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Golden Rice to Eradicate the Vitamin A Deficiency in the Developing Countries

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The development of Golden Rice recently has taken longer than foreseen. Vitamin deficiency is a major medical issue that influences millions of people worldwide. UN Cartagena protocol for biosafety delayed particularly by deferring the determination of phenotypes developed in the open field. In this way, Golden Rice has not possessed the capacity to help with combatting vitamin an insufficiency as golden rice demonstrates fighting hidden hunger, as rice is the dominant crop in most of the Asian countries also staple food so people mostly rely on rice as energy source. Iron, zinc and vitamin A dearth are more dominant in rice consuming countries its named hidden hunger and it affects two billion people worldwide. VAD affect 190 million children and 19 million pregnant women worldwide, 100 grams of uncooked Golden Rice are able to supply up to 57 percent of the

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estimated average requirement (EAR) for vitamin A of pre-school children and from 38-47 percent of the EAR for pregnant and lactating women so far so good Golden rice passed rigorous biosafety assessment in Philippine. To get a working pro-vitamin A (beta-carotene) biosynthetic pathway in rice endosperm, we presented in a solitary, joined change exertion the cDNA coding for phytoene synthase (psy) and lycopene b-cyclase (b-lcy) both from *Narcissus pseudonarcissus* and both under the control of the endosperm-particular glutelin promoter together with a bacterial phytoene desaturase (crtI, from *Erwinia uredovora* under constitutive 35S promoter control). This blend covers the necessities for beta-carotene union and, as trusted, yellow beta-carotene-bearing rice endosperm was acquired in the T0-age.

Keywords: Vitamin A Deficiency (VAD); genetic engineering; golden rice; Average Estimated Value (AEV).

1. INTRODUCTION

Vitamin: vitamin exists an organic compound including a vital nutrient that an organism entails in confined masses. An organic chemical compound is named a vitamin while the organism cannot amalgamate the compound in ample quantities and it must be obtained by diet**.** Vitamin deficiency occurs when there is a deficient or scanty dietary intake of vital vitamins. Ineffectual levels of vitamins may happen in an array of unfortunate conditions and make the body fragile and defenseless to many diseases.

Vitamin A refers to fat-soluble compounds found as preformed vitamin A (retinol) in animal products and as pro-vitamin A carotenoids in fruit and vegetables. The body contains 3 active shapes of vitamin A named retinol, retinal, and retinoic acid. AS is involved in the growth and differentiation of almost all cells in human and play a vigorous function in embryonic development, during the fetal development period and eye development as well as vision. Improved protect from Bacterial and Viral Infections [1,2]. Vitamin A is energetic for healthy surface linings of the eyes, mucous membranes, respiratory, urinary and intestinal tracts [1]. It legalizes the body proper immune system, and play a basic function in white blood cell production to protect the body from infections. Studies proved its anti-cancer property, beta-carotene and vitamin A lower the risk of many types of cancer. This effect could mainly be from a diet high in vegetables and not from supplements. Vitamin A supplements have been shown to increase the risk of cancer [1]. WHO report says a predictable 250 million preschool children are Vitamin A deficient these Area extensive proportion of pregnant women are Vitamin A scarce. A probable 250 000 to 500 000 vitamin A-lacking children develop into blind every year, half of them dying within 12 months of trailing their sight [3] two billion people with anemia worldwide half of them due to iron deficiency. Vitamin A deficiency is most usual among children and women of childbearing age,as it linked with increased susceptibility to infections, as well as to thyroid and skin disorders. Mostly people at all stages can be affected by vitamin A deficit because the body is not able to make it [4,5,6]. The recommended dietary allowance is 700 micrograms of retinol activity equivalents (µg RAE)/day for women and 900 µg RAE/day for men [7]. Research proved that 100 g of uncooked Golden rice are able to provide more than 57% of estimated average vitamin A requirement for pre-school children and 38-47% for lactating and pregnant women. Under-five mortality and population growth rate and latest GDP in China in 2015, it is predictable that 9.36% of the Chinese preschool┼age children were with VAD [8]. This WHO estimation coincides with the 9.23 [9]. Virtually 44-50% of preschool children in the South Asian section were affected by cruel VAD [10]. Study indicated that 178 million children under 5-year age have stunted growth while the global estimate of wasting is ~55 million whom 19 million were severely wasted, another study indicates that approximately 48% children below 5 years in India alone were stunted 43%, in Bangladesh and 37% in Pakistan [11]. furthermore, night blindness in playgroup children was the utmost in South-East Asia (82.4%) in contrast to especially low in Europe (1%) and roughly nil (0%) in America [9].

