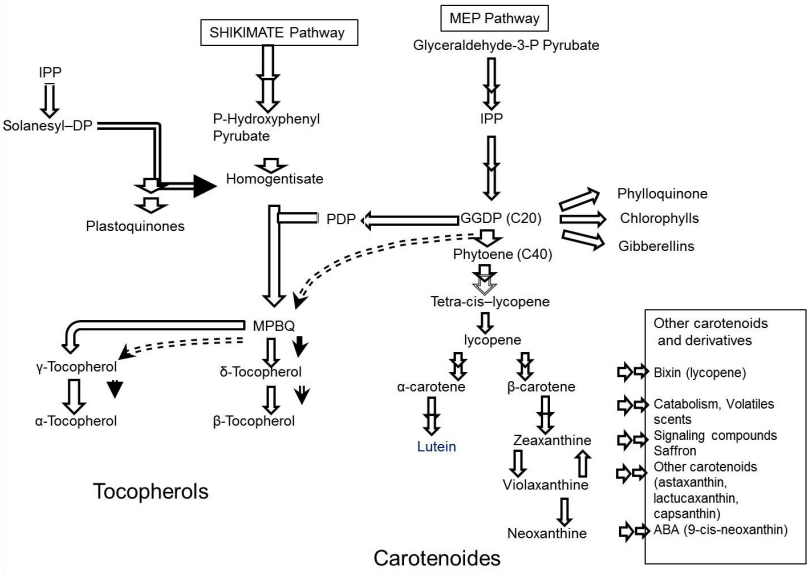

Figure 2. Overview of carotenoid and tocopherol biosynthesis in plants. The 2-C-methyl-d-erythritol-4-phosphate (MEP) pathway provides isopetenylpyrophosphate (IPP) for synthesis of the central intermediate geranylgeranyl diphosphate (GGDP). GGDP can be used for synthesis of phytoene, chlorophylls, and tocotrienols or reduced to phytyl- diphosphate (PDP) used for phylloquinone, chlorophyll, and tocopherol synthesis.

ene Synthase 2) was also reported. The genes were tagged with red fluorescent protein (RFP) for cellular detection in further analysis. The molecular analysis via functionality of the fluorescently tagged proteins revealed that the induced expression of both genes enhanced the carotenoid level in the stable transgenic lines (22). Experiments have also demonstrated the overexpression of the carotenoid biosynthetic pathway genes stacked with the *cauliflower orange (Or)* gene in potato which resulted in enhanced (six-fold) tuber astaxanthin content (23). To fabricate commercially valuable ketocarotenoids in potato, the *4, 4' β-oxygenase (crtW) and 3, 3' β-hydroxylase (crtZ)* genes driven by constitutive promoter were overexpressed in potato, which resulted in alteration of endogenous cellular carotenoids to form an array of hydroxylated and ketolated derivatives (24).

4.2. Transgenic potato with enhanced vitamin E

The vitamin E (α-tocopherol) is only synthesized by photosynthetic organisms which show potent antioxidant activity and vital for human health, however, consumed at the sub-optimal level. A generalized pathway of the vitamin E metabolic biosynthesis in plants is represented in Figure 2. The first report of the development of transgenic tuber over accumulating vitamin E where the transgenic potato lines developed via Agrobacterium mediated transformation using two vitamin-E biosynthetic genes, p- hydroxyphenylpyruvate dioxygenase (At-HPPD) and homogentisate phytyl transferase (At-HPT), isolated from Arabidopsis thaliana. Biochemical and molecular analysis revealed that the over-expression of At-HPPD and At-HPT resulted in a maximum 266% and 106 % increase in alpha-tocopherol respectively, still lesser alpha-tocopherol than leaves or seeds (25). This might be limiting factors for tocopherol accumulation in potato tubers due to physiological and biochemical regulatory constraints. Our laboratory is also engaged in developing transgenic potato lines with an enhanced level of Vitamin E content of tubers.

