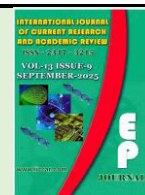




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Golden Rice 2: Augmenting Beta Carotene to Counter Vitamin A Deficiency

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Abstract

Vitamin A deficiency effects millions globally increasing the prevalence of blindness. The situation gets grimmer in the rice consuming population due to lack of variety in food habits. Biofortifying the staple rice can enhance the nutrition level and help in filling the gap. The Phytoene synthase (PSY) gene from *Zea mays*, and carotene desaturase from the soil bacterium *Erwinia uredovora* combined to develop Golden Rice 2. The level of beta carotene from 1.6 $\mu\text{g/g}$ in first generation to 31 $\mu\text{g/g}$ was β -carotene in Golden rice 2. This increased level of beta carotene will help in alleviating the vitamin A related deficiency diseases. Developing Asian countries will be benefited the most as they are the major rice consuming countries.

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Introduction

The rice served on our plates travels a long way from the green pastures to our home's kitchen. The nutrition has seen a depletion over the years due to dehulling and polishing to add to its better looks but compromising the nutrition. The polished rice is less nutritious but the urban population however balances the gap through having other nutritious food like fruits, vegetables nuts etc. But the rural population or ones who are solely dependent on rice as staple always are found nutritionally deficient. Apparently, leafy green vegetable have high amount of beta-carotene but the conversion of beta carotene to Vitamin A remains insufficient.

Therefore, the deficiency of Vit A still remains prevalent in the population having access to green vegetables. The direct consumption of Vit A enriched rice would be a more feasible option (Karapareddy 2021). This deficiency causes various eye conditions from night

blindness to complete blindness and other serious conditions like keratomalacia, corneal scars etc. Various process to alleviate VAD by including supplementation, fortifying food and dietary modification has proved to be futile (ISAAA 2017). Therefore, enhancing or biofortifying rice with certain vitamins would indeed be helpful to meet the deficiency in the populations that are solely dependent on rice.

With similar intentions "Golden rice" got started to reach the desired destination. Golden rice era was started from the year 2000 to produce Golden rice 1 and further modifications gave Golden rice 2 in the year 2005. And after 20 years of field and safety trials it was released in the market. The Golden rice is manipulated genetically with the help of recombinant DNA technology to enhance the level of beta-carotene in rice. With the participation of International Rice Research Institute (IRRI) and Department of Agriculture-Philippine Rice Research Institute "Golden rice" was developed.

Some countries like Australia, New Zealand, Canada and United State of America have already received food safety approvals for Golden rice. However, these countries do not suffer from vitamin A deficiency as compared to South East Asian and African countries. Therefore, the concept and the value of golden rice did not popularize among developed nations. Meanwhile, Philippines became the first nation to get approval for commercial cultivation. Recently on 23 July 2021, Filipino farmers became the first to get approval to cultivate Golden rice with enriched nutrients. Currently the same discussion is taking place in Bangladesh.

In various clinical and subclinical trials, the VAD is found highest in India and South Asia with 62 % of preschoolers registering the said deficiency (Khan 2023). The children under the age of 6 and pregnant women are in the high-risk zone regarding this deficiency (Dubock 2021). Among the Filipino people, one in five children in the marginalized society suffer from Vitamin A deficiency affecting 190 million children. This condition leads to night blindness to complete blindness as well as it also weakens the immune system. Addressing the concern of these people and for the wider acceptance of golden rice Dr John de Leon Executive director of DA-PhilRice reportedly quoted - “we are committed to ensuring the highest quality seed for farmers and a safe nutritious food supply”.

In late 1980s Professor Ingo Potrykus and Peter Beyer created “Golden rice” first time in the history. Norman Borlaug, the Father of Green Revolution contributed the development of dwarf rice and wheat through normal regular breeding in the year 1960. The contribution enabled mostly in the Asian subcontinent to resolve the problem associated with food scarcity in developing countries (Chrispeels and Sadava 1994). Norman Borlaug in 1970 was awarded the Nobel Peace Prize for his participation in food security throughout the globe (Norman Borlaug 1970). This step brought a change in crop yield which is termed as Green Revolution.

The Green Revolution proposed that farmer’s inn one hand adopted the seeds of new cereals, but also were to sign a high-input method for agriculture-including the issue of nitrogen fertilizers, herbicides and pesticides. They also were to be well equipment for tilling and irrigation to get the maximum yields from these varieties. Many small farmers were not able to afford to get best out of the new seeds, whereas the farmers those were well-off produced higher number of crops for less input of labor and therefore started to capture larger land area.

Green Revolution plays a prominent role to enlarge the production of cereal like rice and wheat. The essential step to positive result lies in the evolution of semi-dwarf, productive, winter wheat species adapted to high rainfall conditions in USA after the Second World War. This particular wheat was not suitable for cultivation in the tropics or semi tropical climates since wheat is categorized as Rabi crop that requires cold temperature to grow. It was also found that wheat was vulnerable to rust and fungus (Peng *et al.*, 1999). Meanwhile, The Rockefeller foundation and the Mexican government co-sponsored a project to augment food production in Mexico mainly increase production of wheat. Initially Rockefeller foundation and Norman E Borlaug started to collect wheat germplasms. Then they crossed a number of desirable traits including high yield, rust resistance and warm climatic conditions.

The semi-dwarf spring varieties, matured at a faster pace and were not sensitive to photoperiod. This allowed wheat to be cultivated for more times annually and also showed resistant towards rust and showed better adaptation towards warmer climates. These characteristics meant that yields could be doubled compared with those of traditional varieties under specific experimental conditions.

Simultaneously during that time, various identical breeding programs were carried through for rice. The new variety of rice was produced in Philippines by the International Rice Research Institute (IRRI). Borlaug advanced the production varieties with higher yield like IR8 after famine of 1961 in India. By cross of ‘peta’ (higher yielding variety) and DGWG (Dee-Gee-Woo-Gen) which is semi dwarf variety resulted in semi dwarf (IR8) variety. This had many desirable characters including higher in yield, early maturing, photoperiod independent and better resistant to fungus and greater harvest Index.

