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GENETIC ENGINEERING FOR ENHANCED NUTRITIONAL QUALITY IN POTATO - A REVIEW

Virupaksh U Patil[1*], Rajendra Singh[1], Vanishree G[1], Som Dutt[1], Prashant G Kawar[1], Vinay Bhardwaj[1], and BP Singh[1]

ABSTRACT: Avowed from the reports of FAO, WFP and IFAD, undernourishment alongside vitamin and mineral deficiencies in human diet is the main cause of concern and responsible for the deaths of more than 2.5 million children each year. Moreover, the world population burgeoning at high pace and is expected to reach nine billion by 2050, problems of malnutrition are expected to worsen with the time. Potato is the most important non-grain food crop in the world, ranking 3rd in terms of total production after rice and wheat. Although potato is rich in protein, vitamin C, vitamin B6 and niacin etc., it lacks in providing many other important nutrients. Enhancement of nutrient content of potato through conventional breeding has been found to be a challenging endeavor due to tetraploidy, heterozygosity and lack of variability for the trait of interest. In the recent past, the application of advanced genetic engineering tools to improve the nutritional status of potato has yielded limited success. The review discusses the progress made, challenges faced and lessons learnt from the past studies. It also sets the future agenda for making head way in developing potato as 'complete food' to address the world problem of malnutrition and hunger with the apt use of modern biotechnological tools.

KEYWORDS: Genetic engineering, glycoalkaloids, health compounds, vitamins,

INTRODUCTION

United Nations Food and Agriculture Organization (FAO), records indicate that an estimated 868 million people are undernourished worldwide (FAO, WFP and IFAD, 2012). Undernourishment along with vitamin and mineral deficiencies are responsible for the deaths of more than 2.5 million children per year (FAO, WFP and IFAD, 2012). With a population that is expected to reach nine billion by 2050 (http;//esa.un.org/unpf/index.htm), these problems are only expected to get worse. One solution to this problem is to improve the nutritional quality of staple crops. Although, they provide most of the calories and protein for people in developing countries, staple food crops such as rice, wheat, and the largely consumed tuber crop in the world, potato are not nutritionally complete foods (Hirschi, 2009). As a result, the exclusive consumption of such crops can result in vitamin and/or mineral deficiencies (Bhullar and Gruissem, 2013). Potato is the most important non-grain food crop in the world, ranking 3rd in terms of total production with over 365 million tons per year (FAOSTAT, 2013), after rice and wheat. It is grown in around 125 countries spread across both temperate and tropical regions and at elevations from sea level to 4,000 m. One third of potato production takes place in Asian countries, and over one billion people have potato as their staple diet. It has steadily expanded globally, with 35% increase in overall production since 1960. The increase in production is still higher in developing countries of Asia and Africa (**Fig. 1**) indicating its importance as a staple food source. Therefore, it is need of time to improve the nutritional quality of potato. Though, potatoes are second only to soybean for amount of protein per hectare, with the major storage protein being patatin, of the most nutritionally balanced plant protein

[1]ICAR-Central Potato Research Institute, Shimla 171001, Himachal Pradesh, India
[*]Email: virupakshagouda.patil@icar.gov.in

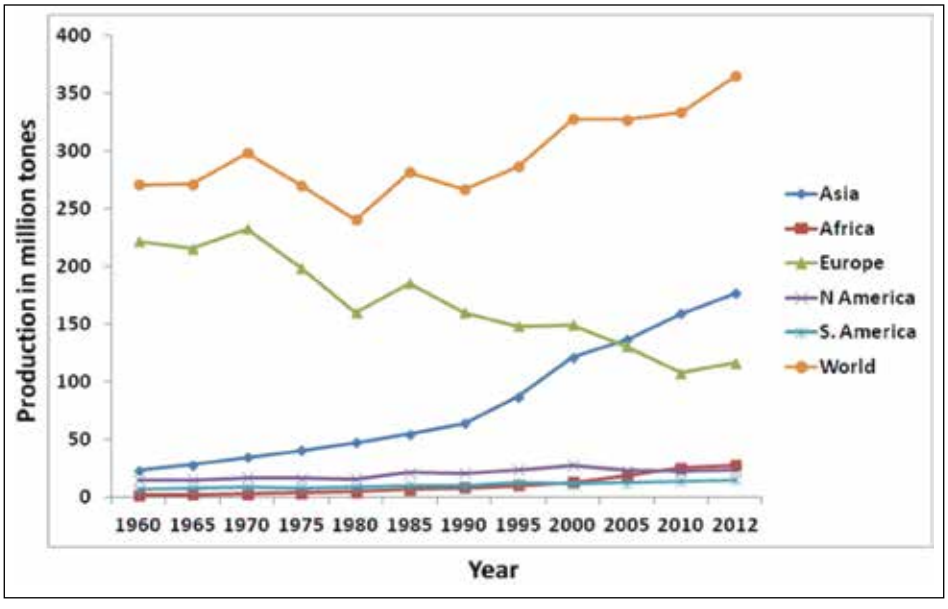

Figure 1: Trends in the world potato production from 1961-2012 (FAOSTAT, 2014)

known till date (Liedl *et al.*, 1987). Though, a single 150g tuber provides up to 45% of recommended daily allowance (RDA) for vitamin C, 10% vitamin B6, 8% niacin, 6% folate with significant amounts of other essential mineral nutrients required, it lacks in providing many other essential nutrients required for human consumption. A detailed nutritional content of potato is presented in table 1.

Though conventional breeding has addressed these problems in potato by developing coloured potatoes and potatoes with improved nutrients, progress is very slow and limited due to the genetic diversity available in a tetraploid potato gene pool (Hirschi, 2009). Of particular significance is the fact that vitamin and nutrient content has not been a focus of most potato breeding programs. Essentially released varieties have been selected for disease resistance, processing quality, and other agronomic traits.

