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Review Article

Folates and *Persea americana* Mill. (Avocado)

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Abstract: In developing countries there has been a rapid increase in the number of cases of metabolic disorders associated to human lifestyle. A rich diet of refined foods, poor in micronutrients has a direct correlation with human metabolic disorders and other diseases. The aim of this study is to reassess the role of indigenous foods, its richness in micronutrients and its re-inclusion in the diet of local man. This cultural factor could minimize the negative impact caused by the lack of micronutrients such as the folates in human health. The Avocado (*Persea americana* Mill.) fruit is native of Central America and is an ancestral food that bear folates. We review the synthesis of folates pathway in plants, cultural and economic importance of the fruit of avocado as ancestral food of the inhabitants of the land bridge that links both the North and South American sub-continents. The important micronutrients such as folates in relieving some metabolic disorders and syndromes associated with human lifestyle are also reviewed in this study.

Key words: Folates, *Persea Americana,* micronutrients

الفولات و (الافكادو) *Persea americana* Mill

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الملخص:إن هناك زيادة سريعة في عدد حالات الاضطرابات الأيضية المرتبطة بحياة الإنسان في البلدان النامية و أن إتباع نظام غذائي غني بالأطعمة المكررة والفقيرة في المغذيات الدقيقة له علاقة مباشرة بالاضطرابات الأيضية وغيرها من الأمراض عند الإنسان. إن الهدف من هذا البحث هو إعادة تقييم دور الأطعمة المحلية، وثراءها بالمغذيات الدقيقة و إعادة إدراجها في النظام الغذائي للسكان المحليين. وهذا العامل الثقافي ممكن استخدامه للحد من الآثار السلبية الناجمة عن نقص المغذيات الدقيقة مثل الفولات في صحة الإنسان. ثمرة الافوكادو الموجودة أصلا في أمريكا الوسطى وغذاء تقليدي و يحتوي على الفولات. قمنا بفحص و مراجعة مسار تصنيع الفولات في النباتات، بالإضافة للأهمية الثقافية والاقتصادية لثمرة الافوكادو (P. americana Mill.)كغذاء تقليدي لسكان الجسر البري الذي يربط بين كل من شبه القارة الأمريكية الشمالية والجنوبية ، و أهمية المغذيات الدقيقة مثل الفولات في تخفيف بعض الاضطرابات الأيضية والمتلازمات المرتبطة مع نمط الحياة البشرية.

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Introduction

From the second decade of the twentieth century came a change in eating habits of the world population mainly in developing countries. A cheap way of to satisfy the body's caloric demand, man increased his food consumption based on refined grains and tubers, which are poor in micronutrients such as minerals and vitamins. At the same time, man divested the expensive native foods rich in micronutrients. The result of the change in dietary habits resulted in the emergence of the epidemic of metabolic diseases related to human lifestyle. This is a reason for analyzing the relationship between man and native foods of plant origin as the source of micronutrients.

A relatively small number of plants have closely accompanied the evolution, development and splendor of the human being. A group of them have been distributed globally; it may include plants used as condiments, ornamentals, and fruit producers, all of them natives from Asia, Europe and North Africa. However, other groups of native plants have contributed to human welfare in its habitat. Of them much of their biology, ecology and phytochemistry are unknown, an example of this is the tree *Persea americana* Mill. A species used by the ancestral pre-Columbian Central American cultures until today, which have lived on the isthmus connecting the mainland portions North and South of the Continent American. Its importance is due to the culinary properties, its use in traditional medicine, and for the physical and mechanical properties of its wood.

Persea americana Mill.

P. americana Mill. belongs to the Family Laureaceae, in nature there exist three landraces. They are, the West Indian (*P. americana* var. *americana*), the Guatemalan (*P. americana* var. *guatemalensis*) and Mexican (*P. americana* var *drymifolia*). In its natural habitat it grows to a height of 10 to 12m, with lateral roots, simple leaves, whole, smooth, leathery and deep green. The flowers are hermaphrodites, symmetrical, and yellow-green. The fruit do not ripe on the tree, are pear shaped, oval, globular or elliptic oblong, with color variation of dark purple to black.

