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Transgenic Approach for Biofortification

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Abstract

Biofortification, the process of breeding nutrients into food crops, provides a sustainable, long-term strategy for delivering micronutrients to rural populations in developing countries. Crops are being bred for higher levels of micronutrients using both conventional and transgenic breeding methods; several conventional and transgenic varieties have been released. The results of efficacy and effectiveness studies, as well as recent successes in delivery, provide evidence that biofortification is a promising strategy for combating hidden hunger. International initiatives, such as the Harvest Plus program and national initiatives, are acting as pillars to achieve these targets.

Introduction

Crop biofortification refers to the development of crops with higher nutritional value. This can be accomplished through traditional selective breeding or genetic engineering. Biofortification differs from fortification in that it tries to make plant foods more nutritive naturally rather than adding nutritional supplements during processing. According to the United Nations Food and Agriculture Organization, there are 792.5 million malnourished people in the globe, with 780 million of them living in developing nations. Aside from that, around two billion people worldwide suffer from "hidden hunger," which is caused by an insufficient intake of key micronutrients in the daily diet despite increased food crop production (Malik and Maqbool, 2020). Apart from that, there is a growing worry about nutrition.

Until now, our agricultural system has been intended solely to increase grain yield and crop productivity, not to enhance human health. This strategy has led in a quick increase in micronutrient deficit in cereal grains, resulting in an increase in micronutrient malnutrition in consumers. Agriculture is now shifting from mass-producing food crops to mass-producing nutrient-dense food crops in sufficient quantities. This will aid in the fight against "hidden hunger" or "micronutrient malnutrition," particularly in poor and emerging nations where staple food crops are deficient in micronutrients (Garg *et al.*, 2018). Biofortification is a one-time investment that provides a cost-effective, long-term, and sustainable method to combating hidden hunger because there are no costs associated with purchasing fortificants and adding them to the food supply during processing once the biofortified crops are produced (Saltzman *et al.*, 2013). One of the main goals of organisations like the World Health Organization and the Consultative Group on International Agricultural Research (CGIAR) is to develop nutritionally enhanced high-yielding biofortified crops.

Methods of Biofortification

Selective Breeding

Selective breeding is one of the most powerful techniques used for biofortification of staple crops, which involves the crossbreeding of existing varieties enriched in micronutrients. The development of the hybrid varieties must be monitored by nutritionists to check whether the improved levels of nutrients can be used by the consumers and how these levels are affected by storage, processing, and cooking of the food crop. However, there are certain limitations in selective breeding, such as low heritability, a lack of genetic diversity for micronutrients, and linkage drag, which makes genetic engineering a more deliberate approach for fortification of staple crops.

Genetic Modification

Altering the genetic makeup of a domesticated crop by introducing genes from the wild crop of same species or other species that code for the increased production of certain nutrients could make the host crop rich in nutrients. Alternatively, different genes which code for different nutrients can also be stacked in a crop to make it rich in a wide variety of nutrients. One of the most glorious examples is that of golden rice which has been enriched with beta-carotene, a precursor of Vitamin A.

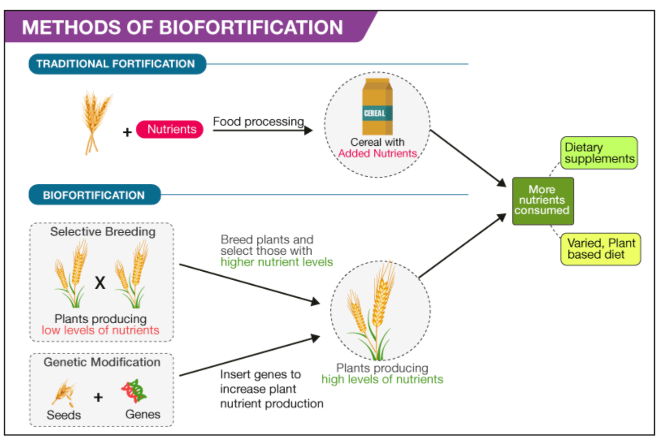

Figure 1: Methods of biofortification

Biofortification through Transgenic Means

When there is little or no genetic variation in nutrient content among plant varieties, the transgenic technique can be a viable option for developing biofortified crops. It is based on having limitless access to the genetic pool for the transfer and expression of desirable genes from one plant species to another, regardless of their evolutionary or taxonomic status. Furthermore, when a micronutrient is not naturally present in crops, transgenic techniques are the only viable alternative for fortifying these crops with the vitamin. The ability to discover and define gene function, as well as use these genes to design plant metabolism, has been critical in the development of transgenic crops. Furthermore, bacteria and other creatures' metabolic pathways can be inserted into crops to leverage alternate metabolic routes.