Limited studies have shown that potato germplasm contains a wide range of phyto-

Table 1: Nutritional composition of potato (source: Lister and Munro, 2000)

Substance	Range (%)	Mean (%)
Dry matter	13.1-36.8	23.7
Starch	8.0-29.4	17.5
Reducing sugars	0.0-5.0	0.3
Total sugars	0.05-8.0	0.5
Crude fibre	0.17-3.48	0.71
Pectic substance	0.2-1.5	-
Total nitrogen	0.11-0.74	0.32
Crude protein	0.69-4.63	2.00
Lipids	0.02-0.2	0.12
Ash	0.44-1.87	1.1
Ascorbic acid	21.7-68.9*	-
Glycoalkaloids	0.2-41**	3-10
Phenolic compounds	5-30**	

*mg/100g, **µg/100g

nutrients and there is ample opportunity for significant increase in the nutrient content of potatoes through conventional breeding. But one of the major difficulties associated with conventional potato breeding is the narrow genetic base of existing potato cultivars because of the limited genetic

stock have been introduced from the South American centre of origin to Europe and Asia. The tetrasomic inheritance and high heterozygosity adds considerable complexity to potato breeding (Conner *et al.*, 1997) resulting the accumulation of desired alleles difficult. Applications of plant biotechnology over the past five decades have helped to facilitate interspecies crosses, and towards augmentation and broadening the cultivated gene pool. Many elite potato genotypes are highly responsive in cell culture and provide opportunities for applications of biotechnology to potato improvement. In addition, there is an over-increasing evidence that the use of transgenic technologies can improve nutrient content still higher and introduce additional vitamins and other beneficial compounds not usually present in potato tubers (Jeff Suttle, 2008).

Genetic engineering of potato

Genetic modification has many advantages for plant breeding, and these advantages are even more striking in crops with complex inheritance such as potato. While conventional breeding manipulates genomes in a largely uncontrolled fashion, requiring generations of selection to assemble and fix the maximum number of desirable traits, transformation offers a direct approach, allowing introgression of a single, distinct gene without linkage drag (Rommens *et al.*, 2004). Thus, genetic modification allows rapid and often powerful improvement of crop plants, and is not limited by compatibility barriers. In cases where genetic diversity among sexually compatible relatives of crop species is insufficient for a particular trait, genetic modification may represent the only possibility for improvement in that trait. Transformation offers a highly effective means of adding single gene to existing elite potato clones with no or very minimal disturbances to their genetic background (Conner *et al.*, 1997).

Like most *Solanaceous* species, potato is readily transformed by *Agrobacterium tumefaciens*. Potato was first transformed in 1987 in order to compare the expression of an organ-specific endogenous gene and a tagged variant introduced by *Agrobacterium* mediated transformation (Stiekema *et al.* 1988). In 1987, De Block and colleagues reported the generation of herbicide resistant transgenic potato expressing the *bar* gene. Soon after these initial transformation experiments, the Monsanto Company announced the creation of transgenic potatoes resistant to potato viruses X and Y (Newell *et al.*, 1991). To date, potato has become one of the model crops for transformation studies. The progress was motivated by the advantages that transformation offered for the genetic improvement in potatoes. Till date various tansformation approaches have been successfully applied in potato like, *Agrobacterium tumefaciens* mediated (Ooms *et al.*, 1986), *Agrobacterium rhizogenes* mediated (Stiekema *et al.*, 1988), direct DNA uptake (Valkov *et al.*, 2011), particle bombardment (Romano *et al.*, 2003), PEG mediated (Craig *et al.*, 2006) and ensife adherence (Wendt *et al.*, 2012). However, among these *Agrobacterium* mediated gene transfer is the most preferred approach and is being routinely performed in laboratories worldwide.

Targets for improvement through genetic engineering

Plants provide a diverse array of chemicals important in the human diet from the nutritional and health point of view (Hirschi, 2009). These phyto-chemicals can be divided into two groups based on their abundance in the plant. Major constituents are present in grams per 100 g of food and include proteins, carbohydrates and lipids. Minor

components are found in micrograms or milligrams per 100 g of food and include vitamins, minerals and health-enhancing secondary metabolites such as antioxidants (Grusak, 2002). Genetic engineering of both types of constituents is possible; however, it is generally considered that alterations in quantities of major constituents are much more difficult than quantitative changes in minor constituents (Grusak, 2002). This is because quantitative changes to major components require the diversion of a substantial amount of precursor(s) from other pathways and may present a storage problem. As a result, modification of proteins, carbohydrates and lipids has largely been confined to qualitative changes. Significant alterations in both quantity and quality of minor components are widely reported in many crops including potato. In the succeeding sections, progress toward engineering various types of major and minor constituents for the improvement of potato nutritional quality is discussed. Major studies carried out in potato to improve its nutritional quality have been summarized in Table 2.

Table 2: Potato transgenics with enhanced nutritional qualities

SN	Nutrient	Subgroup	Gene(s) targeted	Total increase (fold)	Reference
1.1	Vitamins	VitA	EuCrtB, EuCrtI, EuCrtY	114 µg/g DW (20)	Diretto et al., 2007
1.2			BoOr	28.22 µg/g DW (6)	Lopez et al., 2008
1.3			AtZEP	60.8 µg/g DW (5.7)	Romer et al., 2002
1.4			PaCrtB	35.5 µg/g DW (6.3)	Ducreux et al., 2005
1.5		VitC	*StVTC2A*	1.65 mg/g FW (3)	Bulley et al., 2011
1.6			StDGAR	(2)	Hemavathi et al., 2009
1.7			StDHAR	-	Qin et al., 2011
1.8		VitE	*At-HPT*	106	Elizabath et al., 2008
2.1	Minerals	Ca	*Scax1*	1.7 mg/g DW (3)	Park et al., 2005
2.2			*Cax2b* chimeric	2.5 mg/g DW (3)	Kim et al., 2006
3.1	Starch	Starch	*SuSY*	55-85%	Fernandez et al., 2009
3.2			PsGTP, AtNTT1	28%	Zhang et al., 2008
3.3			StGTP	(2)	Claidia et al., 2012
3.4		Waxy starch	*Amylo-sucrase*	(2)	Xing et al., 2014
3.5		Amylose	*SSII, SSIII*	2.88 -29.05%	Du Hong hui et al., 2012
3.6			SBEI, SBEII	60-89%	Scwall et al., 2000
3.7			SBEI, SBEII	(1.5-3)	Andersson et al., 2006
4.1	Protein	Total protein	*AmA1*	48%	Chakraborty et al., 2010
4.2		*Met*	$C_{\mathcal{S}}S_{\partial 90}$	(6)	Dancs et al., 2008
4.3			CAT-HEAAE fusion	0.2-0.35% of tuber protein	Kim, 1992
4.4			StTA	(30)	Michael et al., 2001
4.5		*Met* and *Thr*	AK & DHDPS	(8-*Thr* & 2-*Met*)	Robert et al., 2006
5.1	Health Promoting Compounds	Anthocyanine	St3GT	0.8 to 1.6 µg/mg FW (2-4)	Qing et al., 2012
5.2			St3GT	(3)	Wei et al., 2012
6.1	Reduction of Toxicants	Glycoalkaloids	*GmSTM1*	48-63%	Lisa et al., 2003

