Title: Bioengineered Plants for Essential Vitamins and Minerals

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**ABSTRACT**

Modern biotechnology uses molecular methods to create or enhance commercial goods and processes by using entire or fragments of biological organisms. It is a relatively new and quickly developing field of molecular biology that emerged 30 years ago with the development of the first recombinant gene. By enhancing the foods we eat, the drinks we drink, the clothes we wear, and the medications we take, these strategies are improving how we live in a variety of ways. One of the many facets of biotechnology that has a significant influence on society is the implementation of biotechnology approaches in the food and agriculture industries. There will likely be over ten billion individuals occupying this planet by the year 2050, and it's possible that there won't be enough food to feed everyone. The branch of science with the greatest potential to end hunger now and prevent widespread starvation in the future is biotechnology. Scientists can increase a crop's tolerance to diseases and environmental pressures through the use of biotechnology, enabling crops to be cultivated on relatively unproductive and unsuitable land. New advances in biotechnology will make it possible to produce food that is safer, tastier, and more nutrient-dense. The way we produce and consume food is changing as a result of genetic engineering advancements, and it's likely that within the next ten years a sizable portion of the food we consume will be bioengineered. The development of genes that code for enzymes in the biosynthesis pathway of vitamins, vital amino acids, necessary elements, and micronutrient binding proteins can advance scientific study. With the creation of the first recombinant gene thirty years ago, a relatively young and swiftly growing science of molecular biology was born and these tactics are boosting how we live in a number of ways.

**I. BIOENGINEERED PLANTS**

In order to raise the amount of various nutrients (vitamins, vital amino acids, minerals, and phytochemicals) and improve their availability in plants, genetic engineering techniques have been widely applied. The two primary techniques for introducing genes into plants to create transgenic plants are micro projectile bombardment and transformation mediated by *Agrobacterium*. The transgene is introduced into the plants *via* the *Agrobacterium-*mediated transformation technique, which employs a genetically modified strain of *Agrobacterium tumefaciens*. Some *A. tumefaciens* strains have the innate capacity to introduce a portion of their own DNA into plants in order to cause crown-gall tumours. These *A. tumefaciens* wild-type strains that produce crown galls have a Ti (tumour inducing) plasmid that contains the tumor-causing genes. Agrobacterium distributes a portion of the Ti plasmid, known as T-DNA, to plant cells during the infection process [1]. The Ti plasmid can be modified to form a two-plasmid (binary) system with a tiny plasmid known as a binary cloning vector that contains a "engineered" T-DNA segment and a "disarmed" Ti plasmid in which the T-DNA has been deleted. The designed T-DNA, which has a target gene and a plant selectable marker gene inserted between the T-DNA left and right borders, is helped by the disarmed Ti plasmid, which is kept in an *A. tumefaciens* strain and provides the transfer function. The *Agrobacterium* vir (virulence) gene proteins are produced when the *A. tumefaciens* containing the disarmed Ti plasmid and the binary cloning vector is grown in the presence of acetosyringone [2]. These proteins aid in the transfer of the engineered T-DNA region of the binary cloning vector to the plant cells. For genetically modifying plants, Agrobacterium-mediated transformation is the most popular technique. The microprojectile bombardment technique, sometimes referred to as the gene gun or biolistic transformation technique, entails the direct delivery and expression of alien DNA in individual plant cells [3]. Many plant species, including monocots, which are frequently challenging to transform using A. tumefaciens, have been successfully transformed using it. In this technique, DNA-coated tungsten or gold spherical particles—roughly 4 m in size—are driven into plant cells at high speed utilising a biolistic particle delivery device or a gene gun. Once the DNA has entered a cell, it combines with the plant DNA through an unidentified process. If the microprojectiles must be delivered to the plant nucleus in order for the DNA to be integrated into the chromosome, this is unknown. In a wide variety of plant species, the microprojectile bombardment technique has been utilised to transfer genes into various plant sections used in tissue culture regeneration, calli, cell suspensions, immature embryos, and pollen. The A. tumefaciens-mediated gene transfer is unable to transfer genes into chloroplasts and mitochondria, thus this approach can also do this [4].