In 1984 the idea was first discussed at IRRI international conference in Philippines. Pioneered by Profesor Ingo Potrykus (Emeritus), of the Swiss Federal Institute of Technology, The Institute for Plant Sciences (ETH, Zurich), Professor Peter Beyer, from University of Freiburg, Germany. In 1982 the Rockefeller Foundation first started the initiative for Golden Rice. A meeting of researchers by various research groups was conveyed in 1992 at New York. Both the professors had met for the first time in the meeting and in due course decided to venture a project on the Golden Rice in 1999. Development led to expunge the deficiency of vitamin A

in rice eating countries. For which, they mainly focused on altering the complex pathway named as carotenoids metabolic pathway which is already present on the rice grain. Apparently, they concluded that only two insertion genes were needed to reset the whole carotenoid pathway (Paine *et al.*, 2005). Hence, they both required an additional skillful expertise in order to improvise their invention at large scale. Syngenta actively participated in the Humanitarian Golden Rice project. Many transgenics including Golden Rice with high carotenoids levels and good agrarian characteristics were produced by Syngenta. Performance data and seeds from these plants were gifted to the Golden Rice Humanitarian Board.

In countries consuming more rice Golden Rice 1 was developed to obliterate deficiency due to Vitamin A. Manipulation of only two beta-carotene genes involved in the biosynthesis of 1- phytoene synthase taken from daffodil plant (*Narcissus pseudonarcissus*), (Schledz *et al.*, 1996) 2- carotene desaturase (*crt1*) from the bacterium present in the soil (*Erwinia uredovora*) leads to the development of Golden Rice1 (GR1). This GR1 has approximately 1.6 g of carotenoids in total per gram of dry weight. With a conversion factor 1 µg of retinol to 6 µg of beta carotene. By consuming 200g rice daily, a rough estimate of 70 µg/day need of retinol can be met. The retinol content was very low and not able to fulfil the need of beta carotene (Zimmermann *et al.*, 2002). According to Golden Rice Humanitarian board project, children would have to eat Golden Rice 1 more than 10kg/day to get adequate amount of beta carotene.

In 2005 'Golden Rice 2' (GR2) was created by Syngenta with same construct as Golden Rice 1, only changes were made in the control section of one gene. The gene of Phytoene synthase isolated from *Z. mays* was considered as the most efficacious source. The insertion of phytoene synthase under the seed specific promoter leads to greatest accumulation of carotenoids. In the first trial conducted in 2004, Golden Rice1 grains contained 4 to 8 µg carotenoids per gram of total carotenoid. Comparatively, GR2 yields were approximately with 23 times more carotenoids than GR1 (Paine *et al.*, 2005). Subsequently, Golden Rice 2 was donated to the Humanitarian Project due to high carotene levels (Ye *et al.*, 2000).

By the end of the 20th century, Green Revolution gained advantage over 60% of the agriculture scenario. The paddies of rice were planted by the government and various research institutes. More and more reports started pouring reporting about the genetic manipulation of

crops, stating that it could be critical in contributing to raising the so-called yield ceiling reached by the regular in-practice breeding programs. Nowadays, an estimated 2 billion population suffer from malnutrition and weak immunity in the developing countries. To overcome from the above problem agricultural developments for fortification of the cereal crops has to be considered in third world countries. In this review we cover the of rice grain from morphology to chemical composition. An elaborative discussion on carotenoid biosynthesis pathway extension of lycopene pathway. And including the complete time traveled by golden rice and its advantageous character with Vitamin A as dietary supplements.

Rice as major food crop

Rice (*Oryza sativa*) is an important food crop consumed by around 3.8 billion people as a staple component of diet. Mainly Asian and African people consumed rice as major staple food. In some countries 70-80% calorie intake is from consumption of rice (Zhu *et al.*, 2018; Sasaki and Burr 2000). Rice is categorized as semiaquatic plant cultivated in 95 countries around the world (Coats *et al.*, 2003). It is the most important kharif crop and require heavy rainfall and high humidity and temperature (between 16°C -20°C) during the growing season. China is the substantial producer of rice; it produces 209.6 million metric tons per year. Meanwhile, India became the second largest consumer and producer of rice and produces 116.42 metric tons per year. Almost in the past six decades throughout the world, the production of rice has increased more than three folds from 15,000 metric tons in 1960 to 499,000 metric tons in 2019 (USDA 2020 Report). According to the data released by United States Department of Agriculture in 2019-2020 ~497.9 million tons per year milled rice are produced. Many Asian countries like, Indonesia, China, Pakistan, Vietnam, Bangladesh and Japan produced 90% of world rice (Ellur *et al.*, 2013; Sommers 1988). Population that relies mostly on rice due to financial obligation or cultural preferences golden rice stands a better chance to upscale the nutritional level. Therefore, we can say that by enriching rice a huge population can be targeted and the proper nutrition can be met.

Morphology of rice grain and its Chemical Composition

The grain of rice consists of an outer protective layer, hull and the caryopsis. The major components of rice grain are non-starchy carbohydrate. The rice grain

contains the highest amount of starch about 75% (mostly amylopectin) and 12% of water also contain smaller amount of protein around 7%. Basically, the paddy consists of husk (20%), bran (10%) and 70% milled rice (Lu and Luh 1991). Mostly the pollen grains lands on the stigma (male flower), the male gamete from the pollen, fertilize the ovule and polar nuclei respectively to form embryo and endosperm (Yoshida 1981). The rice kernel (unbroken) being the major product of the rice which is consumed. These kernels are also called as head rice largely determines the best crop quality. Rice grain require particular environmental conditions such as humidity, rainfall, and temperature to attain maturity.

The rice grains begin with the flower of rice and after fertilization approximately it takes 32 days to mature (Sreenivasulu *et al.*, 2018) when the all conditions are fulfilled. Different sections and part of the kernel of rice by weight are discussed below in the Fig: 1 showing the intact grain structure.