Starch engineering

Starch is the predominant storage macromolecule in potato tubers. It consists of two types of high molecular weight D-glucose polymers; amylose- mostly a linear α (1–4) D-glucan polymer with limited branches, and amylopectin-a much larger molecule with extensive branches resulting from both α (1–4) and α (1–6) linkages (Smith *et al.*, 1997). Starch is synthesized in chloroplasts for temporary storage, known as transitory starch, and for long-term storage it is synthesized in the amyloplasts of the non photosynthetic plants parts such as seeds, roots and tubers (underground stem). In plants, storage starch consists of appx. 25-30% amylose and 70-80% amylopectin. However, the ratio of amylose to amylopectin has profound influence on the physico-chemical properties of the starch (Denyer *et al.*, 2001). Potato starch is packed in granules that typically contain amylose and amylopectin in the ratio of 1:3 (Jansen *et al.*, 2001). The branched structure of amylopectin allows for greater digestibility than linear chain structure of amylose, which leads to higher glycemic response. Nutritionally resistant starch (or more slowly digested starch) is considered advantageous as it provides similar health benefits to fermentable fibre, resistant starch degradation products that are not absorbed in small intestine. Higher-amylose starches have greater retrogradation following processing compared with those having more amylopectins. Higher amylose starches also reduce oil penetration, so are favored in processed food (Tarn *et al.*, 2006). Currently, developing potato cultivars with high amylose content is one of the priority research areas of crop biotechnology.

The potato starch has unique physical and chemical properties, such as the low gelatinization temperature, the high degree of white and high degree of polymerization compared with starches from other crop species (Swinkels, 1995). Genes coding for soluble starch synthase (*SSIII*) is responsible for 80% of total soluble starch activity whereas *SSII* accounts for about 10-15% of the activity. Gene silencing of potato *SSIII* resulted in increase of amylose content by 2.68-29.05% in transgenic potato compared to the control. The amylopectin to amylose ratio was also reduced and the phosphorus content of tuber starch was reduced by 34 to 56% which increased the granular structure quality of potato (Du Hong-hui *et al.*, 2012). When, both *SSII* and *SSIII* genes were silenced using antisense technology in potato, resulted in reduction of branches of amylopectin and improving the quality of starch. The freeze-thaw quality of these transgenic potato was better than the maize produced using conventional breeding to manipulate the genes *wx* (waxy) and *su2* (sugary2) (Jobling *et al.*, 2002). In cereals, the high-amylose phenotype is caused by a mutation in the gene that encodes starch-binding enzyme (SBE), which is also known as *'amylose extende (ae)'*. In potato, discovery of corresponding gene and down regulation of its expression in tubers using antisense technology enabled the production of starches that have slightly increased amylose level (Jobling *et al.*, 2002). Whereas, inhibition of two isoforms of the *SBE* gene, *SBE I* and *SBE II* by antisense technology increased the tuber amylose content to 60-89% as compared to 21-29% in wild type (Schwall *et al.*, 2000). This astonishing increase in the resistance starch improved the quality of starch as well. A more efficient method of inhibiting the gene function using single-domain antibodies against SBE II was used to produce starches that had even higher amylose level (Jobling *et al.*, 2003). In another study same *SBEI* and *SBEII* genes were silenced using RNAi technology and transgenic potato lines had 1.5 to 3-fold increase in yields alongside increase

in tuber amylose content also which was up to 60% as compared to 25-30% in control tubers (Andersson *et al.*, 2006). These potatoes with higher amylose content soften during cooking; indicating that swelling pressure generated by the intracellular starch granules has no role in this process, and the texture remains 'succulent' as they contain more free water than normal potato tubers. The average chain length of potato amylose is much greater than that of cereal amylose (Jobling, 2004). So, the new high-amylose potato starches would certainly have improved functionality.

Very recently over expression of *amylo-sucrase* gene from *Neisseria polysaccharea* fused to potato starch binding domain (SBD) in transgenic potato tubers resulted in starch granules with rough surface, a 2-fold increase in median granule size, improved freez-thaw stability, higher end viscosity and better enzymatic digestibility. These altered physico-chemical properties of potato starch called the 'waxy potato starch' has improved paste, clarity and stability and can be expected to find applications in both the food industry and in paper manufacture (Xing *et al.*, 2014). Amylose synthesis requires just a single gene, whereas amylopectin synthesis involves concentrated action of several enzymes including starch synthase, branching enzymes and de-branching enzymes, each of these have multiple isoforms (Jobling, 2004). So, many of the researchers have concentrated their efforts on amylose pathway genes than amylopectin for manipulations.

Potato starch is unique among commercial starches in having a high level of phosphate groups that are covalently linked to the C6 and C3 positions of the glucose monomers. These phosphate groups, coupled with large size of granules give this starch very high swelling power and stable-paste properties (Jobling, 2004). The enzyme responsible for the incorporation of phosphate group is identified as an α-glucan water dikinase (GWD) (Ritte *et al.*, 2002). Antisense inhibition of the gene that encodes this enzyme resulted in starch with low content and viscosity (Lorberth *et al.*, 1998). Sucrose synthase (*SuSy*) is another gene involved in starch synthesis. It is highly regulated enzyme that catalyzes the conversion of sucrose and a nucleoside diphosphate into the corresponding nucleoside diphosphate glucose and fructose. Over expression of *SuSy* gene in tubers increased the starch content by 55-85% than in control tubers. Tuber dry weight, starch content per plant and total yield of *SuSy* over expressing tubers increased significantly over those of control plants (Fernandez *et al.*, 2009).

In another study, transgenic potato plants simultaneously over-expressing pea (*Pisum sativum*) glucose 6-phosphate/phosphate translocator (*GTP*) and *Arabidopsis thaliana* adenylate translocator (*NTT1*) in tubers increased the starch content upto 28% compared to control plants. It was also observed that single gene expression of either gene had no effect on tuber starch content (Zhang *et al.*, 2008). Later the same group increased both starch content and the yield of potato by over expressing endogenous glucose 6-phosphate/phosphate translocator and an adenylate translocator in tubers. Using this source and sink (pull and push) approach the tuber starch content was doubled compared to control (Claidia *et al.*, 2012). Besides, few other genes of importance for starch synthesis have also been sequenced, characterized and transformed.