4.3. Transgenic potato with enhanced vitamin-C

Vitamin C (L-ascorbic acid, AsA) is an important component in nutrition with the property of antioxidant, immuno-protection, cardiovascular function improvement, prevention of ailments associated with connective tissues, and help in iron metabolism. An outline of plant ascorbic acid biosynthesis pathway is represented in Figure 3. The gene D- galacturonic acid reductase (GalUR) associated with Vitamin C biosynthesis was isolated from strawberry and characterized for functional analysis in model plants (26). The transgenic potato lines were developed by overexpression of strawberry D-galacturonic acid reductase (GalUR) and mouse L-gulono-c-lactone oxidase (GLOase) driven by CaMV 35S constitutive promoter. The biochemical analysis showed enrichment in ascorbic acid levels in the potato plant and tubers as well (27, 28). It was also proposed that cellular recycling of AsA could be another strategy to enhance the AsA level in the plant cells. During the recycling, the mono-dehydroascorbate (MDHA) synthesized via oxidation reaction of ascorbate followed by the conversion into the ascorbic acid by the enzyme mono-dehydroascorbate reductase (MDHAR)

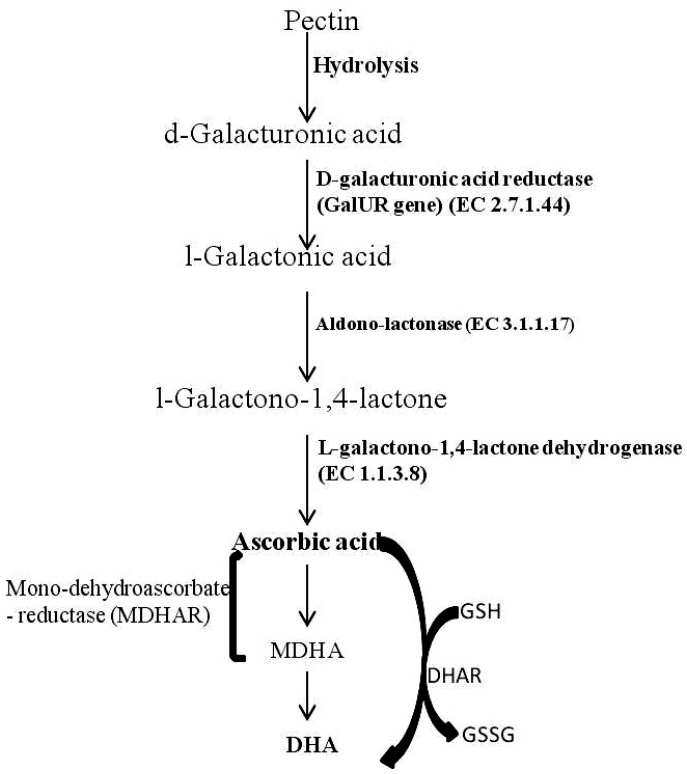

Figure 3. Proposed pathways for ascorbic acid biosynthesis and recycling pathways in plants

or further oxidized forming dehydroascorbate (DHA). The irreversible hydrolysis of this DHA synthesizes the ascorbic acid with the help of the enzyme dehydroascorbate reductase (DHAR) which uses the reduced glutathione (GSH) as a cofactor. It was also observed that overexpression of DHAR gene in plants could result in an increase in ascorbic acid accumulation due to efficient ascorbate recycling process (29, 30). In another report, the potato transgenic lines were developed by overexpressing DHAR gene, driven by the CaMV35S constitutive promoter and a tuber-specific patatin promoter. The AsA level in tubers of Patatin: DHAR transgenic lines showed an enhanced level (up to 1.3. folds) as compared to that of control plants (31). In another report, two independent transgenic potato lines were developed by overexpression of cytosolic DHAR (Cyt-DHAR) gene and chloroplast DHAR (Chl-DHAR) gene (32). The Cyt DHAR gene considerably augmented DHAR activities and AsA contents in potato tubers and leaves, because overexpression of Chl- DHAR gene could only increase DHAR activities and AsA contents in leaves, not in tubers. These results indicated that AsA level of potato enhanced by increasing recycling ascorbate via DHAR overexpression. Similarly, the potato transformation was done using the gene construct with potato isolate GGP (GDP-l-galactose phosphorylase) gene under the control of polyubiquitin promoter (tubers only). The molecular and biochemical study revealed that transgenic potato showed an increase in tuber ascorbate of up to three folds (33). The results confirmed that use of GGP gene to enhance ascorbate content in edible crops.

