Adoption of Golden Rice: A Boon for the Children Suffering from Vitamin A Deficiency

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ABSTRACT
Many poor people worldwide cannot afford nutritious food and depend on rice for their daily food needs. In 2022, Asia was home to 55% (402 million) of the people in the world affected by hunger, while more than 38% (282 million) lived in Africa. India is the world’s second-largest producer of rice, wheat, vegetables, and fruit. Malnutrition is a major problem that causes many immediate and long-term health problems. Over 33 lakh children in India are malnourished, with Maharashtra, Bihar, and Gujarat having a major share. Vitamin A deficiency causes irreversible blindness in 500,000 children annually, which ultimately results in large-scale deaths. In Southeast Asia, due to VAD, every year, five million children develop symptoms that may lead to blindness. VAD is also a matter of concern in several areas of Africa, Latin America, and the Caribbean. It is estimated that vitamin A nutrition may prevent approximately 1 to 2 million deaths every year among children aged 1 to 4 and an additional 0.25 to 0.5 million during the higher age group of children. One of the major causes of vitamin A deficiency is that milled rice does not contain ß-carotene, which is the precursor of vitamin A synthesis. In this scenario, adopting Golden Rice which is genetically modified rice could benefit the population living in developing countries. This review article gives emphasis on the importance of overcoming vitamin A deficiency.

Keywords: Golden Rice, Genetically modified crops, Malnutrition, VAD.

INTRODUCTION
World’s largest number of undernourished people are living in India (24% of the world population). Vitamin A deficiency is a major health problem in developing countries and the leading cause of non-accidental blindness. Foods of plant origin contain pigments of carotenoids, i.e., provitamin A, which are convertible to vitamin A. The most important form of provitamin A is beta-carotene (yellow-orange pigment). Other carotenoids are alpha-carotene, lutein, lycopene, zeaxanthin and beta-cryptoxanthin. Many fruits and vegetables contain a number of different carotenoids. Depending upon the fat in the diet, this absorption varies (5–50%). If fat intake is less, the absorption is less. Carotenoids can be absorbed intact and deposited in adipose tissue or converted to vitamin A in the liver. The retinoids and provitamin A carotenoids are collectively referred to as vitamin A [1].

The retina of the eye consists of sensory elements or specialized cells, i.e., cones and rods. The cones are responsible for the visual process occurring under bright light, translating objects into color images. Rods are responsible for retinal processes that occur in dim light. In rods, the retinol combines with a protein opsin to form rhodopsin. When light strikes rhodopsin in the eye’s retina, the retina undergoes a structural change and detaches from opsin, leading to nerve fibers, i.e., optic nerve stimulation. The optic nerve carries a message of black-and-white vision from the rod cells to the brain. This response to light is called the bleaching process. The bleaching process is the process by which light depletes the rhodopsin concentration in the eye and the fall in rhodopsin concentration allows the eye to become accustomed to bright light. Therefore, when a person from bright light enters a darkened room, his sensitivity to dim light is low, but gradually, it increases because of a process called dark adaptation. This in turn, allows improved vision in the dark. If the diet is deficient in vitamin A for a long time, the retinol in the blood decreases. As a result, the cells in the eye recover from flashes of light more slowly. This condition, called night blindness, reduces the ability of the eyes to adapt quickly to dim light following exposure to bright light. The most common cause of vitamin A deficiency is poor diet intake among families of low-income groups. Children up to 6 months of age are protected due to breastfeeding, but the vitamin content of breast milk depends on the mother’s intake during pregnancy. People below the poverty line cannot buy expensive retinol sources, leading to vitamin A deficiency. In other words, malnutrition is a major problem among children under five in India. A child’s nutritional status is a function of food intake, food absorption, health, and the socioeconomic and ecological environment[1,2]. Vitamin A deficiency can cause symptoms ranging from night blindness to total blindness due to xerophthalmia and keratomalacia [3,9].