Protien engineering

Protein is considered the most important nutrient for humans and animals as manifested by the origin of its name, from the Greek *proteios* for primary. Plant proteins contribute about 65% of the per capita supply of protein

on worldwide basis, with cereal grains, tubers and food legumes as the most important suppliers. A major effort has been to improve the amino acid composition of plant protein because animals, including humans, are incapable of synthesizing 10 of the 21 amino acids required for protein synthesis, and these "essential amino acids" must therefore be obtained from the diet (Chakraborty *et al.*, 2010). Tuber crops and most of the vegetable proteins are deficient in sulphur containing amino acids [metheonine (Met) and cysteine (Cys)]. Protein malnutrition is essentially caused by poor quality diets that include a high intake of staple crops with less protein and/or low-quality proteins in terms of amino acid composition. Protein deficiency lowers resistance to disease, delays physical growth and development, and may cause permanent impairment of the brain in infants and young children. In comparison with meat, plant proteins are much less expensive to produce. Because of the importance of dietary protein and the fact that plants are its major source, development of strategies to increase protein levels and the concentration of essential amino acids in food crops is of primary importance in a crop improvement program. There have been several attempts through mutant selection and engineering genes encoding key amino acid biosynthesis pathway enzymes to increase free essential amino acids in crop plants (Matthews and Hughes, 1993), but with limited success (Falco *et al.*, 1995). Recent advances in biotechnology allow the use of transgenic approach to increase the content of specific essential amino acids in a target plant. It was first demonstrated by the significant enhancement of Met content in tobacco seed proteins through expressing transgene encoding a Met-rich protein from Brazil nut (Altenbach *et al.*, 1989). Several other molecular approaches have been developed, including synthetic protein, modified protein sequence, over-expression of heterologous or homologous protein and metabolic engineering of the free amino acid pool and protein sink. Significant progress has been achieved by applying these approaches and future research directions are emerging from these studies.

Potato protein ranges from 1-1.5% of tuber fresh weight (Ortiz-Medina, 2007). Compared with other, it is negligible a source, potatoes are not typically considered to be good dietary protein sources due to their low overall protein content (Mary *et al.*, 2009). In an attempt to increase the overall protein content of potato (Chakraborty *et al.*, 2010) expressed the *AmA1* gene from *Amaranthus* seed albumin in potato tubers. The transgenic potato lines called "ProTato" had 48% increased protein content than the wild/non-transformed potatoes. The results were consistent in two years bio-safety trials. The paradise nut 2S seed protein is abundant Met residues (16 mol %). To explore the feasibility of further increasing Met content of this protein, modifications were made in the sequence region between the Cys-6 and Cys-7 codons of *PN2S* cDNA to contain 19, 21, and 23 mol% Met, respectively. All the three modified Met-rich *PN2S* were expressed, processed and accumulated in transgenic tobacco seeds (Zuo, 1993). The same modifications were also made in the Brazil nut 2S (BN2S) protein, and the chimeric genes were used to transform potato. Results revealed that the mutated Met-enriched BN2S proteins were expressed and accumulated as well as normal 2S protein in the leaves and tubers of transgenic potato (Tu *et al.*, 1998). In another study attempts were made to increase the Met content in potato tubers through heterologous over expression of *Arabidopsis* cystathionine γ-synthase ($CgS_{\partial 90}$), which is not regulated by Met in potato plants

and a storage Met rich 15-kD zein in Desiree cultivar. There was 6-fold increase in free Met content and in the Met content of the zein-containing protein fraction of the transgenic tubers. In addition, in line with higher Met content, the amounts of soluble isoleucine and serine were also increased. However, all the lines with higher Met content $CgC_{\delta 90}$ expressions were phenotypically abnormal showing sever growth retardation, changes in leaf architecture and 40-60% reduction in tuber yield. Furthermore the color of the transgenic tubers was altered due to reduced amounts of anthocyanin pigments. Consecutive over expression of $CgS_{\delta 90}$ in *Arabidopsis* caused 8- to 20-fold elevation of the Met content (Dancs *et al.*, 2008).

Progress in understanding the structure, function, folding and topology of proteins allow the design and synthesis of a gene encoding a new protein with desirable essential amino acid composition. Jaynes *et al.* (1986) first synthesized a 192-bp DNA encoding a polypeptide composed of 80% essential amino acids. The high essential amino acid encoding DNA (*HEAAE-DNA*) inserted into the chloromphenicol acetyltransferase (*CAT*) coding sequence to generate a CAT-HEAAE fusion protein (Yang *et al.*, 1989). Transgenic study indicated that CAT-HEAAE protein was accumulated at 0.02 to 0.35% of total tuber protein in transgenic potato. Based on the structurally well studied maize zeins, the group later designed and synthesized another artificial storage protein (ASP1) composed of 78.9% essential amino acids and estimated to possess a more stable storage protein like structure in plants (Kim, 1992). The 284-bp *asp1* gene, under the control of *CaMV 35S* promoter, was normally expressed in transgenic tobacco leaves resulting in the accumulation of relatively high levels of ASP1 proteins. Surprisingly, the overall levels of total amino acid and protein were found to be increased remarkably in transgenic potato and sweet potato.

The schematic diagram (Figure 2) of synthesis of lysine, the end product lysine can block the activity of the first key enzyme in the pathway that is common to all of the aspirate-family amino acids aspartate kinase (AK), when it reaches a certain threshold level. In addition, lysine can also inhibit the activity of DiHydroDipicolinate Synthase (DHDPS), the first enzyme of the pathway often the branch point that leads to the synthesis of the lysine. DHDPS is even more sensitive to lysine than AK (Robert *et al.*, 2006).The same group introduced bacterial origin genes AK and DHDPS that are 100 fold less sensitive to feedback inhibition of lysine into potato, which increased lysine by 6-folds, moreover an 8-fold increase in threonine and 2-fold increase of methionine was also observed. Later the same group isolated the potato gene encoding DHDPS and changed one amino acid residue to render the enzyme feedback insensitive. Introduction of this desensitized potato gene back into potato resulted in dramatic increase of lysine content. The lysine level reached upto 15% of the total amino acid level as compared to 1% in the untransformed potato.

Gene silencing by RNAi technology also have been tried in potato to increase the essential amino acid content. The threonine synthase (*TS*) involved in synthesis threonine in potato was targeted for silencing so as to divert the cycle and increase the Met content (Fig 2). A reduction of 6% TS activity levels in transgenic potato which increased the methionine levels upto 30-fold developing on the transgenic line and environmental conditions and had no reduction in threonine (Michaela *et al.*, 2001).

