Title: Bioengineered Plants for Essential Vitamins and Minerals

M.BHUMIKA (1st Author)
Department of Applied science
Shri Rawatpura Sarkar University,
Raipur, Chhattisgarh
bhumikaawww@gmail.com

Ramautin Kurre (2st Author)
Department of Life science
Shri Rawatpura Sarkar University,
Raipur, Chhattisgarh
Kurrerama94@gmail.com

Dr. Rupal Purena (3rd Author)
Department of Applied science
Shri Rawatpura Sarkar University,
Raipur, Chhattisgarh
drrupalpurena@sruraipur.ac.in

ABSTRACT

Modern biotechnology uses molecular techniques to create or improve products and processes using whole or parts of biological organisms. It is a new and rapidly growing field of molecular biology that emerged 30 years ago with the development of genetics. These ideas improve the way we live in many ways by improving the food we eat, the things we drink, the clothes we wear, and the medicines we take. 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 machines will produce safer, tastier and better food. Advances in genetic engineering are changing the way we produce and eat food, and much of our food will be bioengineered within the next decade. The development of enzyme genes in the biosynthetic pathway of vitamins, amino acids, essential elements and micronutrient-binding proteins is increasing research and improving our lives.

I. BIOENGINEERED PLANTS

Genetic engineering techniques are widely used to improve and enhance the availability of various nutrients (vitamins, essential amino acids, minerals and phytochemicals) in plants. Agrobacterium-mediated transformation and micro projectile bombardment are two important methods of introducing genes into plants to produce genetically modified plants. The Agrobacterium-mediated transformation method uses genetically modified Agrobacterium tumefaciens strains to insert genes into plants. The gall-producing wild-type strain of Agr*obacterium tumef*aciens contains a Ti (tumor-inducing) plasmid carrying tumor-inducing genes. During infection, Agrobacterium transfers T-DNA, part of the Ti plasmid, into plant cells [1]. The Ti plasmid can be modified to create a two-plasmid (binary) system consisting of a “deleted” Ti plasmid from which the T-DNA has been removed and a smaller plasmid called a binary cloning vector with the “engineered” T-DNA DNA fragment. The disarmed Ti plasmid is stored 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 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. Be one. Rhizobium bacteria carrying unarmed Ti plasmids and binary cloning vectors were cultured in the presence of acetosyringone to produce virulence gene proteins from *Agrobacterium* [2]. With the help of these proteins, the modified T-DNA region of the binary cloning vector is transferred to plant cells. Agrobacterium-mediated transformation is the most widely used method for genetic modification. The microprojectile bombardment method (also known as the genetic ball or bioprojectile transformation method) involves the direct delivery and expression of foreign DNA into the same cells [3]. A. Rhizobium bacteria have been used to transform many plant species, including monocots, which are often difficult to transform. This 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 microprojectiles need to reach the plant cell nucleus to integrate DNA into chromosomes. Microprojectile 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.

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 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].

I.I.A 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 diverse nutrition and other micronutrient treatments [6].

I.I.B VITAMIN

A Most of the world's population (almost two-thirds) rely on vitamin A as a staple food, and 300 million people are considered vitamin A deficient.Some (WHO 1997). It is an important health problem in many countries, especially in the densely populated regions of Asia, Africa and Latin America. The starchy part of rice grains, called endosperm, does not contain beta-carotene (the precursor of vitamin A). The visual color of the rods and cones in the retina is produced by vitamin A, and its deficiency can cause symptoms ranging from night blindness 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, a genetically modified bean was developed by researchers Peter Beyer of the University of Freiburg and Ingo Potrykus of the Swiss Federal Institute of Technology in Zurich, Switzerland. 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 to β-carotene production. GGPP can be converted to beta-carotene in four different ways. Bacterial phytoene desaturase (EC 1.14.99.30), produced by the CrtI gene, can replace the enzymes phytoene desaturase and β-carotene desaturase [9]. To reduce the number of genes in the β-carotene conversion pathway in rice, researchers used the crtI gene of Erwinia uredovora [10]. It appears that the gene encoding phytoene synthase (psy) and the gene encoding lycopene cyclase (lcy) come from the narcissus plant. The plant psy gene (cDNA) and the bacterial crtI gene were controlled by the endosperm-specific wheat gluten (Gt1) promoter and the 35S CaMV promoter, respectively, and then 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 transformed into immature embryos using Agrobacterium-mediated transformation. All hygromycin-resistant transformants were checked for the psy, crtI, and lcy genes by Southern hybridization. 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.

