BIOENGINEERED PLANTS FOR ESSENTIAL VITAMINS AND MINERALS

Abstract

New generation biotechnology involves modern bio molecular techniques using whole or part of biological organism to create or improve products and process. Rapidly growing field of modern molecular biology has emerged 30 decades before along with the development of genetics. These ideas improve our life style in many ways by improving the food, the drink, the clothes, and the medicines. One of the ways biotechnology affects people is through the use of biotechnological methods in the food industry and agriculture. The greatest potential of biotechnology lies in its ability to improve crop tolerance to diseases and environmental stress, thereby providing greater yields and suitable soil for cultivation. These techniques will produce healthier, tastier and better food. Emerging genetic engineering technology is changing the product quality of food, and much of our food will be bioengineered within the next decade. The insertion of advanced genes which codes for enzymes involved in the biosynthetic pathway of micro molecules like essential amino acids, vitamins, essential elements and micronutrient-binding proteins is increasing research and improving our lives.

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I. BIOENGINEERED PLANTS

Genetic engineering techniques are widely used to improve and enhance the availability of various nutrients like essential amino acids, vitamins, minerals and phytochemicals in plants products. In plants transformation of genes to produce genetically modified plant is either done by Agrobacterium-mediated gene transformation and micro projectile bombardment method. The former method uses genetically modified *Agrobacterium tumefaciens* strains to insert genes into plants. The wild-type strain of Agr*obacterium tumef*a*ciens* contains a Ti plasmid also known as tumor-inducing plasmid which carrying tumor-inducing genes. While infecting plant cells, Agrobacterium transfers T-DNA, which is the part of the Ti plasmid, into the plant cells [1]. The Ti plasmid can be modified to create a binary plasmid system or two plasmid system which is made up of a Ti plasmid from which the T-DNA has been deleted and a smaller plasmid -binary cloning vector with the "engineered" T-DNA fragment. The disarmed Ti plasmid is transfered in the *Agrobacterium tumefaciens* strain and provides transformation function by promoting the production of T-DNA containing target genes and plant selectable markers located at the left border and right relaxed border of the T-DNA of the *Agrobacterium tumefaciens* strain. It has a natural structure can inject small amounts of autologous DNA into plants to induce crown gall tumors. *Rhizobiu*m bacteria carrying unarmed Ti plasmids and binary cloning vectors were cultured in the presence of acetosyringone to produce virulence proteins from *Agrobacterium* [2]. With the help of these proteins, the modified T-DNA region of the vector is transferred. In plant cells Agrobacterium-mediated gene transformation is the most widely used method for genetic modification. However, the other method is the microprojectile bombardment/ bioprojectile method. This method involves the direct transfer of foreign DNA into the same plant cells [3]. *A. Rhizobium* bacteria have been used to transform many plant species, also to monocot plant cells, to which transfer of gene is often difficult. Microprojectile bombardment method involves firing a seed gun, or biomissile particle, that is sent into the plant at high speed to replace DNA-coated tungsten or gold spheres approximately 4 m in size. Once the DNA enters the cell, it interacts with the plant's DNA in unknown ways. It is not yet clear whether micro-projectiles need to reach the plant cell nucleus to integrate DNA into chromosomes. Micro-projectile bombardment methods have been used to implant seeds into callus, cell suspensions, immature embryos, pollen, and different plants used in tissue culture. This method can also transfer genes into chloroplasts and mitochondria, something the A. tumefaciens-mediated gene transfer is unable to achieve.

1. **Essential Vitamins:** By regulating metabolic pathways and supporting the molecular processes that involves release of energy from meals, multiple vitamins play a critical role in improving human health. 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 health problems can be caused by vitamin deficiency. Through biotechnology, scientists can increase the vitamin content of some crops, enabling many people around the world to enjoy their health benefits [5].

   - **Biofortication:** Biofortification technology requires genetic modifications using contemporary biotechnology or best traditional breeding method to create food crops rich in micronutrients. This preventive measure focuses on increasing the nutritional value of crops as they grow and mature, rather than manually adding nutrients during food processing. Through biofortification, micronutrients can be efficiently and cost-effectively delivered to people with limited access to various nutrition and micronutrient treatments [6].