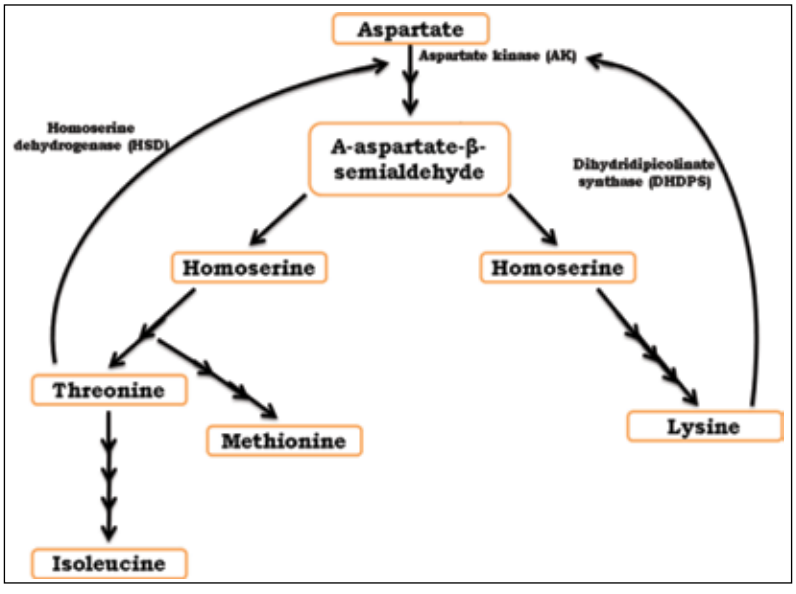

Fig 2: Schematic overview of family amino acid biosynthesis pathway. Only the key enzymes are indicated. Curved arrows indicate the feedback inhibition by the end-product amino acids.

Vitamins engineering

Vitamins are a class of organic compounds, absolutely required for the maintenance of healthy life processes. Though they are required in small amounts, their capability of sustenance and their ability to perform biochemical functions is remarkable. Vitamins are the compounds that cannot be synthesized by humans and thus need to be taken up in the diet. The study of vitamins is very important in understanding the biological processes. Based on the solubility, vitamins have been grouped into water soluble vitamins and fat soluble vitamins. Fat soluble vitamins are A, D, E and K and the rest are water soluble. Most of the vitamins have been found to act as coenzymes, some act as growth regulators and most of them as antioxidants. Well known human vitamin related disorders include blindness (Vitamin A), beriberi (Vitamin B1), pellagra (Vitamin B3), anemia (Vitamin B6), neural defects in infants (Vitamin B9), scurvy (Vitamin C), sterility related diseases (Vitamin E), and ricketsia (Vitamin D).

Vitamin in plant derived foods can be increased through fortification in processed foods, conventional breeding or through use of transgenic techniques, a process known as biofortification (Amparo and Sergi, 2010). Predominant vitamin in potatoes is vitamin C (AsA), which ranges from 84 to 145 mg per 100g DW depending on cultivar and soil composition (Camire *et al.*, 2009). Potato also contains several B vitamins (Folic acid, niacin, pyridoxine, riboflavin and thiamin). The different vitamin composition of potato is given in table 3. L-ascorbic acid (AsA, Vitamin C) is required for prevention of scurvy and maintenance of healthy skin, gums and blood vessels, and also known to have many biological functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other radicals (Brown, 2008). More than 90% of AsA in human diets is supplied by fruits and vegetables. It has been suggested that 100-200

Table 3: Different vitamin content of potato (Source: Christelle *et al.*, 2008)

Substance	Quantity (µg/100g tuber)
Ascorbic acid (As A, Vitamin C)	10,000-25,000
Vitamin B1 (Thiamin)	100
Vitamin B2 (Riboflavin)	70
Vitamin B6 (Pyridoxin)	-
Vitamin B3 (Niacin)	1000
Vitamin B5 (Pantathonic acid)	190-320
Vitamin B9 (Folic acid)	5-33
Vitamin B7 (Biotin)	0.6
Provitamin A (β-carotene)	11-56
Phytonadione	60-80
Vitamin B12 (cobalamine)	-
Vitamin D (Calciferol)	-

mg AsA should be supplied by human diets and this quantity is expected to be increasing because of increasing stress in modern life. Therefore, it is valuable to increase AsA content in edible products of plant. In India the available supply of vitamin C is 43mg/ capita/day, and in the different states of India it ranges from 27 to 66mg/day which is far below the recommended dose of 400 mg/day by the WHO.

Although little is known about the AsA biosynthatic pathway in plants, modifications of vitamin C content in crops was achieved in several way. In the very first study, a rat L-gulone-γ-lactone oxidase gene was transformed into lettuce to obtain a 7-fold increase in vitamin C (Jain and Nessler, 2000). The rat gene was also constitutively expressed in potato giving rise to 40% higher ascorbate accumulation and increased abiotic stress tolerance (Hemavathi *et al.*, 2010). Over expression of D-galacturonic acid reductase from strawberry was used to increase vitamin C levels in *Arabidopsis* by two- to three-fold (Agius *et al.*, 2003). This enzyme reduces D-galacturonic acid or L-galactonic acid in the pathway for ascorbic acid biosynthesis via uronic acids. Its over expression in potato gave rise to two-fold increase in tuber ascorbate content, accompanied by increased drought, salt and oxidative stress tolerance with respect to wild type plants (Hemavathi *et al.*, 2009). Qin *et al.* (2011) transformed potato with its native cytosolic and chloroplastic targeted *DHAR* cDNAs, each under the control of the *CaMV 35S* promoter. Over-expression of cytosol-targeted *DHAR* led to increased ascorbate content in both tubers and leaves while over-expressing the chloroplastic enzyme also affected leaf ascorbate content.

Cultivated potato is extremely poor in pro-vitamin A, another very important vitamin required for life. Vitamin A deficiency (VAD) is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections. An estimated 250 million preschool children are vitamin A deficient and it is likely that in vitamin A deficient areas a substantial proportion of pregnant women are vitamin A deficient. An estimated 250 000 to 500 000 vitamin A-deficient children become blind every year, half of them dying within 12 months of losing their sight. The carotenoid content of tubers in most potato cultivars ranges between 0.5 and 2.5 µg per gram FW. The main carotenoids are the xanthophylls lutein and violaxanthin, which are devoid of pro-vitamin A activity. The main pro-vitamin A carotenoid, β-carotene, is present only in trace amounts, from undetectable levels in most cultivars and breeding lines, up to 0.03 µg/g FW. Even, the β-carotene to retinol conversion efficiency is very low (21 µg of β-carotene per 1 µg retinol) entailing that there is ultimately no vitamin A in potato (Van *et al.*, 2010).

