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Scientists create rice variety with high folate stability

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Researchers from Ghent University succeeded in stabilizing folates in biofortified rice in order to prevent their degradation upon long term storage. They used two strategies: by linking folates with folate binding proteins and by extending the tail of the folate molecules. These approaches can offer a solution to serious health problems caused by folate deficiency in developing countries.

The human body is unable to make vitamin B9, better known as folate. Adults need approximately 400 microgram of folates per day to remain healthy, a number which is increased to 600 microgram for pregnant women. Folates are abundant in green leafy vegetables (folium is Latin for leaf), such as spinach and legumes (e.g. beans). Most staple crops, such as rice and other cereals, contain very low amounts of this vitamin.

Inadequate folate intake can have severe effects on human health. In addition to certain forms of anemia, folate deficiency in pregnant women can result in an impaired development of the neural tube (the precursor of the spinal cord) of the embryo. These developmental problems often result in spina bifida: the so-called "cleft spine". Folate deficiency is also associated with Alzheimer disease, cardio-vascular diseases and the development of a range of cancers. Due to the marginal levels of folate in rice, consumed by about half the world population as sole energy source, folate deficiency is highly prevalent in developing countries. Several studies show that in certain regions of e.g. China and India the occurrence of neural tube defects is at least 10-fold higher than in Western countries.

Unstable molecules

Vitamins are unstable molecules, that degrade easily upon contact with oxygen, light, humidity, increased temperatures and changes in acidity. For this reason, it is important to consume food products, such as vegetables and fruit, as fresh as possible. A lot of vitamins get lost, not only during food processing and preparation, but also during storage. In biofortified corn, for instance, provitamin A levels decrease 70% upon six months of storage; vitamin C content in guava nectar even 89%. Evidently, these problems occur in harvest products that are stored for a longer period, such as rice grains. These stability problems become more severe in developing countries where the storage in high temperature and high humidity is inevitable.

In 2007, a research team from Ghent University (Belgium), coordinated by prof. Dominique Van Der Straeten, reported the development of a first generation of rice lines with 100-fold higher folate levels as compared to normal rice. This result was achieved through metabolic engineering, the modulation of the biosynthesis pathway of a plant compound. Their new study shows that about half of the folate content in these rice lines degrades after half a year.

Assemblage lines

To tackle this problem, researchers from this lab developed a new rice prototype, in which the folate content remains stable upon long term storage. Again, metabolic engineering was applied. Folate is produced in a plant cell by specific enzymes (molecular machines) that add consecutive changes to a certain start product until a folate molecule is formed. This process is comparable to a car assembly line. By stimulating the production of two enzymes in the folate biosynthesis, researchers created the first generation of rice lines containing high folate levels.

Two strategies

Now, they were able to stabilize this high folate content in a new rice prototype. They applied two strategies. A first strategy comprised the binding of folates with a folate binding protein. This protein is unknown in plants, but well studied in mammals. It occurs in e.g. milk and protects folate from degradation. This is also the way intact folates are passed on from the mother to her infant, to support its development. By expressing a synthetic gene, based on a folate binding protein from bovine milk, in the rice grain, the same principle is applied and folate content remains stable upon long term storage.

A second strategy consisted of the stimulation of the last step in folate production. This step extends the tail of the folate molecule. This promotes cellular retention and binding to folate dependent proteins. Besides enhancing folate stability, the new gene combinations also resulted in folate levels that are up to 150 fold those found in normal rice. Since all genes which were used in this study, were placed next to each other on a single piece of DNA, this piece of genetic material can easily be transferred to edible rice varieties. Moreover, it is fairly easy to make combinations with other interesting traits, such as the enhancement of other vitamins or certain minerals, such as iron. This technology can also be used in other crops, both cereals (e.g. wheat, sorghum) and non-cereals (e.g. potato, banana).

This investigation is the result of a close collaboration between the labs of prof. Dominique Van Der Straeten (development and characterization of the new rice prototypes), prof. Willy Lambert and prof. Christophe Stove (development of analytical methods to measure folates) and dr. Hans De Steur and prof. Xavier Gellynck (study of the socio-economic impact of folate rice). These results were published yesterday in the renowned journal *Nature Biotechnology* (Blancquaert et al., 2015).

The stability issue is often underestimated or even neglected in biofortification programs. It is obvious that not only high, but also stable vitamin levels are important to tackle vitamin deficiencies. Not only does this study describe the effect of long term storage on the folate levels in the first rice prototypes, it also provides an elegant solution to the stability problem. This solution can be applied, in a customized form, to other crops and vitamins and opens the door for awareness and consideration of vitamin stability in future biofortification studies.

More information: "Improving folate (vitamin B_9) stability in biofortified rice through metabolic engineering." *Nature Biotechnology* (2015) DOI: 10.1038/nbt.3358

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