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 utilizes 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

Vitamin E is the collective name for the eight lipid-soluble antioxidants in the tocotrienol and tocopherol families, which are mostly produced by plants and other photosynthetic organisms [17]. The four different forms of tocotrienol and tocopherol families may be distinguished by the number and location of methyl groups inside the aromatic ring [18]. Plants are protected from oxidative stress by tocotrienols and tocopherols, and their antioxidant characteristics provide food products extra beneficial qualities [19]. According to Theriault et al. (1999), excessive vitamin E consumption has been associated with a number of therapeutic advantages, such as lowered cholesterol levels, the inhibition of breast cancer cell growth in vitro, a lower risk of cardiovascular disease, and a lower incidence of many degenerative diseases in humans. Although tocotrienols are less readily absorbed than tocopherols, they are more potent antioxidants [20]. The two primary forms of vitamin E present in seeds and leaves, respectively, are tocotrienol and -tocopherol, according to Munne'-Bosch and Alegre (2002) [21]. Even though the precise genes that encode the different enzymes in the process have just recently been discovered, the creation of tocopherols and tocotrienols has long been understood. In the quest to develop plants with increased vitamin E content, several encouraging advancements have already been accomplished. The first step in the route for the biosynthesis of tocopherols and tocotrienols is the creation of homogentisic acid (HGA), which is catalysed by the enzyme p-hydroxyphenyl-pyruvate dioxygenase (HPPD) (EC 1.13.11.27). Tocotrienol and tocopherol are produced in plants using two different precursors. Tocotrienols are produced by HGA geranylgeranyl transferase (HGGT) (EC 2.5.1.32), while tocopherols are produced by HGA phytyl transferase (HPT) (EC 2.5.1.62). Scientists from Germany's Institute of Botany documented the results of constitutive expression of HPPD cDNA from barley (Hordeum vulgare) in tobacco plants. The HPPD gene was cloned into the pBinAR binary vector using a SmaI cloning site between the 35S CaMV promoter and the octopine synthase (EC 1.5.1.11) polyadenylation signal. The construct was then introduced into the tobacco explant-transforming Agrobacterium GV3101. The results showed that transgenic lines generated twice as much vitamin E in the seeds and had a higher overall capacity for homogentisic acid synthesis [24]. The vitamin E content of the leaves was unaltered. Another technique of modifying plant vitamin E content includes the last enzyme in the process that converts -tocotrienol and -tocopherol into -tocotrienol and -tocopherol, respectively. This happens at the end of the biosynthetic pathway for tocotrienols and tocopherols. The enzyme -tocopherol methyltransferase (-TMT) (EC 2.1.1.95) is responsible for catalyzing this process [25].

I.II 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 created by living things. They are divided into two groups: macronutrients and micronutrients. We need many foods called micronutrients, which include calcium, phosphorus, sodium, magnesium, chlorine, iron, sulfur and silicon [26].

I.II.A IRON

Although iron requirements are only numerical, the most common foodstuff worldwide is iron. According to the World Health Organization's estimate in 1992, approximately 30% of the world's population faces serious nutritional problems due to malnutrition. Myoglobin, which helps muscle cells 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). Hemoglobin and myoglobin in meat, fish 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 of the iron-binding protein ferritin and (2) reducing phytate, which inhibits iron absorption. It is important to be able to store and release iron in a controlled manner because although 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 under the control of a specific promoter gene, GluB-1 (from the gluten-producing wheat storage protein gene). This supplement has two advantages: It likes to collect iron from rice endosperm and can increase ferritin levels. Ferritin cDNA was isolated from soybean leaves using Agrobacterium, inserted into the pGPTV-35S-bar binary vector, and then transferred into rice. 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 biolistic 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 needed to develop an endogenous phytase that is heat stable and maintains high activity in tissues.

I.III IMPORTANT AMINO ACIDS

Amino acids combine to form chemical molecules called proteins. Proteins are broken down by the digestive system into individual amino acids so they can enter the bloodstream. Amino acids are then used by cells as building blocks to create structural and enzymatic proteins. There are two types of amino acids: essential amino acids and selective amino acids. Animals, including humans, cannot produce essential amino acids; Therefore, they must get them from food. Histine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are the nine essential amino acids. When sufficient amounts of amino acids and calories are consumed, the body can produce non-essential amino acids. Different proteins are found in foods, and some foods are considered high protein because they contain all nine essential amino acids. Most animal products, such as meat, dairy and eggs, provide protein. On the other hand, many essential amino acids are generally absent or unavailable in plants. For example, legumes are often deficient in methionine, while grains are often deficient in lysine [37].