**I.I ESSENTIAL VITAMINS**

By regulating metabolism and supporting the molecular processes that release energy from meals, vitamins play a critical role in human health. They play a crucial role in the production of genetic material, blood cells, nervous system chemicals, and hormones. Vitamins and proteins interact to form metabolically active enzymes that are crucial in a variety of chemical processes. Only vitamin D, one of the 13 well-known vitamins, can be produced by the body; other vitamins, including vitamins A, C, and E, must be obtained through diet. Many different health issues may result from inadequate vitamin intake. Scientists can enhance the amount of vitamins in some crops through biotechnology, enabling a larger spectrum of the global population to benefit from their health advantages [5].

**I.I.A BIOFORTIFICATION**

The technique of biofortification entails either genetic alteration utilising contemporary biotechnology or the best conventional breeding practises to create food crops that are rich in micronutrients. Instead than manually adding nutrients to the food as it is being processed, this method of fortification concentrates on enhancing the nutrient profile of the food crop as it grows and matures. The delivery of micronutrients to populations with limited access to various diets and other micronutrient therapies can be accomplished realistically and affordably through biofortification [6].

**I.I.B VITAMIN A**

The majority of the world's population—nearly two-thirds—depends on rice as a primary food source, and 300 million of them are thought to be vitamin A deficient in some way (WHO 1997). This is a significant public health issue in many nations, especially densely populated regions of Asia, Africa, and Latin America. There is no ß-carotene, a precursor to vitamin A, in the starchy centre of the rice grain known as the endosperm. The visual pigments of rod and cone cells in the retina are made up in part of vitamin A, and a lack of it can result in symptoms ranging from night blindness to complete blindness. It is believed that this dietary shortage causes a quarter of a million youngsters in Southeast Asia to lose their vision every year. ß-carotene is an antioxidant found in plant foods like carrots and many other vegetables. In the intestine, each ß-carotene molecule undergoes an oxidative cleavage to produce two molecules of retinal, which can then be reduced to produce retinol or vitamin A [7].

Recently, transgenic rice was created by Peter Beyer from the University of Freiburg and Ingo Potrykus from the Swiss Federal Institute of Technology, Zurich, Switzerland. This rice expresses genes for ß-carotene production in rice grains [8]. Geranylgeranyl pyrophosphate (GGPP), a precursor of the pathway for the manufacture of ß-carotene, is a substance that naturally exists in rice endosperm. In four processes, GGPP can become ß-carotene. Phytoene desaturase and ß-carotene desaturase are plant enzymes that are both capable of being replaced by the bacterial phytoene desaturase (EC 1.14.99.30) enzyme produced by the crtI gene [9]. Researchers employed the crtI gene from the bacteria *Erwinia uredovora* to lower the quantity of genes for the -carotene pathway that were converted into rice [10]. The psy gene, which codes for phytoene synthase, and the lcy gene, which codes for lycopene -cyclase, both came from the daffodil plant. The endosperm-specific rice glutelin (Gt1) promoter and the 35S CaMV promoter, respectively, were used to regulate the plant psy gene (cDNA) and the bacterial crtI gene, which were then inserted into the binary plasmid pZPsC. A different plasmid, pZLcyH, was created by combining the aphIV gene for hygromycin resistance with the lcy gene from daffodils under the control of rice Gt1 promoter. By using *Agrobacterium*-mediated transformation, the plasmids pZPsC and pZLCyH were co-transformed into immature rice embryos. Southern hybridization was used to check all hygromycin-resistant transformants for the presence of the psy, crtI, and lcy genes. The endosperm of a handful of the changed plants generated -carotene, which gave the kernel its yellow colour. The chosen line was designated as "golden rice" because it had an endosperm concentration of 1.6 g of beta-carotene.