- **Vitamin:** Almost two-thirds two third of the world's population depend on vitamin A as a staple food, and according to WHO 300 million people are vitamin A deficient (WHO 1997). It is an important health problem in many under developed countries, especially in the densely populated regions of Africa, Asia and Latin America. The starchy part of rice grains, called endosperm, does not contain beta-carotene which is a Vitamin A precursor .The disease called visual color of the rods and cones in the retina is produced by vitamin A deficiency. The symptoms of above disease are night blindness which leads to blindness. Malnutrition is believed to cause 250,000 young people in Southeast Asia to lose their eyesight every year. Beta-carotene is an antioxidant found in foods such as carrots and many other vegetables. In the intestine, each β-carotene molecule undergoes oxidative cleavage to produce two retinal molecules, which can be reduced to produce retinol or vitamin A [7]. Recently, Peter Beyer and Ingo Potrykus developed genetically modified rice. This rice variety displays the genes required to produce β-carotene [8]. Rice endosperm contains geranylgeranyl pyrophosphate (GGPP), a natural chemical. GGPP is the precursor of β-carotene. GGPP can form to beta-carotene in one of the four different ways. Bacterial enzyme phytoene desaturase which is encoded by the CrtI gene, can replace the enzymes phytoene desaturase and β-carotene desaturase [9]. For the reduction of the number of genes in the β-carotene conversion pathway in rice, crtI gene of *Erwinia uredovora* is used by researchers[10]. It appears that the gene for plant phytoene synthase (psy) and the gene for lycopene cyclase (lcy) taken from the narcissus plant species. The plant psy gene controlled by the endosperms wheat gluten (Gt1) promoter and the crtI gene of bacteria controlled by the the 35S CaMV promoter inserted into the binary plasmid pZPsC. The hygromycin-resistant aphIV gene was combined with the Icy gene of narcissus under the control of the rice Gt1 promoter to create a new plasmid pZLcyH. The pZPsC and pZLCyH vectors were transfected via Agrobacterium mediated transmormation into the immature embryo cells. All the transformed hygromycin-resistant were screened by Southern hybridization technique. Some plants are designed to produce beta-carotene in their endosperm, which gives grains their yellow color. It is called "golden rice" because the endosperm of the selection contains 1.6 grams of beta-carotene.

- **Vitamin C:**Vitamin C (ascorbic acid) is a essential 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 Vitamin C, humans are lacking the enzyme L-gulono-1,4-lactone oxidoreductase which is 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 genetically engineered plants that synthesize Vitamin C at considerably higher quantity was made possible by the latest discovery of Vitamin C biosynthesis routes in cell of plant. Ascorbic acid is biosynthesized differently in plants and animals. In plant cells there are two methods through which vitamin C can be synthesized. Firstly, D-galacturonic acid is converted into L-galactonicacid via the enzyme D-galacturonic acid reductase. L-galactono-1,4-lactone (precursor of Ascorbic acid) is then easily converted from L-galactonic acid [13,14]. GalUR, a strawberry gene which was discovered and characterized by Spanish researchers is a 956 bp gene that encodes for D-galacturonic acid reductase enzyme. The fragment of galUR gene was amplified using polymerase chain reaction (PCR) and cloned into a binary vector with 35S CaMV promoter. 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. Compared to wild type the bioengineered plants improve ascorbic acid production by two to three folds in *A. thaliana*. The second method involves recycling of used ascorbic acid by which plants produce vitamin C. Ascorbic acid undergoes oxidation in the first step of this cycle, creating the radical monodehydroascorbate (MDHA).The monodehydroascorbate reductase (MDHAR) enzyme may easily convert MDHA back into ascorbic acid after it has been produced, or it can be further oxidised to produce dehydroascorbate (DHA). The enzyme dehydroascorbate reductase (DHAR) utilizes reduced glutathione (GSH), to 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]. *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].