Over the last decade public interest is growing in the field of genetically modified crops (maize, wheat, rice) mainly focused on staple foods. Genetically modified crops can be defined as altering DNA sequence using molecular technique to increase nutritional, physiological value of the product. In developing countries where human populations are fully dependent on staple food such as rice, a lot of trouble is faced to improve vitamins deficiency (normal rice lacks vitamin A). Therefore, researchers focused to produce genetically engineered rice with better and enhanced nutritional aspects for example Golden rice 1 and 2 (Lu and Luh 1991) fortified with vitamin A.

Molecular Genetic Engineering Over Cross Breeding

Plant genetic engineering was first revealed in 1983. Also known as plant genetic modification or manipulation (Goldberg 2001). It opens up the doors for introducing the crops with valuable traits to produce crops with better nutritional value. It involves assigning the specific stretch of DNA into the plant genome, conferring it new or distinct trait. Later, in the early 1990s first GM crops were introduced.

Molecular genetic technique is more controlled and explicit as compared to traditional plant cross breeding. Genetic engineering permits the relocation of a specific gene into a bioengineered product. Plant breeding lacks specificity of several hundred genes that can be pass on from the parent plant to offspring.

Molecular biology precisely selects the specific traits that can be transferred from one plant to another. Here, the transferred genes are known but in plant cross breeding, there is no control on the number and specificity of genes transferred. Hence there can be a huge genomic scale change in plant cross breeding but in molecular genetic technique, appreciable changes in the protein expression of only the desirable gene are observed in the progeny as compared to parent plants (Lack *et al.*, 2002). Molecular biology technique has higher capability for producing greater diversity because it allows interspecies transferring of gene (Lack *et al.*, 2002). Genetic engineering has gained much importance as compared to traditional cross breeding as the specific genes are targeted and transferred. Cross breeding takes lots of trials and chances of error are higher in producing the desired phenotypes

Plant Cross Breeding V/S Genetic Engineering (Adapted from Lack *et al.*, 2002)

US Department of Agriculture (USDA) has approved more than 40 genetically modified crops for animal and human consumption worldwide (Bonneta 2001). In the first instance GM plants are designed to benefit the producers as well as consumers. Bio-engineered crops are designed in such a manner that they carry many desirable traits like herbicide resistance, increased productivity and yield. After few initial years emphasis was laid to develop various food crops with increased nutritional value such as increased vitamins levels to eradicate the vitamin deficiency (Falk *et al.*, 2002).

Figure: 2 Shows that contrasting features of Genetic engineering over plant cross breeding.

GM crops had not gained much importance in public till 2001. People still are not much aware about genetically modified crops. According to the survey of 2001 only 44% have 'heard' some information regarding genetically modified food (Pew Initiative 2003). But researchers kept on working in the field of GM crops to gain much more knowledge and to get the most of the desired traits in the crops of interest.

Importance of Vitamin A

Vitamins are major micronutrient that cannot be synthesized endogenously in sufficient amount. Humans meet the need for vitamins through the diet that they consume. Vitamin A is categorized as fat soluble vitamin (Ezzati *et al.*, 2004). This is an essential complex organic

molecule which is required for overall growth and development including maintaining healthy vision, for embryonic development and for healthy immune system (West 2018). Vitamin A plays a key role to develop immunity because it helps in WBC production to protect body from infection. Retinal, Retinol, and Retinoic acid are various kinds of Vitamin-A present in human body (West 2018). Dietary vitamin A which we take from plants is converted into retinol in the human body. Mainly green leafy vegetables, yellow vegetables, orange-colored fruits and broccoli are the good natural source of provitamin A. Vitamin A is also present in dairy, meats, cheese, and butter though quite rarely the elevated levels of vitamin A can also cause nausea, coma or respiratory failure and become toxic to human health (Oltikar 2001).

The deficiency of Vitamin A or VAD is an important nutritional challenge in regions rice is consumed as main food. VAD is most common in Southeast Asia because it is considered as rice eating region of the world. VAD has been recognized as significant public health issue in many United Nation countries. Night blindness due the deficiency of vitamin A and higher risk total blindness and result in xerophthalmia and keratomalacia as well. VAD is most prevalent among young children at the age of 5 and the developing fetus (pregnant women). Around 11 million children are dying due to malnutrition every year, deaths are increasing due to micronutrient deficiency. The deficiency of vitamin A is roughly seen in around 124 million children, of which about 500, 000 children go blind every year. According to the estimated data of World Health Organization (WHO) VAD prevalently affects preschool children (190 million) also affects pregnant and nursing women. Infants with VAD die prematurely due to poor health.

The lack in vitamin A also induces other health issues such as diarrhea, respiratory diseases and childhood disease like measles, etc. plays a vital role in the retina to form a pigment called Rhodopsin to promote a good vision in day and in dim light also (Ross 2010). Increased level of vitamin A also protect us from many genetic disorders like *Retinitis pigmentosa* (mainly affects the rod cells) in which patients are unable to seen in dim light and may get permanent blindness. As a result, it can be said that improved vitamin A nutrition could prevent 1-2 million childhood death per year. VAD deficiency also relates with keratinization of mucous membrane, gastrointestinal and urinary tract (Oltikar 2001). Study proved that beta carotene and vitamin A has anti cancerous properties. VAD are categorized

under the nutritionally Acquired Immuno Deficiency Syndrome (Semba 2012). At that time scientist already started to advocate focusing on the poor developing countries who are fully dependent on the starchy food such as rice and lacks crucial micronutrients such as fat-soluble vitamin (A) is particularly susceptible to VAD. In 2000 the number of blind people were estimated to be 18. 7 million in India. It was assumed the number of blind persons would increase to 24. 1 million to 31. 6 million till 2020 in India. India was reported to be home to the largest number of blind people in the world as per Times of India Oct 11, 2017

Fig: 3 above graph shows that proportion of children with severe visual impairment due to Vitamin A deficiency in different state of India. (Archives of disease in childhood 1995; vol -72 One of the causes of vitamin A deficiency in regions.

