Biofortification of Vitamin B_6 in Seeds

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The Versatile Functions of Vitamin B_6

Water-soluble pyridoxine, pyridoxal, and pyridoxamine are collectively called vitamin B_6. These vitamers are remarkably versatile, providing essential functions in a large number of biological reactions. Approximately four percent of all classified enzymatic activities are pyridoxal 5'-phosphate-dependent\. Pyridoxal 5'-phosphate (PLP), the coenzyme form of vitamin B_6 that can be converted from pyridoxine, pyridoxal, and pyridoxamine, is required by numerous enzymatic reactions, including transaminations, aldol cleavages, beta- or gamma-eliminations or replacements, decarboxylations, and racemizations that are involved in the biosynthesis and/or catabolism of amino acids, structural compounds, neurotransmitters, histamine, hemoglobin, lipids, and carbohydrates.[1] Recently, vitamin B_6 has also been shown to function as a potent antioxidant, equivalent to vitamins C and E, and is particularly active in quenching singlet oxygen and the superoxide anion. Moreover, PLP is involved in enhancing or suppressing the expression of certain genes. PLP conjugates to a lysine residue in the nuclear repressor RIP140, and such conjugation is essential for RIP140's interaction with histone deacetylases, nuclear retention, and subsequent transcriptional repression.

Vitamin B_6 provides a great number of health benefits and is essential for all cellular organisms. It is particularly known in the medical field for its involvement in more bodily functions than any other single nutrient. Vitamin B_6 is required for the maintenance of mental as well as physical health[2] and is essential for normal brain development and function. Vitamin B_6 is especially important for maintaining healthy muscle cells, absorption of vitamin B_{12}, synthesis of vitamin B_3, and the production and proper function of red blood cells and cells of the immune system.[3] Moreover, vitamin B_6 can inhibit platelet aggregation, lower blood pressure, and protect against the development of diabetic neuropathy.

Consequently, vitamin B_6 deficiency will result in high levels of homocysteine, muscle weakness, skin inflammation, nervousness, irritability, fatigue and sleepiness, difficulty in concentration, and short-term memory loss. Deficiency of this vitamin has thus been associated with depression, epilepsy, impaired cognitive functions, Alzheimer's disease, cardiovascular diseases, and different types of cancer.[3] Although primary vitamin B_6 deficiency is rare in developed countries, it is estimated that 14% of people in America fall below the estimated average requirement[4] for vitamin B_6. In addition, certain populations such as infants and children, elderly people, and people who consume excessive amounts of alcohol or smoke cigarettes are at a higher risk of vitamin B_6 deficiency.

Seeds Are a Good Target for Elevating Vitamin B_6

The vitamin B_6 biosynthesis pathway, in which pyridoxine is synthesized from 1-deoxy-D-xylulose-5-phosphate and 4-phosphohydroxy-L-threonine through Pdx2A and Pdx2J, is well-characterized in eubacteria such as *E. coli*. Recently, another distinctive vitamin B_6 biosynthesis pathway was discovered in fungi, archaeabacteria, certain eubacteria, and plants. In these organisms, pyridoxal 5'-phosphate is directly synthesized from glutamine and ribose 5-phosphate or ribulose 5-phosphate through a highly conserved bi-enzyme complex consisting of PDX1, the acceptor/synthase, and PDX2, the glutaminase domain. In contrast to bacteria, fungi, and plants, which have the ability to synthesize their own supply of vitamin B_6 *de novo*, animals cannot synthesize the vitamin and need to obtain it from their diet. Unlike fat-soluble vitamins, which can be stored in the liver, water-soluble vitamin B_6 is not or barely stored in the body, and the excess is excreted through the urine. Therefore, animals need a continuous supply of the vitamin included in their daily diet. Since plants are the major source of vitamin B_6 for animals either directly or indirectly, it is of great interest to increase vitamin B_6 levels in plants for improved nutrition value.

Thus far, there is only one recent report of research to engineer vitamin B_6 content in plants. Herrero and Daub overexpressed *PDX1* and *PDX2* genes from the fungi *Cercospora nicotianae* in tobacco. However, only one of all transgenic plants tested had an ~21% increase in vitamin content. One problem with this heterologous expression system is that it suppressed endogenous *PDX* genes.[5]

In our study, we initially generated transgenic Arabidopsis using endogenous *PDX* genes under control of the CaMV 35S promoter. Although *PDX1* or *PDX2* transcript levels are greatly increased in these 35S promoter-driven overexpression lines, no dramatic increase in vitamin B_6 content was detected in either shoots or roots. It seems that vitamin B_6 homeostasis is more tightly regulated in these vegetative parts. In contrast to shoots and roots, a significantly higher amount of pyridoxine accumulated in the seeds of all these transgenic plants. Since the CaMV 35S promoter has a low activity during early embryogenesis, and its activity greatly decreases during seed development, we decided to over-express the

PDX genes specifically in seeds using a seed-specific promoter. We made constructs consisting of the *PDX1* or *PDX2* cDNA under control of the Arabidopsis 12S seed storage protein gene promoter individually or in tandem and transferred them separately into wild-type Arabidopsis plants. We found that all lines have significantly increased pyridoxine and pyridoxamine contents in dry seeds. The total vitamin B_6 level in some transgenic lines is three times that of wild type.[6] Our results indicate that the seed is a suitable target organ for further engineering high levels of bio-available vitamin B_6.

Recently, a concern has been raised about the decline in nutrient quality of crops with increased yield, and their subsequent effect on nutrient quality of meat and dairy products when they are used as animal feed. Because seeds are relatively rich in vitamin B_6, the two-fold increase of the vitamin in engineered seeds demonstrated in our work is particularly valuable for the crop plants whose seeds are a major source of food and feed. In addition to the nutritional advantage, elevated vitamin B_6 in seeds may help seeds in combating the pathogenic fungus *Cercospora nicotianae*. This pathogen produces a strong singlet oxygen-generating toxin called cercosporin, and vitamin B_6 is indispensible for quenching the radicals from this toxin. In addition, enhanced vitamin B_6 levels in seeds may also inhibit lipid peroxidation induced by reactive oxygen species (ROS) and preserve the seed quality during the long-term storage.

It is expected that in the near future vitamin B_6 levels in seeds can be further increased by several other means once the biosynthesis and catabolism pathways as well as their regulation are better understood. For example, one may combine the use of mutant lines that contain elevated glutamine or other precursors of vitamin B_6 biosynthesis with PDX transgenic lines, employ other seed-specific promoters that are active during earlier stages of embryo and endosperm development, reduce the catabolism of the vitamers, and relieve possible end-production inhibition on the biosynthetic pathway.

References

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