Rice is main vital food crop in the world and it feeds half of world population. According to USDA 2019/2020 globally Rice production forecast ~497.9 million tons of milled rice is produced annually, 80% of total rice is produced by small farmer for chiefly to meet family needs. 90% of World Rice produced by Asian country China, India, Indonesia, Bangladesh, Pakistan, Vietnam and Japan [12,13] World Rice production in metric tons [14].

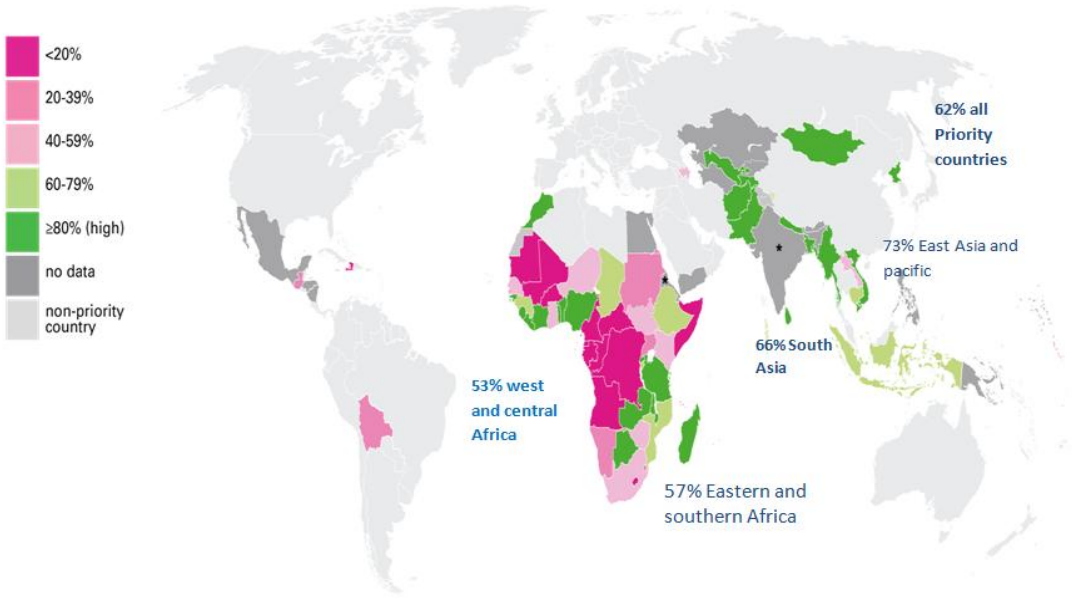

Fig. 1. Rice productive areas of world

*Figure SEQ Figure * ARABIC 1; % Children age 6-59 months that received too high dose vitamin A supplement in 2017 source. UNICEF global nutrition database, 2019, based on administrative reports from countries for the 2017 calendar year*