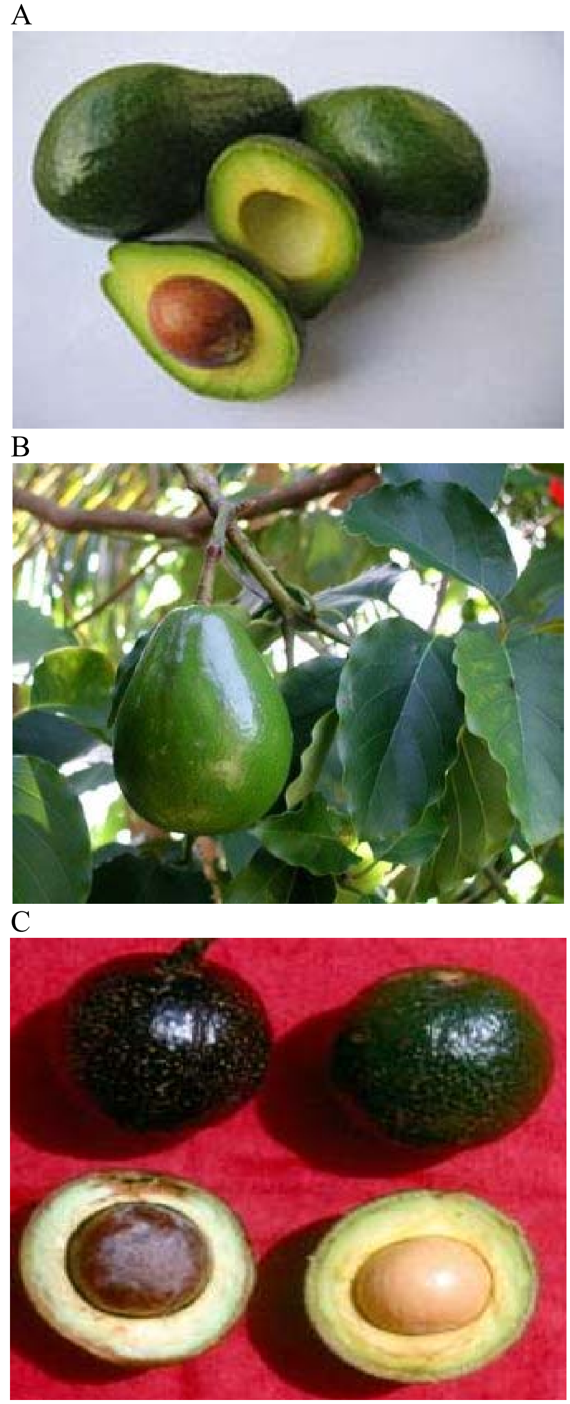

Figure 1. Images of *Persea americana* Mill fruits. A) *P. americana* var. *americana*. B) *P. americana* var. *drymifolia.* C) *P. americana* var. *guatemalensis.*

The shape, color, structure and consistency of the fruit are special characteristic of each of the varieties. It is estimated that *P. americana*

Mill (avocado) is native of the highlands of central and eastern Mexico and Central America, the areas domesticated (Williams, 1997).

Archaeological and linguistic evidence for the avocado domestication is date of 8000 to 7000 B.C. which were found in Coxcatlan, Puebla, Mexico (Smith, 1966). Another source of information is the Florentine Codex, a database of Mexican traditional medicine written in the nahuatl language in the XVI century, there is a registry of three types of *P. americana* which in accordance to its morphological description, the "aoacatl" might be *P. american* var *drymifolia* (Mexican variety), "tlacacolaocatl" is *P. americana* var. *americana* (West Indian variety) and "quilaoacatl" is *P. americana* var. *guatemalensis* (Guatemalan variety).

P. americana (avocado tree) is of importance in the ethnopharmacopeas in use today by most Mexican ethnic groups. The first report of avocado with medicinal use, also, is reported in the Florentine Codex, there it is mentioned that the use of ground seed for curing dandruff, scabies, headaches, sore ears and the fruit is edible. Leaves, seeds, bark, fruit and herbal extracts all mixed with other herbs are used to relieve symptoms and signs associated with parasites (worms), bacterial and fungal infections. Additionally, to cure inflammation, body aches, and postpartum. Is used to relieve signs and symptoms related to sexuality, such as: to cure female infertility and to enhance virility, anti-abortion, contraception, and emmenagogue. Another use is for the treatment of the psychosomatic illness called "cultural diseases" such as bad-air, shock, loss-of-soul and evil eye (BDMT-UNAM).