I.III. A LYSINE

Meat is one of the most important staple products consumed 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 development. Lysine is an essential amino acid that helps absorb calcium, form collagen, and produce antibodies, hormones, and enzymes. Lysine deficiency can cause fatigue, difficulty concentrating, irritability, bloodshot eyes, delayed growth, hair loss, anemia, and growth problems. Zheng et al. Transgenic rice with increased lysine content was developed in 1995 [40]. Scientists achieved this by expressing Phaseolin, a seed storage protein from legumes (Phaseolus vulgaris), in genetically modified crops. Wheat gene-specific glutenin Gt1 promoter or normal phaseolin promoter was used to check the genomic and cDNA sequence of the gene at the gene level. The vector containing the β-phaseolin gene was introduced into the rice chromosome by protoplast-mediated transfection. In genetic modification, phaseolin accounts for 4% of the total endosperm protein and increases the lysine content in rice [40]

I.III.B METHIONINE AND TYROSINE

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 consumption [41]. According to Chakraborty et al. (2000) [41] indicate that there are four main markets for potatoes: fresh food, animal feed, food processing and non-food products such as starch and alcohol. Although potatoes are a good source of potassium, iron, vitamin C and vitamin B, they are low in protein. The nutritional value of potato protein is limited due to the lack of lysine, methionine and tyrosine [42]. Methionine deficiency affects growth and development by preventing the absorption of other amino acids. Methionine is an important source of sulfur and a natural form of heavy metals; it can prevent hair, skin and nail problems, lower cholesterol and lower blood pressure by promoting the production of phospholipid lecithin in the liver. [43].

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 genetic engineering in the food industry is not limited to manipulation of the plant genome. 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.

REFERENCES

[1] Zambryski, P., Joos, H., Genetello, C., Leemans, J., Van Montagu, M., & Schell, J. (1983). Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. The EMBO journal, 2(12), 2143-2150.

[2] Zambryski, P. (1988). Basic processes underlying Agrobacterium-mediated DNA transfer to plant cells. Annual review of genetics, 22(1), 1-30.

[3] Klein, T. M., Wolf, E. D., Wu, R., & Sanford, J. C. (1987). High-velocity microprojectiles for delivering nucleic acids into living cells. Nature, 327(6117), 70-73.

[4] Vain, P., De Buyser, J., Trang, V. B., Haicour, R., & Henry, Y. (1995). Foreign gene delivery into monocotyledonous species. Biotechnology advances, 13(4), 653-671.

[5] Kraemer, K., Semba, R. D., Eggersdorfer, M., & Schaumberg, D. A. (2012). Introduction: the diverse and essential biological functions of vitamins. Annals of Nutrition and Metabolism, 61(3), 185-191.

[6] Sharma, P., Aggarwal, P., & Kaur, A. (2017). Biofortification: A new approach to eradicate hidden hunger. Food Reviews International, 33(1), 1-21.

[7] Graham, R. D., Welch, R. M., & Bouis, H. E. (2001). Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps.

[8] Potrykus, I. (2001). Golden rice and beyond. Plant physiology, 125(3), 1157-1161.

[9] Armstrong, G. A., & Hearst, J. E. (1996). Genetics and molecular biology of carotenoid pigment biosynthesis. The FASEB journal, 10(2), 228-237.

[10] Ye, X., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P., & Potrykus, I. (2000). Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science, 287(5451), 303-305.

[11] Hallberg, L., Brune, M., & Rossander, L. (1989). Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. The American journal of clinical nutrition, 49(1), 140-144.

[12] Davey, M. W., Montagu, M. V., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., ... & Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture, 80(7), 825-860.

[13] Wheeler, G. L., Jones, M. A., & Smirnoff, N. (1998). The biosynthetic pathway of vitamin C in higher plants. Nature, 393(6683), 365-369.

[14] Smirnoff, N., Conklin, P. L., & Loewus, F. A. (2001). Biosynthesis of ascorbic acid in plants: a renaissance. Annual review of plant biology, 52(1), 437-467.

[15] Washko, P. W., Welch, R. W., Dhariwal, K. R., Wang, Y., & Levine, M. (1992). Ascorbic acid and dehydroascorbic acid analyses in biological samples. Analytical biochemistry, 204(1), 1-14.

[16] Chen, F., D'Auria, J. C., Tholl, D., Ross, J. R., Gershenzon, J., Noel, J. P., & Pichersky, E. (2003). An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. The Plant Journal, 36(5), 577-588.

[17] Hess, J. L. (2017). Vitamin E, α-tocopherol. In Antioxidants in higher plants (pp. 111-134). CRC press.

[18] Kamal-Eldin, A., & Appelqvist, L. Å. (1996). The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 31(7), 671-701.

[19] Andlauer, W., & Fürst, P. (1998). Antioxidative power of phytochemicals with special reference to cereals. Cereal foods world, 43(5), 356-360.

[20] Theriault, A., Chao, J. T., Wang, Q. I., Gapor, A., & Adeli, K. (1999). Tocotrienol: a review of its therapeutic potential. Clinical biochemistry, 32(5), 309-319.

[21] Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences, 21(1), 31-57.