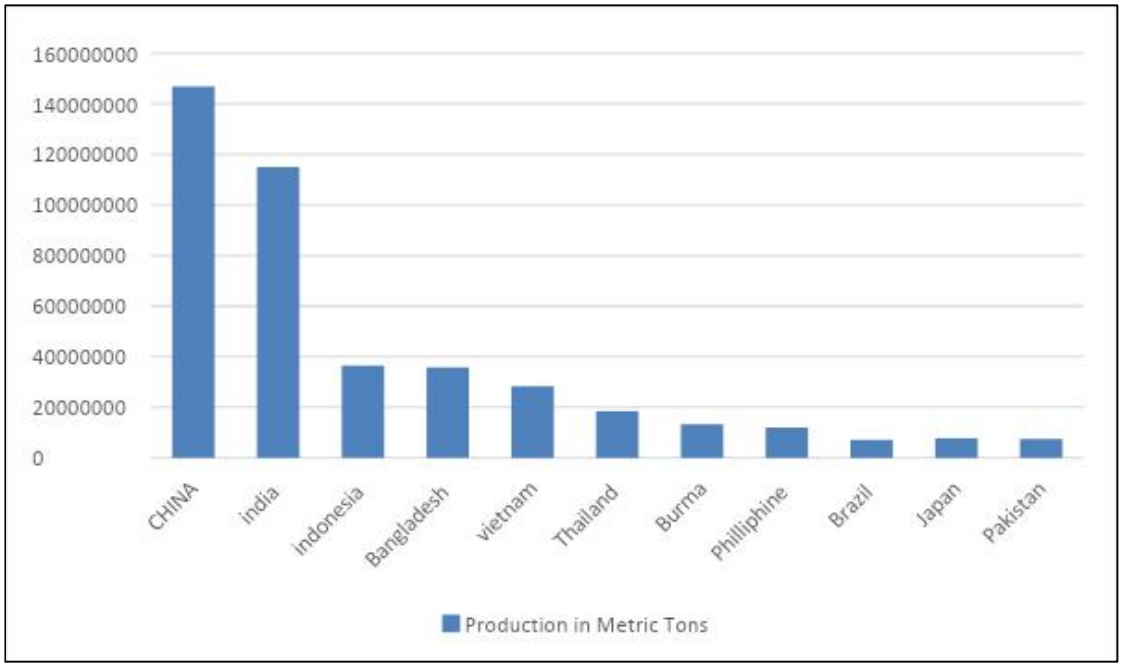

Fig. 2. Globally rice production in metric tons

Source: Statista 2020(51)

Due to inherent lack of vitamin A in rice endosperm cause severe VAD deficiency in countries eating rice as staple food together 26 rice eating countries mostly Asia and Africa [13,15]. Golden Rice was formerly conceived by Professors Ingo Potrykus and Peter Beyer and

their craving to bestow Golden Rice as a present to resource-poor farmers in developing countries. The research for Golden Rice is initiative in 1982 as a Rockefeller Foundations [16]. First time scientifically details of Golden Rice were published by the science in 2000 [17]. The product of eight-year project and the field trial of Golden Rice begin in 2004 by Louisiana State University Agricultural Center [18]. First time trials were conducted in Philippine, Taiwan and Bangladesh in 2015 [19]. Furthermore in 2005 Syngenta researcher created Golden Rice which possessed two genes and they shared the phytoene synthase gene as of maize with *crt1* as of the original golden rice so Golden Rice 2 construct 23 time more carotenoids as compare to Golden Rice 1, [20] One gram of GR contains up to 35 µg b-carotene per gram of rice [21]. 144 g of GR would be enough to obtain suggested dose of Vitamin A. Golden Rice is 7th winners of the 2015 Patents for Humanity Awards by the United States Patent and Trademark Office [22] Golden Rice field trial first held in Bangladesh has yielded promising results, and it is predictable so as to Golden Rice will be unconfined there near the beginning as 2018 [22]. American Journal of Clinical Nutrition study in 2012 about Golden Rice specified that beta–carotene in Golden Rice was create to be effective as pure beta-carotene in capsule even more effective than spinach.

2. METHODS USED TO INTRODUCE PRO-VITAMIN A (BETA-CAROTENOID) IN RICE KERNEL

Pro-Vitamin deficiency in Rice endosperm of all cultivar can attain only by Recombinant DNA techniques. After examined the Biosynthetic pathway it acts practicable to introduce the complete pro-Vitamin (beta-carotenoid) biosynthetic pathway keen on Rice endosperm by genetic engineering. Immature Rice Endosperm produces early transitional Geranyl geranyl diphosphate that is precursor of vitamin A biosynthetic pathway [23].

There are four beta carotene pathways enzymes to engineer the pathway towards beta carotene formation, namely phytoene synthase, phytoene desaturase, -carotene desaturase and lycopene beta-cyclase. Transformation efforts can be cut down by reducing the no of enzyme required and via bacterial carotene desaturases capable of introducing all 4 double bonds required. Agrobacterium-mediated transformation of pre-cultured rice immature embryo was designed to install the entire beta-carotene biosynthetic pathway into Rice endosperm in a single transformation effort. Three vectors designated pB19hpc combine the sequences for a plant phytoene synthase (psy) originating from daffodil (*Narcissus pseudonarcissus*; accession no. X78814) [24].