- **Vitamin E:** Vitamin E is group of eight fat-soluble antioxidants compound in the tocotrienol and tocopherol groups produced by photosynthetic plant and animals. Four different types of tocotrienols and the tocopherol family can be distinguished based on the number and position of the methyl group in the aromatic ring [18]. Tocotrienols and tocopherols protect plants against oxidative stress, and their antioxidant properties provide additional nutritional benefits [19]. According to Theriot et al. (1999), excessive consumption of vitamin E has many benefits, such as lowering cholesterol, inhibiting breast cancer growth in vitro, reducing cardiovascular disease risk, and reducing the consequences of various degenerative diseases. Although tocotrienols are less absorbed than tocopherols, they are potent antioxidants [20]. According to Munne'-Bosch and Alegre (2002), the two main forms of vitamin E found in seeds and leaves are tocotrienols and tocopherols [21]. The production of tocopherols and tocotrienols has been known for a long time, but the precise genes that understand the different enzymes involved in the process have only recently been discovered. There have been some encouraging successes in producing plants with higher vitamin E content. The first step in the tocopherol and tocotrienol biosynthetic pathway is the production of homogenization (HGA), catalyzed by p-hydroxyphenyl-pyruvate dioxygenase enzyme. Tocotrienols are produced by HGA geranylgeranyl transferase (HGGT) precursor molecule, while tocopherols are produced by enzyme HGA phytyl transferase (HPT). Researchers at the German Botanical Institute have documented the results of expression of HPPD cDNA from barley in tobacco plants. The HPPD gene was cloned into the pBinAR binary vector using the Small cloning site of the 35S CaMV promoter and the octopine synthase (EC 1.5.1.11) polyadenylation signal. This construct was introduced into tobacco transformed with *Agrobacterium tumefaciens* GV3101. The results showed that the transgenic lines produced twice as much vitamin E in seeds and had higher homogenization synthesis capacity [24]. The vitamin E content in the leaves does not change. Another process to alter the vitamin E content of plants involves the final enzyme in the process of converting alpha-tocotrienol and beta-tocopherol to alpha-tocotrienol and beta-tocopherol, respectively. This occurs at the end of the tocotrienol and tocopherol biosynthetic pathway. Tocopherol methyltransferase (-TMT) (EC 2.1.1.95) is responsible for catalyzing this progression [25].

2. **Essential Minerals:** People need to consume 17 essential minerals every day to keep their bodies healthy and able to function properly. Minerals are naturally occurring inorganic ions that cannot be synthesized by living organisms. Minerals are divided into two groups: macronutrients and micronutrients. We need many foods called micronutrients, which include phosphorus, calcium, sodium, chlorine, magnesium, sulfur, iron and silicon [26].

   - **IRON:** Although iron requirements are only numerical, the most common foodstuff worldwide is iron. According to the WHO estimate in 1992, approximately 30% population of world faces serious malnutrition problems. Myoglobin, which helps muscle cells to store oxygen, and hemoglobin, which carries oxygen from the blood, contains plenty of iron. The occurrence of iron deficiency anemia can be attributed to iron deficiency. Low oxygen in the blood of people with diabetes can cause many health problems, including growth retardation in babies [27], pregnancy problems [28], affecting physical weakness [29], and fatigue [30]. Foods contain inorganic metals (ferric and ferrous) and biogenic metals (heme and non-heme). Hb protein and myoglobin protein in fish, meat and chicken are the main sources of heme iron and are easily absorbed. People absorb reduced iron (ferrous iron) more easily than oxidized iron (ferric iron). Food products, good nutrition, and food preparation and exercise are used to combat iron deficiency [31]. None of these treatments have been shown to be effective in treating iron deficiency, especially in developing countries. Biotechnology is a new tool to help supplement important crops with essential nutrients in the fight against food insecurity. Genetic engineering can now be used to achieve this goal in two ways: (1) increasing the concentration ferritin (a iron-binding protein and (2) decreasing the level of phytate, which inhibits absorption of iron. It is important to control storage and release of iron because as such iron intake is necessary for human health, it can also cause problems. According to research, ferritin can be used orally and works well in the treatment of diabetes in rats [32]. This suggests that increasing the ferritin content in grains may be a solution to iron deficiency in humans. Japanese researchers [33] applied liquid ferritin cDNA to rice plants with specific promoter gene, GluB-1 (from the gluten-producing wheat storage protein gene). This supplement has 2 advantages: It likes to collect iron from rice endosperm and can increase ferritin levels. Ferritin cDNA from soybean leaves using Agrobacterium, inserted into the pGPTV-35S-bar binary vector, and transferred into rice plant cells. Rice seeds from genetically modified plants have three times the iron content of seeds from wild non-GMO plants. As the phytate content in staple foods decreases, the bioavailability of iron and other essential minerals should increase. Luca et al. (2002) [34] introduced fungal (Kojima fumigatus) phytase cDNA into wheat to accelerate the degradation of phytic acid. The phytase gene from Aspergillus fumigatus was used for transformation of immature zygotic embryonic rice suspension cells. Phytase from *Aspergillus fumigatus* was chosen as the enzyme because it is thermostable and can be converted to the active form after thermal denaturation [35]. The main aim of this study was to enhance phytase activity during seed germination and maintain its activity even after food processing and human digestion. Although the scientists were able to produce more phytase in the rice endosperm thanks to tissue-specific globulin supplementation, the temperature tolerance of the modified soybeans was not as high as they had hoped. The thermostability of Kojima fumigatus phytase in transgenic rice is abnormal and is believed to be due to the effect of the endosperm in regulating the enzyme status [36]. Further research is in need to create an endogenous phytase enzyme that is heat stable and together with high activity in tissues.