Although a staple food crop, potato is significantly poor in β-carotene (Breithaupt and Bamedi, 2002). However, metabolic

engineering efforts to accumulate high levels of β-carotene in potato tubers proved successful (Diretto *et al.*, 2006, Diretto *et al.*, 2007, Lopez *et al.*, 2008). Two potato cultivars were selected for work to increase the carotenoid content of potato tubers. *S. tuberosum* cv Desiree, which typically accumulates 5.6 µg per g DW carotenoids with negligible β-carotene content and *S. phureja* cv. Mayan Gold which typically accumulates 20 µg per g DW carotenoids (Ducreux *et al.*, 2005). Both cultivars were transformed with the *crtB* gene (for phytoene synthase) from *Erwinia uredovora*. Transgenic potato showed an accumulation of 35 total carotenoids and 11µg per g DW β-carotene in developing tubers of Desiree and 78 µg per g DW in Mayan Gold tubers. In another study with a similar objective, the gene encoding *Lcy-e* was targeted with a tuber specific antisense construct in order to suppress epsilon cyclization of lycopene and direct the flux towards β-β-carotenoid branch (Diretto *et al.*, 2006). Results indicated a tuber-specific increase in the accumulation of β-carotene (up to 14-fold) and β-β-carotenoids (up to 25-fold) with a decrease in accumulation of lutein. When the β-carotene hydroxylation step of the β-β-carotenoid branch was targeted by tuber specific antisense silencing of the hydroxylase *chy1* and *chy2*, a 38-fold increase in tuber β-carotene content was achieved (Diretto *et al.*, 2007). Some other successful examples include a profound production of β-carotene in golden rice (Paine *et al.*, 2005), orange tomato (Brigelius and Traber, 1999).

Vitamin E is another essential nutrient for human health, but is consumed at suboptimal levels. The importance of vitamin E for reproductive health was recognized as early as 1922 (Brigelius and Traber, 1999). Humans and other animals are not capable of synthesizing tocopherol (vitamin E) autonomously and must be obtained from their diet. The enzymes involved in vitamin E synthesis are all membrane bound and localized to the chloroplast inner membrane, with exception of p-hydroxyphenylpyruvate dioxygenase (HPPD), which has been shown to be cytosolic (Garcia *et al.*, 1999) and all of the genes in Vitamin E pathway have been cloned, many of which were identified using a genomic approach based on sequence homology between the model plant *Arabidopsis thaliana*. Over expression of *Arabidopsis At-HPPD* and Homogentisate phytyl transferase (*At-HPT*) genes in potato transgenics was carried in an attempt to increase vitamin E content of potato. *At-HPPD* resulted in maximum 266% increase in α-tocopherol and over expression of *At-HPT* yielded a 106% increase in potato (Elizabeth *et al.*, 2008).

Mineral content

Human require various minerals to maintain health and for proper growth (Welch, 2002). For example Iron and Zinc deficiencies result in decreased immune function and can interfere with growth and development (Zimmerman and Hurrell, 2002). It is estimated that Iron deficiency alone affects one third of the world's population and causes 800,000 deaths worldwide each year (Masuda *et al.*, 2012). Plants are essential source of such minerals (Welch, 2002). The minerals present in greatest concentrations in raw potato (Buckenhuskes, 2005) **(**Table 4). Skin on potatoes is considered a good dietary source of potassium. Potatoes contain relatively little phosphorous in the form of phytate. Biofortification has been attempted for micronutrients in potato (White and Broadley, 2009).

Because plants cannot synthesize these minerals, they must be acquired from soil. As a result, engineering of plant mineral content is quite different from modifications

Table 4: Mineral composition of tuber ash (Source: Christelle *et al.*, 2007)

Element	Quantity (mg/100g DW)
Potassium (K)	1400-2500
Phosphorous (P)	120-600
Chlorine (Cl)	45-800
Sulphur (S)	40-400
Magnesium (Mg)	45-220
Calcium (Ca)	27.1-109.3
Silicon (Si)	5-89
Iron (Fe)	2.9-15.7
Alluminium (Al)	0.2-35
Manganese (Mn)	0.5-8
Zinc (Zn)	1.2-2.9
Copper (Cu)	0.06-2.8

of compounds like vitamins that the plant itself synthesizes. Transport of minerals within the plant presents an additional level of complications. Although most minerals are transported through xylem, some minerals are transported by phloem (Grusak, 2002). The xylem moves minerals to those tissues/organs with the greatest water loss-mostly to leaves. In contrast the phloem preferentially transports compounds from source (photosynthesis) organs to sinks such as seeds, fruits, and tubers. Thus, depending on the method of transport and the consumed part of the plant mineral content improvement may also require modification of the plant's normal transport system.

Research to improve the mineral composition of crop plants has mostly focused on Iron content. Several reports exist in this particular area, most of which describe research that was performed on Iron biofortification in rice crop (Drakakaki *et al.*, 2000, Vasconcelos *et al.*, 2003). Very less research in this regard in potato is carried out worldwide. An attempt was made to over express *Arabidopsis sCAX* (Cationic Exchanger 1) and H+/Ca2+ transporter genes in potato. Transgenic tubers expressing *sCAX1* displayed up to three-fold more calcium content compared to wild type without significant alteration in growth and development. The trait was also found to be stably inherited when monitored over three generations (Park *et al.*, 2005). In other work, a chimeric, N-terminus truncated *Arabidopsis* cation transporter (*CAX2B*) that contains a domain from *CAX1* for increased substrate specificity was over expressed in potato to improve calcium accumulation. The transgenic plants had 50% to 65% improved tuber calcium content relative to wild type, with stable inheritance and no deleterious effects on plant growth or development (Kim *et al.*, 2006).

Antioxidants and other health promoting compounds

Diets rich in antioxidant flavonoids and carotenoids have been associated with a lower incidence of atherosclerotic heart disease, certain cancers, muscular degeneration and severity of cataracts (Kruezer, 2001). Arguments for the health benefits of antioxidants are largely correlated to diet composition v/s disease and morbidity in populations. The consumption of antioxidant-rich foods results in the maintenance of higher antioxidant levels in blood serum (Mazza *et al.*, 2002). Phenolic acid and polyphenols play important role in plant health, cooking properties and human health (Friedman, 1997). Chlorogenic acid is the predominant phenolic compound in potatoes, as other solanaceaus species such as eggplant (Prohens *et al.*, 2007).