5. PATHWAY ENGINEERING FOR THE LIPIDS BIOSYNTHESIS

The investigations on lipid biosynthesis and its limiting factors in potato were done using the functional genomics and genetic engineering approaches. Proposed pathway represented for plant starch and lipid biosynthesis in Figure 4. Potato has high content of starch and limiting or rare in lipids. The over-expression of the 14-3-3 protein isolated from *Cucurbita pepo* has been reported in transgenic potato with 69% increase in total fat as compared to the wild-type plants (34). The overexpression of acetyl- CoA-carboxylase from *Arabidopsis* in the amyloplasts of potato has also been demonstrated, which led to 4-5 folds increase in fatty acid triacylglycerol biosynthesis (35). However, the changes in lipid content achieved in this study represent only the first steps towards the attempt to redirect a substantial amount of carbon from starch to lipid. The overexpression of Des-A gene encoding the acyl-lipid Δ12-desaturase isolated from the *Cyanobacterium synechocystis* was also reported in potato (36). Lipid analysis revealed that lipid content and the unsaturation level of their

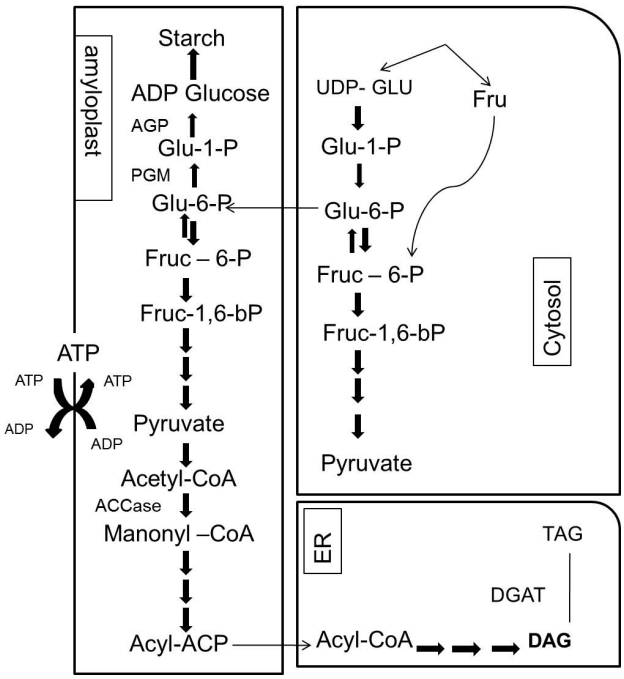

Figure 4. Generalized scheme of starch and lipid synthesis in potato (*Solanum tuberosum* L.) tubers. Sucrose is converted to UDP-glucose and fructose in tuber cells by *SuSy*. A large fraction of carbohydrate is imported into the amyloplast and used to produce ADP-glucose. A smaller amount of carbohydrate is metabolized via glycolysis or converted to acetyl-CoA and malonyl-CoA for fatty acid de novo synthesis in the amyloplastic. Fatty acyl groups are exported to the endoplasmic reticulum for lipid biosynthesis.

