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VİTAMİN VE MİKROELEMENT İÇERİĞİ GENETİKSEL OLARAK ARTIRILMIŞ BİTKİLER

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ÖZET

Bitkiler beslenmemiz için gerekli olan vitamin ve mikroelementlerin temel kaynağıdır. Fakat her gün temel olarak tükettiğimiz bitkiler çoğunlukla eksikliğinde ciddi sağlık problemlerine sebep olabilen önemli vitamin ve mikroelementler yönünden fakirdir. Biyoteknolojik araştırmalar yiyeceklerimizin besin değerinin artırılmasında sahip olduğu potansiyeli çoktan göstermiştir. Bu noktada provitamin A, C vitamini, E vitamini ve ferritin miktarı artırılmış transgenik bitkiler insan sağlığı için beslenme problemlerinin giderilmesinde ümit vericidir. Bu derleme ticari olarak büyütülen transgenik bitkileri kısaca özetlemekte olup yakın gelecekte ticari amaçlı kullanılabilecek besin değeri artırılmış yeni transgenik bitkiler üzerine odaklanmıştır.

Anahtar kelimeler: Besin değeri artırılmış bitkiler, Genetik mühendisliği, Demir eksikliği, Vitamin eksikliği, Vitamin biyosentezi.

PLANTS GENETICALLY ENHANCED with VITAMINS and MICROELEMENTS

ABSTRACT

Plants are a major source of vitamins and micronutrients that required in human diet. However main food crops are usually deficient in important nutrients such as vitamins and microelements that deficiencies can cause series health problems. Biotechnology research has already demonsrated its potantial in enhancing the nutritional quality of our food. At this point, transgenic plants with enhanced provitamin A, vitamin C, vitamin E and ferritin content are promising for solving nutrition problems for human health. This review briefly summarise commercially grown transgenic crops and concentrates on new transgenic crops with enhanced nutritive value that might commercialized in near future.

Keywords: Biofortified plants, Genetic engineering, Iron deficiency, Vitamin deficiency, Vitamin synthesis.

1. INTRODUCTİON

Genetic engineering can most simply be defined as the transfer of genetic materyal from a different species (plant, bacterial or animal) or from a chemically synthesized gene into a target plant [1]. The application of this technique to agricultural production has resulted in a number of transgenic, or genetically modified (GM), crops that have increased features of herbicide tolerance, insect or virus resistance [2, 3, 4]. These GM crops were first commercialized in 1996 and there are about 12-15 major GM crops such as soybean, maize, cotton, conola produced word-wide [5]. Today they are being grown on nearly 81 million hectares in 17 countries (Tablo 1.1) and food ingredients produced from GM crops are found in thousands of food products [2, 5, 6, 7]. During the nine-year period 1996 to 2004, herbicide tolerance has consistently been the dominant trait followed by insect resistance.

Today most of the research and commercial trends are towards the generation of transgenics with improved product quality and multiple gene inserts [3, 8, 9]. Especially the recent application of genetic engineering to improve the nutritional content of staple food crops has the greatest potential to benifit global health [10, 11]. Because poverty limits food access for much of the devoloping world's population, it is important that affordable staple foods be as nutritious as possible. Plants are a major source of vitamins and micronutrients in the human diet. Due to their significance for human health and development, research has been initiated to understand the biosynthesis of vitamins in plants. The scientists have recently succeeded in transfering genes into food crop species to increase the quantities of vitamin A, C, E and micronutrient iron [12, 13, 14, 15]. This review will concentrate on biosynthesis of above vitamins and iron uptake mechanisms together with currently results obtained from these transgenic plants.

Table 1.1. Global Area of GM Crops in 2004 by Country [5].

Country	GM planted area (million hectares)	% of global total	Planted GM crop(s)
USA	47.6	%59	Soybean, Maize, Cotton, Canola
Argentina	16.2	%20	Soybean, Maize, Cotton
Canada	5.4	%6	Canola, Maize, Soybean
Brazil	5.0	%6	Soybean
China	3.7	%5	Cotton
Paraguay	1.2	%2	Soybean
India	0.5	%1	Cotton
South Africa	0.5	%1	Maize, Soybean, Cotton
Uruguay	0.3	<%1	Soybean, Maize
Australia	0.2	<%1	Cotton
Romania	0.1	<%1	Soybean
Mexico	0.1	<%1	Cotton, Soybean
Spain	0.1	<%1	Maize
Philippines	0.1	<%1	Maize
Colombia	0.05	<%1	Cotton
Honduras	0.05	<%1	Maize
Germany	0.05	<%1	Maize