Transgenic techniques can also be used to incorporate genes involved in increasing the concentration of micronutrients, their bioavailability, and lowering the concentration of antinutrients, which limit nutrient bioavailability in plants. Furthermore, genetic alterations can be used to redistribute micronutrients throughout tissues, increase the concentration of micronutrients in edible parts of commercial crops, improve the efficiency of biochemical pathways in edible tissues, or even reconstruct specific pathways. Unlike nutrition-based organisational and agronomic biofortification programmes, the development of transgenically biofortified crops requires a significant amount of time, effort, and investment during the research and development stage, but it is a cost-effective and sustainable approach in the long run. Furthermore, there are no taxonomic restrictions when it comes to genetic engineering, and even synthetic genes can be created and employed. Transgenic crops with higher levels of micronutrients have the potential to reduce micronutrient deficiency among their consumers, particularly the poor in developing nations. Several crops have been genetically modified to increase the amount of micronutrients they contain. Vitamins, minerals, essential amino acids, and essential fatty acids are among the micronutrients that have been targeted through the application of numerous genes from various sources to improve the nutritional status of food crops. PSY, carotene desaturase, and lycopene-cyclase have all been identified as candidates for biofortification, as have ferritin and nicotinamine synthase for minerals, albumin for necessary amino acids, and Δ^6 desaturase for vital fatty acids (Garg *et al*., 2018). The following are some successful transgenic rice and maize examples:

1. Transgenic Rice (Oryza sativa)

The idea of transgenic rice were first discussed at international conference at IRRI in Philippines in 1984. In 1999, Team of scientists, including Ingo Potrykus, Swiss Federal Institute of Technology, successfully genetically engineer rice to produce carotenoids, precursors to Vitamin-A. *Golden Rice* technology is based on the simple principle that rice plants possess the whole machinery to synthesis of β-carotene, and while this machinery is fully active in leaves, parts of it are turned off in the grain endosperm.

By adding only two genes, a plant phytoene synthase (psy) and a bacterial phytoene desaturase (crt I), the pathway is turned back on and β-carotene consequently accumulates in the grain.Golden rice was created by transforming rice with only two β-carotene biosynthesis genes:

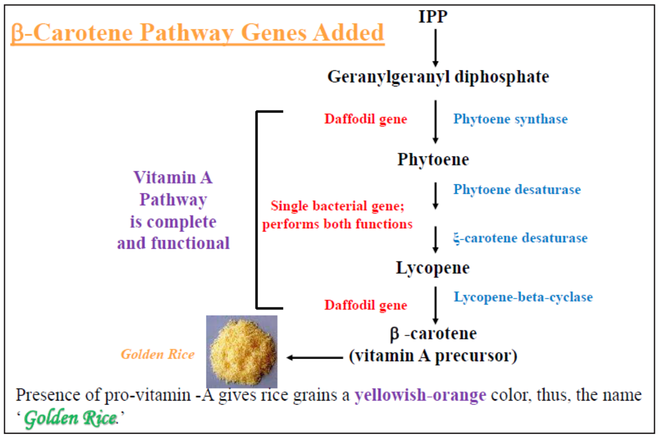

Figure 2: Science behind golden rice

i) psy (Phytoene synthase) from daffodil (*Narcissus pseudonarcissus*)

ii) crtI (Carotene desaturase) from the soil bacterium (*Erwinia uredovora*)

The psy and crt1 genes were transformed into the rice nuclear genome and placed under the control of an endosperm-specific promoter, so they are only expressed in the endosperm. The exogenous lyc gene has a transit peptide sequence attached so it is targeted to the plastid, where geranylgerany diphosphate formation occurs. The bacterial crt1 gene was an important inclusion to complete the pathway, since it can catalyze multiple steps in the synthesis of carotenoid, while these steps require more than one enzyme in plants.The end product of the engineered pathway is lycopene, but if the plant accumulated lycopene, the rice would be red.Recent analysis has shown the plant's endogenous enzymes process the lycopene to beta-carotene in the endosperm, giving the rice, the distinctive yellow color for which it is named. The original golden rice was called SGR1, and under greenhouse conditions it produced 1.6 μg g^{-1} of carotenoids.