Rice is the principal food of many living in India. Rice grain is rich in starch but has low protein content (7%). It also contains fat,
minerals, and vitamins. The aleurone layer is rich in nutrients like protein, fat, calcium, and phosphorus. The rice endosperm is highly digestible [4].

**Rice Growing Regions**

*In world*

Countries like India, Bangladesh, China, Japan, Korea, the Philippines, Malaysia, Indonesia, Thailand, North and South Vietnam, Cambodia, Sri Lanka, etc., produce and consume about 95% of the world’s rice. Other rice-producing countries are Brazil, the USA, Egypt, West Africa, Spain, and Italy (Fig. 1) [4].

*In India*

Rice occupies a vast cultivated area (44 MH). The total production of Kharif rice during 2022–23 is estimated at 104.99 million metric tons. It is higher by 4.40 million metric tons than the previous five years (2016–17 to 2020–21) average Kharif rice production of 100.59 million metric tons [4]. It is mainly confined to the wetter parts of the country. It is chiefly grown in Assam, West Bengal, Bihar, UP, Orissa, MP, Punjab, Maharashtra, Karnataka, Tamil Nadu, and Kerala (Fig. 2). The production of rice is about 92 million tonnes [4].

India has five rice-growing regions based on rainfall, growing season, etc.

- **The northeast zone**
  The rice crop is sown from June to July and November to December. It includes Assam, West Bengal, Orissa, and the southern districts of Bihar [4].
- **The northern zone**
  The rice crop is sown from May–June to September–October. This zone comprises Jammu-Kashmir, Himachal Pradesh, Punjab, UP, and North Bihar [4].
- **The central zone**
  The rice season is from June to July and from November to December. The central zone includes MP, Maharashtra, eastern Karnataka, and the Telangana region of Andhra Pradesh [4].
- **The west coast zone**
  The rice-growing season is usually from May–June to November-December, but irrigated crops can also be grown elsewhere. This zone includes coastal Gujarat, Maharashtra, Karnata, and Kerala [4].
- **In the southern zone**
  The rice is grown throughout the year. The southern zone comprises parts of Karnataka, AP, Tamil Nadu, and the deltaic regions of the Krishna, the Kaveri, the Godavari, and the Tambraparni rivers [4].

Source: www.usda.gov (2022-2023)

*Fig. 1: Major rice producing countries in the world*

**Genetically Modified Crops**

Genetically modified (GM) crops like rice and mustard, described as ‘Golden Rice’ and ‘Golden Mustard,’ are rich in vitamin A. Crops are modified genetically by altering an organism’s genes. It may be a plant, animal or microorganism. These modifications of crops have been done to develop various traits such as herbicide tolerance, disease resistance, insect pest resistance, altered nutritional profile, enhanced storage life, etc [3].

For the first time in 1981, the genetically modified organism was produced; consequently, in the USA and France, the first field trials of herbicide-resistant tobacco were conducted in 1986. In the following 15 years (1986-2001), more than 5,000 field trials of more than 100 transgenic crop varieties (representing only 8 species) were conducted on more than 20,000 sites in at least 40 different countries. Most of these trials were conducted in the USA and Europe. After safe field trials, transgenic crops were approved for commercial cultivation in 15 countries from 1990 to 2001. The area covered by transgenic crops increased from 1.7 million hectares in 1996 to 52.6 million hectares in 2001 and 58.7 million hectares in 2002 [5].

Transgenic tobacco and tomato were commercialized for the first time in China in the early 1990s. In 1996, 2.5 million acres of transgenic tobacco and tomato were grown in China. In the USA, the first transgenic crop for food was FlavrSavr™ tomato, which was characterized for delayed ripening, thus improving its flavor and nutritional value. Several transgenic crops were cultivated commercially, including tomato, cotton, soybean, corn/maize, canola/rapeseed, potato and squash. The global market’s value for transgenic crops in 2000 exceeded 3 million US dollars, which increased to 6 billion US dollars in 2005. This proves that genetically modified crops are going to be commercialized in the future and, therefore, will play a major role in agriculture at a global level [5].