1.1.Transgenic Plants With Enhanced Provitamin a Content

Vitamin A compounds, consisting of retinol and β-carotene (provitamin A), are found in animal tissues and plants. The animal based substance, known as retinol, is ready for use by the human body. It is derived from break down of β-carotene which can be synthesized naturally by plants and microorganisms. Therefore humans and animals are depend on dietary carotenoids for making their retinol. The recomemded dietary allowance for vitamin A is 1000 retinol equivalents, equal to 6 mg β-carotene, per day. The widespread occurrence of vitamin A deficiency is well documented [16] and emphasizes the requirement for staple foods enhanced in carotenoids exhibiting provitamin A activity [17].

Carotenoids are synthesized by all photosynthetic organisms, where they participate in light harvesting and photoprotection from excess light energy. In plants carotenoids accumulate together with chlorophyll in leaf chloroplasts and in the chromoplasts of many fruits, seeds and flowers. A wide range of different carotenoids can be found in chromoplasts: lycopene in tomato fruits, β-carotene in carrot roots, lutein and zeaxantine in maize endosperm [17]. Geranylgeranyl diphosphate (GGPP), derived from the ubiquitous isoprenoid pathway, is the precursor of carotenoids as well as tocopherols and phylloquinone (Figure 1.1.1). The conversion of GGPP to phytoene is the first committed step in carotenoid biosynthesis, catalyzed by the enzyme phytoene synthase. The next two enzymes (phytoene and ζ-carotene desaturases) desaturate phytoene and ζ-carotene, respectively. The product of ζ-carotene desaturase is lycopene, which is responsible for the characteristic red color of ripe fruits. It can undergo cyclization to either β- or α-carotenes followed by hydroxylation to yield xanthophylls such as lutein. [17, 18].

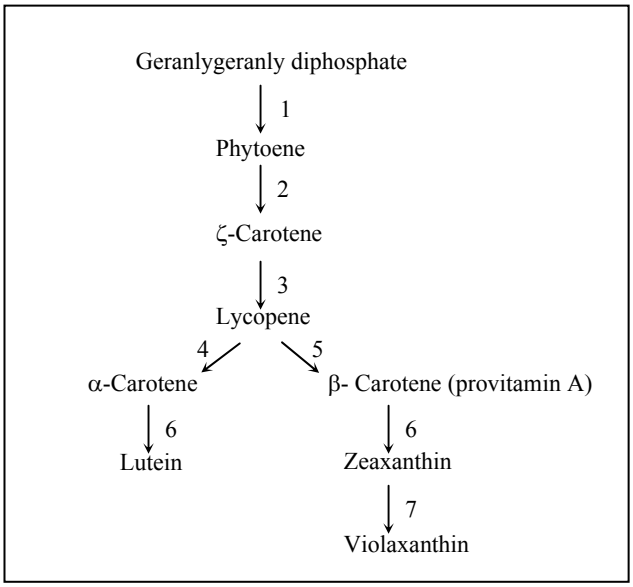

Figure 1.1.1. Biosynthetic Pathways of Carotenoids in Plants (adapted from Giuliano et al., [17]). Enzymes catalyzing the numbered reactions are; 1, phytone synthase; 2, phytone desaturase; 3, ζ-carotene desaturase; 4, ε cyclase / β cylase; 5, β cylase; 6, hydroxylase; 7, epoxidase.

Several approaches have been performed resulting in increased levels of β-carotene in different plant species and tissues (Table 1.1.1). The best known example of carotenoid metabolic engineering in plants is of course the synthesis of β-carotene in rice endosperm (Golden Rice), which normally accumulates GGPP but lacks the subsequent enymes in the pathway [12]. The expression of phytoene synthase (*psy*) and lycopene cyclase (*lcy*) both from *Narcissus pseudonarcissus* together with a bacterial phytoene desaturase (*crt*I) from *Erwinia uredovora* in rice endosperm increased β-carotene content with levels up to 200 μg β-carotene per 100 g [19] Additionally the researches found that expression of *psy* and *crt*I alone were sufficient not only for the synthesis of lycopene but also for that of β-carotene and zeaxanthin [20].

