ECOCYCLES
Scientific journal of the European Ecocycles Society
2017

Ecocycles 3(1): 1-5 (2017) **ISSN** 2416-2140
DOI: 10.19040/ecocycles.v3i1.67

MINIREVIEW

From golden rice to drought-tolerant maize and new techniques controlling plant disease - can we expect a breakthrough in crop production?

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Abstract – This Minireview paper summarizes the views of the authors on the history and current status of golden rice – the highly promising concept of introducing new genes into existing rice strains to make them capable to accumulate beta carotene (the biochemical precursor of vitamin A) in the grain. Based on the questionable success of genetically modified organisms to achieve a significant increase in food and feed production we speculate on the possibility of a breakthrough in this area via the latest discoveries in molecular biological techniques.

Keywords – golden rice, genetically modified organisms (GMO), genome editing, CRISPR, food security, nutrition, fortified rice

Received: January 16, 2017 Accepted: February 6, 2017

This rice could save a million kids a year.

J. Madeleine Nash
Cover story, *Time Magazine*
July 31, 2000

Few GM crops are discussed as much - and misunderstood as much - as "Golden Rice".

Glenn D. Stone
Blog post on *fieldquestions.com*
August 28, 2015

Introduction

The last two centuries brought about revolutionary discoveries in agriculture: the use of crop rotation, mineral fertilizers, pesticides, and the development of new varieties of crops. In case of domestic animals the use of inbreeding combined with strict selection was successful (original idea by Festetics, 1819). Further contributions were the application the Mendelian rules of inheritance (Mendel, 1866), hybrid vigor (heterosis), as well as the natural dwarf mutants in wheat breeding (a major factor in the success of Green Revolution). Application of these methods led to a major rise in agricultural productivity that, in turn, resulted in a continuous decrease in food prices all over the world up until the beginning of the 21st century (FAO, 2016).

In contrast, there is a surprisingly great number of important scientific discoveries in agricultural development, which failed to achieve their full potential in the practical life. To mention a few: application of bacteriophages (virus pathogens) against bacterial infections, bioproduction of human enzymes in transgenic plants, male sterile insects against harmful pests in the field, antibiotics in animal husbandry and crop production, and biological control of weeds, plant pests and pathogens in the field.

We have been wondering, whether the technology based on the use of transgenic organisms (GMOs) in agriculture belongs to the first or the second category, and would like to illustrate the cause of our hesitation by taking the example of golden rice (IRRI, 2017). The primary cause of our concern is the apparent inability of the world to feed its population with nutritious food that is free from pollutants and toxins. Lack of sufficient amounts of food led dramatic price increases (FAO, 2016).

Advent of molecular biology

With the discovery of the structure of DNA and the development of methods to its sequencing and manipulation by restriction enzymes, molecular biology took giant leaps after the middle of the last century. Recombinant DNA technology (fusing genetic material from different organisms) created formerly unavailable research tools and found its way to industrial, medical, and agricultural biotechnology.

Scientific discussions on the possible hazards of recombinant DNA technology at the Asilomar Conference in 1975 led to a 16-month halt in research. Interestingly, although unknowingly, this halt in research set the first example of the precautionary principle. This principle was developed only in the 1980s as a strategy to cope with possible risks where scientific understanding is yet incomplete by taking actions to avoid or diminish scientifically plausible but uncertain harm.

'Flavr Savr' tomato

The biotech revolution led to the formation of many university spin-off/start-up companies. In 1994, one of these, Calgene, registered and started to market the 'Flavr Savr' tomato. This fruit was characterized by enhanced shelf life (as a result of the addition of an antisense construct which interferes with the production of the enzyme polygalacturonase) and also as the first transgenic food for human consumption. Unfortunately, the product was not successful: in 1996 Calgene ran into financial problems and was acquired by Monsanto (already a biotech giant at that time), and 'Flavr Savr' tomatoes were withdrawn from the market during the next year.