India has the world’s fourth largest GM crop acreage, surpassing China’s 3.0 million hectares (mh), while equal to Canada’s 11.6 mh. This is mostly based on GM Cotton, the only GM crop allowed in the country. In 2014, farmers in India planted a total of 11.6 mh under transgenic. Significantly, the entire 11.57 mh GM crop area in India consists of Bt cotton, most of which (about 96%) is now covered by Bt hybrids [5,6].

In a genetic modification of a plant, a foreign gene (called “transgene”) is inserted in the plant’s own genes. This gene may be introduced from one plant to another plant, from a plant to an
Table 1: List of biotech crops in field trials in India (2008)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Organization (transgene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brinjal</td>
<td>IARI, New Delhi (cry1Aa, cry1Ba,Sungro Seeds, New Delhi (cry1Ac); Mahyco, Mumbai (cry1Ac)</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Nunhems India Pvt Ltd (cry1Bacry1Ca)</td>
</tr>
<tr>
<td>Castor</td>
<td>Directorate of Oilseeds Research, Hyderabad (cry1Aa, cry1Ec)</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Sungro Seeds, New Delhi (cry1Accry1Ba, cry1Ca); Nunhems India Pvt Ltd</td>
</tr>
<tr>
<td>Corn</td>
<td>(cry1Ac, cry1Ba, cry1Ca) Monsanto, Mumbai (cry1Ab)</td>
</tr>
<tr>
<td>Groundnut</td>
<td>ICRISAT, Hyderabad (chitinase gene from rice), DREB</td>
</tr>
<tr>
<td>Okra</td>
<td>Mahyco, Mumbai (cry1Ac, cry2Ab)</td>
</tr>
<tr>
<td>Potato</td>
<td>CPRI, Shimla (RB gene delivered from Solanum bulbocastanum)</td>
</tr>
<tr>
<td>Rice</td>
<td>IARI, New Delhi (cry1B-cry1Ba fusion gene); Mahyco, Mumbai (cry1Ac cry2Ab); TNALI, Coimbatore (rice chitinase (chilI)/tobacco osmotin gene) IARI, New Delhi (antisense tomato leaf curl virus); Mahyco, Mumbai (cry1Ac)</td>
</tr>
<tr>
<td>Tomato</td>
<td>IARI, New Delhi (antisense tomato leaf curl virus): Mahyco, Mumbai (cry1Ac)</td>
</tr>
</tbody>
</table>

animal, or from a microorganism to a plant. For example, a gene from a bacterium resistant to pesticides can be inserted.

Steps involved in genetic modification in transgenic plants (Fig. 3)

Locating genes for plant traits
The most important steps are identifying and locating genes for agriculturally important traits. An organism with the desired trait is identified and from the desired trait, the specific gene is located and cut out from the plant’s DNA [7].

Gene cloning
The desired gene is obtained by attaching the carrier to the desired gene in the cells of the plant being modified. ‘Plasmid’, which is a bacterial piece of DNA, is joined to the gene to act as a carrier. For proper functioning of the gene ‘Promoter’ and carrier is combined with the gene. A ‘selectable marker gene’ is also used to identify the ‘transformants’ [7].

Transformation
The bacterium is to be inserted with Transformants/a proper host that will generate multiple copies of the gene package. There are two techniques of transforming plant cells and tissues.

The “Gene Gun” technique is especially useful in transforming monocot species like corn and rice. Tiny particles of gold or tungsten are attached to the gene packages and bombarded with high speed into the plant tissue. Gold or tungsten is used over others, as they are chemically inert. In the second method, a soil bacterium, Agrobacterium tumefaciens, is used to infect the plant tissue with the desired gene. The genes are incorporated into the plant genome when a vector enters the cell [7].

Selection
To ensure that the genes inserted are working properly, GM plants must be monitored extensively. The cells incorporating the vector will grow on a selective antibiotic medium. These cells are then allowed to grow in a medium containing plant growth factors [7].