Protein Sequence:

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  1 mvvailrvvs aieipirlgf seanwrfssp kydnlgrkks rlsvyslytt skyacvgfea
 61 enngkflirs slvanpagea tisseqkvyd vvlkqaalvk dqtkssrkst dvkpdivlpg
121 tvyllkdayd rcgevcaeya ktfylgtllm tperrraiwa iyvwcrrtde lvdghnashi
181 tpsaldrwea rledlfagrp ydmfdaalsd tvsrfpvdiq pfmdmvegmr mdlkksrykn
241 fdelylycyy vagtvglmsv pvmgiapesl aeaesvynaa lalgianqlt nilrdvgeda
301 rrgriylpqd elaeaglsde dvftgkvtdk wrsfmkrqik rartffeqae kgvtelsqas
361 rwpvwaslll yrqildeiea ndynnftkra yvskvkrlaa lplaygksll iplslrppsl
421 ska
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Bacterial phytoene desaturase sequence (crtI) originating from *Erwinia uredovora* (accession no. D90087), the two being controlled by endosperm-specific glutelin (Gt1) and the constitutive CaMV 35S promoter, respectively [25]. The phytoene synthase cDNA controlled a 5-sequence coding for a functional transit peptide [26], while the crtI gene was fused to the transit peptide sequence of the pea Rubisco small subunit (tp), as constructed by Misawa et al. [27]. The development of lycopene in endosperm plasmids which is site of GGPP construction co-transformation was carried out utilizing two vectors, one (pZPsC) carrying psy and crtI, like in pB19hpc but lacking the expression cassette for the selectable marker aphIV and the other (pZCycH) providing, under glutelin promoter control, the sequence coding for the enzyme lycopene beta-cyclase, originating from *N. pseudonarcissus* (accession no. X98796) [28]. Like phytoene synthase, lycopene beta-cyclase carried a functional transit peptide, allowing plastid-import [26]. Plasmid combination can direct beta- carotene formation in rice endosperm. 800 pre-cultured rice immature embryos were inoculated with Agrobacterium LBA 4404/ pB19hpc.50 hygromycin-resistant plants

then were analyzed by Southern hybridization analysis. All tested lines carried the transgenes and most of the plants showed single insertions, but in some cases, multiple insertions were observed. For co-transformation, 500 pre-cultured immature embryos were inoculated with an Agrobacterium mixture of LBA4404/pZPsC carrying the psy and crtI genes and LBA4404/ pZCycH containing lcy together with aph IV as the selectable marker. Co-transformed plants were identified by Southern hybridization. All 60 randomly selected regenerated lines were positive for lcy, among which 12 plants were co-transformed with pZPsC. Like the transformation above, 1–3 transgene copies were predominant in co-transformed plants. Ten plants harboring all four introduced genes were transferred into the greenhouse for setting seeds. All plants from all transformations described here showed a normal phenotype as well as normal fertility [25].

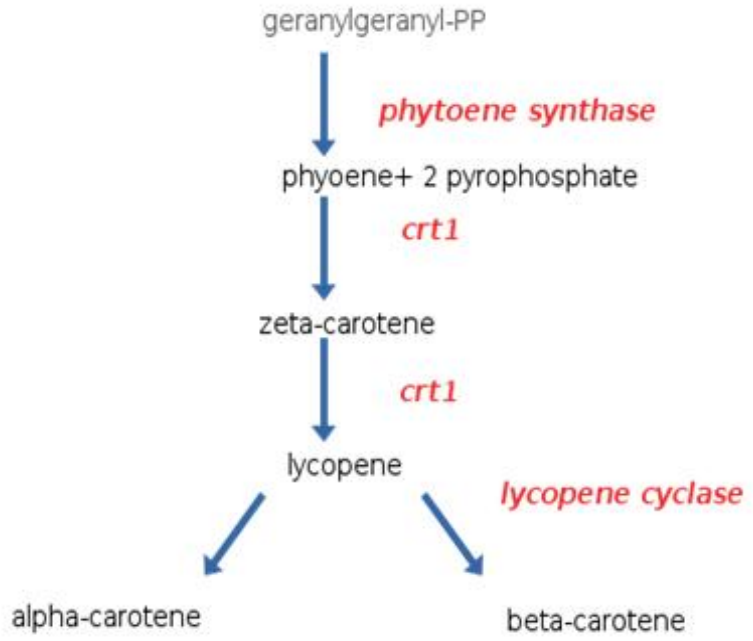

Fig. 3. Beta-carotenoid biosynthetic pathway in rice endosperm

Fig. 4. Milled rice and golden rice

1. One gram seed of each line was ground into powder to extract to depolarization by acetone. By photometrically quantified and HPLC qualitatively analyzed shows pathway completion to beta-carotene with additional lutein and zeaxanthin production at some level carotenoid pattern seems to be qualitatively quite analogous to that one present in green leaves. The