For decades recombinant DNA has been a promising avenue of research with the potential to do miracles traditional plant breeders could only dream of, such as herbicide resistant crops, pest and disease resistant plants (thereby eliminating the need for pesticides), engineering non-legumes to make them capable of biological nitrogen fixation, increasing plant tolerance to environmental stress (drought, salt, heat, etc.), making foods and feeds free from toxins and allergens, and increasing shelf life of produce. Although a very large number of transgenic bacteria, plants, and animals were developed for different (mostly research) purposes, only a few of the promises of this astonishingly powerful tool became reality (such as crops resistant to some herbicides and insect attack, and limited success with disease resistance) (Jones et al., 2014). As a result, criticism related to the underperformance of the technology made recently its way from fringe media to the mainstream, such as the *New York Times* (Hakim, 2016) and the *Economist* (Carr, 2016).

Golden rice: the origins

In the literature the concept of golden rice is often attributed to a Rockefeller Institute initiative in 1982. Unfortunately, this initiative is not readable on the web. However, there is reference to an International Program in Rice Biotechnology (IPRB) launched by the Rockefeller Foundation in 1984 in order to help solve some intricate problems of the nutritional content of rice (Rockefeller Foundation, 2016). When IPRB ended in 2000, by that time more than 700 scientists from 30 countries received financial support, including the laboratories of Ingo Potrykus (Swiss Federal Institute of Technology) and Peter Beyer (University of Freiburg).

Golden rice: development

Potrykus and Beyer took part in IPRB for eight years beginning in 1990. Their goal was to develop transgenic rice plants that produced high levels of beta-carotene (a precursor of vitamin A in humans) in amounts sufficient for the necessary daily intake of this vitamin. Vitamin A deficiency (VAD) is common in poor countries and claims the life of hundreds of thousands of children a year. Successful development of beta-carotene producing rice ('golden rice') was announced in 2000 (Ye et al., 2000) and received worldwide acclaim. Golden Rice 2 is an improved biotype characterized by higher accumulation of beta-carotene (Figure 1). In support, major biotech companies waived their patent rights for the methods used in the project.

Figure1. Kernels of wild type rice, Golden Rice 1, and Golden Rice 2 (picture from http://www.goldenrice.org/Content2-How/how1_sci.php, reproduced with permission of the Golden Rice Humanitarian Board www.goldenrice.org)

Golden rice: very slow progress

Unfortunately, the praised launch of golden rice was followed by disappointingly slow progress. Its field tests began only in 2004, and, according to the latest update (IRRI 2014) on the webpage of the International Rice Research Institute (IRRI) golden rice is "still under development and evaluation" and there is "…a research and development delay that is currently being assessed" (IRRI 2017).

On the positive side, golden rice (together with six other innovations) won the "2015 Patents for Humanity Awards" by the United States Patent and Trademark Office. On June 30, 2016, a letter, signed by more than 100 Nobel Laureates, urged Greenpeace and other environmental activists to end their opposition to GMOs and cease their efforts to block introduction of golden rice (Achenbach, 2016). Greenpeace's response was published on the same day, denying any role in blocking golden rice and claiming that golden rice has failed as a solution. Greenpeace also pointed out that golden rice has not been proven to address VAD (Greenpeace, 2016), in spite of the fact that the improved variety of golden rice has markedly elevated levels of beta-carotene.

Golden rice: future prospects

Luckily, during the more than two-decades long struggle to improve rice by genetic modification, alternative solutions for helping VAD sufferers were also developed. Of these, the low-cost intervention of periodically given high-dose vitamin A supplementation, the use of 'fortified rice' (the kernels are coated to include several critical micronutrients, including vitamin A; Kuong et al., 2016), and golden bananas containing naturally high concentrations of carotenoids (Buah et al., 2016) are the most promising.