fatty acid moieties increased in leaves of transgenic plants as compared to wild type control plants. There are reports of isolation and cloning of full-length cDNA of SADs (*ScoSAD*, *SaSAD*, *ScaSAD* and *StSAD*) from four Solanum species which were also overexpressed in potato under the control of patatin promoter (37). Biochemical analysis revealed that the overexpression of the ScoSAD gene in transgenic potato could enhance the linoleic acid content which in turn also enhanced the freezing stress tolerance. Our research group is also involved in enhancing the total lipids content in tubers via genetic transformation with already developed transgenic potato over-expressing the vitamin E pathway genes. Potato tubers are known for high level of starch accumulation, however, only a small amount of triacylglycerol (TAG) are present. In a recent study, the transgenic potato lines were developed by overexpression of three genes including *WRI1* (wrinkled 1), *DGAT1* (diacylglycerol acyltransferase 1) and *OLEOSIN* driven by the tuber specific *Patatin* and constitutive *CaMV 35S* promoter. Biochemical analysis revealed that the transgenic tubers could accumulate a higher amount of TAG accumulation drastically in the tuber tissues. The concentrations of phospholipids and galactolipids were also significantly augmented in the potato tuber as confirmed by the HPLC analysis. However, a significant reduction in starch content and an increase in soluble sugars were also observed due to carbon reallocation in non-photosynthetic underground storage organs.

6. PATHWAY ENGINEERING FOR THE CARBOHYDRATES QUALITY

Genetically modified potato with modified carbohydrate important in the food industry has been reported. For the first time, the transgenic potato storing modified carbohydrates were developed by overexpressing the bacterial fructosyl transferase genes (39). This modification affected photosynthetic partitioning in tubers and leaves which increased non-structural carbohydrate content in the leaves. The non-structural carbohydrates enhanced the quality of fries and chips of potato. In another study, the transgenic potato developed by the over-expression of phosphofructokinase, isolated from bacterium Lactobacillus bulgaricus, resulted in breakage of simple sugars via glycolytic pathway and transgenic tubers also showed lower reducing sugar (40). There was a great advantage of the overexpression of the bacterial phosphofructokinase in potato. The transgenic potato could be stored longer in cold temperature without any change in the sweetness in potato. The transgenic potato not only has lower sugar content, but the chips prepared from such transgenic potatoes are lighter in color than those prepared from untransformed control tubers. Another novel transgenic potato tubers were developed by the overexpression of SnRK1 (sucrose non-fermenting-1-related protein kinase-1) gene under the control of a PATATIN (tuber-specific) promoter which resulted in a considerable increase (20%−30%) in starch content and a reduction in glucose level (41).

The synthesis of reducing sugar in cold storage tuber is the cause of acrylamide formation during the potato frying which is a major health issue. To reduce the acrylamide content and reducing sugars in cold storage potato, knockout technology was used for silencing the *VInv* gene through TALENs (transcription activator-like effectors nucleases) in the commercial potato ariety (42). Molecular and biochemical analyses of knockout potato revealed that the potato tuber from full *VInv*-knockout plants had undetectable levels of reducing sugars and processed chips accumulated lesser acrylamide with lighter in color. The results successfully provided an outline for using TALENs to rapidly develop important traits in commercially significant auto-tetraploid potato lines. The *Starch Branching Enzyme II* (*SBEII*) gene involved in amylopectin branching was overexpressed in potato (43). Analysis of starch from these transgenic tubers showed an improved degree of amylopectin branching as compared with wild-type. This report showed that the overexpression of *SBEII* using a simple single-intron hybrid intragenic is an effective way to modify potato starch physicochemical properties.

7. PATHWAY ENGINEERING OF POTATO FOR PRODUCTION OF VACCINES

Expression of antigens as vaccines against pathogens in transgenic plants has already been proved a suitable and economical resource for immune-therapeutic molecules. Different antigens, epitopes, adjuvants were over-expressed effectively in plants and shown to keep up their natural functional aspects. The recombinant plant proteins are helpful due to safety level in comparison to other techniques, easier storage without any material costs, cheaper production and large-scale biosynthesis of the biopharmaceuticals and potential elimination of a purification process (44). Lower protein yield and its stability, as well as uncontrolled post-translational changes of proteins affecting the activity and immunogenicity of the recombinant, are the major bottleneck of this technology hampering the use of the plant as bioreactors for biopharmaceuticals. The transgenic plants represent unconventional tools for recombinant protein production. Banana plants were used to develop edible vaccines (human vaccines) due to its property of natural raw eating, popularity as fruit in a larger population. However, the foreign proteins have also been successfully expressed in other plants such as maize, tomato, and potato as a model system.