Economic importance of avocado in Mexico

From crosslinking of these landraces or via grafting were obtained cultivars such as the Anaheim, Bacon, Fuerte, Hass and Zutano. All are widely accepted by consumers by the high quality of avocado fruit and resulted in extensive global commercial success. The avocado fruit of cultivar "Hass" is the highest volume of production and marketing in Mexico and in the world. The avocado is a fruit that has great importance in agriculture and regional economy. Mexico is the major producer, consumer and exporter of avocado fruit, and holds the first place in production with 34% in global ranks (SAGARPA, 2009).

In various areas of Mexico there are orchards with high production of tree and avocado fruit in a systematic way, emphasizing that the state of Michoacán alone produced 1,028,291 tons of the total volume of avocado fruit produced in Mexico in the year 2010 (1,162,606 tons total production). In Michoacan, the avocado orchards cover a land surface of 101,946.29 hectares and it is the major avocado fruit producing region in the worldwide, as shown in Figure 2. Only, few orchards of this area are certified for the exportation of avocado fruit, they are located in so the called "strip avocado fruit" (APEAM, 2010).

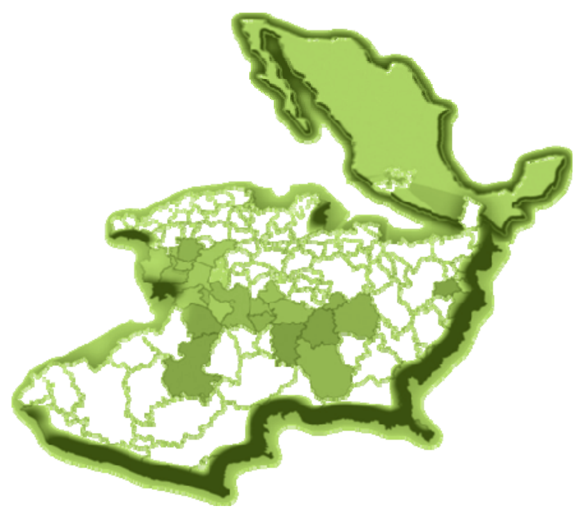

Figure 2. Map of the Michoacán State. Color highlights avocado producing municipalities and the area is known as the "strip of Michoacan avocado".
http://www.apeamc.com.mx/

The Mexican avocado fruit has various uses: 19% is processed in the transformation industry and 69% is for domestic in fresh consumption (FAO-STAT, 2003). Only 12% of avocado fruit is exported (400 thousand tons) generating an income annually for the country of 600 million dollars. The main countries where the fruit is exported are: Canada, Japan, United States and some

European and Central American countries. From 2005 to 2010 years the trend of exports to these countries were steady, but United States was the exception, it imported 270,215 tons in 2010, which makes this country the major importer of Mexican avocado.

Avocado is a fruit that is eaten fresh and in large quantities, it has been reported that based on the content of vitamins and folates, the fruit of avocado varieties “Fuerte”, “Anaheim”, and “Hass” produced in the State of California, USA, are a rich source of soluble B vitamins (Hall et al., 1958). One ounce Hass avocado fruit contains 50 calories, 3g monounsaturated fat, 0g cholesterol, 1g fiber and about 20 essential vitamins and minerals, α and β-carotene, lutein (1 ounce contains 81mg of lutein).

Functions performed by folates

Bacteria, fungi and plants synthesize folates *de novo*, but mammals have an incomplete folate biosynthetic pathway. In mammals, the folates supplementation necessary comes from bacteria, fungi and plants. For man, the folates supplementation is acquired mostly from fresh plant foods such as avocado fruit (Cossins et al., 1997; Green et al 1996, Rebeille and Douce, 1999, Scott et al., 2000).