Basically, all of calories taken in comes from milled rice that has no Beta- carotene (provitamin A). This issue can be addressed by the insertion of specific gene responsible for the production of β - carotene through Genetic Engineering attain increased level of vitamin A in the rice endosperm by the successful manipulation of phytoene synthase gene in the rice endosperm. That described as Golden Rice with yellow/orange in colour, this has higher provitamin A precursor of β - carotene.

The Carotenoid Biosynthesis Pathway Extension of Lycopene Pathway

Therefore, through genetic engineering the genes responsible for enhancing the production of carotenoids precursor of vitamin A was introduced in rice. Basically, carotenoids are the pigments presents in plants, photosynthetic bacteria and algae. These pigments are responsible to produce yellow, orange and red colour in vegetables like apricots, broccoli, corn, carrots, are the major source of β -carotene.

Figure: – 4 Carotenoid biosynthesis pathway. As shown, the synthesis of lycopene and beta carotene originate from the isoprenoid pathway. The sequential addition of five - carbon isoprenoid units (isopentenyl diphosphate; the dimethyl diphosphate, DMADP) to form geranyl diphosphate; GDP (10 carbon), farnesyl diphosphate; FDP (15 carbon) and (geranylgeranyl diphosphate; GGDP (20 carbon) units an important initiation points for the synthesis of compounds that include steroids, carotenoids, xanthophylls, and anthocyanins (Ye *et al.*, 2000).

The plant pigment beta carotene is changed to vitamin A inside human body. Carotenoids are 40 carbon containing compounds. Juvenile rice endosperm is capable to produce geranylgeranyl diphosphate (GGDP), responsible for beta carotene biosynthesis are inserted in rice endosperm through genetic engineering.

Geranyl diphosphate produced in rice endosperm, is a precursor of beta carotene. It is not produced naturally in rice endosperm; it produces 20 carbon containing GGDP is a primary element of beta carotene. Consequently, it was obligatory to use genetic approaches to develop a rice that would synthesize beta carotene in the edible part of rice (Potrykus 2001). GGDP vital intermediate in isoprenoid biosynthesis, carotenoids also belong to the isoprenoid class. Isopentenyl- diphosphate are the precursor of GGDP synthesized in glycolysis. Number of isoprenylation and cyclisation of GGDP leads to huge essential products like tocotrienols, carotenoids, tocopherols and few phytohormones (ABA- are categorized as plant growth regulator responsible for seed dormancy and GA- for cell elongation).

In case of plants in order to convert geranylgeranyl diphosphate to beta carotene basically three succeeding steps are necessary to convert phytoene to beta carotene: (1) phytoene desaturase (2) ζ - carotene desaturase (3) lycopene β cyclase. Phytoene desaturase ζ -carotene desaturase both are required to introduce a double bond to form lycopene and lycopene β cyclase is needed to form the ring in beta- carotene. To synthesize 40 carbons

containing β carotene from GGDP, mainly three enzymes are involved. Plant enzymes named as-phytoene synthase, carotene desaturase and lycopene β cyclase isolated from various plants and bacterium. Phytoene synthase (PSY) gene is isolated from *Zea mays*, and carotene desaturase from the soil bacterium *E. uredovora* combined to develop Golden Rice 2 with increased level of beta carotene.

Thus, carotenoid gets oxidized and prone to degradation it has 25 days of half-life after harvesting. Such oxidative degradation of carotenoids is seen to occur in almost all carotenoid synthesizing tissues. There are only few enzymes that catalyze beta-carotene that are identified recently (Koschmieder *et al.*, 2021: 2022).

Some other factors can reduce beta carotene content for example poor storage practice and choice of cultivars used. To augment the improvement of Golden Rice or to reduce the probability of damage to Golden rice 2 we can adopt few strategies. One by amplifying the production of beta carotene during the development of rice endosperm. Secondly, to make the process of oxidation of beta carotene slow some alternative way should be looked for. Another provision could be to distribute the fortified rice from fields to homes directly. A lesser storage period can help the beta carotene to be intact and prior getting oxidized the rice can be consumed. This would need to be implemented from farmer to household in a smooth manner through concerned government authorities and right policies.