Transgenic tomotoes have also been described expressing phytone desaturase, β-cyclase or phytone synthase [18, 21, 22]. The expression of phytone desaturase in tomato fruits resulted in a %50 decrease in total carotenoids, mainly at the expence of lycopene while β-carotene increased about threefold from about 270-520 μg/g dry weight [21]. Furthermore, expression of phytone synthase in tomato plants resulted in a two fold increase in total carotenoid levels and an enrichment of 2.4-, 1.8-, and 2.2-fold for lycopene, β-carotene and lutein, respectively, in the fruit [18]. Recently, potato tubers containing enhanced levels of β-carotene and lutein has been produced [23]. Carotenoid content of developing tubers of transgenic potato plants increased to 35- 78 μg g^{-1} dry weight from which 5.6- 20 μg g^{-1} dry weight in untransformed control tubers. Although control tubers contained negligible amounts β-carotene in the transgenic tubers it reached to 11μg g^{-1} dry weight [23].

Table. 1.1.1. Metabolic Engineering Approaches for Increased β-carotene Levels in Transgenic Plants.

Genes	**Source**	**Promotor**	**Plant**	**Tissue**	**Main Products**	**Ref.**
Phytone desaturase (*crtI*)	*E. uredovora*	CaMV 35S	Tobacco	Leaves	Violaxanthin, β-carotene	[24]
Phytoene synthase (*crtB*)	*E. uredovora*	Brassica napin	Canola	Embryo	β-carotene, α-carotene	[25]
Phytoene synthase Phytone desaturase (*crtI*) Lycopene β-cyclase	Daffodil *E. uredovora* Daffodil	Rice glutein CaMV 35S Rice glutein	Rice	Endosperm	Lutein, zeaxantine, β-carotene, small amounts of α-carotene	[19,26]
Phytone desaturase (*crtI*)	*E. uredovora*	CaMV 35S	Tomato	Fruit	50% total reduction, β-carotene	[21]
β-cyclase	Arabidopsis	Tomato *Pds*	Tomato	Fruit	β-carotene	[22]
Phytoene synthase (*crtB*)	*E. uredovora*	PG	Tomato	Fruit	lycopene, β-carotene, lutein	[18]
Phytoene synthase (*crtB*)	*E. uredovora*	Potato patatin	Potato	Tuber	β-carotene, lutein	[23]

Abbreviations: CaMV= Cauliflower mosaic virus; PG= polygalacturonase
Taxonomic names: *Brassica napus* *Nicotiana tobacum* *Erwinia uredovora* *Oryza sativa* *Lycopersicon esculentum* *Solanum tuberosum* *Narcissus pseudonarcissus*

1.2.Transgenic Plants With Enhanced Vitamin C Content

Plants, algea and majority of animals are able to synthesize L-ascorbic acid (vitamin C). However, humans cannot synthesize L-ascorbic acid because the gene encoding L-gulono-1,4-lactone axidase, the last enzyme of L-ascorbic acid pathway, is mutated and non-functional, and require ascorbic acid as an essential micronutrient. Therefore L-ascorbic acid in fruits and vegetables is an essential component of human nutrition but the biosynthesis of L-ascorbic acid in plants is not completely elucidated and its regulation is largely unknown [27].

Two distinct patways for L-ascorbic acid in plants proposed (Figure 1.2.1), [27]. The first pathway involves pectine-derived D-galacturonic acid that is reduced to L-galacturonic acid by the D-galacturonic acid reductase [28] , and the resulting L-galactono-1,4-lactone is oxidized to L-ascorbic acid by the mitocondrial L-galactono-1,4-lactone dehydrogenase [27]. The second pathway is energy-dependent biosynthesis that involves the conversion of GDP-D-mannose either to GDP-L-galactose or GDP-L-gulose catalyzed by GDP-D-mannose-3,5-epimerase [29, 30].