Golden Rice
Conventional rice has been genetically modified with beta-carotene genes from maize to have high levels of beta-carotene, which is the precursor to vitamin A. The search for Golden Rice started as a Rockefeller Foundation initiative in 1982. Steps involved, sources of genes and important components of gene constructs, used for the development of Golden Rice (Fig. 3).

In 1990, Potrykus and his colleague Peter Beyer of the University of Freiburg started genetically modifying a metabolic pathway into a variety of Oryza sativa, so that the plant’s edible kernels would contain beta-carotene. Since the successful manipulation of beta-carotene synthesis in the rice grains gives them a characteristic yellow/orange color, which is genetically enriched in provitamin A, it has been described as Golden Rice. In 1999, they successfully developed it. Louisiana State University conducted the first field trials in 2004. Subsequently, additional field trials were conducted in the Philippines, Taiwan, and in Bangladesh [3].

So far, the two versions of Golden Rice developed are Golden Rice 1 and 2, both Japonica (sticky, dry land) rice. The first nutritionally enhanced genetically modified rice varieties are GR2E Golden Rice, which is yet to receive regulatory approval for use in food. Because rice is already widely grown and eaten, these rice varieties have the potential to reach many people. The Bangladesh Rice Research Institute and the Philippine Rice Research Institute are developing the GR2E Golden Rice trait [2]. To produce it, 2 enzymes are introduced into the endosperm [phytoene synthase (psy) and phytoene desaturase (crtl)] via an endosperm-specific glutelin (Gtl) promoter (15), to establish a beta-carotene biosynthetic pathway in the rice grains [8,9].

GR2 is produced by Syngenta (Biotech Company) using phytoenesynthetase gene (psy) from maize and the carotene desaturase gene (crtl) from Erwinia uredovora. The daffodil psy: rice gluten promoter construct was inserted into the vector pZPsc, along with the bacterial carotene desaturase gene (crtl) from Erwinia uredovora controlled by the 355 promoter. Both enzymes were targeted to the plastid (the site of GGDP synthesis) the psy gene by its own transit peptide, and the crtl gene by fusion to a pea ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit (RCBs) transit peptide sequence. The lycopene B-cyclase gene from daffodil with a functional transit peptide was inserted into the vector pZLcyH under the control of the rice endosperm specific glutelin promoter, along with a hygromycin resistance selectable marker gene [3]. List of biotech crops in field trials in India (2008) (Table 1).

A mixture of agrobacterium LBA4404 containing each of the two plasmids are inoculated in the immature embryo of rice. A total of 60 hygromycin-resistant regenerated lines were selected randomly, all shown to contain the pZLcyH construct. Out of these, 12 were also found to contain the pZPsc cassette. Most of the seeds from these transgenic lines containing both constructs were found to be yellow, indicating carotenoid synthesis. Some of these lines found A range of carotenoids, whereas beta-carotene was only carotenoid in others. A mixed population of segregating grains in the endosperm contained 1.6 µg β-carotene g⁻¹. It was calculated that the homozygous grains of this line would produce about 2 µg g⁻¹ provitamin A, which responds to a daily intake of 100 µg retinol equivalents, assuming that rice consumption is 300 g per day. Since the initial demonstration of this
approach’s scientific feasibility, several technical improvements have been made to it. These include the following [3].

The phosphomannose isomerase (pmi), also known as mannose-6-phosphate isomerase mannose selection gene was substituted using the hygromycin-resistance gene to avoid antibiotic selection.

The Golden Rice trait was genetically modified into Indica rice cultivars. Indica rice is consumed by 90% of the Asian population, whereas the original Golden Rice was produced using the japonica variety Taipei 309 [3].

Subsequent research confirmed results obtained during the initial phase of development, indicating that the lycopene β-cyclase transgene was not required to produce β-carotene in the endosperm. Further work by Syngenta to optimize β-carotene production showed that the daffodil phytoene synthase was rate limiting and that a psy gene from maize was much more effective. Transformation of rice with the construct pSYN12424 resulted in 1 23 fold increase (up to 37µg^-1) in carotenoids compared with the original Golden Rice and has been named Golden Rice 2 [3].

