JUST AGRICULTURE
multidisciplinary e-Newsletter

Transgenicplant with improved nutritional quality

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ARTICLE ID: 68

INTRODUCTION

GM technology could help tackle both poverty and health problems facing developing countries. If only those who oppose GM crops would relax their stance and weigh up the technology costs

and benefits.

Transgene: A gene that is taken from the genome of one organism and introduced into the genome of another organism by artificial techniques.

Transgenesis: Being or used to produce an organism or cell of one species into which one or more genes of another species have been incorporated a transgenic mouse transgenic crops also: produced by or consisting of transgenic plants or animals.

Transgenic plants: Transgenic plants are plants that have been genetically engineered, a breeding approach that uses recombinant DNA techniques to create plants with new characteristics. They are identified as a class of genetically modified organism (GMO).

Golden rice is now within reach

Global staple food and is especially important in Asia. Cultivated for over 10,000 years. Rice provides as much as 80 percent or more of the daily caloric intake of 3 billion people, which is half the world's population. To provide pro-vitamin A to third world i.e., developing countries where malnutrition and vitamin A deficiency are common. It is generally consumed in its milled form with outer layers removed. The main reason for milling is to remove the oil-rich aleurone layer, which turns rancid upon storage. As a result, the edible part of rice grains consists of the endosperm, filled with starch granules and protein bodies, but it lacks several essential nutrients such as carotenoids exhibiting pro-vitamin A-activity. Vitamin A deficiency is a serious health problem in at least 26 countries in Asia, Africa and Latin America**.**

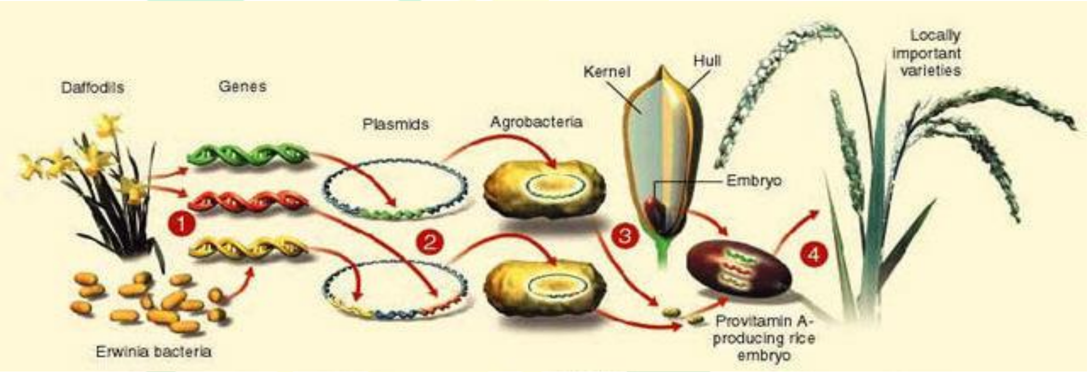
Golden Rice – A golden opportunity?

- Vitamin A deficiency often occurs where rice is the staple food since rice grain does not contain pro-vitamin A i.e., β-carotene.
- Rice produces β-carotene in the leaves but not in the grain, where the biosynthetic pathway is turned off during plant development.
- The resulting transgenic rice 'golden rice' contains good quantities of βcarotene, which gives the grain a golden colour.

How does it work?

- The addition of 3 genes in the rice genome will complete the biosynthetic pathway. Phytoene synthase (psy) gene –derived from daffodils (Narcissus pseudonarcissus) fused to rice endosperm-specific glutelin (Gt1) promoter.
- (Phytoene synthase is a transferase enzyme involved in the biosynthes of carotenoids. It catalyzes the conversion of geranyl pyrophosphate to phytoene).
- Three steps required to convert: phtoene to β-carotene Phytoene desaturase (pds) and ζ-carotene desaturase to introduce double bonds to form lycopene.
- Lycopene cyclase – from soil bacteria Erwinia uredovora form rings in the beta-carotene (biosynthesis of carotenoids in the endosperm). Bacterial carotene desaturase capable of introducing all four double bonds can be substituted for the Phytoene desaturase and ζ-carotene desaturase.

- Manipulation of Golden rice would require the introduction of 3 genes: Phtoene synthase, Carotene desaturase, Lycopene beta-cyclase.
- The daffodil psy gene rice glutelin promoter construct was inserted into the vector pZPsC, along with the bacterial carotene desaturase gene, (crt1) controlled by the 35S promoter.
- Both enzymes were targeted to the plastid (the site of GGDP synthesis): psy gene by its own transit peptide and the crt1 gene by fusion to a pea ribulose-1, 5-bisphosphate carboxlase/oxygenase small subunit (rbcs) transit peptide sequence.
- The lycopene β-cyclase gene with a functional transit peptide was inserted into vector pZLcyH under the rice endosperm-specific glutelin promoter along with hygromycin resistance marker gene.



A new Golden Rice generation Golden Rice 2

Further work by Syngenta to optimize beta-carotene production showed that the daffodil phytoene synthase was rate limiting and psy gene from maize was much more effective (resulting in the greatest accumulation of total carotenoids and β-carotene). After trying with psy genes from different sources it turned out that the maize and rice genes gave the best results. In the process, Golden Rice lines were obtained that accumulated up to 38 µg/g carotenoids, of which 32 µg/g was β-carotene (as compared to the first generation Golden Rice (original golden rice was called GR1) where only 1.7 µg/g were obtained.