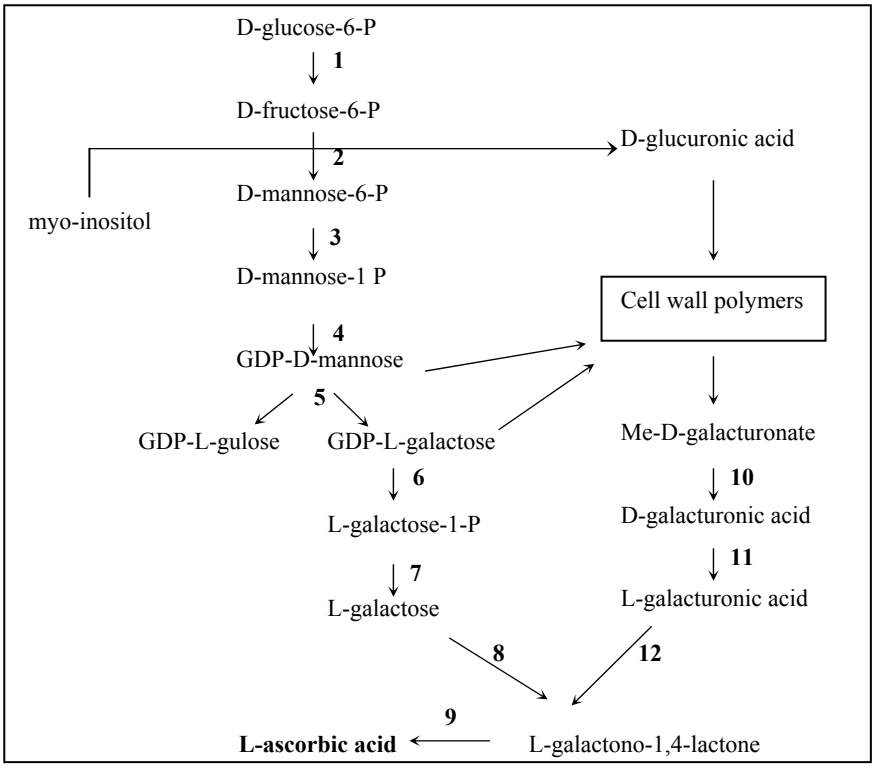

Figure 1.2.1. Proposed Biosynthetic Pathways of L-Ascorbic Acid in Plants (adapted from Vulpuesta and Botella [27]). Enzymes catalyzing the numbered reactions are; 1, glucose-6-phosphate isomerase; 2, mannose-6-phosphate isomerase; 3, phosphomannomutase; 4, GDP-mannose phyrophosphorylase; 5,GDP-mannose-3',5'-epimerase; 6, phosphodiesterase;

7, sugar phosphatase; 8, L-galactose dehydrogenase; 9, L-galactono-1,4-lactone dehydrogenase; 10, metlyesterase; 11, D-galacturonate reductase; 12, aldono-lactonase.
L-galactose released from GDP-L-galactose through some poorly understand steps, is then oxidized to L-galactono-1,4-lactone by an L-galactose dehydrogenase; the latter compound is converted to L-ascorbic acid by L-galactono-1,4-lactone dehydrogenase. However the following steps for GDP-L-gulose in the branch point have not been described yet. In addition, *myo*-inositol also proposed to be an important precursor of L-ascorbic acid biosynthesis. But the contribution of this sugar to L-ascorbic acid biosynthesis *in vivo* has yet to be determined [27].

Recent studies indicate that multiple L-ascorbic acid biosynthetic pathways are functioning in plants. These findings are also offering additional tools to increase L-ascorbic acid content of plants via genetic engineering, in view of improving the nutritional value of crops, but also potentially exploited for the industrial production of L-ascorbic acid [31].

Overexpression of the gene encoding D-galacturonic reductase from strawberry in *Arabidopsis thaliana* enhanced L-ascorbic acid content two-to threefold, demonsrating the potantial of engineering increased L-ascorbic acid levels in plants using this gene [28]. Beside the synthesis, catabolism of L-ascorbic acid also important for controlling L-ascorbic acid content in some plant tissues. The first catabolic step is oxidation, which produces the monodehydroascorbate and dehydroascorbate (DHA) in a reaction catalyzed by dehydroascorbate reductase (DHAR). DHAR allows the plant to recycle DHA before L-ascorbic acid is lost. The overexpression of the enzyme DHAR in tobacco and maize increased foliar and kernel ascorbic acid levels 2- to 4 fold. It was demonstrated that vitamin C content of plants can be elevated by increasing expression of the enzyme responsible for recycling ascorbate [13].