carotenoid content of 1.6 g/g dry rice endosperm was determined [25]. Two lines were parted one with beta carotene and another one produces a certain amount of zeaxanthin and lutein. Research proof as xanthophyll's is existing in eyes and deficiency basis macular degeneration that cause blindness [29]. Stability of Beta-carotene checked in cooked rice by adding 150% water of rice weight and 30 minutes duration prove beta-carotene amount same as in uncooked rice (0.99 or 1.53 mg b-carotene in a dose). 50 g uncooked Golden Rice, are rational serving size for children aged 4–8 year in rice-eating regions, who eat 130–200 g rice/d [24], would be able to provide >90% of vitamin A and EAR(275 μg retinol/d) or >60% of the RDA (400 μg retinol/d) [30].

A study published in 2012 American Journal of Clinical Nutrition distributed [31] about research including Chinese kids and the bioconversion of beta carotene from a solitary serving of spinach, beta-carotene in oil or Golden Rice. The field looks into had been finished in 2008, 4 years already. The information proved that a bowl of $<$100 to 150 g cooked Golden Rice (50 g dry weight) can give $<$60% of the Chinese Recommended Nutrient Intake of vitamin A for 6-8-years old children and its greater than spinach and proportional of beta–carotene getting from oil. August 30th, 2012 Greenpeace come out with a statement disapproving GMO Golden Rice with Chinese children as 'guinea pigs of American specialists.' Dr. Tang with 25 years' understanding of comparative research, and associates had beforehand led comparative research with Golden Rice in the USA with adults [32] and with children in China with other, non-GMO-edit wellsprings of beta-carotene [33] Only Tang's 2012 research with GMO Golden Rice was examined by Greenpeace. In 2001 Greenpeace issued a press released about Golden Rice in which they mentioned lack of vitamin in golden rice to acquire suggested vitamin A measure should eat 12 times more as 3.6 kg golden rice every day. Unquestionably in 2012, Tang research proved that Golden Rice can help with its Vitamin A deficiency. Over 20 years since its crated Golden rice has not been ended available to those for whom it was created. Vitamin A deficiency is a matter of life or death in developing countries as its still unfamiliar by the west because they can get it from different sources. If it had been allowed in those countries suffering from vitamin A deficiency millions of children would not go blind and can save millions of lives lost to malnutrition. Green leaves like vegetables, corn, cassava, sweet potato and sorghum ought to be produced to serve vitamin A and utilization of such a green product are helpful to battle Vitamin A deficiency each of them can add vitamin A to the human body.

3. GOLDEN RICE CHALLENGING

Technology for Golden rice is free but its acceptance depends on national government and governments do not want to keep on benefits-cost matric. No factor effect to introduction of GMO crops by different lobby groups [34] and the media role has urged and the Anti-GMO strategy has successfully fundraised for Greenpeace or friend of the earth. These lobbies have solid control over decision making people especially in developing countries they use the political systems to keep their concern [34] at the end decision maker come out with the decision delay of approval.

4. GOLDEN RICE CONFLICTING BY GREEN PEACE

Greenpeace launch worldwide movement is anti-GMO with the help of partners they claimed GMO food is Harmful, unverified and inefficiently synchronized. But GM crops and foods are safe and have been checked by enormous practice. As in 2018 Canada and USA food and drug administration (FDA) approved Golden rice safe for human consumption [35]. Since 1996 human has eaten billion of GMO created food from the past 20 years without any single case of harm to human health because of GMO consumption. After a long biosafety assessment process, Philippine approves golden rice for food, feed and different processes. Worldwide billions of animals ate GM feed since 1996. GMOs are safe at any rate crops formed with added breeding methods. The only time a safety disparity has been found the GMOs have been safer.

5. GREENPEACE CLAIMED

GE 'Golden Rice' being developed more than 20 years ago AS Greenpeace claimed food fortification, food supplement and cultivation of nutrition-rich like vitamin A and other nutrients at home level can eradicate VAD.

1: GE 'Golden Rice 'able to contaminate other non-GMO rice by different agronomic practices.