Improve level of iron and zinc in rice grains

- Iron deficiency is the most widespread micronutrient deficiency world-wide.
- Affecting an estimated one-third of the world's population and causing 0.8 million deaths annually worldwide.

- Anemia caused by iron deficiency triggers serious disorders such as abortion, brain damage in infants, increase susceptibility to infection.
- Rice is a poor source of most of many essential micronutrients, especially iron (Fe) and zinc (Zn), for human nutrition.

- According to the World Health Organization (2010), approximately two billion people suffer from iron deficiency. May, 2021/ Issue-13/Page | 54
- The polished rice contains an average of only 2 mg kg-1 Fe and 12 mg kg-1 Zn (IRRI, 2006), whereas the recommended dietary intake of Fe and Zn for humans is 10-15 and 12-15 mg per day, respectively.
- Rice actually has a lot of iron, but only in the seed coat. Because unpeeled rice quickly becomes rancid in tropical and subtropical climates, the seed is removed for storage.
- A major cause is the poor absorption of iron from cereal and legume-based diets high in phytic acid.
- Besides having inherently low levels of Zn, wheat grain is also rich in substances limiting utilization (bioavailability) of Zn in the human digestive tract, such as polyphenols and phytic acid.
- Phytic acid is the major storage compound of phosphorus in grain. By binding Zn, phytic acid reduces solubility of Zn in food and restricts its utilization and retention in human body.

Approaches for increasing the amount of iron absorbed from rice-based meals

- Introduced a ferritin gene from *Phaseolus vulgaris* into rice grains, increasing their iron content up to twofold.
- To increase iron bioavailability, introduced a thermotolerant phytase from *Aspergillus fumigatus* into the rice endosperm.

- As cysteine peptides are considered a major enhancer of iron absorption, over expressing the endogenous cysteine-rich metallothionein-like protein.

Plant Genes Help to Mobilize and Store Iron

One gene encodes nicotianamine synthase, the enzyme that produces nicotianamine. Nicotianamine chelates (metal ion) iron temporarily and facilitates its transport in the plant. Nicotianamine synthase is expressed under a constitutive promoter. The second gene encodes the protein ferritin (consists of 24 subunits), which functions as a storage depot for up to four thousand iron atoms per protein molecule in both plants and humans. Since the ferritin gene is under the control of an endosperm specific promoter, ferritin comprises a sink for iron in the center of the endosperm. The synergistic action of these two genes allows the rice plant to absorb more iron from the soil, transport it in the plant, and store it in the rice kernel. A third gene encoding phytase was also engineered into this rice line. Phytase degrades phytate, a compound that stores phosphate and binds divalent cations like iron and thus inhibits their absorption in the intestine.

Feed Crops with Improved Proteins and Amino Acids

- Seeds of higher plants contain large quantities of storage proteins.
- These proteins have been classified on the basis of their solubility in various solvents.
- Albumins (soluble in water) Globulins (soluble in salt solution) Prolamins (alcohol soluble) Glutelins (soluble in acidic or basic solution) Wheat, barley, maize, sorghum accumulate major storage proteins which are low in lysine.
- Storage proteins of legumes are insufficient in sulfur containing amino acids.
- Barley, rice, wheat, sorghum are also low in threonine and maize in tryptophan.

Three molecular approaches are being used in altering amino acid sequence

1. Identification of naturally occurring seed storage plant with high levels of desired amino acids, followed by cloning the corresponding gene and expressing it at high levels in the species distinctly differ from the sources of genes.
2. Modification by recombinant DNA technologies so that they encode proteins similar to wild type proteins but possess higher levels of desired amino acids.

3. Modification in the pool size of the desired amino acids for the synthesis of seed storage proteins by an alternative metabolic pathway.

Examples of expression of recombinant storage proteins with desirable amino acid profiles:

1. The expression of sunflower seed albumin, which is rich in methionine, in the laboratory model lupin.
2. Expression of pea (*Pisum sativum*) legumin, which has high lysine content, in rice and wheat grains.

Protein-rich potato

- In India, a genetically modified potato has been developed by a coalition of charities, scientists, government institutes and industry as part of a 15-year plan to combat malnutrition amongst India's poorest children.
- The 'potato', contains a gene AmA1 from the South American amaranth plant, resulting in an increased protein content of 2.5 per cent. AmA1 gene from the Prince's feather (*Amaranthus hypochondriacus*), which encodes seed albumin, was expressed in potato and was shown to double the protein content and increase the levels of several essential amino acids.

- The potato has high levels of essential amino acids, lysine and methionine.
- GM maize with increased lysine (LY038) was developed by inserting a cordap A gene from a common soil bacteria *Corynobacterium glutamicum*.
- 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.

- 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 kDa zein promoter.
- This protein called the high sulphur zein (HS 7) was 21 kDa and contained 37% Met.
- Biotechnology offers great potential for the production of novel design crops, which are the sole solution to safeguard the supply of sufficient quantities of safe & healthy food tomorrow.

Conclusion

Genetically-modified foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. Yet there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labelling. Many people feel that genetic engineering is the inevitable wave of the future and that we cannot afford to ignore a technology that has such enormous potential benefits. However, we must proceed with caution to avoid causing unintended harm to human health and the environment as a result of our enthusiasm for this powerful technology.