7.1. Production of vaccines against viral diseases

The transgenic potato over-expressing a gene for the capsid protein of *Norwalk Virus – NVCP* (which causes epidemic gastroenteritis in humans) driven under the tuber specific patatin promoter, was the first report of the development of vaccines in potato (45). Analysis of transgenic potato immunogenicity was done by feeding tubers to the mice in the controlled laboratory conditions, and the IgG antibodies against recombinant Norwalk Virus were readily detected in mice. Later, the same result on the human volunteer and was found to activate the immune system (46).

The rotaviruses are the major causative agents for diarrheal diseases in children throughout the world (47). The transgenic potato developed by over-expressing the recombinant capsid protein (VP6) of rotavirus. Analysis of immunogenicity of recombinant protein by the administration of the potato extract into the mouse enables to detect the antibodies against VP6 protein in all the tested mice (48). The oral immunization by feeding the transgenic potato to mice stimulated the IgG and IgA antibodies against rotavirus capsid protein, and thus represented the advancement in the rotavirus edible vaccine by means of transgenic potato (49). The transgenic potato overexpressing the gene for surface antigen of hepatitis B – HBsAg developed in the laboratory (50). The stimulation of primary immune response was also observed in the mouse fed on this transgenic tuber overexpressing the hepatitis B surface antigen. However, the immunogenicity of antigen reduced in cooked potato which is not a good sign for the use of potato as edible vaccine at least for this disease. Human Papilloma Viruses (HPV) causing cervical cancer is relatively frequent in women; hence, development of a preventative vaccine against this disease is also reported using the genetic engineering. The transgenic potato lines carrying the structural protein of L1 HVP virus (type HVP-16) showed prophylactic vaccination against cervical cancer. An oral administration to mice of these transgenic tubers overexpressing the HVP antigen protein led to induction of immune response proved a novel approach for the management of this disease (51).

The transgenic potato developed by the over expression of the hantavirus nucleo-capsid protein gene from Puumala virus (causative agent *Nefrophatia epidemica*) and the ability to induce the immune response tested in rabbits through intraperitoneal and intramuscular inoculation of tuber extract (52). Positive results in these rabbits proved the advantage of this technique as an alternative for vaccines production due to its stable expression in potato plants. The viral coat proteins (VP1) of Foot & Mouth Disease Virus which causes severe disease in cattle were also expressed in transgenic potato (53). Experiments showed successful detection the IgG antibodies in immunized mice in the controlled laboratory conditions with resistance to foot and mouth disease. High titers value of antibodies in mice also proved that use of transgenic potato tubers is useful for oral immunization. The transgenic potato developed by *Agrobacterium* mediated transformation via the over-expressing the

S1 glycoprotein of the contagious bronchitis virus (IBV). This virus belongs to class coronavirus which is highly infectious affecting the respiratory system in animals. The laboratory mice and chickens immunized with the extract of these tubers were completely protected against bronchitis viruses proving induction of virus neutralizing antibodies in the animals (54). Over expression of full-length S-protein of IBV in transgenic potato and intramuscular immunization in experimental animals with the transgenic tuber extract showed a high titer of anti-IBV antibodies which protected the experimental animals from infection of the virulent IBV (55).

