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Golden Rice and 'Golden' crops for human nutrition

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Abstract

Micronutrients are essential for a healthy life. Humans do not produce micronutrients, and hence they must obtain them through the foodchain. Staple crops are the predominant food source of mankind, but need to be complemented by other foodstuffs because they are generally deficient in one or the other micronutrient. Breeding for micronutrient-dense crops is not always a viable option because of the absence of genetic variability for the desired trait. Moreover, sterility issues and the complex genetic makeup of some crop plants make them unamenable to conventional breeding. In these cases, genetic modification remains the only viable option. The tools to produce a number of micronutrients in staple crops have recently become available thanks to the identification of the genes involved in the corresponding biochemical pathways at an unprecedented rate. Discarding genetic modification as a viable option is definitely not in the interest of human wellbeing.

Contents

Nutritional genomics

Owing to the rapid development of sophisticated molecular techniques over the past decades, progress in gene discovery has been extraordinary, and the knowledge of biosynthetic pathways has grown tremendously. The main reason is that all organisms share a good proportion of their genes and metabolic functions, making it possible to draw conclusions from one species to another and to do research on model organisms with short life cycles while taking advantage of an exhaustive genetic infrastructure already available, such as sequenced genomes and gene expression data [1,2].

The elucidation of plant metabolic pathways and their interconnections still remains a significant challenge, not least because of sheer numbers. Plants are extremely versatile chemical factories. An Arabidopsis leaf contains no fewer than 2000 different substances, and the entire plant kingdom can produce 200 000 or more different chemical compounds [3]. Many of these are so-called secondary metabolites and relevant for the plant's adaptation to specific environments. However, some of these are also relevant from a human nutritional perspective. Being essential

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components of the diet, these are termed 'vitamins' when organic, and 'trace minerals' when inorganic, and together are referred to as 'micronutrients'. 'Nutritional Genomics' is a discipline of modern biology that focuses on elucidating the biochemical pathways (vitamins) or physiological processes of uptake and mobilization (minerals) that are needed in plants to sustain human health.

Unequal distribution of micronutrients in plant tissues

As a whole, plants can provide all human dietary macro and micronutrients needed to live a healthy life as a vegetarian. However, micronutrients are usually unevenly distributed not only between plant species but more importantly also within different tissues of a given plant. Leaves often contain all necessary micronutrients, while the specialised storage tissues of grain, roots, and tubers – which make up most staple foods – show only limited biochemical diversity and at levels often insufficient for human nutritional needs.

For example, in the rice endosperm, the edible part of the rice grain, the micronutrients iron, folate, provitamin A, and vitamin E are present only at minimal levels while in the rice leaf they are present in quantities which would be adequate if rice leaves were apt for human consumption. For rice endosperm to meet human nutritional requirements it is, therefore necessary to modify the plant so that the endosperm accumulates the required nutrients. Alternatively, supplementation and fortification can and are being used to cope with the deficiency problems that arise from an unbalanced diet owing to reliance mainly on micronutrient deficient food staples. Predominant consumption of staples is a consequence of extreme poverty. Unfortunately, large parts of the worlds population survive on less than two dollars a day and hence can neither diversify their diets nor buy supplements.

Biofortification: Breeding vs. genetic modification

Micronutrient malnutrition is a continuing and serious public health problem in many countries. Supplementation and fortification-based interventions have been applied for decades. Although such interventions have shown considerable success in the past, they have not been able to eradicate the problem, mainly owing to the significant and expensive distribution logistics required which are insufficiently developed in poorer countries. Moreover, such interventions are not economically sustainable, requiring ongoing funding which may falter upon economic crisis or political turmoil. Experience with vitamin A supplementation programs, for instance, revealed that coverage achieved over the last decade in 103 priority countries has stagnated at 58%, with high year-to-year fluctuation [4]. In India, the 'Nutritional Anaemia Control Programme', mostly designed to address iron deficiency, has been in place since 1970 with little impact, because of mismanagement, underfunding, logistical problems, and poor compliance [5]. In recent years, coverage with iron folate supplements was around 30% for pregnant women and 10% for adolescent girls [6].