Cellular critical functions of vitamins and minerals obtained in the diet are to serve as coenzymes in a variety of enzymatic reactions in different cellular processes that are vitamin dependent. Biochemical understanding of these coenzymes is still incomplete due to the complexity of cellular processes, for example, in human the genome there are 30,000 genes and about 3,800 enzymes have been catalogued, of which 22% require a coenzyme to carry on the catalytic process, which in many cases is a vitamin. Even less is understood as vitamins can protect genes from the damage caused by radiation and oxidants and are involved in repairing genetic damage (Eydallin et al., 2007).

Aging is an inevitable differentiation stage through which all living organisms pass, in the case of man it is accompanied by a slowness of movement and rhythm of life, there are manifestations of aches and pain, vision problems, and initiation of the process of loss of cognitive ability. However, in the late twentieth century and this decade there have been scientific observations that have helped us understand some of the symptoms of aging, how it can be mitigated, and delay its onset through the daily practice of individual health behavior to improve quality of life.

An important aspect is the kind food consumed by the man that helps to maintain optimal health. They are the source from which they draw carbon, nitrogen, minerals, fatty acids and antioxidants, as well as a variety of phytochemicals that are essential in many enzymatic reactions, such as vitamins found between folates (acid molecules derived from folic acid), vitamin B12 and B6.

Some features of folic acid and folates is that they form part of the B complex vitamins, are essential for health, are necessary for the production of red blood cells are essential to absorb fats and proteins are very important to maintain digestive tract muscle tone. Folic acid promotes a healthy nervous system, is important in periods of high growth such as infancy, adolescence and during pregnancy. Folic acid along with vitamins B6, B12, and metabolites betaine and S-adenosylmethionine help to control in blood levels of homocysteine, an amino acid linked to certain chronic heart diseases, depression, Alzheimer syndrome and cervical cancer.

The synthesis of many metabolites and the regulation of many metabolic processes require the addition or removal of 1-carbon units (C_1 metabolism). These C_1-reactions have a crucial role in important cellular processes, such as, synthesis of nucleic acids, methionine and pantothenate. Also, they are involved in the biogenesis of the choline, lignin and chlorophyll (Hanson and Roje, 2001; Jabrin et al., 2003). Tetrahydrofolate (H_4F) and S-adenosylmethionine mediate C_1-reactions, however, the synthesis of both depends on the presence H_4F, implying the importance of this metabolite in the availability of these cofactors. In addition, folates and vitamins B2, B6 and B12, are involved in enzymatic processes of DNA methylation and synthesis of phosphorylated compounds and the *de novo* synthesis and recycling of folates in plant (Hanson and Gregory, 2002).

A deficiency in the uptake of folates results in an increased incorporation of uracyl radicals (over one million molecules) into DNA which increase the breaking of chromosomes and DNA hypermethylation of both double and single strands (Fenech and Ferguson, 2001; Hori et al., 2005). Folic acid deficiency during pregnancy can lead to health problems and neural tube defects in the fetus, it also causes poor growth of the infant, inflammation of the tongue, gingivitis, loss of appetite, diarrhea, irritability, distractibility and temporary memory loss (Riggs et al., 1996).

A deficiency in folates has been associated with elevated levels of blood homocysteine and coronary infarction, as well as, with vascular and neurological mechanisms involved in cognitive decline. Even less is known whether the uptake of folates could prevent cognitive decline (Riggs et al., 1996; Verhoef et al., 1999). This topic is an exciting area of research that offers hope in the future to promote better quality of life for our people of which the group of the elderly is increasing.

Folate biosynthesis

Both bacteria and plants possess the same enzymatic steps of folate biosynthesis. However, the plants have a fascinating feature that is the unique spatial organization (Figure 3). Also tetrahydrofolate molecule (H4FH4FH4FH4F) is formed from the following molecular segments: a pterinyl ring, a *p*-amino benzoyl (p-ABA), and stem polyglutamyl of one to eight residues, see Figure 4. The folate enzymes and genes reported in plants are in Table 1.