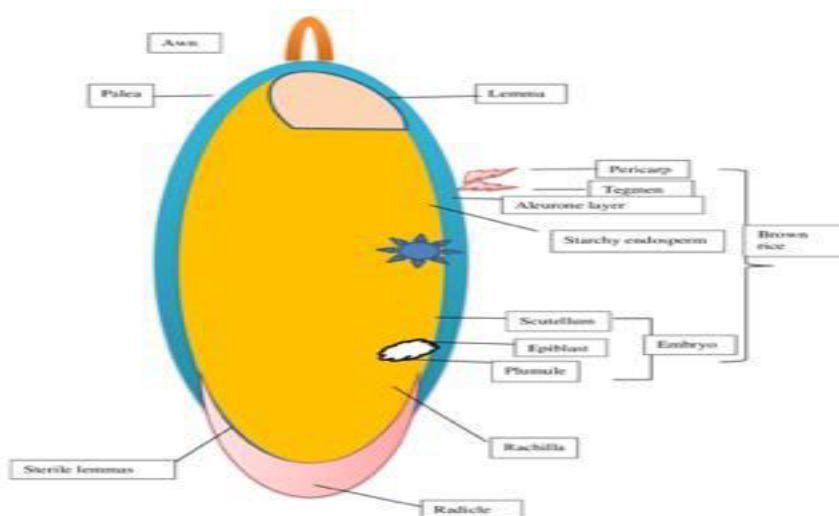


Fig. 1 The intact grain structure of rice

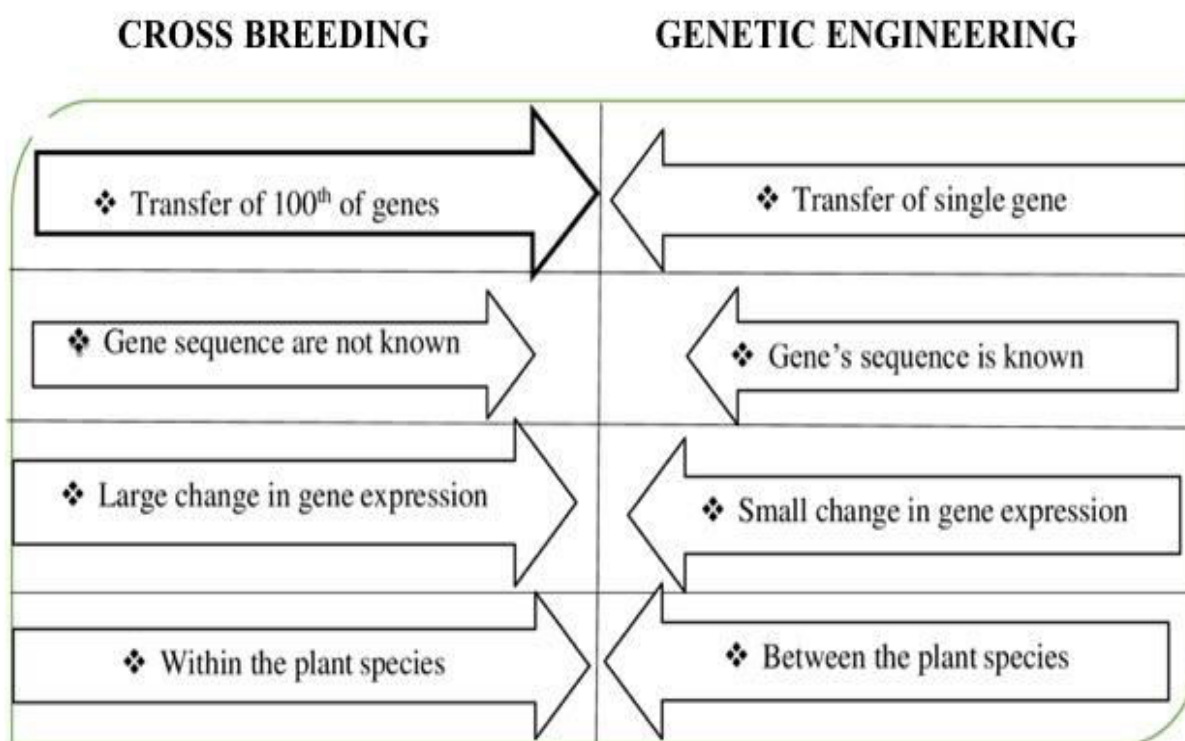


Fig. 2 Shows that contrasting features of Genetic engineering over Plant cross breeding.

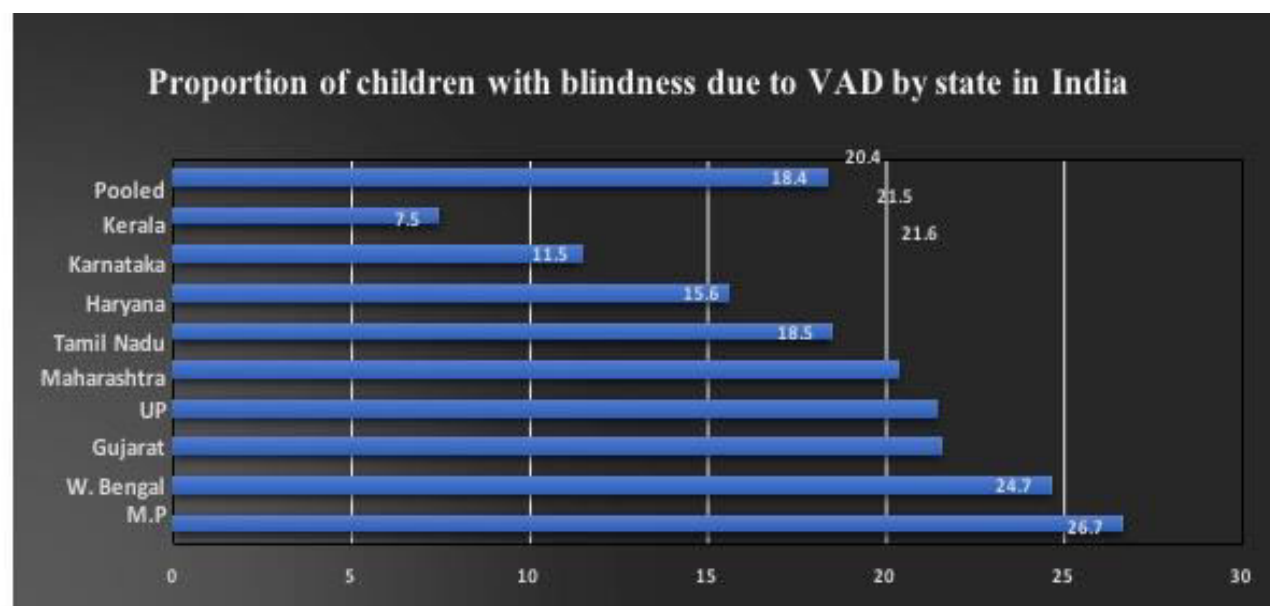


Fig. 3 The graph shows that proportion of children with severe visual impairment due to Vitamin A deficiency in different states of India. (Archives of disease in childhood 1995; vol -72 Rahi et al.