7.2. Production of vaccines against bacterial diseases

E. coli enterotoxin (LT) is a multimeric protein which is structurally like cholera toxin (CT) in terms of function and antigenicity. This enterotoxin has two subunits including the subunit A (LT-A; 27 kDa) and a pentameric subunits B (LT-B; 11.6. kDa). The binding of non-toxic LT-B pentamer to the epithelial cell surface gangliosides allows entry of the toxin LT-A subunits into the cells. The LT-B and CT-B are both potent oral immunogens. The transgenic potato developed by overexpression of thermo-labile enterotoxin B gene of *E coli* driven under the constitutive promoter. The transgenic potato tubers fed to the mouse under laboratory condition and existence of IgA in mouse fecal matter and serum anti-LT-B IgG proved the immunogenic response; however, full immunity against the bacterial disease was not observed during the experiment (56).

8. PATHWAY ENGINEERING FOR THE FUNCTIONAL SECONDARY METABOLITES

Potato steroidal glyco-alkaloids (SGAs) occur throughout the plants but limited to leaves or other areal parts. The SGAs are toxic secondary metabolites and an important part for plant defense which are synthesized by the sterol branching via the mevalonic acid/isoprenoid pathway in metabolism(57). The enzymes involved in the conversion of cholesterol into the various SGAs via mevalonic acid/isoprenoid pathway are still not completely known; however, various glycosylation and glycosyl-transferases genes are well known and characterized in plants (58). The steroidal transferase gene isolated and cloned from soybean were over expressed in potato. The HPLC analysis of the tubers showed a drastic change in total SGAs content (59). This study proved that the SGA content can be modulated in tubers by the genetic engineering of a single gene. Similarly, concentrations of the SGAs in transgenic potato were also modulated by the antisense and sense expression of steroidal glycosyl-transferases genes (57, 59). The SGAs were also modulated in transgenic potato by the over expression of the HMG1 (3-Hydroxy-3-methylglutaryl-coenzyme-A- reductase 1) or SQS1 (squalene synthase 1) genes, and the transgenic potato lines showed a higher content of SGA (steroidal glycoalkaloids) as compared to untransformed controls (60).

The over-expression of flavonoid biosynthetic pathway gene encoding chalcone synthase (*CHS*), chalcone isomerase (*CHI*), and dihydroflavonol reductase (*DFR*) resulted in a significant increase of measured phenolic acids and anthocyanins in transgenic potato (61). The increase in phenolic compounds also resulted in a decrease in starch and/or glucose level of transgenic potato. The flavonoids-enriched plants showed improved antioxidant capacity too. These results finally proved that the most efficacious way to meet this goal was the overexpression of the dihydroflavonol reductase gene (*DFR*) in potato. This transgenic potato fed to the laboratory rat resulted in better health of the rat also proved the nutritional benefits (62). In another study the transgenic potato were developed by the over expression of *ubiC* gene, which encodes chorismate pyruvate-lyase (CPL) that converts chorismate to 4- hydroxybenzoate. The transgenic showed higher accumulation of 4- hydroxybenzoate-glucosides as new, artificial secondary metabolites (63). Interestingly, the 4-HB glucoside content reached up to 4.0. % of dry weight in the leaves of potato shoots without any change in the physical parameters of the plant growth and development.

9. FUTURE PROSPECTIVE AND CONCLUSIONS

Plants have the potential to rapidly produce nutritional supplements, valuable therapeutic compounds and recombinant proteins on a large scale at a relatively low cost compared to other production systems; however, concerns about biosafety, human health (allergenic response to plant-specific glycans), and other factors need to be adequately addressed. Recent advances in the field of structural and functional genomics of crops and the ability to integrate genes of interest into genome have also revolutionized the potato research. An effort to maximize the nutritional and therapeutic potential of potato is still at early stages. This is possible only through genetic transformation technology and its integration with plant breeding program. However, the main obstacle will be consumer approval and acceptance of genetically modified potato as food. Scientific information about various transgenic crops and their introduction in agriculture around the globe will change the perception towards transgenic potato.

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Send correspondence to: Chandrama Prakash Upadhyay, Department of Biotechnology, Dr Harisingh Gour University, Sagar, Madhya Pradesh, PIN Code 470003, India, Tel: 91-7587194330, Fax: 91-7582-261910, E-mail: cpupadhyay@gmail.com