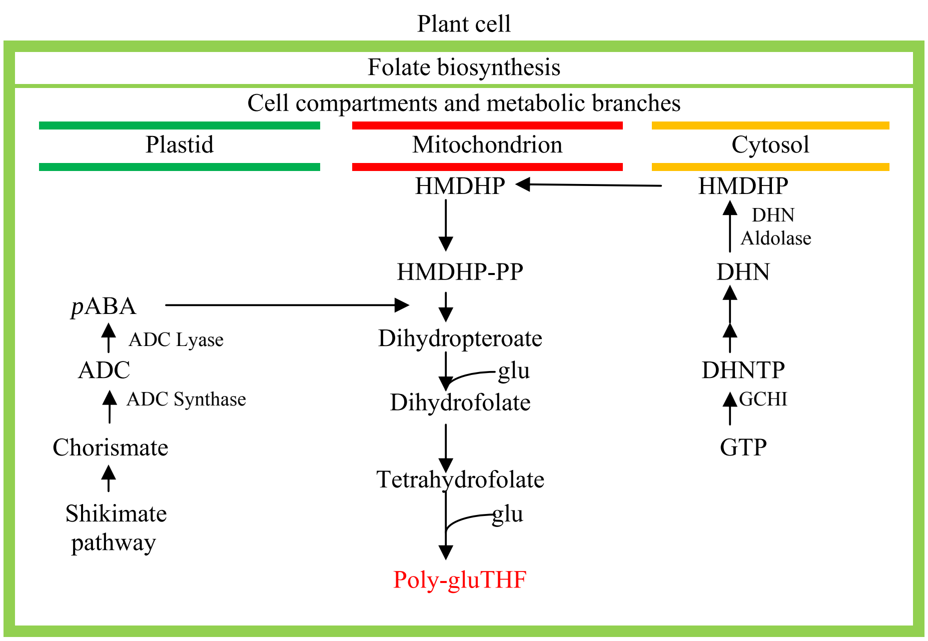

Figure 3. Special distribution of enzymatic synthesis of folate in plants. ADC aminodesoxychorismate, DHN, Dihydroneopterin; DHNTP, dihydroneopterin triphosphate; GCHI, GTP cyclohydrolase I; HMDHP, hydroxymethyildihydropterin: glu, glutamate;-PP, pyrophosphate; *p*-ABA, *p*-aminobenzoate; THF, tetrahydrofolate.

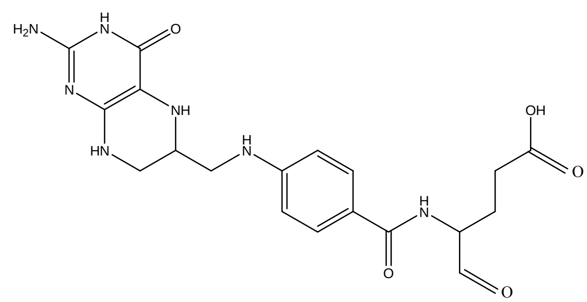

Figure 4. Chemical structure of mono glutamyl tetrahydrofolate (H4F-Glu$_1$).

Table 1. Enzymes and genes in the biosynthesis of folates in plants.

	Genes				
Enzymes	**At**	**Gm**	**Ls**	**Ps**	**So**
GTP cyclohydrolase I	AF489530	-o-	AY069920	-o-	-o-
Dihydro neopterin triphosphate pyrophospho hydrolase: nudB (*E. coli*)	-o-	-o-	-o-	-o-	-o-
Dihydro neopterin monophosphate dephosphorylase: (*E. coli K-12*)	-o-	-o-	-o-	-o-	-o-
Dihydro neopterin aldolase	FOLB1		AY422466	-o-	-o-
Dihydropterin pyrophospho kinase/dihydro pteroate syntase	-o-	-o-	-o-	Y08611	-o-
Dihydro folate synthetase	DHFS	-o-	-o-	-o-	-o-
Dihydro folate reductase/thymidylate synthase	THY-1, THY-2	DHFR-TS	-o-	No EC#	-o-
Folyl polyglutamate synthetase	DHFS/FPGS2 DHFS/FPGS4DH FS/FPGS3	-o-	-o-	-o-	-o-
γ-glutamyl hydrolase	-o-	GGH	-o-	-o-	-o-
Amine deoxy chorysmate synthase	AY096797	-o-	AY425708	-o-	-o-
Amine deoxy chorysmate lyase	AY099783	-o-	AY547289	-o-	-o-
UDPG:*p*-aminobenzoate glycosyl transferase	-o-	-o-	-o-	No EC#	-o-
Methenyl tetrahydrofolate cyclohydrolase	-o-	-o-	-o-	AF030516	-o-
10-formyl tetrahydro folate synthetase	THFS	-o-	-o-	-o-	SFS1