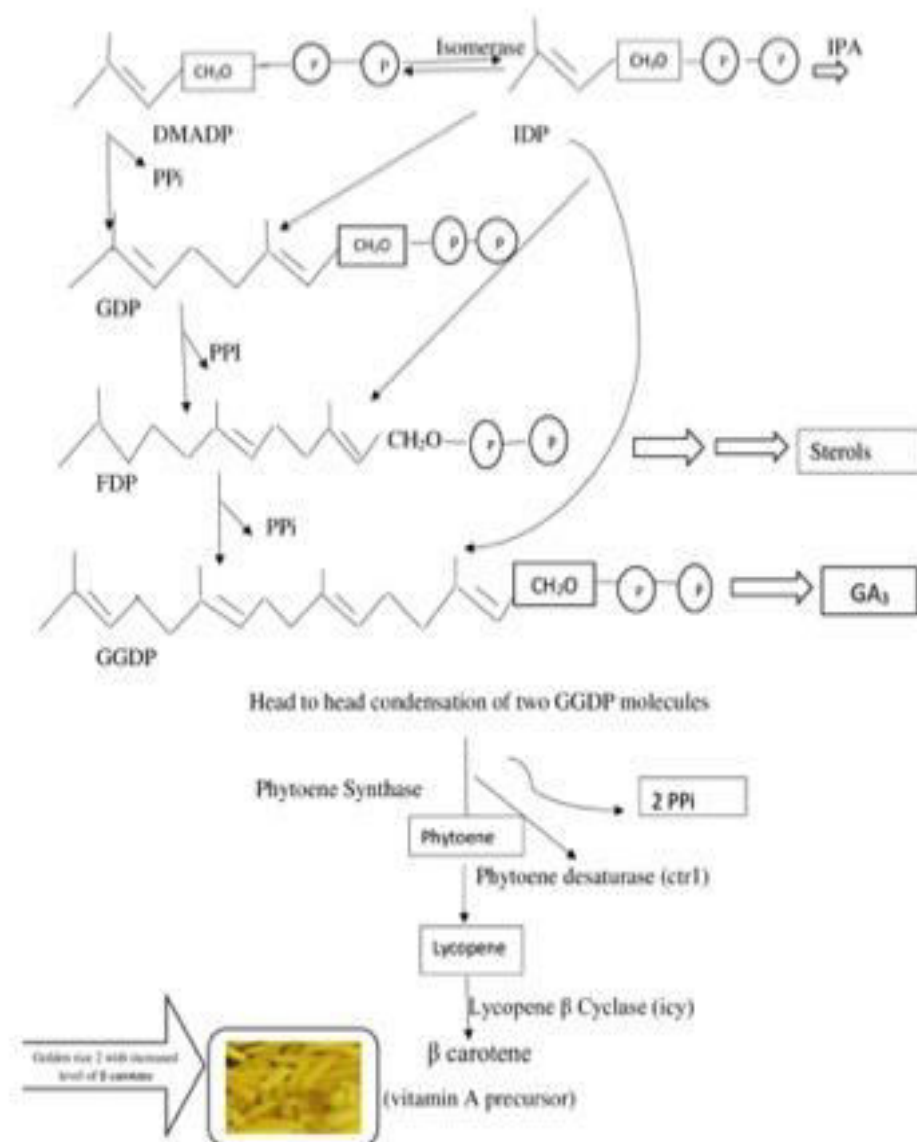


Fig. 4 Carotenoid biosynthesis pathway

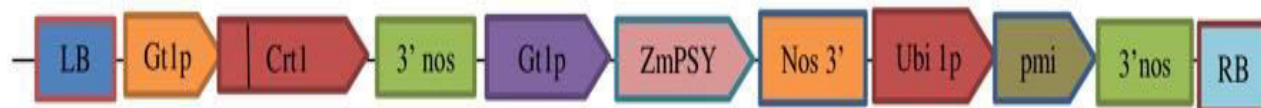


Fig. 5 Figure shows the constructs for the production of Golden rice 2 - GRE2 is produced by transformation with the improved construct pSYN12424

