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Folate biofortification in food crops

Simon Strobbe and Dominique Van Der Straeten

Folates are essential vitamins in the human diet. Folate deficiency is still very common, provoking disorders such as birth defects and anemia. Biofortification via metabolic engineering is a proven powerful means to alleviate folate malnutrition. A variety of metabolic engineering approaches have been successfully implemented in different crops and tissues. Furthermore, ensuring folate stability is crucial for long-term storage of crop products. However, the current strategies, shown to be successful in rice and tomato, will need to be fine-tuned to enable adequate biofortification of other staples such as potato, wheat and cassava. Thus, there is a need to overcome remaining hurdles in folate biofortification. Overall, biofortification, via breeding or metabolic engineering, will be imperative to effectively combat folate deficiency.

Address
Laboratory of Functional Plant Biology, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Corresponding author: Van Der Straeten, Dominique (Dominique.VanDerStraeten@ugent.be)

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Folates are a group of water soluble B-vitamins (vitB9), consisting of a pteridine ring, a para-aminobenzoate moiety (*p*-ABA) and a γ-linked tail with one or more L-glutamates (Figure 1) [1]. Folates are labile compounds, prone to (photo-)oxidative cleavage [2]. Specific folate entities are chemically distinguished by three different structural modifications. First, folates exist in varying oxidation states, with tetrahydrofolate (THF) being the most reduced form. THF is the bioactive vitamin, functioning as an essential co-enzyme in numerous metabolic reactions. Second, folates can harbor a range of one-carbon (C1) units on the pteridine (N5) and *p*-ABA (N10) moiety, influencing their stability. Third, the length of the glutamate tail is highly variable [3]. A longer glutamate tail facilitates binding of the vitamin to folate-dependent enzymes, as well as ensuring its cellular retention [4]. Polyglutamylated folates can therefore be considered more stable than monoglutamates *in vivo*.

The chemical diversity of folates reflects a perfect adaptation to their varied biological function as C1-donors and acceptors, rendering them a pivotal role in primary metabolism of nearly all organisms. Folate-dependent enzymes play a key role in thymidylate and purine synthesis, as well as pantothenate (vitB5) formation [3]. 5-methyl-THF donates its methyl group to homocysteine to form methionine by the action of the cobalamin (vitB12)-dependent methionine synthase [5]. In plants, folates have an additional essential role in photorespiration, as well as in chlorophyll, plastoquinone, tocopherol, pectin and lignin synthesis [6].

Due to their central role in primary metabolism, detrimental physiological effects arise upon folate deficiency [7]. Animals, unable to synthesize folates *de novo*, rely primarily on their diet for an adequate folate supply. Decreased folate levels result in impeded erythrocyte development, causing megaloblastic anemia. During embryogenesis, folate deficiency provokes aberrant neurulation, leading to the onset of neurodegenerative disorders such as anencephaly and spina bifida [8]. Together, folate deficiency induced Neural Tube Defects (NTDs) are estimated to account for over 150 000 birth defects each year, predominantly in the developing world [9].