**I.I.C VITAMIN C**

Ascorbic acid, also known as vitamin C, is a vital part of human nutrition and is present in a wide variety of plants. It contains anti-oxidant qualities, enhances immune cell and cardiovascular functions, guards against connective tissue illnesses, and is necessary for iron utilization [11, 12]. While the majority of animals and plants can produce ascorbic acid, humans lack the enzyme L-gulono-1,4-lactone oxidoreductase required for the last stage of ascorbic acid biosynthesis. Because of this, ascorbic acid must be obtained through diet, mainly from plants [11]. The creation of bioengineered plants that synthesize ascorbic acid at considerably higher levels was made possible by the recent discovery of ascorbic acid biosynthesis routes in plants. The creation of bioengineered plants that synthesize ascorbic acid at considerably higher levels was made possible by the recent discovery of ascorbic acid biosynthesis routes in plants. Ascorbic acid is biosynthesized differently in plants and animals. There are two methods through which vitamin C can be synthesized in plants. First, the enzyme D-galacturonic acid reductase converts D-galacturonic acid, which is generated during the degradation of pectin (a significant component of the cell wall), into L-galactonicacid. The immediate precursor of ascorbic acid, L-galactono-1,4-lactone, is then easily converted from L-galactonic acid [13,14]. GalUR, a strawberry gene that encodes the enzyme D-galacturonic acid reductase, was discovered and characterized by Spanish researchers. A 956-bp fragment of the galUR gene was amplified by PCR and cloned into a binary vector in front of the 35S CaMVpromoter.Triparental mating was used to transport the resultant plasmid to *Agrobacterium* and transform it into *E.coli*. Finally, *Agrobacterium*-mediated transformation was used to introduce the GalUR gene into *Arabidopsis thaliana* seedlings. The bioengineered plants were able to improve ascorbic acid production by two to three times compared to the wild-type plants thanks to the expression of the strawberry GalUR gene in *A. thaliana*. GalUR, a strawberry gene that produces the enzyme D-galacturonic acid reductase, was discovered and studied. A 956-bp fragment of the galUR gene was amplified by PCR and cloned into a binary vector in front of the 35S CaMV promoter. Through triparental mating, the resultant plasmid was transferred into *E. coli* and given to *Agrobacterium*. Finally, *Arabidopsis thaliana* plants were transformed using an *Agrobacterium* to insert the GalUR gene. When compared to wild-type plants, the bioengineered plants were able to increase ascorbic acid production by two to three times thanks to the expression of the strawberry GalUR gene in *A. thaliana*. The recycling of spent ascorbic acid is the second method by which plants produce vitamin C. Ascorbic acid undergoes oxidation in the first step of this cycle, creating the radical monodehydroascorbate (MDHA).The enzyme monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4) may easily convert MDHA back into ascorbic acid after it has been produced, or it can be further oxidised to produce dehydroascorbate (DHA). Dehydroascorbate reductase (DHAR) (EC 1.8.5.1), an enzyme that utilises the reductant glutathione (GSH), can then either recycle DHA back into ascorbic acid or undergo irreversible hydrolysis [13,14,15]. Theoretically, by increasing DHAR expression in plants, researchers from the University of California, Riverside, could boost ascorbic acid synthesis by achieving a more effective ascorbate recycling mechanism [16]. They extracted DHAR cDNA from wheat and expressed the gene in tobacco and maize plants to verify their theory. *Agrobacterium* was used to alter tobacco plants. DHAR was given a His tag before being inserted into the binary vector pBI101, which was subsequently promoted by the 35S CaMV gene. A DHAR lacking a His tag was inserted into the pACH18 vector for maize and put under the control of the shrunken 2 (Sh2) or maize ubiquitin (Ub) promoters. By introducing the embryogenic callus to particle bombardment, transgenic maize was produced. DHAR expression was raised up to 100 times in maize and up to 32 times in tobacco, leading to up to a four-fold rise in ascorbic acid levels in the bioengineered plants [16].

**I.I.D VITAMIN E**

The tocotrienol and tocopherol families of eight lipid-soluble antioxidants, mostly produced by plants and other photosynthetic organisms, are together referred to as vitamin E [17]. Based on the quantity and placement of methyl groups within the aromatic ring, the tocotrienol and tocopherol families can be divided into four distinct types [18]. Tocotrienols and tocopherols give plants protection from oxidative stress, and their antioxidant properties give food items additional useful features [19]. Theriault et al. (1999) found that excessive intake of vitamin E has been linked to a number of therapeutic benefits, including lowered cholesterol levels, inhibition of breast cancer cell growth in vitro, a lower risk of cardiovascular disease, and a lower incidence of many degenerative diseases in humans.Tocotrienols are less easily absorbed than tocopherols but have stronger antioxidant effects [20]. According to Munne'-Bosch and Alegre (2002) [21], tocotrienol and -tocopherol are the two main forms of vitamin E found in seeds and leaves, respectively. While the specific genes that code for the various enzymes in the pathway have only recently been found, the production of tocopherols and tocotrienols has been known for a very long time. Some promising progress has already been made in the effort to create plants with higher vitamin E content.The production of homogentisic acid (HGA), which is catalysed by the enzyme p-hydroxyphenyl-pyruvate dioxygenase (HPPD) (EC 1.13.11.27), is the first step in the pathway for the biosynthesis of both tocopherols and tocotrienols [22]. In plants, two distinct precursors are used in the production of tocotrienol and tocopherol. HGA geranylgeranyl transferase (HGGT) (EC 2.5.1.32) catalyses the condensation of HGA and geranylgeranyl diphosphate (GGDP) to produce tocotrienols, and HGA phytyl transferase (HPT) (EC 2.5.1.62) catalyses the condensation of HGA and phytyl diphosphate to produce tocopherols [23]. The consequences of constitutive expression of HPPD cDNA from barley (*Hordeum vulgare*) in tobacco plants were described by scientists from the Institute of Botany in Germany. A SmaI cloning site between the 35S CaMV promoter and the octopine synthase (EC 1.5.1.11) polyadenylation signal was used to clone the HPPD gene into the pBinAR binary vector. After that, the construct was added to the tobacco explant-transforming *Agrobacterium* GV3101. The outcomes demonstrated that transgenic lines produced two times as much vitamin E in the seeds and had a better potential for homogentisic acid production overall [24]. The vitamin E content of leaves remained unaffected. Another method of changing the vitamin E content of plants involves the last enzyme in the process that transforms -tocotrienol and -tocopherol into -tocotrienol and -tocopherol, respectively. This process occurs in the final step of the biosynthetic pathway for tocotrienols and tocopherols. The enzyme -tocopherol methyltransferase (-TMT) (EC 2.1.1.95) is responsible for catalysing this process [25].