Biofortification, much advocated by the HarvestPlus research consortium (see http://www.harvestplus.org), is potentially a cost-effective and sustainable intervention, either as a stand-alone solution or in combination with supplementation and fortification. Biofortification denotes the 'fortification' of agronomically important crop plant tissues by means of their own biochemical capacity. Once the crops are developed the costs of biofortification – including development costs – constitute only a fraction of supplementation costs. Biofortified crops are intended to be distributed through the traditional pathways of agriculture and local trade. Golden Rice, for instance, should require little more than the costs of the normally reliable seed production systems for its continued deployment following introduction. Production costs will be no different from any other rice.

The costs of breeding are moderate – in the range of $4m per variety spread over 10 years – amounting to approximately 0.2% of the global vitamin A supplementation expenditures in the scenario described above over the same period [7]. The development and regulatory approval of a transgenic crop can be 5–8 times higher [8,9], still representing only a fraction of the sustained costs necessary for a classical public health intervention. These higher costs have the potential to be substantially reduced once a more scientifically based regulatory requirement is introduced.

Given these advantages, the often-debated question is whether all of the biofortification needs can be achieved by breeding techniques alone, including 'precision (smart) breeding' relying on genetic markers associated with the desired trait. The simple answer is 'no'. There are two main reasons for the need for genetic modification. In cases where the genetic variability for a given trait is too low to meet the target levels, breeding is not an option and one needs to resort to the knowledge gained in nutritional genomics research (see above) in order to apply recombinant DNA technology. The other case is with crop plants which are impossible or very difficult to breed.

In other words, biofortification of staple crop plant tissues can be achieved through breeding where this is possible, while recombinant DNA technology must be applied in all other cases. Some examples will be given in the subsequent paragraphs.

Some plants do not show adequate trait variability: the case of provitamin A rice (Golden Rice)

Accumulation of provitamin A carotenoids in rice grains cannot be achieved through breeding. Germplasm screening conducted at the International Rice Research Institute (IRRI) and elsewhere did not reveal any cultivar of rice capable of accumulating provitamin A in the grain that could be used as a parental line for further breeding. Consequently, genetic engineering needed to be applied to biofortify rice, with the aim of helping combat vitamin A deficiency (VAD) induced diseases, which are widespread in the tropics.

Vitamin A denotes a group of C_{20} carotenoid derivatives (retinal, retinol and its esters, and retinoic acid), which play an essential role in vision, immune response, epithelial cell growth, bone growth, reproduction, maintenance of the surface linings of the eyes, embryonic development, and regulation of adult genes. In humans, provitamin A carotenoids are cleaved in the centre of the molecule and converted into vitamin A retinoids. An early symptom of vitamin A deficiency is night blindness. Structural alterations of the conjunctiva and the cornea (xerophthalmia, keratomalacia) may follow, and subsequent inflammation and infection result in irreversible blindness. Depression of the immune system increases the severity of measles and diarrhoea, leading to a nine-fold increase in child mortality, which is apparent even before the appearance of xerophthalmia. According to WHO, an estimated 127 million preschool children are affected by vitamin A deficiency, with 250 000–500 000 becoming blind every year, half of whom die within 12 months of losing their sight (WHO database of vitamin A deficiency; http://www.who.int/vmnis/vitamina/data/en/index.html).