1.3.Transgenic Plants With Enhanced Vitamin E Activity

Tocols (vitamin E) are an important class of lipid-soluble compounds with antioxidant activities that are synthesized only by plants and other photosynthetic microorganisms. The two major classes of tocols , tocopherols and tocotrienols, have a common structure consisting of a polar chromanol head group and a non-polar prenyl tail. In tocopherols the polar chromanol headgroup derived from homogentisic acid and the hydrophobic prenyl tail derived from phytol (Figure 1.3.1). However in tocotrienols only prenyl tail derived from geranylgeranyl diphosphate (GGDP) instead of phytol. There are four naturally accurring forms of tocopherols and tocotrienols (α, β , γ, δ), which differ only in the position and number of metly groups on chromanol ring. Although all tocopherols and tocotrienols are potent antioxidants *in vitro*, α-tocopherol is the most active in terms of vitamin E activity, partly because it is retained the human body in preference to other tocopherols and tocotrienols [32, 33].

The primary sources of dietary vitamin E drived from plants, in which the quantity and composition varies between tissues and species. Oilseeds are the richest source of vitamin E, having total tocol levels ranging from 330 to 2000 μg per gram of oil [34]. Unfortunately, many plant oils accumulate γ-tocopherol, which has one-tenth the vitamin E activity of α-tocopherol (Table 1.3.1). Beside, another good source of vitamin E, green vegetables, has low tocol yields but high proportions of α-tocopherol. Although green plant

tissues produce only between 20 and 50 μg of total tocols per gram tissue, the tocol pools comprise almost entrely α-tocopherol [34].

Tocopherol and tocotrienol represents essential micronutrients for humans. α-Tocopherol in particular has been reported to have benificial effects on human health when vitamin E supplements have been taken at therapeutic doses (100 to 1000 International Units, I.U. [34]. However, these vitamin E levels are greatly in excess of the recommended daily allowance (40 I.U.) and cannot be obtained from the average plant-derived diet. Therefore, much more effort is currently aimed at identifying the genes involved in tocopherol biosynthesis to improve vitamin E levels in crop plants by metabololic engineering [32, 33, 36].

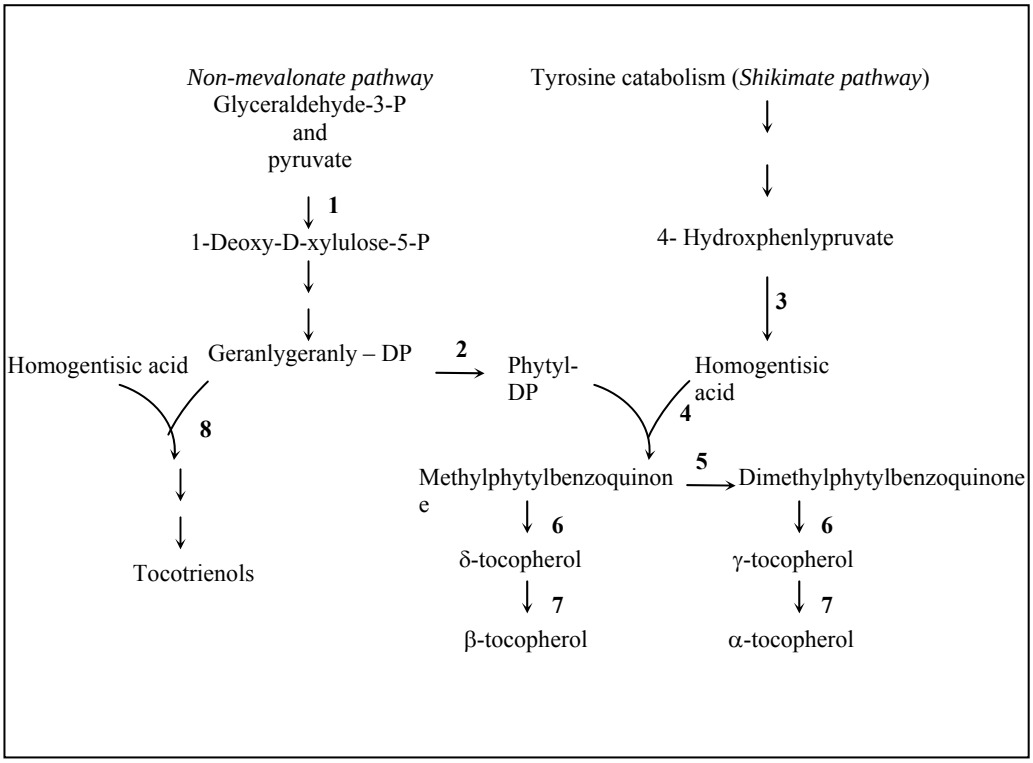

Figure 1.3.1. Tocopherol Biosynthesis in Plants (adapted from Ajjawi and Shintani [41]). Enzymes catalyzing the numbered reactions are; 1, 1-deoxy-D-xylulose-5-phosphate synthase; 2, geranylgeranly diphosphate reductase; 3, p-hydroxyphenyl-pyruvate dioxygenase; 4, homogentisate phytyltransferase; 5, methyphytylbenzoquinone methytransferase; 6, cyclase; 7, γ-tocopherol methytransferase; 8, homogentisate geranylgeranyl transferase.