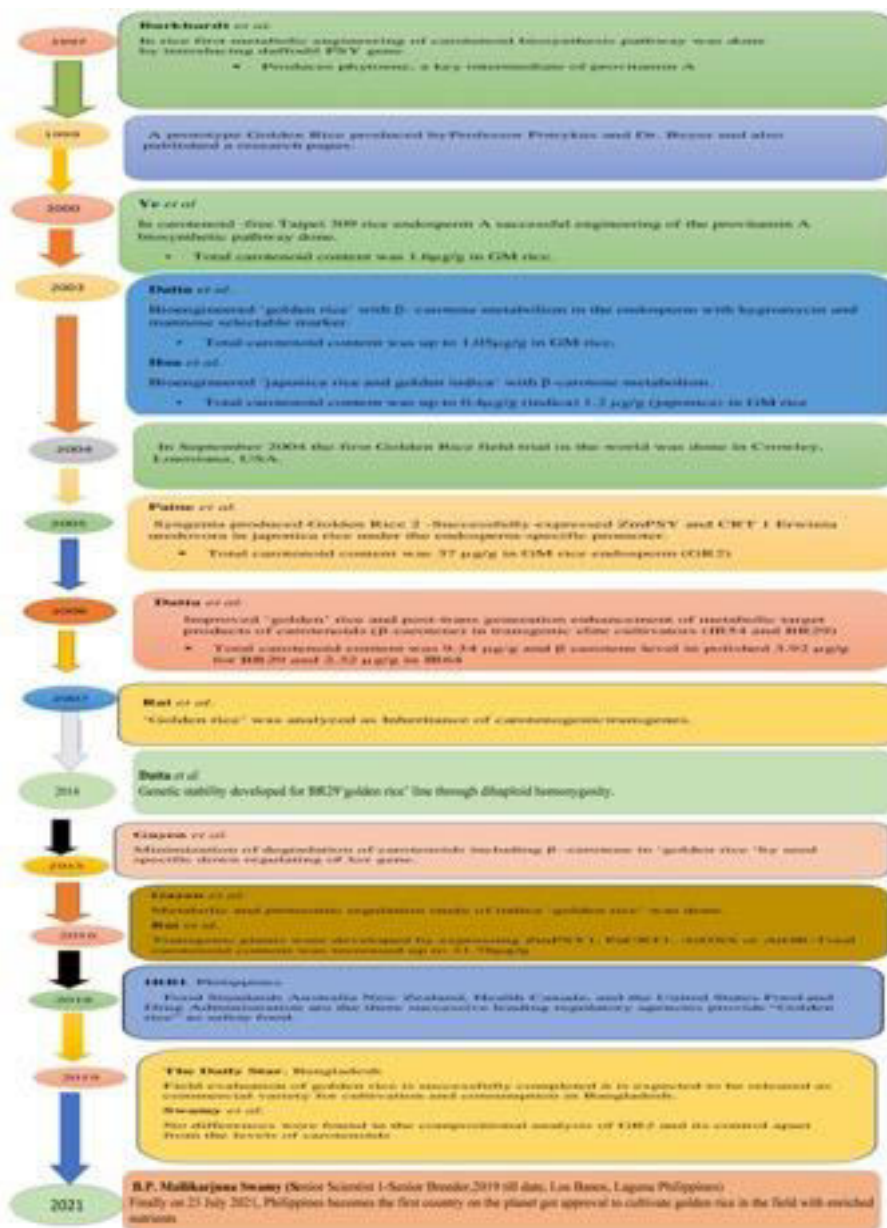


Fig. 6: A Timeline achievement of Golden Rice

Genetic manipulation to develop golden rice 2 (enriched with Vit A)

Golden rice 2 was constructed to exalt the level of beta carotene precursor of provitamin A in the edible part to combat the deficiency of vitamin A. Because no rice agronomist could produce carotenoids in the rice endosperm but cultivators are able to produce the precursor, GGDP. Golden Rice 2 are homologous to the Golden Rice 1 because similar genes were used in

Golden Rice 2 construct and enzymes that participate in the carotenoid biosynthesis pathway are similar in both constructs. Only the mode of isolation of genes varied. The gene encoding phytoene synthase was isolated from *Zea mays* and carotene desaturase from *E. uredovora*. Golden Rice 2 construct with genetic controlled element are discussed below in the Figure 5. To introduce the DNA construct into *Oryza sativa*, *Agrobacterium tumefaciens*-mediated transformations was used Pmi (phosphomannose isomerase; also known as mannose-6-

phosphate isomerase used as selectable marker for the transformation. Further work by Syngenta proved that a *psy* gene from maize was much effective than the daffodil phytoene synthase. Subsequent research confirmed that lycopene β cyclase was not required to produce beta carotene in the endosperm only insertion of two genes is required to construct Golden rice.

Figure: – 5 above figure shows the construct for producing Golden rice 2 - GRE2 with the improved construct pSYN12424. It has endosperm specific-glutein promoter, Phytoene desaturase (*crt1*) and maize phytoene synthase (*ZmPSY*) genes both driven by *Gt1p* and phosphomannomutase (*pmi*) as selectable marker gene combined to the ubiquitin gene promoter (*ubi1p*), Pea Rubisco small subunit (*rbcS*) chloroplast transit peptide sequence, neomycin phosphotransferase is used as terminator (Ye *et al.*, 2000)

People involved in the Golden Rice project and humanitarian board have focused beyond Golden Rice to the development of a biofortified rice in which the levels of micronutrients and vitamins are enhanced. They targeted to develop a rice variety with increased level of iron and zinc content. Iron deficiency estimated about 30% world's population leads to major global disorder. In polished rice, removal of aleurone layer and outer bran layer which are the site for accumulation of high content of vitamins.

Removal of outer layer of rice endosperm in the polished rice to avoid spoilage due to oxidation of fats and oils present in the outer layer. Due to this, zinc and iron accumulation is low inside the grain. Zinc and iron are essential as they assist the absorption of provitamin A in human gut. Due to this genetic manipulation done through genetic engineering in rice to enhance the production of Beta- carotene in the edible part of rice. Another growing concern of the decade that almost every child is malnourished in developing countries and therefore are more vulnerable to diseases.

This causes learning dysfunction, weak immunity, mental retardation, blindness and premature death. In general, high rates of malnutrition are seen in the region where the people consume less than 2, 000 calorie per day. In 1999 Golden Rice was constructed through conventional breeding. Golden Rice serves as the first purposefully created biofortified crop. In 2002 the term biofortification was used for the first time and in 2004 it was defined for the first time (Ye *et al.*, 2000; Welch and Graham 2004).

In 2003 a not-for-profit public-sector program “Harvest Plus” was started to biofortify staple through GM approaches. To benefit the poor, in 2016 biofortification through conventional breeding was rewarded by the World Food Prize (Welch and Graham 2004). Biofortification designed as a nutrition specific intervention to elevate the amount of micronutrient in food by the use of agronomic practices.

The aim of biofortification is to promote the development of staple crops such as rice competent to accumulating the increased level of micronutrients in the edible portion of staple foods. Golden Rice specifically mentioned as biofortified crops. In Golden Rice biofortification was obtained by genetic manipulation in the plant through genetic engineering to produce β carotene provitamin A in the rice endosperm. The manipulation of particular genes permits the rice plant to alter a certain metabolic pathway mainly carotenoid biosynthesis pathway to produce β -carotene in the grain. In the late 1990's after several years of works this feat was accomplished by professor emeritus Ingo Potrykus, of the Federal Institute of technology, Switzerland and Peter Beyer of the university of Freiburg, Germany (WFP 2016). This was considered as a technical milestone in Agrobiotechnology (The World Group Report 2017). The biofortified products presently being employed to expunge malnutrition.