Ingo Potrykus, has pointed out the following benefits of golden rice.

• The poor and disadvantaged will benefit.
• It will be distributed free of charge to farmers;
• It can be resown every year from the saved harvest,
• No advantages for rich landowners;
• It was not developed for the benefit of the biotechnology industry,
• It fulfills urgent needs by complementing (rather than displacing) traditional interventions.
• It is a cost-free solution to vitamin A deficiency.
• The unfortunate negative side effects of the Green Revolution are avoided.
• It creates no new dependencies.
• No additional inputs is required,
• It does not reduce agricultural biodiversity;
• It does not affect natural biodiversity;
• Environmental impact is negligible;
• It was impossible to develop the trait by traditional methods [3].

### Issues and Challenges with Golden Rice

#### IPRs and technology transfer

The Golden Rice Humanitarian Board has been established for the technology transfer process. This board ensures that, for each region, there is a proper assessment of needs and a framework for the optimal use of it. It also coordinates the bioavailability, food safety, and allergenicity assessments required before the rice is made available for human consumption. It is given to research institutions to ensure proper handling and use of GM plants according to local rules and regulations. These institutions will transfer the Golden Rice trait into the best locally adapted lines by conventional breeding methods and/or de novo transformation of elite varieties. Socioeconomic and environmental impact studies will also be conducted to ensure that the technology reaches the poor without damaging the environment.

India plays a model role in this technology transfer process for other countries [3].

The main problem is that the Golden Rice is developed in public laboratories with public funding for humanitarian purposes. However, it was discovered that the research development it had involved the use of procedures and technology protected by 70 IPRS and technology protection rights belonging to 32 different companies and universities [3].

#### Qualitative and quantitative issue

In 2017, a study by the Indian Council of Agricultural Science discovered abnormalities in golden rice traits and lower productivity in these traits, both qualitatively (lower Vitamin A content) and quantitatively (yields).

It does not provide enough Vitamin A; it can provide 1.6 ug of vitamin A per gram of rice. A daily intake of 3300 grams of rice is required to get adequate vitamin A. This amount would be too much for people living in the areas that need rice as food.

Vitamin A is fat-soluble, so fat in the diet is needed to be able to intake vitamin A. Unfortunately, adequate protein and fat are not readily available in developing nations like India [8].

<table>
<thead>
<tr>
<th>Steps involved</th>
<th>Genes utilized</th>
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<tbody>
<tr>
<td>Germinal mylphosphate (GGDP) (20 carbon compound)</td>
<td>(i) phytoene synthase gene from narcissus (psy) + endosperm specific promoter + transit peptide for plastid import</td>
</tr>
<tr>
<td>psy</td>
<td>(ii) phytoenesaturase gene from Promoter + transit peptide for plastid import</td>
</tr>
<tr>
<td>phytoene (40 carbon compound)</td>
<td>(iii) lycopene β-cyclase gene from Narcissus (ley) + specific promoter + transit peptide for plastid transport</td>
</tr>
<tr>
<td>crtE (crtE) + constitutive</td>
<td>lycopene (40 carbon compound)</td>
</tr>
<tr>
<td>lycopene (40 carbon compound)</td>
<td>β-carotene</td>
</tr>
<tr>
<td>o-carotene</td>
<td>zeaxanthine</td>
</tr>
<tr>
<td>lutein</td>
<td>Source: (8)</td>
</tr>
</tbody>
</table>
Risk to the environment
Genetically modified crops are a risk to the environment due to the impact of imparted traits on other related species. Transgenic crops can accidentally cross with traditional varieties through pollen transfer and contaminate traditional local varieties with transgenes. The transfer of genes into a crop can actually remain in the environment, leading to environmental problems, e.g., Bt cotton. Transgenic crops may interact with other organisms in the environment [11].