Table 1.3.1 Tocopherol Contents (mg/Kg) in Some Seed Oils [35].

Plants	Alpha-T	Beta-T	Gamma-T	Delta-T
Palm	89	-	18	-
Soybean	100	8	1021	421
Sunfower	282	54	1034	54
Maize	670	27	11	1
Rapeseed	202	65	490	9

The strategies for increasing the vitamin E content in plant foods could be either by increasing the flux through the tocopherol biosynthetic pathway to produce elevated levels of tocols or altering the tocol composition in favor of α-tocopherol. Increasing pathway flux would be especially useful in increasing the vitamin E content of green vegetable crops, and altering the tocol composition in favor of α-tocopherol would be significanly increase the vitamin E content of oilseeds.

The first potential flux control points in tocopherol synthesis involve the enzyme hydroxphenyl pruvate dioxygenase (HPPD) (Figure 1.3.1). It has been shown that when the gene encoding HPPD was overexpressed in Arabidopsis and tobacco there was a small increase in tocopherol content both in leaves (10%) and seeds (30%). This result shows that increasing HPPD activity is not sufficient to increase tocopherol content [37, 38]. Furthermore, to investigate the importance of homogentisate phytyltransferase (HPT) activity on the flux regulation of tocopherol biosynthetic pathway, the gene encoding Arabidopsis *HPT1* was constitutively overexpressed in Arabidopsis. In leaves, *HPT1*overexpression resulted in a 10-fold increase in HPT specific activity and a 4.4-fold increase in total tocopherol content relative to wild type. In seeds, *HPT1* overexpression resulted in a 4-fold increase in HPT specific activity and a total seed tocopherol content that was 40% higher than wild type, primarily because of an increase in γ-tocopherol content [39, 40].

The enzymes catalyzing the later steps of the tocopherol biosynthetic pathway, specifically the methylphytlbenzoquinone methyltransferase (MPBQMT) and γ-tocopherol methyltransferase (γ-TMT) are important in determining tocopherol composition. Transgenic plants with elevated α-tocopherol content has already been reported [14, 41]. The genes encoding MPBQMT and γ-TMT were overexpressed in soybean seeds. The overexpression of MPBQMT and γ-TMT resulted in 95% conversion of δ- and γ-tocopherol forms of vitamin E to α-tocopherol and five fold incerase in vitamin E activity.

1.4 Transgenic Plants With Enhanced İron Concent

Iron is essential micronutrient for human health and is found in red meat and breast milk, and, in a less absorable form, in grains, legumes and vegetables. Iron deficiency is estimated to affect about 30% of the world population, making iron by far the most widespread nutrient deficiency world-wide [16]. Options to improve iron intake can include the universal fortification of widely consumed commercially processed foods (e.g. wheat and corn flours, and rice) or changes in dietary practices to increase intake of available food sources that naturally contain iron and absorption enhancers.

There are genetic engineering approaches that could be used to increase Fe content and bioavailability in plant foods. The Fe content could be increased by increasing soil Fe uptake or Fe storage within the edible plant. Dicots and nongramineous monocots must reduce Fe(III) to Fe(II) before transporting it into the cell [42]. The reduction of ferric iron to ferrus iron by ferric chelate reductase (FRO2) is the rate limiting step in Fe uptake at the root surface. Expression of a yeast ferric reductase gene in tobacco roots led to enhanced constitutive reductase activity and leaf Fe by 50% [43]. Plant genes encoding putative ferric chelate reductases have also been identified [44]. When the Arabidopsis *FRO2* gene was overexpressed in transgenic Arabidopsis plants the FRO2 activity was only elevated under conditions of Fe deficiency. Although Fe content of plants were not increased it was suggested that overexpression of *FRO2* gene might be usuful for the construction of plants capable of thriving on low-iron soils, because approximately one-third of the word's soils are considered iron deficient [45].