3. **Important Amino Acids:** Amino acids are organic compounds they combine to form chemical molecules called proteins. Although over 500 amino acids exist in nature and the most important are 22 α- amino acid incorpoted into proteins. Proteins are broken down into individual amino acids by the digestive system, so they can enter the bloodstream. Amino acids are then used by cells as building blocks to create structural and enzymatic proteins. There are three groups of amino acids: essential amino acids, non-essential amino acid and conditionally essential amino acids. Animals, including humans, cannot produce essential amino acids; therefore they must be obtained from meal. Histine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan are the nine essential amino acids. When sufficient amino acids and calories are consumed, the body can produce non-essential amino acids. Protein foods vary, and some foods are considered high protein because they contain all nine essential amino acids. Animal products, such as meat, dairy and eggs, provide protein. On the other hand, many essential amino acids are frequently absent in plants. For example, legumes are often deficient in methionine, while grains are often deficient in lysine [37].

- **Lysine:** Lysine is an α-amino acid that precursor for many proteins. Meat is one of the most important foods fascinated daily by 65% of the world's population [38]. It is rich in vitamins B1 (thiamine), B2 (riboflavin) and B3 (nicotinic acid), but lacks the amino acids lysine and isoleucine, which are essential for human growth and also play essential role in th production of carnitine. Lysine is an essential amino acid that helps absorb calcium, form collagen, and produce antibodies, hormones, and enzymes. Lysine deficiency can cause fatigue, depression, irritability, bloodshot eyes, growth delay, hair loss, anemia and growthp roblems.Z henge t al .A genetic modification with increased lysine content was developed in 1995 [40]. The scientists achieved this by expressing Phaseolin, a seed storage protein from legumes (*Phaseolus vulgaris*), in transgenic c rops. Genomic and cDNA sequences of these genes were examined at the gene level using the wheat-specific glutenin Gt1 promoter or normal promoter levels. The vector containing the β-phaseolin gene was introduced into the rice chromosome via protoplast-mediated transformation. In genetic modification, Phaseolin accounts for 4% of the total endosperm protein and increases the lysine content in rice.[41].

- **Methionine and Tyrosine:** Methionine cannot be made by the body ,so it must consumed in diet.In terms of global food production, potatoes (*Solanum tuberosum*) are second only to rice, wheat and maize in the list of the most important crops for human utilization[41]. According to Chakraborty et al. (2000) [41] noted that there are four main markets for potatoes: fresh food, pet food, food processing, and non-food products such as starch and alcohol. Although potatoes are a good source of potassium, iron, vitamins B C, minerals and fiber they are low in protein, calories fat and cholesterol. The nutritional value of potato protein is limited due to the lack of lysine, methionine and tyrosine [42]. Methionine deficiency will affect ocysteien re methylation pathway,growth and development by affecting the absorption of other amino acids. Methionine is an antioxidant it is an important form of sulfur and a natural form of heavy metals; It may help protect the body from damage by ionizing radiation, hair and nail problems, lower cholesterol and lower blood pressure by promoting the production of phospholipid lecithin in the liver [43].

II. CONCLUSION

Advances in genetic engineering will make it possible to develop genetically modified plants to produce more food needed now and in the future. The application of bioengineering in the food industry is not only limited to manipulation of the plant genome but also there is also intense research on using animals and organisms to produce better food. Biotechnology is important for creating new products and improving existing products, as well as ensuring food safety.

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