The Golden Rice technology currently being developed – known as GR2 – employs two provitamin A pathway genes (see [10], for review). One codes for the enzyme phytoene synthase, which has turned out to be the major rate-limiting step in carotenoid production in most plant tissues. The essential improvement in the development of GR2 over the previous GR1 version was to exchange the phytoene synthase from daffodil with the one from maize, thus leading to increased provitamin A formation [11]. The second gene (which codes for a carotene desaturase) is CrtI from the bacterium *Pantoea ananatis* (formerly named *Erwinia uredovora*). CrtI was used because plants utilise four genes (two carotene desaturases and two carotene *cis–trans* isomerases) to carry out the same reaction sequence leading to the provitamin A precursor lycopene. One single bacterial transgene can thus substitute for four plant transgenes. The enzyme activities upstream of phytoene and downstream of lycopene in the carotenoid pathway are all actively expressed in wild-type rice endosperm. Thus all that is needed is to reconstitute the overall biosynthetic sequence is to 'bridge the gap' [12].

During the past few years the trait has been transferred from the original cultivar into selected locally adapted rice varieties. This was done by a consortium of partner institutions in the Philippines, Vietnam, and India. Concomitantly, the event to be carried through the regulatory process was determined based on the datasets collected during the breeding process and instructed by the bioconversion numbers obtained, which showed that bioavailability of the provitamin A in GR2 is very high [13].

The case of folate and iron in rice

Folate, or vitamin B9, is an essential coenzyme involved in one-carbon metabolism. Folate deficiency is associated with a higher risk to newborns of neural tube defects, spina bifida, and anencephaly, and an increased risk of cardiovascular diseases, cancer, and impaired cognitive function in adults. Mandatory fortification of wheat flour with folic acid in the United States in 1998 was followed by a significant reduction in the prevalence of neural tube defects [14]. Folate deficiency also causes widespread megaloblastic anaemia during pregnancy and often exacerbates already existing iron deficiency anaemia [15], see also [17–19].

As with provitamin A, folate levels are very low in rice endosperm and so is the genetic variability of the trait. Thus, folate biofortification is another case for which genetic modification is required. A successful proof-of principle rice has recently been reported [16]. Here, the transformation of two pathway genes from *Arabidopsis thaliana* shifted folate production to levels in the grains which can be expected to meet the requirements necessary to combat its deficiency. Product development would be required but, inexplicably, has so far not met the interest of donor agencies. Other results demonstrate that recommendations for folic acid supplementation alone did not appear to succeed in reducing the incidence of open NTDs in Nova Scotia, whereas the fortification of grain products with folic acid did result in a significant reduction in the incidence [20].

Iron is a redox-active constituent of the catalytic site of heme and non-heme iron proteins. More than one-third of the world's population suffers from anaemia; half of it caused by iron deficiency [21]. Endemic infectious diseases exacerbate the incidence of iron deficiency anaemia in developing countries. Iron deficiency adversely affects cognitive development, resistance to infection, work capacity, productivity, and pregnancy. Children of anaemic mothers have low iron reserves, requiring more iron than is supplied by breast milk, and suffer from physical growth and irreversible mental development impairment [21]. It is estimated that 800 000 deaths are attributable to iron-deficiency anaemia annually.

Rice endosperm is a very poor source of iron, the variability between cultivars ranging from ca. 1 to 8 ppm. Although a study conducted with rice containing 6 ppm can have a positive impact on nutritional status [22], there is consensus that significantly higher levels would be very desirable. The lack of adequate variability in iron content of seed calls for transgenic approaches capable of increasing iron partitioning in favour of the grains. However, the knowledge base for this trait is still meagre; 39 genes are thought to control iron homeostasis in rice [23] of which the rate-limiting ones are currently unknown. More research in the field of nutritional genomics is required to answer this question.

In contrast to iron, a significantly higher variability has been found for the zinc content of polished rice grains (G. Barry, P. Virk, IRRI, personal communication) which makes this trait a likely candidate for precision breeding.