In graminaceous plants ferric Fe is chelated to one of several organic molecules, known as phytosiderophores, which are synthesized and secreted by the roots prior to their reabsorption as an Fe-phytosiderophore complex [46]. In addition the overexpression of barley nicotianamine aminotransferase genes in rice roots resulted in enhanced phytosiderophore synthesis by these roots [47, 48]. By increasing the availability of Fe at the root surface, both strategies enabled the plants to accumulate more Fe in leaf (and root) tissues.

The Fe content of plants could be also increased by increasing Fe storage within the edible plant. In plants and other organisms, the spesific sequestration of Fe ions is carried out by ubiquitous proteins, termed ferritins. Ferritins are typically present in plastids, both in non-photosynthetic tissues and in photosynthetic tissues, and form complexes with up to 4500 Fe atoms per ferritin molecule, making it a likely target for improving the iron content of plants (Table 1.4.1) [15, 49, 50].

Table 1.4.1 Iron Content (atoms/molecule) of Ferritins From Some Legume Seeds [50].

Taxon	Fe per molecule
Pea (*Pisum sativum*)	1800-2100
Soybean (*Gylcine max*)	2500
Lentil (*Lens esculenta*)	2100
Jackbean (*Canavalia ensiformes*)	900
Black gram (*Vigna mungo*)	1100

Metabolic engineering of plants to elevate the level of ferritin has been reported. The gene encoding *ferritin* isolated from *Phaseolus vulgaris* [15] and *Glycine max* [51] were introduced into rice grains and overexpressed. The expression of *Glycine max ferritin* gene led to higher Fe and Zn levels in transgenic rice grains. The removel of outer layers of the rice seed by commercial milling dramatically reduces the level of Fe in grains because most of the Fe is accumuted in the aleurone layer. However, in transgenic rice Fe and Zn content was increased not only in brown grains but also in polished grains [51].

In addition grain and legume staples are high in phytic acid, which is a potent inhibitor of Fe absoption. The manipulation of phytase activity is an important approach to enhancing mineral bioavalilability. Phytic acid, a storage form of phosphorus in plant tissue, binds Fe and Zn very strongly. As it is poorly digested and thus prevents absoption of these minerals, phytic acid may represent the most important factor restricting the uptake of these minerals [52]. To increase Fe bioavailability a thermo-tolerant phytase gene from *Aspergillus fumigatus* was transferred into the rice endosperm. This resulted in 130- fold increase in the expression of this enzyme and giving a phtase activity sufficient to completely degrate phytic acid in a sitimulated digestion experiment [15].

2. DISCUSSION

Vitamin and micronutrient deficiencies are one of the most important health problems for human being especially in developing countries. Despite improvements over the last 50 years, some 800 million people have insufficient food, and over a billion people, particulary women and children, are affected by specific nutrient deficiencies [53]. Although plant foods contain almost all of the mineral and organic nutrients established as essential for human nutrition but often these are not present in sufficient amounts. Improving the nutritional quality of our food crop species through plant biotechnology may be a more sustainable strategy to combat deficiencies in human populations. Current results already show that provitamin A, vitamin C and vitamin E biosynthestic pathways in plants can be manipulated to produce more nutritive plants. However, bioavailability of the nutrients from these transgenic crops is an important issue. For example, because of the low bioavailability of β-caroten some researchers suggest that rice containing β-caroten is unlikely to alleviate vitamin A deficiency [54]. This issue remains to be demonsrated whether the provitamin A will be available in human studies. A similar problem also exits for transgenic plants with enhanced Fe content. Because phytic acid content of the plants effect the bioavailabity of Fe. Although in transgenic rice with increased phytase activity phytic acid was completely degraded in uncooked rice the activity was destroyed when the rice was cooked. The influence of these changes on Fe bioavailabity remains to be investigated [10, 11]. Acceptance of this new transgenic crops or its products by public is also another important point.

Beside these limitations there are also benifits that these transgenic plants offer. For examle, the current world production of L-ascorbic acid is estimated at 80 000 tons per annum with a global market in excess of US$ 600 million. At present, the majority of commercially manufactured sythetic L-ascorbic acid is synthesized via the seven step Reichstein process using D-glucose as a starting point. The yield of L-ascorbic acid from D-glucose abtained by Reichstein process is about 50% and it is higly energy comsuming requiring high temperatures and/or pressures for many steps [31]. Production of vitamins and micronutrients in transgenic plants is not competitive to the currently available production systems. But these plants are more likely to become as factories of vitamin or micronutrient in near future.

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