[22] Kobayashi, N., & DellaPenna, D. (2008). Tocopherol metabolism, oxidation and recycling under high light stress in *Arabidopsis*. The Plant Journal, 55(4), 607-618.

[23] Soll, J., Kemmerling, M., & Schultz, G. (1980). Tocopherol and plastoquinone synthesis in spinach chloroplasts subfractions. Archives of biochemistry and biophysics, 204(2), 544-550.

[24] Falk, J., Andersen, G., Kernebeck, B., & Krupinska, K. (2003). Constitutive overexpression of barley 4-hydroxyphenylpyruvate dioxygenase in tobacco results in elevation of the vitamin E content in seeds but not in leaves. FEBS letters, 540(1-3), 35-40.

[25] Shintani, D., & DellaPenna, D. (1998). Elevating the vitamin E content of plants through metabolic engineering. Science, 282(5396), 2098-2100.

[26] Morris AL, Mohiuddin SS. Biochemistry, Nutrients. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2022. PMID: 32119432.

[27] Stoltzfus, R. J., Mullany, L., & Black, R. E. (2004). Iron deficiency anaemia. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors, 1, 163-209.

[28] Viteri, F. E. (1994). The consequences of iron deficiency and anemia in pregnancy. Nutrient regulation during pregnancy, lactation, and infant growth, 127-139.

[29] Murakawa, H., Bland, C. E., Willis, W. T., & Dallman, P. R. (1987). Iron deficiency and neutrophil function: different rates of correction of the depressions in oxidative burst and myeloperoxidase activity after iron treatment.

[30] Basta, S. S., Karyadi, D., & Scrimshaw, N. S. (1979). Iron deficiency anemia and the productivity of adult males in Indonesia. The American Journal of Clinical Nutrition, 32(4), 916-925.

[31] Maberly, G. F., Trowbridge, F. L., Yip, R., Sullivan, K. M., & West, C. E. (1994). Programs against micronutrient malnutrition: ending hidden hunger. Annual Review of Public Health, 15(1), 277-301.

[32] Beard, J. L., Dawson, H., & Piñero, D. J. (1996). Iron metabolism: a comprehensive review. Nutrition Reviews, 54(10), 295-317.

[33] Goto, F., Yoshihara, T., Shigemoto, N., Toki, S., & Takaiwa, F. (1999). Iron fortification of rice seed by the soybean ferritin gene. Nature biotechnology, 17(3), 282-286.

[34] Lucca, P., Hurrell, R., & Potrykus, I. (2002). Fighting iron deficiency anemia with iron-rich rice. Journal of the American College of Nutrition, 21(sup3), 184S-190S.

[35] Wyss, M., Pasamontes, L., Rémy, R., Kohler, J., Kusznir, E., Gadient, M., ... & van Loon, A. P. (1998). Comparison of the thermostability properties of three acid phosphatases from molds: Aspergillus fumigatus phytase, A. niger phytase, and A. niger pH 2.5 acid phosphatase. Applied and Environmental Microbiology, 64(11), 4446-4451.

[36] Holm, P. B., Kristiansen, K. N., & Pedersen, H. B. (2002). Transgenic approaches in commonly consumed cereals to improve iron and zinc content and bioavailability. The Journal of nutrition, 132(3), 514S-516S.

[37] Miflin, B., Napier, J., & Shewry, P. (1999). Improving plant product quality. Nature Biotechnology, 17(2), BV13-BV14.

[38] Li, Z. K., Yu, S. B., Lafitte, H. R., Huang, N., Courtois, B., Hittalmani, S., ... & Khush, G. S. (2003). QTL× environment interactions in rice. I. Heading date and plant height. Theoretical and Applied Genetics, 108, 141-153.

[39] Fickler, J., Kirchgessner, M., & Roth, F. X. (1995). The effect of dietary arginine supply on the N balance of piglets, 4: Importance of non-essential amino acids for protein retention. Journal of Animal Physiology and Animal Nutrition (Germany).

[40] Zheng, Z., Sumi, K., Tanaka, K., & Murai, N. (1995). The bean seed storage protein [beta]-phaseolin is synthesized, processed, and accumulated in the vacuolar type-II protein bodies of transgenic rice endosperm. Plant Physiology, 109(3), 777-786.

[41] Chakraborty, S., Tiedemann, A. V., & Teng, P. S. (2000). Climate change: potential impact on plant diseases. Environmental pollution, 108(3), 317-326.

[42] Jaynes, J. M., Yang, M. S., Espinoza, N., & Dodds, J. H. (1986). Plant protein improvement by genetic engineering: use of synthetic genes. Trends in biotechnology, 4(12), 314-320.

[43] Cooper, T. G. (1996). Allantoin degradative system—an integrated transcriptional response to multiple signals. Mycota, 3, 139-169.