**I.II ESSENTIAL MINERALS**

Humans need to consume 17 different important minerals daily to keep their bodies healthy and operating properly. Minerals are inorganic ions that occur naturally and cannot be produced by living things. There are two categories that they fall under: macronutrients and micronutrients. The minerals we require in substantial amounts are known as micronutrients, and they include calcium, phosphorus, sodium, magnesium, chlorine, iron, sulphur, and silicon [26].

**I.II.A IRON**

The most common nutritional shortfall worldwide is iron, despite the fact that it is only necessary in trace levels. According to estimates by the WHO from 1992, roughly 30% of the world's population experiences severe nutritional issues as a result of inadequate iron intake. Both myoglobin, which aids in the storage of oxygen in muscle cells, and hemoglobin, which carries oxygen through the blood, includes significant amounts of iron. The emergence of iron deficiency anemia can be attributed to low iron levels. Many health issues, such as child retardation [27], pregnancy complications [28], weakened immune system [29], and fatigue [30] are caused by low oxygen levels in the blood of anemic people. Both inorganic (ferric and ferrous) and biological (heme and nonheme) types of iron are found in food. The hemoglobin and myoglobin found in flesh meals such meats, fish, and poultry are the main sources of heme iron, which is highly accessible (Taylor et al. 1986). Reduced iron (ferrous) is more easily absorbed in humans than oxidized iron (ferric). Nutraceutical supplements, food fortification, and various food preparation and processing techniques have all been utilized in the fight against iron deficiency [31]. None of these methods, particularly in underdeveloped nations, has so far proved successful in curing iron deficiency. Biotechnology is a new weapon in the fight against nutrient insufficiency, helping to increase the nutrition of vital minerals in basic crops.Genetic engineering can now be employed for this aim in essentially two different ways: (1) by increasing the concentration of the iron-binding protein ferritin, and (2) by decreasing the amount of the iron-absorption inhibitor phytic acid. The capacity to store and release iron in a controlled manner is essential since, despite the fact that iron intake is needed for human health, it can also be poisonous. According to research [32], ferritin may be given orally and is efficient in treating rat anemia. This suggests that raising the ferritin content of cereals may be the answer to the problem of dietary iron shortage in people. Under the direction of a seed-specific promoter, GluB-1, from the rice seed-storage protein gene producing glutelin, Japanese researchers [33] inserted soybean ferritin cDNA into rice plants. This promoter has two benefits: it can induce ferritin at a high level and accumulates iron selectively in the endosperm of rice grains. Through the use of Agrobacterium, the ferritin cDNA was extracted from soybean cotyledons, put into the binary vector pGPTV-35S-bar, and then introduced into rice. The transgenic plants' rice seeds have a three-fold higher iron content than the wild-type, non-transformed plants.