Note 1: *Spinacia oleracea* (So), *Arabidopsis thaliana* col (At), *Lycopersicon esculentum* (Le), *Glycine max* (Gm), *Pisum sativum* (Ps),

Note 2: *Arabidopsis thaliana* col, *Glycine max*, *Lycopersicon esculentum*, *Pisum sativum*, *Spinacia oleracea*

-o- = Not information into MetaCyc database.

No EC # = No evidence of the gene

Dihydropterin (H_2p) is synthesized from GTP in three steps and none of the enzymes involved have a signal peptide, indicating that they have a cytosolic location. The first reaction is carried out by the enzyme GTP-cyclohydrolase I (GTPHI) that form the triphosphate dihydroneopterin. The GTPHI is an enzyme that is present in organisms that synthesize H_4F *de novo* and in mammalian cells that synthesize tetrahydrobiopterin a coenzyme involved in redox reactions. The protein primary structure of plant GTPHI is unique, the active enzyme is a dimer and each monomer contains two domains in tandem

GTPHI (Basset et al., 2002). None of the domains has all essential residues for catalysis, it is interpreted that the tandem arrangement is necessary for catalysis. Since GTPHI is the first enzyme in the biosynthesis of dihydropterin, the enzyme is considered a step in the control flow within this channel.

The triphosphate chain of dihydroneopterin triphosphate molecule is removed to produce dihidroneopterin, it is a reaction that occurs in two stages, and the pyrophosphate group is removed by a NUDIX hydrolase, followed by the action of a nonspecific phosphatase (Klaus et al., 2005; Suzuki and Brown, 1974). At the end the Aldolase dihydroneopterin (DHNA) shortens the side chain to form the dihydroneopterin dihydropterin. The enzyme is a homo octadecamer DHNA very similar to its bacterial counterpart (Goyer et al., 2004; McLennan, 2006).

The *p*-amino benzoic acid (*p*-ABA) is synthesized from chorismic acid, in plants is carried out by an enzyme having the activity of amino deoxy chorismate synthase (ADC) and which has a transit signal peptide that is located in the chloroplast. The last step in the synthesis of *p*-ABA is catalyzed by Deoxy amino chorismate lyase (ADC lyase), a protein located in plastids (Basset et al., 2004a,b).

Tetrahydrofolate (H_4F-Glu1) is synthesized from the dihydropterin, *p*-ABA and glutamate, through four reactions in which the enzymes are signal peptides for their location in the mitochondrion. First, the pyrophosphate dihydropterin is activated and is combines with the p-ABA in a second reaction, in plants the two reactions are carried within a single bifunctional protein the Hydroxy methyl dihydropterin pyrophosphokinase (HPPK) and dihydropteroate synthase (DHPS) that only has been detected in mitochondria. The glutamylation process of *p*-ABA is dependent on ATP for the formation of the dihydrofolate-Glu ($H_2F\text{-}Glu_1$) by dihydrofolate synthetase (DHFS). Then the $H_2F\text{-}Glu_1$ is reduced to H4F-Glu_1 by dihydrofolate reductase (DHFR) using NADPH as electron donor.

Both plants and protozoa have a bifunctional enzyme bear the activities of DHFR and Thymidylate synthase (TK), the latter catalyzes the methylation of deoxy uridine monophosphate to form the deoxy thymidine monophosphate in the presence of $H_4P\text{-}Glu_n$.

In all organisms the polyglutamic stem of H_4PGlun is formed by the sequential addition of the γ-linker to glutamate residue of $H_4P\text{-}Glu_1$, a reaction catalyzed by Folyl polyglutamate synthase (FPGS). The glutamylation is a mechanism for the retention of folate in a given cell compartment by increasing the ionic charge in folate, which prevents their diffusion through hydrophobic barriers. It is likely that folate have the polyglutamyl stem in all subcellular compartments, since once that the $H_4P\text{-}Glu_1$ it is formed in mitochondrion, this is immediately exported to other cell sites. Although other enzymes can modify to $H_4P\text{-}Glu_1$ to stop or prevent the escape of this coenzyme, see Table 2.