Some plants cannot be bred or breeding is very difficult

Banana (cooking banana: 'plantain') is the staple food in more than 50 countries; in Uganda, for instance, consumption is approximate to 220 kg per person per year. However, banana fruit as eaten contains only low levels of vitamins and minerals. Bananas constitute sterile triploids selected from the wild. This makes conventional breeding extremely difficult, which explains why bananas are propagated vegetatively. For this reason the species has been genetically static for thousands of years and is consequently susceptible to changing environments and biotic stresses, such as attack by fungal diseases, as well as by bacteria and viruses. The banana variety 'Gros Michel' has been lost almost entirely to the so-called 'Panama Disease' caused by the fungus *Fusarium oxysporum* because breeding for resistance was not possible. Because banana-based diets are deficient in provitamin A, iron, and vitamin E, a project employing genetic transformation is underway to increase the level of these micronutrients. The project is being led by the Queensland University of Technology, Australia, in partnership with scientists at the National Agricultural Research Organization of Uganda (see http://www.grandchallenges.org).

Cassava ranks number five among human staple foods. In sub-Saharan Africa, however, it is number one because of its ability to provide food security in drought conditions. However, the micronutrient content is low and there are other problems with this crop such as the content of toxic cyanogenic glycosides and post-harvest deterioration, which minimises shelf-life. Cassava is vegetatively propagated, like banana. By contrast, however, it can be bred but has never been pushed to produce genetically uniform inbred lines. Cassava plants therefore, possess a very complex, heterozygous genetic makeup, which renders varietal recovery very difficult. Long breeding cycles and asynchronous flowering represent further hindrances to breeding cassava for specific traits [24]. Consequently, projects employing genetic modification are underway at several institutions, such as The Danforth Plant Science Center (USA) partnering with several Universities and African institutions (see http://www.grandchallenges.org), and at CIAT (Colombia), partnering with the University of Freiburg, Germany. All of these have made significant progress (Richard Sayre, Danforth Center, personal communications) on all fronts and have developed proof-of-principle results showing that nutritionally improved versions of cassava can be produced through genetic modification.

Potato, as a vegetatively propagated crop plant, shares the issue of genetic complexity with cassava and has also been targeted to increase provitamin A content. This was successful; however the installation of a 'mini-pathway' was required to arrive at high provitamin A levels [25].

Needs for the development of nutritionally improved crop plants in the public sector

The examples given above are not at all comprehensive; the list could be extended by further cases of product-focused transgenic research conducted by public-sector scientists both in industrialised countries as well as in developing nations.

The public sector needs to resource these developing country targeted projects as they do not represent commercially valuable targets and therefore cannot be a commercial priority for the private sector. However, the private sector does have a potentially valuable role to play in pro-poor projects if skill is applied to structuring public–private partnerships so that all parties benefit. Golden Rice is an example where crucial phases of technology development have been mastered for pro-poor purposes by partnering with Syngenta AG (Basle) and by carefully defining complementary commercial and non-commercial (humanitarian) exploitation rights of combined technologies originating from both the public and the private sector.

Public-sector research funding is needed in basic science to further improve the arsenal of identified genes, together with molecular and genetic knowledge of pathways involved in the biosynthesis and biodegradation of nutritionally important micronutrients or in mineral homeostasis. However, the detailed knowledge gained from model systems must also be more effectively extended to crop plants, such as by understanding rate-limiting steps of metabolic pathways and pathway regulation. Where genetic modification is needed, better toolsets need to be developed for many crop plants grown in the tropics, such as promoters conferring precise tissue specificity of gene expression or more effective plant transformation protocols.

With genetically modified plants, the public sector also needs to learn and apply the skills required to proceed beyond the purely scientific or proof-of-principle state of their work. A complete set of highly specialised skills, not currently common in the public sector, is required to drive a genetically modified plant through the processes involved in regulatory approval and beyond. For example, more specialised knowledge is needed in the areas of community engagement, communication, social marketing, and nutritional extension and education, requiring partnerships across disciplines as well as considerable management skills.

These public–private and multidisciplinary partnerships and their management require sustained funding, given the fact that for many traits and crop plants genetic modification is not an option, but rather a necessity to deliver sustainable nutrition in staple crops in developing countries.

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