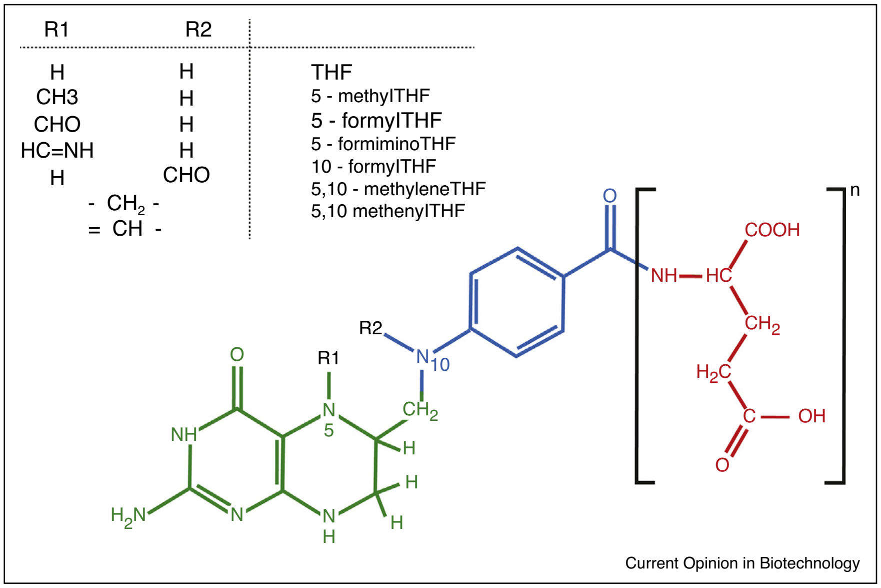
Fermented foods, leguminous and leafy vegetables can be considered rich sources of folates. However, some massively consumed staple crops, such as rice, corn, wheat, potato and cassava, contain inadequate folate levels (Table 1). The recommended daily intake (RDI) of folate is 400 μg for an adult, increasing to 600 μg during pregnancy [4]. Unfortunately, many diets, in developing as well as developed countries, fail to reach these standards.

A combined strategy of technical, socio-economical and biotechnological solutions will be essential to relieve this global burden. In this review, current state-of-the-art on biotechnological approaches for folate biofortification will be discussed.

Biosynthesis

In plants, folate biosynthesis is characterized by subcellular compartmentation (Figure 2) [3]. The pterin branch of folate biosynthesis takes place in the cytosol, yielding 6-hydroxymethyldihydropterin (HMDHP). Secondly,

Figure 1



Chemical structure of folates.
Folates consist of three moieties: a pteridine (green), a *p*-aminobenzoate molecule (blue) and a glutamate tail (red). The green/blue transition reflects a (photo)-oxidation-labile bond. The folate shown is a polyglutamylated tetrahydrofolate (THF). Plant folates carry up to eight glutamates [4,67]. Different folate forms are distinguished by different C1-substituents, at different levels of oxidation, on N5 or N10.

the *p*-ABA branch resides in plastids, consuming chorismate as a substrate. The resulting *p*-ABA, together with HMDHP, are assumed to enter the mitochondria by passive diffusion and carrier mediated transport, respectively [2]. Condensation of the two moieties occurs in mitochondria, followed by polyglutamylation of the resulting folate [10]. However, polyglutamated folates are retained in mitochondria, as they are intracellularly transported as monoglutamates, with vacuolar import being the only known exception [11].

Table 1

Folates in foods. Different food products are ranked according to folate content. Most staples contain inadequate levels of folate (RDI: 400 µg for adults; 600 µg for pregnant women). Brie cheese is an example of a fermented food. Data on folate content were derived from the USDA National Nutrient Database for Standard Reference (Release 28, September 2015, revised in May 2016). Based on these data, the fold increase, needed to obtain a sufficient amount of folate to reach the RDI in 100 g of raw food material, was calculated. As adequate folate levels are most critical during pregnancy, 600 µg was set as the target level. Possible losses during processing and variation of folate bioavailability – both shown to notably decrease the amount of bioeffective folate, as for instance in rice endosperm [35••,69] – are not accounted for)

Food	Folate content (µg/100 g)	Fold increase to reach RDI in 100 g	Global supply[a] (g/capita.day)
Rice, white, long-grain, regular, raw	8	75	148.2
Tomatoes, red, ripe, raw	15	40	55.4
Potatoes, flesh and skin, raw	15	40	94.9
Corn grain, yellow	19	32	48.2
Plantains, raw	22	27	9.6
Cassava, raw	27	22	40.3
Lettuce, green leaf, raw	38	16	/
Wheat, soft white	41	15	178.8
Cheese, Brie	65	9	/
Spinach, raw	194	3	/
Beans, white, mature seeds, raw	388	2	6.8
Lentils, raw	479	2	/
Turkey, liver, raw	677	–	/

[a] Data on average global supply (if available) of the corresponding (wet) crop product are derived from FAOSTAT, 2011 (http://faostat.fao.org)(Food Supply—Crops Primary Equivalent). For rice, milled equivalent is presented.