Several attempts have been made to re-design pteridine synthesis, such as the heterologous expression of GTPCHI from *Escherichia coli* in *Arabidopsis thaliana*; it was observed that this enzyme is not subject to metabolic regulation. The transgenic lines contained 1000 times more pterine and two to four times more folate, indicating that the GTPCHI is indeed a limiting enzyme, but that other factors were regulating the folate synthesis. Also, it was noted that mammalian-GTPCHI over-expressed in bio-fortified tomato, *in planta* this enzyme was not under the control of final product (Diaz de la Garza et al., 2004; Hossai et al., 2004). Folate biosynthesis is a target for metabolic design and has sought to increase the levels of this vitamin in Arabidopsis, rice, tomato and tobacco.

Table 2. Enzymes and genes in the synthesis of Formyl THF in plants.

	Genes				
Enzimas	**At**	**Gm**	**Le**	**Ps**	**So**
10-formyl tetrahydrofolate synthetase	THFS	-o-	-o-	-o-	SFS1
Serin hydroxy methyl transferase	No EC #	-o-	-o-	-o-	-o-
Glycin decarboxylase	AT2G35370 AT2G35120	-o-	-o-	-o-	-o-
5,10-methylen tetrahydro folate reductase	AtMTHFR-2 AtMTHFR-1	-o-	-o-	-o-	-o-
Dihydro folate reductase /thymidylate synthase	THY-1 THY-2	DHFR- TS	-o-	No EC #	-o-
5,10-methylen tetra hydro folate dehydrogenase	-o-	-o-	-o-	AF030516	-o-
Methenyl tetra hydro folate cycle hydrolase	-o-	-o-	-o-	AF030516 ¿??	-o-
5-formyl tetra hydro folate cycle ligase	AtFCL	-o-	LeFCL	-o-	-o-
Serin hydroxy methyl transferase	No EC #	-o-	-o-	-o-	-o-
Folyl poly glutamate synthetase	DHFS/FPGS2 DHFS/FPGS3 DHFS/FPGS4	-o-	-o-	-o-	-o-

Note: *A. thaliana* col (At), *G. max* (Gm), *L. esculentum* (Le), *P. sativum* (Ps), *S. oleracea* (So)

-o- = Not information into into MetaCyc database.

No EC # = No evidence of the gene

It was reported that in plant the synthesis of *p*-ABA is a potential limiting step for folates accumulation (Diaz de la Garza et al., 2004). Diaz de la Garza and coworkers were able to increase the folates content in tomato by manipulating the branch pteroate synthesis. It was observed that after the expression of GTPCHI, the levels of *p*-ABA decreased and an accumulation of pteridine, with a tenfold increase in folate content. It has also been reported that *Arabidopsis* contains a cytosolic bifunctional isoenzyme HPPK-DHPS (Mouillon et al., 2002). Indicating that this function should be investigated to find out how this protein is involved in the *de novo* folate synthesis. The mitochondrial DHPS is strongly regulated by the H_2P and H_4F products, suggesting that this enzyme is a point of regulation of mitochondrial branch of folate synthesis.

Perspectives

Deciphering the metabolism of folate in plants and understanding of the biochemical properties of its enzymes is important for least two reasons: First, folate deficiency in humans is a worldwide public health problem and because plant foods are main source of folates, this coenzymes have chemical instability, so it is important to understand how the folates content are synthesized in plants can be improved. Second, several enzymes involved in folate biosynthesis are not present in animals and are a potential target for new herbicides.

With the chemical instability of folates due to light, oxygen and heat, its content is lost in the cooked plant foods, as vegetables, fruits and seeds that are eaten raw and fresh are the source of these vitamins. It is in this context that avocado fruit should be include into the diet because is a good folate source and is consumed fresh, and could help to cover the body's demand of micronutrients and reduce the number of people with "hidden hunger". Also, it is important to establish government measures at the economic, cultural and health levels for the control of micronutrient malnutrition in the population of developing countries; it is an investment that will bring social benefits.

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