Golden rice with advantageous character – Vitamin A as dietary supplements

Golden Rice can be advantageous for the human populations because it serves as major source of dietary supplements required for the human body. Vitamin A and beta carotene have been associated with declining many health issues like respiratory disease, cancers and macular degeneration (Romer *et al.*, 2000). Beta carotene work as good antioxidant, consequently it can defend the body from free radical formation (Milone 2003)

Figure: 6 A TimeLine Achievements in Golden Rice

Hunger serves as major macronutrient/micronutrient deficient, in the world wide 1995, 800 million population depend on food choices that are not appropriate in macronutrients like carbohydrates, lipids and proteins and micronutrients include minerals and vitamins (DellaPenne 1999). Vitamin A, Iron, Iodine and alpha Tocopherol deficiencies may lead to major disorders. Especially, VAD become the major cause of Night blindness, premature death and Xerophthalmia (severe

eye disorder described by pathologic dryness of the conjunctiva and cornea) (Romer *et al.*, 2000) Even industrial workers also suffer from VAD and mineral deficiencies due to poverty and malnutrition diet (DellaPenne 1999). Hence, staple food such as rice, widely consumed in many developing countries can serve be used to address the VAD. Therefore, the Golden Rice enhanced the nutritional quality of rice and widely dispersed to eliminate VAD.

Golden rice and intellectual property rights (IPR)

The successful engineering of carotenoid biosynthetic pathway in Golden Rice has led to the subsequent expression of beta-carotene. This is due to the specific involvement of the obsolete complexity of carotenogenic pathway along with the plant metabolic systems that interrelates with this. These major complexities both scientific and technical ones utterly involve the intellectual/technical property of Golden Rice (Kryder *et al.*, 2000). The management of Agri-biotech IPR must be taken into account for the whole procedure to fulfill the idea of supplying the product to subsistence farmers without any charges and restrictions especially in the developing countries.

The successful relocation of golden rice to the rice eating countries is again a major challenge for the Agri-biotech industries to face. Inspite, of being synthesized in public laboratories by possible funding's for the cause of humanity, delivery of golden rice faced an immense contradiction. It was found that the research behind this project involved the use of scientific and technical procedures secured by about seventy IPRs and TPRs that belonged to thirty-two different universities & companies. Despite the fact of involvement of number of scientific and technical patents in the production of Golden Rice. Syngenta, a commercial partner was able to negotiate with the inventors of technologies i. e., public scientists. The agreement intended to meet the humanitarian cause provided the Humanitarian Board with the opportunity to sublicense the agencies working on the Golden Rice Project in developing countries free of costs.

It was very clear during the onset of Golden Rice Project that once the research gets success, the product should be developed which meets the deficiency of Vitamin A among the recipients of developing countries. Therefore, this should be a stringent humanitarian project. However, the institutes/ universities could not develop products. On the other hand, industries can develop the research

into products. Hence, in the university of Freiburg and ETH Zurich the industrial partner Syngenta took over the charge of converting the results generated in into commercially deliverable products.

The benefaction was grounded on the fact that Syngenta would retain commercial exclusivity for the technology involving the huge agricultural practices in developing nations.

The Sublicensing Agreements

Inventors have authorized the exclusive rights to Golden Rice Technology. Furthermore, Syngenta put on few additional technologies for which the licenses were arranged with other companies. Syngenta also have given license to the inventors with the right to sublicense the public research areas & subsistence farmers of developing nations. The agreement also denied the export (except for the license) as the aim was to comply with the daily demand of Vitamin A among the poor sections. It was negotiated that golden rice cannot be released in countries that lacks biosafety rules. It was also taken into account that a proper assessment of food safety, bioavailability and allergenicity is done before the human consumption. Besides, the golden rice material was made available to only public researches with a compulsion of proper use and handling of genetically modified plants as per the local guidelines and other rules and regulations. Further the research institutes will transfer the traits of golden rice into the locally adapted lines using conventional breeding approach or other transformation methods. The environmental impacts and other socio-economic impacts are also to be taken care of so as to make sure that Golden Rice is benefiting the poor section without causing any environmental destruction.

In conclusion, day by day the deficiency of Vitamin A has become a crucial issue. Deficiency of macro and micronutrient are majorly seen in rice eating regions, (zinc and iron deficiency). To overcome these kind of deficiencies Golden Rice came as biofortified rice. The necessity and utilization of Golden Rice 2 are similar as Golden Rice 1. Construct for designing Golden Rice 2 was homologous to Golden Rice 1; though, in Golden Rice 1, the gene isolated from daffodil has been replaced by a more efficient gene analogue isolated from *Zea mays*. Golden Rice 2 is proved to have increased carotenoid level as compared to Golden Rice 1. According to the recent published data in many journals Golden Rice 2 is considered to as safe to be used as

staple food in rice consuming countries. Golden Rice 2 confers the increased level of beta carotene that helps to eliminate vitamin A deficiency. By the virtue of the traits incorporated in golden rice, it has gained enormous importance and advantages in the field of genetic engineering. It comes under second generation GM crops which has a high amount of micronutrient and helps to reduce nutritional deficiency among the poor and underprivileged (Khan 2023).

Hence, for the Asian sub-continent mostly rice consuming countries Golden rice 2 can be an answer to pervading situation of Vit A deficiency. Government policy makers should quickly adopt this technology in order to promote the cultivation of this biofortified rice. Also, along with adaptation the thought process that comes with the hitch regarding the implementation of GM crops also needs to be addressed. A flow of positive mindset counting on the proven scientific advantages should be emphasized more for larger acceptance. The unnecessary reluctance in the adaptation process though anti-campaigning by opposing parties has to take a back seat.

A thoughtful debate rather than magnified negativity towards a better technology is the need of time. This is important as this will decrease the overall negativity and rejection attached with GM and increase the acceptability. This will be a thrust to the adaption of more biofortified crops in days to come. Instead of framing a burden, one should be concentrating that golden rice will address a deficiency problem that has many detrimental effects. So, the dissemination of golden rice can be a preferred way to augment the need of Vitamin A in otherwise deficient rural population. The burden of micronutrient deficiency is high and a need to switch over to balance healthy diets is needed. The challenge of providing the weaker section with required doses of complete nutrition is ever demanding. Equipping the staple diet with such biofortified nutrients will add value to the overall diet on a daily basis.

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Conflicts of Interest

The authors declare there is no conflict of interest.

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