2. Transgenic Maize (Zea mays)

- GM maize with increased lysine (LY038) was developed by inserting a *cordapA* gene from a common soil bacteria *Corynobacteriumglutamicum*.
- Enhanced production and accumulation of free lysine (Lys) in the GM corn kernel made body weight gain, feed conversion and carcass yields of experimental poultry and swine comparable with animals fed with Lys supplemented diets, and higher than those fed with conventional maize diets.
- Lys-enriched maize with the gene sourced from potato, was also found to be safe as conventional maize.
- LY038 has been commercialized and incorporated in feed meals since 2006.

In all higher plants, lysine, threonine and methionine are synthesized from aspartic acid *via* a pathway that is highly branched and under complex **feedback control**.

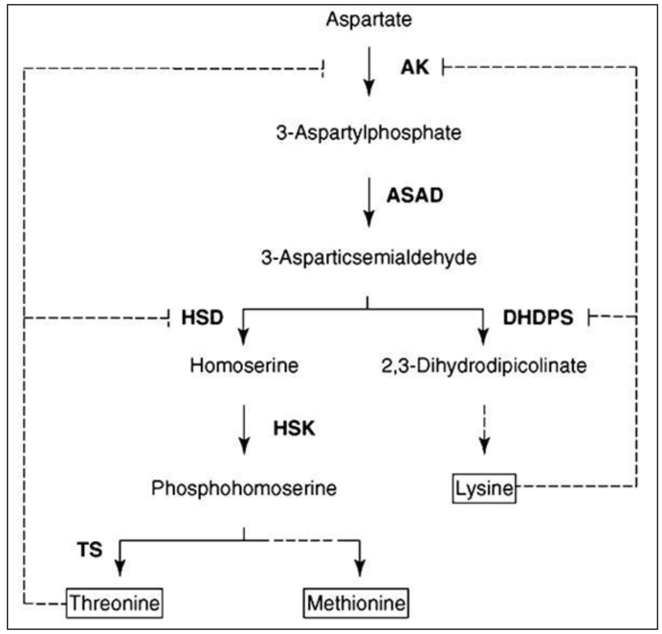

Figure 3: Development of Lysine rich transgenic maize

- Two key enzymes are aspartate kinase (AK), which functions early in the pathway and is inhibited by both lysine and threonine.
- Dihydrodipicolinate synthase (DHPS), which functions in the lysine-specific branch and is inhibited by lysine alone.
- **Feedback-insensitive versions** of the bacterial enzymes have been expressed in plants with promising results:
  - The free lysine content of *Arabidopsis* seeds was increased either by expressing a bacterial, feedback-insensitive DHPS transgene.
  - Or by knocking out the lysine catabolism pathway, resulting in 12-fold or fivefold gains in lysine, respectively.
- Where both the transgene and knockout were combined in the same *Arabidopsis* line, increases of 80-fold over wild-type levels were achieved.
- Protein-enriched soybean event M703 was found to contain more digestible amino acids lysine, methionine, threonine, and valine, and had a higher level of metabolizable energy.
- A maize γ-zein gene encoding a sulphur amino acid rich protein was used to transform alfalfa and trefoil (*Lotus corniculatus*) under CaMV 35S promoter and RUBISCO small subunit promoter.
- Expression level was rather low to the extent of 0.05% of alcohol soluble protein.
- To increase methionine level, a new methionine-rich zein, normally expressed at low levels was expressed at a high level using the 27 kDazein promoter.
- This protein called the high sulphurzein (HS 7) was 21 kDa and contained 37% Met.

The amino acid methionine is a common protein building block that is also important in other cellular processes. Its content has been increased in maize by modifying *cis*-acting site for *Dzs10*. Amino acid balance of maize has also been improved by expressing milk protein α-lactalbumin (Garg *et al*., 2018).

Conclusion

Traditional breeding methods have been utilised to improve the nutritional quality of meals and have found great approval. Although transgenic means are being emphasised more, the success rates of breeding-based approaches are much higher, as transgenically fortified crop plants must overcome barriers such as consumer acceptance and various expensive and time-consuming regulatory approval processes used by different countries. Aside from these obstacles, biofortified crops have a promising future since they have the ability to eliminate micronutrient malnutrition in billions of people, particularly in poorer nations.

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