It could breed with weeds and can contaminate wild rice forever. GM crop production requires pesticides and fertilizers. Another risk is that it can eliminate variety among rice grains because transgenes promote the use of monocultures and the farmers would be required to create large areas of monoculture crop land [5,12].

Transgenic Crops and Biosafety Regulations
Biosafety regulations are separate in different countries and are prescribed for conducting laboratory/greenhouse and field experiments and for commercializing transgenic crops. Field experiments (field releases) of transgenic crops are designed to contain transgenic crops to safeguard the environment [8].

Regulations for field releases
USA first developed the regulations for field trials of transgenic crops. There it is regulated by three federal agencies, the US Department of Agriculture (USDA) and the Animal and Plant Health Inspection Service of USDA. APHIS (1993) introduced a ‘Notification System’ for six transgenic crops (corn, tomato, soybean, potato, cotton and tobacco). This system was introduced to simplify the process and it did not require the applicant to obtain a permit. After reviewing the notification, the applicant was allowed to conduct a trial if APHIS did not have any objection. In May 1997 this notification system was extended, permitting almost all field trials to be conducted. The rule also allowed previous deregulation decisions to be extended to additional genetically modified products already approved for commercial release [8].

Regulations governing commercialization
Commercialization of transgenic crops are regulated by independent approvals from several agencies, depending upon the nature of the crop, the trait improved through genetic engineering and the ultimate use of the product (food/feed, etc.). In the USA, approval from the Environment Protection Agency (EPA) is also required if the crop contains a gene for pesticide. Additional clearance from the Food and Drug Administration (FDA) is required if the crop is used for food/feed. Similar regulations are being exercised in India also [8].

Regulations of biosafety in India
In India, through the Ministry of Environment and Forests (MOEF), the central Government has enacted environmental protection laws under the Environment Protection Act (EPA). The DBT prepared biosafety guidelines for the first time in 1990 and subsequently revised them in 1994 and 2008. Under these guidelines, every organization involved in R & D using GMOs is required to set up its Institutional Biosafety Committee (IBSC) with a nominee from DBT. This committee is the nodal point for interaction with the Government through a national committee called the Review Committee for Genetic Manipulation (RCGM), which functions under the charge of DBT. RCGM reviews all approvals of ongoing R & D projects on GMOs, undertakes field visits of experiments and issues clearance for import/export of etiologic agents, vectors, germplasm, organelles, etc., needed for experimental work, training and research. On the recommendation of RCGM, trial permits are issued by DBT. Experiments are monitored by RCGM, in addition to the IBSC.

For any large-scale use of GMOs, MOEF has constituted an inter-ministerial committee called the Genetic Engineering Approval Committee (GEAC), which reviews applications and approves approvals for large-scale trials, leading to commercial cultivation.(8)

CONCLUSION
It is clear that the genetic modification of crops can increase the yield in comparison to conventional methods. It has now been proved by research that beta carotene in food is a safe source of vitamin A. Multiple tests done on Golden Rice confirm it is neither toxic nor allergenic. Beta carotene content found in other foods is the same as in Golden Rice. The body only converts beta carotene according to the requirement; any excess is safely flushed out of the body.

Other food products can supplement vitamin A but are difficult to afford for poor people. The major concern is that rice does not have many essential nutrients but it is a part of the diet. In countries like India, where many people live below the poverty line and depend on rice for food, it could bring the gift of life and sight.

CONFLICT OF INTEREST
The authors report no financial or any other conflicts of interest in this work.

SOURCE OF FUNDING
None declared

REFERENCES
2. The Indian express, October 14, 2023, Accessed on 20th October 2023
5. The Indian Express, February 2, 2015 accessed on 25th August 2023
10. Anonymous, Annual Report ,Department of Agriculture & Farmers Welfare, Government of India, Krishi Bhawan, New Delhi,2022-2023,
11. The Indian express, December 20, 2022, Accessed on 12th August 2023