As rice is a self-pollinated crop here is a way of cross-pollination makes it possible to contaminate. If supplementary studies reveal unsafe properties of GE 'Golden' rice to communities/countries wherever rice is a staple, they may have their food and nutrition safety at risk to contamination and incapacity to recall and clean-up the crops [36]. Letter release by 109 Nobel Laureates questioned Greenpeace to "dump their campaign against 'GMOs [37]. The letter stated that Greenpeace has "misrepresented [the] risks, benefits and impacts" of engineered crops and avow that numerous studies have resolute that GMOs pretense no greater hazard to human health or the environment than conventional crops. VAD is particularly prevalent among the world's poor who exist roughly entirely on rice, as conventional rice does not enclose any vitamin A and foods that do are inaccessible to those in extreme poverty. Golden Rice seems like a potentially promising food that could help alleviate the suffering of literally millions of people, most of whom are impoverished children. So, what does Greenpeace have to say in response to this letter? Greenpeace's legitimate reaction starts with the reproach: "Allegations that anybody is blocking hereditarily built 'Brilliant' rice are false" [38] I am somewhat confounded in the matter of what they mean by this since around a half year prior the Philippine Supreme Court managed to support Greenpeace and set an entire ban on the container to utilize, import, commercialization, and proliferation of hereditarily designed harvests in the country [39]. Greenpeace's site even has an official statement praising the success and guarantees the peruser that the boycott "incorporates the very questionable 'Brilliant' rice" [40]. The International Rice Research Institute (IRRI) is situated in the Philippines and is the essential main thrust behind the advancement and kept testing of Golden Rice. Accordingly, a total ban on the utilization and spread of GMOs in the Philippines, as the consequence of legitimate move made by Greenpeace, viably obstructs the advancement of Golden Rice. The latest salvo is from Glenn Davis Stone, a Washington University in St. Louis anthropology professor. In an academic study released last month, "Disembedding Grains: Golden Rice, the Green Revolution and Heirloom Seeds in the Philippines," Stone not only pokes holes in virtually every feature of Golden Rice, he claims to address the role of international anti-GMO activists who have been criticized for contributing to the delay in the crops commercialization.

6. ACCORDING TO STONE

> *"Golden Rice is still not ready for the market, but we find little support for the common claim that environmental activists are responsible for stalling its introduction. GMO opponents have not been the problem."*

Different studies indicated that one bowl Golden Rice can supply 60% vitamin A dose recommended to a child's daily needs [41]. As WHO, the American medical association and national academy of science have accepted officially GMOs are safe as conventional food [42]. A study done on 900 individuals from the last two decades proved that there's no evidence of human health risk regarding existing genetically engineered crops and there is no environmental problem issued regarding GMO crops [43]. As beta-carotene found identical in golden rice compared to carrot [44] additional studies found that Golden Rice does not enclose any toxins or allergens [45]. Regarding Golden Rice the expenditures of resistance to GMO-Crop in India alone have been computed at $200 million every year for as long as a decade [46] 2010 worldwide vitamin A deficiency killed children more than even HIV/aids, malaria and TB [47]. 2012-13 field trial at different area held by Philippines rice research institute and IRRI as a major administrative process of GR2R golden Rice estimated trial area was distributed online and was monitored by a high-security fence and monitor 24 hours. As they monitored some area destroyed by GMO demonstrators later found that all demonstrator were not agriculturists. Philippine farming experts embraced to find and indict the people involved [48]. Field trial destruction was condemned by the local community [49]. Regularly business cultivators embrace new product assortments and attribute simply because of expanded gainfulness, and additionally simplicity of development or preparing both of which have monetary advantages [50] So far so good now Philippine is the first country to announce golden Rice for direct use also in 2018 Canada and USA declare it safe for consumption.

7. CONCLUSION

Golden Rice strain is a great achievement in GE era its design hereditary to supply vitamin A in rice-eating countries to eradicate vitamin A deficiency. It has had a long way of testing against human health and environment and improvements according to actual vitamin A requirement. Golden Rice's contentions against

progression and arrival do not have strong evidence in science to prove it harmful. Nutritional and financial studies shown that Golden rice can diminish mortalities and diseases related to vitamin A at a lower cost compared to other approaches. Delay over the last 10 years in India caused 1,424,680 lives losses.in India's annual perceived cost of US$199 million per year from the last decade to delay technology approval [51]. Dr. Tang's project proves that beta-carotene is the same in golden rice as available in spinach and oil [52]. Golden Rice is now at a third stage as it had done successfully research, laboratory and field experiments now standing at the last stage of large scale farming. Philippine government is the first to take the step for allowing golden rice for consuming and other rice practices thanks to the Philippine government for saving millions of children from VAD. The global community must insist on the dependence on science they are trying to improve nutritional qualities of the crop through conventional methods, breeding and modern biotechnology to approach for fortified crops for farmers and humans to make nutritional independence and improving life quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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