Cultivated potato tuber skin contains 2000-5000 pg per g FW phenolic acids and 200-300 ~tg of flavonoids whereas, the tuber flesh contains lower concentrations ranging from 100-600 pg of phenolic acids and 0 to 30 ~tg of flavonoids (Lewis *et al.*, 1998).

Purple- and red-skinned tubers contained twice the concentration of phenolic acids and flavonoids as white-skinned tubers. Flavonoids in order of abundance are catechin, epicatechin, erodictyol, kaempeferol, and naringenin. The predominant phenolic acids are chorogenic acid, protocatechic acid, vanillic acid, and p-coumaric acid (Lewis *et al.*, 1998). Carotenoids are a class of flavonoids with red, orange, and yellow pigments that are widely distributed in flowers, fruits and vegetables. These are synthesized in nearly all types of plastids in plants, but accumulate in high levels in chromoplasts and chloroplasts (Howitt and Pogson, 2006). Chromoplasts develop a unique mechanism to accumulate massive amounts of carotenoids by generating novel carotenoid sequestering structures (Vishnevetsky *et al.*, 1999). These structures probably serve as a metabolic sink to sequester carotenoids and may also prevent the end-products of the carotenoids biosynthetic pathway from overloading chromoplast membranes, the site of carotenoid biosynthesis (Rabbani *et al.*, 1998). Thus creating such a metabolic sink can exert a positive effect on carotenoid accumulation. Carotenoids and their derivative xanthophylls are diverse lipid-soluble pigments, in potato xanthophylls are the most abundant carotenoids (Brown, 2008). Two of these pigments, present in low concentration in potato (β-carotene and lutein), have an important role to play in eye health. Carotenoid content ranges from 57-750 µg per 150g FW, there are more carotenoids in colored potatoes than white fleshed potatoes (Buckenhuskes, 2005). A promising genetic tool for carotenoid biofortification is the novel orange (*Or*) mutation isolated from cauliflower (*Brassica oleracea* var. *Botrytis*) (Zhou *et al.*, 2008). The mutant orange phenotype is due to the accumulation of carotenoids caused by the differentiation of proplastids into chromoplasts. The mutant phenotype was also confined in potato tubers carrying the *Or* transgene (Lu *et al.*, 2006, Lopez *et al.*, 2008).

Phytochemicals are secondary products of plant metabolism, many of which are implemented in human health as antioxidants. These vary in amount and composition among potato cultivars (Brown, 2008). Improvement of potato antioxidant capacity through improving tuber anthocyanin pigmentation has also been a focus of research. Tissue specific accumulation of red and purple pigments in the tuber skin requires a dominant allele of locus *D* (*developer*), a gene that has been known since 1910 (Salaman, 1910). D maps to a region of chromosome 10 (Van *et al.*, 2010), that harbors *MYB* transcription factor homologs that regulate anthocyanin biosynthesis. The pigmentation of tuber flesh (*pf*) locus is tightly linked to locus *D* (De Jong 1987), and QTLs identified on chromosome 5, 8 and 9 (Zhang *et al.*, 2009) also regulate tuber flesh pigmentation. A candidate gene for locus *D* was identified and constitutively expressed in white skinned and light red-colored cultivars and two white skinned diploid clones that lack dominant allele of the *D* locus. Transgenic tubers had uniform skin pigmentation, accompanied by pigmentation of tuber flesh, peals, and foliage (Jung *et al.*, 2005). However, flesh anthocyanin pigmentation was neither complete nor uniform, implying that a functional allele of *D* is insufficient for complete coloration of tuber flesh and that specific alleles of QTLs that influence flesh coloration are also required. Anthocyanidin 3-o-glucosyltransferase is a key enzyme in anthocyanin biosynthesis with a critical role in anthocyanin stability and water solubility (Wei *et al.*, 2012).

Anthocyanins are potential source of natural coloring in potato. Anthocyanidin 3-o-glucosyltransferase (*3GT*) is a key enzyme in anthocyanin biosynthesis, and it catalyzes the transfer of the glucosyl moiety from UDP-glucose to 3-hydroxyl group of anthocyanidin. This is critical for improving the stability and

water solubility of anthocyanins (Yoshihara *et al.*, 2005). Transgenic potato plants over expressing the anthocyanin biosynthetic gene dihydroflavonol 4-reductase (*DFR*) showed an increase in tuber anthocyanin content, and inhibiting the expression of the gene caused a significant decrease in the anthocyanin levels (Stobiecki *et al.*, 2003). Jung *et al.* (2005) introduced an anthocyanin biosynthetic gene flavonoid 3′, 5′-hydroxylase (*F3′5′H*) into the red skinned potato Desiree by genetic transformation and generated transgenic plants with purple colored tubers and stems. Over expression of endogenic *3GT* driven by *GBSS1* promoter in a red skinned cultivar Desiree resulted in increase in anthocyanin content and varied from 0.8 to 1.6 µg per mg FW as compared to 0.4 µg per mg FW in the control (Qing *et al.*, 2012). The over expression of the *3GT* gene did not affect expression of other genes in the anthocyanin biosynthetic pathway *CHS, CHI, DFR*, and *ANS* in the transgenic and the expression levels of these genes were at par with control indicating that the over expression of *3GT* do not affect the expression of other upstream genes in the anthocyanin pathway, thus directly increases the anthocyanin accumulation (Qing *et al.*, 2012). In another study aimed to enhance anthocyanin synthesis in potato tubers, the same enzyme (3GT) was over expressed in the light red skinned cultivar Desiree (Wei *et al.*, 2012). Skin of the transgenic tubers had an up to three-fold improved anthocyanin content, which was visible to the eye as a deeper color with respect to wild type controls. Transgene expression in the organs of the plants on the plants depends on the characteristics of the promoter used and the genetic background of the plant receiving the gene. Dihydroflavonol 4-reductase gene (*dfr*) was introduced into the potato cultivar Prince Hairy (white tuber) under the control of double *CaMV 35S* promoter, resulting in a change in the color of the flower of the transgenic plant from light blue to purple, but no change in the tuber color was observed (Zhang *et al.*, 2009).