The bioavailability of iron and other important minerals is predicted to increase significantly with a decrease in the amount of phytic acid in staple meals. To speed up the breakdown of phytic acid, Lucca et al. (2002) [34] inserted a fungus (*Aspergillus fumigatus*) phytase cDNA into rice. The *A. fumigatus* phytase gene was employed to biolistically convert immature zygotic embryonic rice suspension cells. Because it is heat stable and can refold into an active state after heat denaturation, phytase from *A. fumigatus* was chosen as the enzyme [35].The main goal of this study was to boost the activity of the enzyme phytase during seed germination and to keep it there even after food processing and in human digestion. The powerful tissue-specific globulin promoter used by the researchers allowed for significant levels of phytase expression in the rice endosperm, but the transgenic rice's thermotolerance was not as great as anticipated. According to speculation [36], the endosperm's interference in maintaining the enzyme in an active form may be the cause of the unexpectedly low thermostability of A. fumigatus phytase in transgenic rice. To create an endogenous phytase enzyme that is thermostable and sustains high activity in plant tissues, more research is required

**I.III ESSENTIAL AMINO ACIDS**

Amino acids combine to make chemical molecules called proteins. Proteins are disassembled into individual amino acids by the digestive system so that they can be absorbed into the bloodstream. The amino acids are subsequently utilised by cells as a building block to create structural and enzyme proteins. Amino acids come in two varieties: essential and optional. Animals, including humans, are unable to synthesise essential amino acids; as a result, they must be obtained through nutrition. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are the nine essential amino acids. As long as enough necessary amino acids and calories are consumed, the body can produce non-essential amino acids. Proteins can be found in foods in varied levels, and some foods are considered to as complete proteins because they include all nine essential amino acids. The majority of animal products, such as meat, milk, and eggs, provide a rich source of complete proteins. On the other hand, several necessary amino acids are typically lacking or insufficient in vegetable sources. For instance, pulses are typically deficient in methionine while grains frequently lack lysine [37]. It is crucial to boost the amount of essential amino acids in the protein found in seeds and tubers in order to give superior nutrition derived from plant sources. This is crucial for nations where specific crops, like rice, potatoes, and maize, serve as the primary source of food.

**I.III.A LYSINE**

One of the most significant staple crops, rice is consumed everyday by 65% of the world's population [38]. It is an excellent source of vital nutrients such as vitamins B1 (thiamin), B2 (riboflavin), and B3 (niacin), but it is deficient in the amino acids lysine and isoleucine, which are necessary for human growth and development [39]. Lysine is a crucial amino acid for the body, performing a variety of vital tasks includes assisting calcium absorption, forming collagen, and producing antibodies, hormones, and enzymes. Lysine insufficiency can cause fatigue, difficulty focusing, irritability, bloodshot eyes, growth retardation, hair loss, anaemia, and reproductive issues An increased lysine content transgenic rice was created by Zheng et al. in 1995 [40]. They achieved this by expressing in the grain of transgenic rice the seed storage protein -phaseolin from the common bean (*Phaseolus vulgaris*). A rice seed-specific glutelin Gt1 promoter or the native -phaseolin promoter was used to control the genomic and cDNA sequences of the -phaseolin gene from P. vulgaris. Through transformation mediated by protoplasts, the vectors harbouring the -phaseolin gene were inserted into the rice chromosome. Phaseolin made up 4% of the total endosperm protein in the transgenic rice, which significantly raised the amount of lysine in the grain [40].

**I.III .B METHIONINE AND TYROSINE**

On the list of crop species that are most crucial for human nutrition globally, the potato (*Solanum tuberosum*) is only second after rice, wheat, and corn in terms of global food production [41]. According to Chakraborty et al. (2000) [41], there are four main markets for potatoes: the fresh food market, animal feed, the food processing industry, and non-food industrial uses such the manufacturing of starch and alcohol. While potatoes are a significant source of potassium, iron, vitamin C, and vitamin B, they are not particularly high in protein. The lack of the amino acids lysine, methionine, and tyrosine in potato proteins limits their nutritional value [42]. Lack of methionine in the diet can affect how other amino acids are absorbed, which can slow down growth and development. As the body's primary source of sulphur, methionine also protects against problems with the hair, skin, and nails, lowers cholesterol by boosting the formation of the phospholipid lecithin by the liver, and functions as a natural chelating agent for heavy metals [43].

**CONCLUSION**

Genetic engineering developments will make it possible to develop transgenic plants that manufacture more nutritional plant products that are the need in present and future perspectives. The use of genetic engineering in the food business is not just restricted to plant genome manipulation. In-depth research has also been done on using animals and microbes to create better food products. Biotechnology is crucial for ensuring food safety in addition to producing new substances and improving existing ones.

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