At this point we would like to mention similar efforts to develop other genetically modified food crops with increased antioxidant power, such as the blue tomatoes (Zhang et al., 2013), potatoes and apples (FDA Newsletter, 2016) that do not turn brown when cut (both created by silencing polyphenol oxidase enzymes), and the "consumer quality" fruit pink pineapples (modified to accumulate high lycopene levels by increasing the rate of its synthesis and reducing its conversion to beta-carotene; FDA, 2016).

It is worth to note that sorghum plants containing elevated levels of vitamin E accumulate higher amounts of beta-carotene (Che et al., 2016): this finding may lead to improved varieties of golden rice.

We would like to add that a speedy registration of golden rice is further complicated by the possible teratogenic effects of all retinoids (including vitamin A) and derivatives (Lee et al., 2012). As a result, extensive food safety testing will be necessary when high-yielding golden rice varieties will eventually become available.

For the control of plant diseases a very new and perhaps revolutionary procedure has been published recently from different laboratories applying a gene silencing technique. Koch and Kogel (2014) silenced genes that are required for the biosynthesis of ergosterol. Suppression of ergosterol in a host plant (barley) seemed not harmful at all, while the pathogen (*Fusarium graminearum*) basically requires the synthesis of this particular compound. Therefore, following the gene silencing treatment, plants turned to be perfectly resistant to the fungal infection. Recently, it was also demonstrated that this gene silencing procedure can also be successful by spraying plant leaves with double-stranded RNAs against fungal (Koch et al., 2016) and viral (Mitter et al. 2017) pathogens. It is important to note that application of this technique allows the modification of plant traits without altering the plant's DNA.

Conclusions

We think that for the moment it is impossible to tell the future of golden rice: seeds may be available sometime during this year, but their widespread production may also take much longer time (Stone and Glover, 2016). This is true, because the exact causes of the delay in the development of golden rice are not known: they could be financial, biological, technical, agronomical, and many more. It is hypothesized that the genetic modification negatively influenced the ability of the development of the roots of rice plants, thereby reducing their yield under field conditions (Dubock, 2014). If indeed this is the case, the newly developed genome editing techniques (such as CRISPR) may save the golden rice project, because they target very specific regions in the DNA, in contrast to the 'classical' GMO method, where the point of gene introduction is random (Wang et al., 2016). The CRISPR technique is based on the immune procedure of bacteria or many archaea against viral (phage) infections and relatively simple and inexpensive. Many scientists believe that this is a revolutionary new method. At the moment, only a few crop modifications were carried out by this technique and its applicability in crop breeding remains to be seen (Wang et al. 2014). The very first commercial example of the use of this technique in the modification of a food crop is represented by the non-browning white button mushrooms (*Agaricus bisporus*) developed by knocking out the enzyme polyphenol oxidase (Waltz, 2016). The importance of this enzyme in plant tissue browning was first described by Szent-Gyorgyi and Vietorisz (1931).

We would like to mention that in our opinion the fair evaluation of gene modifying methods would be based on the results instead of the applied procedures themselves. For example, resistance to a particular herbicide can be achieved by introducing a transgene into the crop (GMO, e.g. soybean), or with the gene editing procedure (CRISPR, e.g. maize), or selecting a plant which has a natural resistance mutation against the herbicide (e.g. sunflowers). Although the end-product is a new herbicide resistant crop in all three cases, regulations in many countries treat them differently: GMOs plants are unacceptable, natural mutation is accepted, and the product of the CRISPR method yet waits for the decision of the experts or the uneducated public.

Finally, we find it regrettable that during the last decades academic research and private enterprises failed to develop technologies that could solve the world's ever-increasing food security problem. Here we propose that international organizations and governments should provide dramatically increased support for this vastly important goal.

Acknowledgement

We thank five unknown referees for their comments

that significantly improved this paper.

Open access statement

Public interest statement

Using the example of golden rice, in this paper we discuss the possibilities whether the recently developed molecular biology techniques may contribute to solve the ever increasing problem of world food security.

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