Potatoes are also used as very good bioreactors to produce several health promoting important compounds, enzymes and vaccines (edible vaccines). Transgenic potato plants producing human lactic β-casein might also be significant for nourishment. Human β-casein produced by plants might be used in future for the production of human milk proteins such as lactoferrin and lysozyme or for preparation of baby food with increased nutritional value and preventive effects against gastric and intestinal dysfunctions in children (Chong *et al.*, 1997). In the same manner fructons, another important health compound are linear or branched polymers of repeating fructose residues connected by β (2-1) and/or β (2-6) fructosyl fructose linkage, optionally including one terminal glycosyl unit. Inulin, the best characterized fructan contains predominantly linear molecules with β (2-1) linkage. It is generally believed that inulin biosynthsis in plant occur through two vacuolar enzymes 1-SST (sucrose:sucrose 1 fructosyl transferase) and 1-FFT (fructan:fructan 1-fructosyl transferase). Stoop *et al.* (2007) expressed the *1-sst* and *1-fft* gene isolated from Jerusalem artichoke in transgenic potato. Inulin of DP 3 to DP 8 at 1.8 mg per g tuber with a mean DP between 3 and 4 was accumulated in transgenic potatoes. Similar studies in potato by Hellwege *et al.*, (2000) produced inulin molecules of DP>60 similar to the inulin profile found in the globe artichoke.

Reduction of toxicants and allergens compounds

Glycoalkaloids are found throughout the *Solanaceae* and among cultivated crops they occur in potato, eggplant and tomato (Maga, 1994). They are found in every plant organs

(roots, tubers, stolans, stems, foliage, flowers and fruits) with fresh weight concentrations in potato plants ranging from 10 mg per kg (fresh weight) in tubers to 5,000 mg per kg (fresh weight) in the flowers (Smith *et al.*, 1996). In potatoes, they have been a particular concern, due to their toxicity to humans (Friedman and McDonald, 1997). The increased use of wild potato germplasm to improve the pest resistance, yield, quality and processing characteristics of cultivated potato presents the possibility that glycoalkaloid levels may increase to unsafe levels or that new, more toxic glycoalkaloids might be introduced (Friedman and McDonald, 1997). Solanine and chaconine, derived from the aglycone solanidine are the most prevalent glycoalkaloids found in cultivated potato (Dale *et al.*, 1993). Solanine and solasonine have a common sugar moiety (solatriose) while chaconine and solamargine have chacotriose in common (Sinden *et al.*, 1991). The alkamine steroidal skeletons (aglycones) of the glycoalkaloids are classified into two groups, the spirosolanes and solanidanes, of which solasodine and solanidine are representatives, respectively. These compounds are derived from mevolonic acid, sharing a common biosynthetic pathway to cholesterol (Osman *et al.*, 1986). Several reports suggest that suppression of glycoalkoloid is probably dominant and that multiple recessive alleles are required for the expression of elevated glycoalkaloid levels (Sanford *et al.*, 1995). Glycoalkaloid content in tubers of potato cultivars has been shown to be genetically controlled with broad-sense heritability ranging from 86 to 89% (Sanford *et al.*, 1995). The use of wild germplasm in potato breeding is extensive and the main source of transmission of unusual SGAs (Vaananen *et al.*, 2006).

Elimination of solanidine glycosylation would also decrease toxicity of edible tuber. Antisense DNA constructs of *SGT1* coding for solanidine galactosyl transferase involved in α-solanine biosynthsis (McCue *et al.*, 2005), *SGT2* coding for solanidine glucosyltransferase involved in α-chaconine biosynthsis (McCue *et al.*, 2006) or *SGT3* coding for sterol rhamnosyl transferase, the last step in the triose formation of α-chaconine and α-solanine (McCue et al,. 2007), reduced the corresponding glycoalkaloids in transgenic potato plants. Antisense silencing of a potato gene encoding a sterol alkaloid glycosyl transferase (*sgt1*) resulted in complete inhibition of α-solanine accumulation. But this decrease was compensated by elevated levels of α-chaconine and resulted in wild type total steroidal glycoalkalids (SGA) levels in transgenic lines (McCue *et al.*, 2005). Lisa *et al.* (2003) over expressed soybean (*Glycine max*) type 1 sterol methyl transferase (*GmSTM1*) in potato (cv. Desiree) in an attempt to reduce glycoalkaloids. The transgenic potato showed decrease glycoakaloid levels in leaves and tubers, down to 41% and 63% of wild type levels, respectively.

Allergies to potatoes appear to be relatively uncommon. Patatin (*Solt 1*) is the primary storage protein (Shewry, 2003) and the major allergen in potatoes. Patatin may be cross reactive for persons with allergy to latex, and children with atopic dermatitis appear to have increased sensitivity to this potato protein (Schmidt *et al.*, 2002). Boiling of potatoes reduce or nullify the allergic reaction, which was studied on 12 children of 4 years age (Lee *et al.*, 2006). Similarly potato polyphenol oxidase [PPO] are the enzymes responsible for enzymatic browning reaction observed in impacted, damaged or sliced tubers (Thygesen *et al.*, 1995). These oxidative deterioration reactions alter the organoleptic properties of food and greatly affect potato tuber quality. Llorente *et al.* (2011) silenced the *PPO* gene in transgenic potato which reduced

the enzymatic browning and enhanced the shelf life of potato.

CONCLUSIONS

Biofortification offers a long-term, sustainable, food-based solution for a world population that will reach eight billion in coming years. This poses a challenge to scientists developing biofortified potato that is seeking a sustainable increase in calorie production and establishing as a staple food crop especially in developing countries of Asia and Africa where despite major concerted international efforts, eradication of micronutrient malnutrition (MNM) has remained a widespread and persistent health problem and where it continues to exert an enormous toll on individuals, populations, and society. In measuring up to this challenge, biofortification through genetic engineering also addresses socio-economic and socio-political needs, thus contributing towards equitable development. Even though biotechnology has proven successful in improving the nutritional quality of potato in past in certain aspects, the concentrated and focused efforts of scientific community are needed in developing 'potato as complete food' through improvements in resistant starch, enrichment of vitamins, major and minor minerals, antioxidant and by reducing the toxicants and allergens through learning the lessons from previous studies and taking the real insights from rice and tomato and other successful stories of nutrient rich, healthy and safe foods. The aim of future potato biofortification strategy needs to be addressed in relation to develop 'Potato', a nutrient rich, health promoting and safe complete staple food for the alleviation of nutrient-related health problems and malnutrition persisting in the developing countries of Asian and African subcontinents in consideration with regulatory approvals.

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